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Hydrogen peroxide-mediated cell-wall stiffening in vitro in maize coleoptiles

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Abstract. It has recently been proposed that H_2O_2-dependent peroxidative formation of phenolic cross-links between cell-wall polymers serves as a mechanism for fixing the viscoelastically extended wall structure and thus confers irreversibility to wall extension during cell growth (M. Hohl et al. 1995, Physiol. Plant. 94: 491–498). In the present paper the isolated cell wall (operationally, frozen/thawed maize coleoptile segments) was used as an experimental system to investigate H_2O_2-dependent cell-wall stiffening in vitro. Hydrogen peroxide inhibited elongation growth (in vivo) and decreased cell-wall extensibility (in vitro) in the concentration range of 10–10000 $\mu mol \cdot l^{-1}$. In rheological measurements with a constant-load extensiometer the stiffening effect of H_2O_2 could be observed with both relaxed and stressed cell walls. In-vitro cell-wall stiffening was a time-dependent reaction that lasted about 60 min in the presence of saturating concentrations of H_2O_2. The presence of peroxidase in the growth-limiting outer epidermal wall of the coleoptile was shown by histochemical assays. Peroxidase inhibitors (azide, ascorbate) suppressed the wall-stiffening reaction by H_2O_2 in vitro. Hydrogen peroxide induced the accumulation of a fluorescent, insoluble material in the cell walls of living coleoptile segments. These results demonstrate that primary cell walls of a growing plant organ contain all ingredients for the mechanical fortification of the wall structure by H_2O_2-inducible phenolic cross-linking.

Key words: Cell-wall stiffening (coleoptile) – Coleoptile growth – Hydrogen peroxide – Peroxidase – *Zea* coleoptile (cell-wall stiffening)

Introduction

The cell walls of growing plant organs such as the coleoptile of grass seedlings represent a complex composite material consisting of cellulose microfibrils embedded in an amorphous, gel-like matrix. The extraordinary mechanical stability of this material, that resists tensile stresses of the order of 10^3 MPa, is provided by various types of intermolecular cross-links among which the coupling of phenolic side-chains may play a dominant role (Fry 1986; liyama et al. 1994). However, despite their high tensile strength, these walls are still able to undergo stress relaxation and expansion without obvious impairment of their mechanical stability. This indicates that the mechanical properties of primary cell walls are the product of a highly dynamic state established by an interplay of controlled wall-loosening and wall-stiffening processes which are not yet well understood at the biochemical level (Ray 1987; Talbot and Ray 1992; Bolwell 1993; Carpita and Gibeaut 1993; Roberts 1994). In this context it has been suggested that the interpolymer dehydrogenative coupling of phenolic residues by wall-bound peroxidase using H_2O_2 as an electron acceptor plays a major role in cell-wall stiffening. Specifically, it has been proposed that the formation of phenolic cross-links serves as a mechanism for fixing the viscoelastically extended wall structure and thus confers irreversibility to wall extension during cell growth (Hohl et al. 1995).

Potentially, there are at least three phenolic substrates for peroxidase-catalyzed oxidative cross-linking in the cell wall: (i) lignin precursors such as cinnamoyl alcohols, (ii) tyrosine residues of certain glycoproteins, and (iii) ester-linked hydroxycinnamic-acid residues of pectic or hemicellulosic polysaccharides (Fry 1986; liyama et al. 1994). In the primary wall of graminaceous plants, the presently available evidence points to feruloylated glucuronoarabinoxylans as the major phenol-substituted wall polymers available for coupling by peroxidase (Carpita and Gibeaut 1993). Without touching the problem of the biochemical nature of the coupling substrate I have examined the hypothesis that such a substrate is present in the cell wall of growing maize coleoptiles and can be cross-linked by endogenous peroxidase upon supplementation with H_2O_2, resulting in cell-wall stiffening.

Materials and methods

Coleoptile segments (1 cm long, 3 mm below the tip) were prepared from etiolated 4-d-old *Zea mays* L. seedings (cv. Brio, from Asgrow,

Buxtehude, Germany) as described previously (Kutschera and Schopfer 1985). Coleoptiles were abraded with polishing cloth before preparation of segments (Schopfer 1993). After cutting, segments were preincubated for 1 h in aerated distilled water in order to obtain fully turgid, non-growing material. Auxin (IAA) solutions have been applied according to Kutschera and Schopfer (1985).

For measuring long-term growth kinetics, segments were strung on a plastic rod and incubated in a test tube with 18 ml of aerated incubation solution. Length changes were determined with a ruler ($\pm$ 0.5 mm). Short-term growth kinetics were determined with a growth recorder (linear-displacement transducer) using single segments as described by Kutschera and Schopfer (1985).

Cell-wall extensibility was measured with a custom-built constant-load extensiometer (Kutschera and Schopfer 1986). One-centimeter segments were fixed in the instrument, killed by freezing with liquid nitrogen and thawed in water for 15 min (25 °C). Then the water in the incubation chamber was replaced by 3 ml of experimental solution. Cell walls were extended by applying a load of 20 g for 15–120 min, followed by unloading for observation of the relaxation kinetics.

Osmolality of cell sap obtained by high-speed centrifugation of frozen/thawed segments was measured with a vapour-pressure osmometer (model 5100 C; Wescor, Logan, Utah, USA).

Histochemical peroxidase assays were basically performed after Griffing and Fowke (1985). Hand-cut coleoptile sections (approx. 100 µm) were incubated in citrate buffer (50 mmol·l^{-1}, pH 5.5) containing 3,3'-diaminobenzidine (1 mg·ml^{-1}) and H_2O_2 (10 mmol·l^{-1}) at room temperature. The sections were photographed after 5 min of incubation. Tissue prints were prepared by gently pressing freshly cut coleoptile sections on nitrocellulose paper (Cassab et al. 1992) which was subsequently developed in citrate buffer containing either 3,3'-diaminobenzidine of *p*-diphenylenediamine (1 mg·ml^{-1}) and H_2O_2 (10 mmol·l^{-1}).

Fluorescence of unfixed, hand-cut sections mounted in water was analyzed by epifluorescence light microscopy using the following filter combination (Zeiss, Oberkochen, Germany): 390–420 (excitation)/FT 425 (colour splitter)/LP 450 (emission) and Zeiss Neofluar objectives. Photographs were taken on Kodak Tri X 400 black and white film using identical exposure times.

All experiments were repeated at least four times on different occasions. Data points represent means ($\pm$ estimated standard errors where appropriate).

Results and discussion

Effect of H_2O_2 on elongation growth. Initial orienting experiments indicated that the waxy cuticle covering the coleoptile surface prevents the penetration of H_2O_2 into the segments effectively. Therefore, all subsequent experiments were performed with abraded segments which are readily and homogeneously accessible for low-molecular-weight solutes from the incubation medium (Schopfer 1993). Figure 1 shows that IAA-mediated elongation growth of abraded coleoptile segments, as well as endogenous growth in the absence of added IAA, can be inhibited by H_2O_2 concentrations above 10 µmol·l^{-1}. A reduction in growth rate by 85% was observed at 10 mmol·l^{-1} H_2O_2. As the segments contain substantial amounts of peroxidase, partly released into the medium, it was necessary to test whether H_2O_2 causes a destruction of IAA in these experiments. For this purpose, segments were incubated for 6 h in a solution containing 10 µmol·l^{-1} IAA and 10 mmol·l^{-1} H_2O_2 as in Fig. 1a. The incubation medium (18 ml) was recovered, treated with 130 000 units of catalase for 30 min and subsequently used for a second growth experiment. The growth of fresh

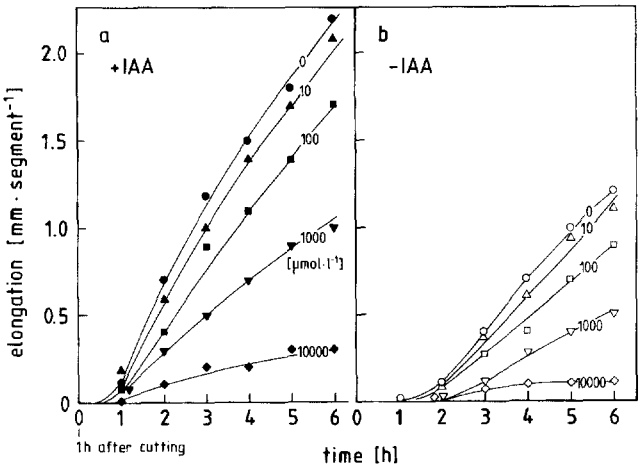

Fig. 1a, b. Effect of H_2O_2 on IAA-mediated elongation growth of maize coleoptile segments. After a 1-h preincubation in water, segments were incubated in H_2O_2 solutions (0–10000 µmol·l^{-1}) in the presence (**a**) and absence (**b**) of 10 µmol·l^{-1} IAA. The segments were transferred to fresh medium every hour in order to minimize the effect of H_2O_2 breakdown

segments incubated in this medium was not significantly different from the growth of control segments incubated in freshly prepared IAA solution (data not shown). Thus, the inhibitory effect of H_2O_2 shown in Fig. 1a cannot be attributed to a destruction of IAA in the incubation medium.

As H_2O_2 is a strong oxidant that can potentially damage semi-permeability of membranes, its inhibitory effect on elongation growth could be due to induced solute leakage from the segments and thus to a decline of osmotic pressure and turgor. This possibility was tested by measuring the changes in osmolality of the cell sap from H_2O_2-treated segments. Table 1 shows that even 10 mmol·l^{-1} H_2O_2 has no significant effect on cell-sap osmolality. The fact that turgor changes can be excluded as a cause of growth inhibition has led to the conclusion that H_2O_2 affects growth by impairing directly or indirectly the extensibility of the cell wall.

Figure 1 demonstrates that H_2O_2 inhibits IAA-induced growth and endogenous growth – which is presumably due to a rise in the endogenous IAA level – in a similar manner. In order to test whether this inhibition affects IAA-dependent growth specifically, the effect of H_2O_2 on acid-mediated growth of maize coleoptiles was investigated. Figure 2 shows that the growth response induced by citrate buffer of pH 4 is also strongly and rapidly inhibited by H_2O_2. A similar inhibition can be observed if H_2O_2 is applied simultaneously with or before the acid treatment (data not shown). It appears, therefore, that H_2O_2 affects one (or more) of those processes that are common to IAA- and acid-mediated growth.

In summary, the inhibition of growth by H_2O_2 documented in Figs. 1 and 2 is in agreement with a wall-stiffening effect of H_2O_2 in vivo but does not exclude other inhibitory effects of H_2O_2, e.g. on wall-loosening reactions.

Table 1. Effect of H_2O_2 on cell-sap osmolality of maize coleoptile segments. Segments were preincubated for 1 h in water followed by 3 h in solutions $\pm H_2O_2$ (1 or 10 mmol·l^{-1}) and $\pm$ IAA (10 μmol·l^{-1}) as in Fig. 1

Incubation	Osmolality	
	mosmol·kg^{-1}	%
1 h H_2O	264 ± 3	100
+ 3 h H_2O	238 ± 6	90
+ 3 h IAA	225 ± 6	85
+ 3 h H_2O_2 (1 mmol·l^{-1})	238 ± 3	90
+ 3 h H_2O_2 (10 mmol·l^{-1})	226 ± 3	86
+ 3 h IAA + H_2O_2 (1 mmol·l^{-1})	229 ± 5	87
+ 3 h IAA + H_2O_2 (10 mmol·l^{-1})	220 ± 4	84

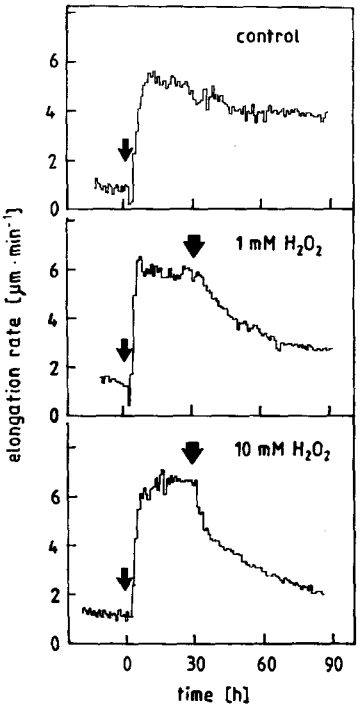

Fig. 2. Effect of H_2O_2 on acid-mediated elongation growth of maize coleoptile segments. After a 1-h preincubation in water, segments were incubated in 5 ml citrate buffer (10 mmol·l^{-1} citrate, pH 4.0) for induction of an acid-growth response (*small arrows*). After 30 min H_2O_2 was added (final concentrations 1 or 10 mmol·l^{-1}, *large arrows*). Growth rates of single segments were measured with a displacement transducer in 1-min intervals (steps of the curves)

Effect of H_2O_2 on cell-wall extension in vitro. Recently, it was shown that maize coleoptile segments undergo cell-wall stiffening under certain experimental conditions. In the living tissue, wall stiffening can be inhibited by anoxia and this inhibition can be alleviated by H_2O_2. These observations have led to the hypothesis that the wall-stiffening reaction is caused by metabolically controlled peroxidative cross-linking of phenolic side-chains of cell-wall polymers such as feruloylated glucuronoarabinoxylans (Hohl et al. 1995; Hohl and Schopfer 1995). There are numerous lines of mostly correlative evidence in the literature suggesting that phenolic cross-linking of polysaccharides and/or proteins by wall-bound peroxidase can take place in aging or pathogen-infected plant tissues and can lead to increased wall stability (e.g. Goldberg et al. 1987; Kim et al. 1989; Kamisaka et al. 1990; MacAdam et al. 1992; Shedletzki et al. 1992; Tan et al. 1992; Zheng and van Huystee 1992; Brisson et al. 1994). However, the operation of this type of wall-stiffening reaction is difficult to demonstrate unequivocally in vivo and has so far not been shown to occur in the isolated, native cell wall. The following experiments were conducted in order to test if H_2O_2-dependent, peroxidase-catalyzed cross-linking can cause stiffening of maize coleoptile cell walls in vitro.

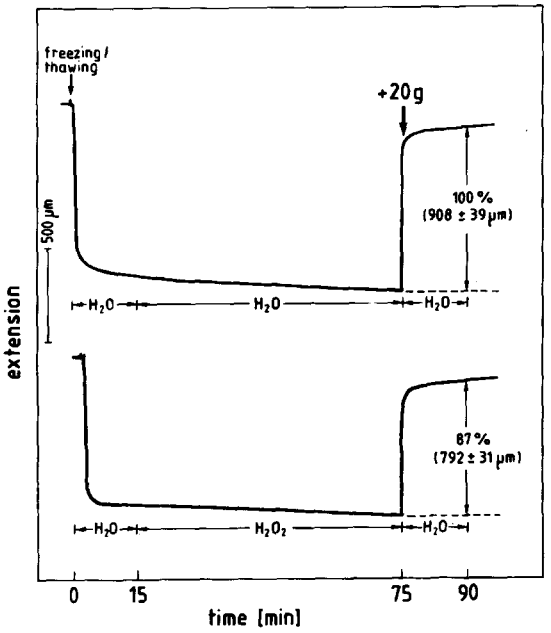

Fig. 3. Extensiometer kinetics demonstrating stiffening by H_2O_2 in isolated cell walls of maize coleoptile segments in the relaxed state. Single segments were fixed in the extensiometer, frozen/thawed and, after 15 min of relaxation in distilled water, incubated without load in distilled water (*upper curve*) or 1 mmol·l^{-1} H_2O_2 (*lower curve*). After 60 min, the medium was replaced by distilled water and a load of 20 g was applied. Extension was measured after 15 min as indicated

Figure 3 shows a basic experiment demonstrating the effect of H_2O_2 on cell-wall extensibility in vitro. If a coleoptile segment killed by freezing and thawing is incubated for 1 h in H_2O_2 and then stretched by applying a constant load, it extends significantly less than a control segment kept for 1 h in water. This wall-stiffening reaction also works in the wall subjected to tensile stress. Figure 4 shows that a segment stretched in the extensiometer by a constant load for 1 h in the presence of H_2O_2 does not return to its original length upon unloading, i.e. part of the extension has become irreversible whereas the extension of control segments in water remains reversible. This experiment indicates that the H_2O_2-induced wall-stiffening reaction can be used to generate irreversible extension on the expense of reversible extension in the presence of tensile stress. We have previously argued that a reaction of this kind is necessary for fixing wall-loosening events and

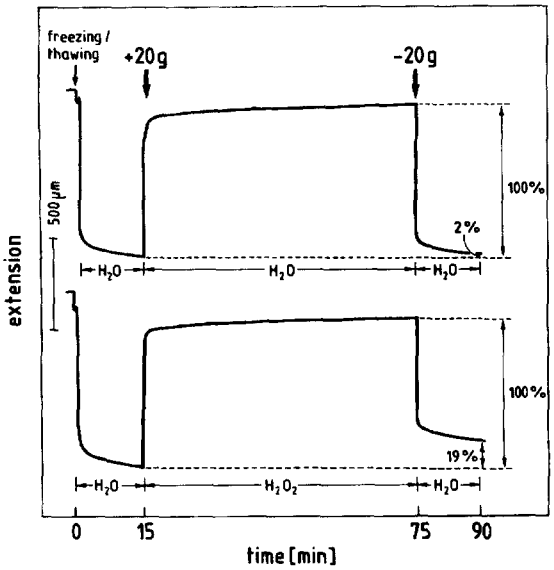

Fig. 4. Extensiometer kinetics demonstrating stiffening by H_2O_2 in isolated cell walls of maize coleoptile segments in the stressed state. Single segments were fixed in the extensiometer, frozen/thawed and, after 15 min of relaxation in distilled water, extended by a load of 20 g in the presence of distilled water (*upper curve*) or 1 mmol·l^{-1} H_2O_2 (*lower curve*). After 60 min of extension the load was removed and the cell wall allowed to relax for further 15 min in distilled water

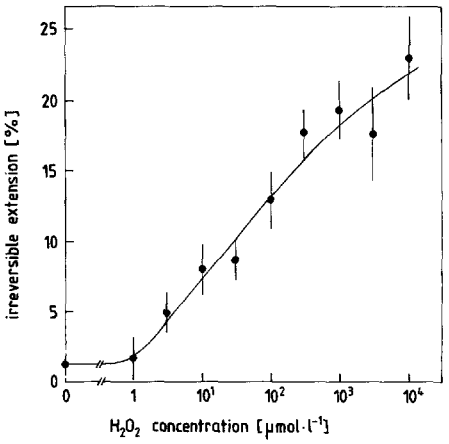

Fig. 5. Dependence of stiffening of isolated maize coleoptile cell walls on H_2O_2 concentration. Frozen/thawed segments were treated for 60 min with various concentrations of H_2O_2 and the percent irreversible extension produced was measured as depicted in Fig. 4

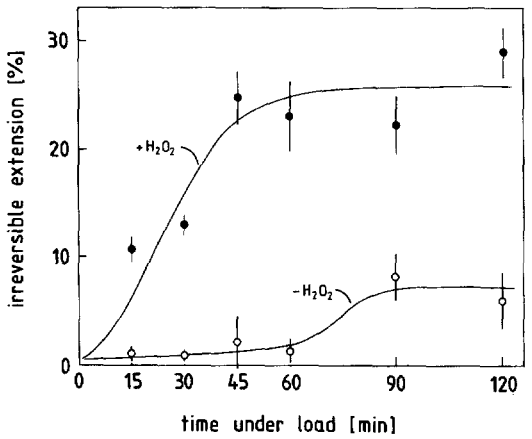

Fig. 6. Time course of the stiffening reaction elicited by H_2O_2 in isolated maize coleoptile cell walls. Segments were treated with or without 10 mmol·l^{-1} H_2O_2 for 0–120 min and the percent irreversible extension produced was measured as depicted in Fig. 4

is thus an integral component of cell growth (Hohl et al. 1995).

Using the generation of irreversible extension as defined in the experiment of Fig. 4 as a measure of induced wall stiffening, the dependence of this response on the concentration of H_2O_2 was investigated. Figure 5 shows that the wall-stiffening reaction in vitro can be elicited by H_2O_2 in the range of 10–10 000 µmol·l^{-1}, i.e. in the same concentration range where H_2O_2 causes inhibition of extension in vivo (see Fig. 1). Figure 6 shows that cell-wall stiffening in vitro is a time-dependent reaction that starts within less than 15 min and lasts for about 60 min at 10 mmol·l^{-1} H_2O_2. This time course agrees with the existence in the isolated wall of a limited pool of phenolic substrates which becomes exhausted after about 60 min in the presence of saturating concentrations of H_2O_2.

Demonstration of peroxidase activity in the cell walls. The H_2O_2-dependent formation of phenolic cross-links between wall polymers requires the catalytic activity of peroxidase. Thus, it was necessary to examine the presence of this enzyme in the cell walls of the maize coleoptile. Figure 7a, b shows the results obtained with a histochemical peroxidase assay using 3,3′-diaminobenzidine as the phenolic substrate. After a reaction time of 5 min, black deposits of reaction product indicating peroxidase activity were visible in the cell walls at the inner and outer surface of the coleoptile (outer epidermal walls). After prolonged reaction times the walls of the parenchyma and the vascular bundles also became stained, indicating that smaller enzyme levels are also present in the inner tissue walls. Tissue prints on nitrocellulose paper (Cassab et al. 1992) indicated that at least part of the peroxidase is present in extractable form (Fig. 7c, d). Interestingly, blots obtained with different peroxidase substrates demonstrated specific histological patterns. Peroxidase isoforms reacting with 3,3′-diaminobenzidine were released from the outer and inner epidermis and, to a lesser extent, from the inner tissues of the coleoptile (Fig. 7c). In contrast, peroxidase isoforms reacting with *p*-phenylenediamine were released only from the outer epidermis and the vascular bundles. It can be concluded from these results, that active peroxidase is present in the walls of the maize coleoptile, with particularly high levels in the growth-limiting outer epidermal wall.

Effects of inhibitors of peroxidase activity on cell-wall stiffening. If the cell-wall stiffening response mediated by H_2O_2 is caused by peroxidase-catalyzed cross-linking of phenolics, it should be inhibited by peroxidase-inactivating agents. This prediction was tested by measuring the effect of azide and ascorbate on the H_2O_2-dependent

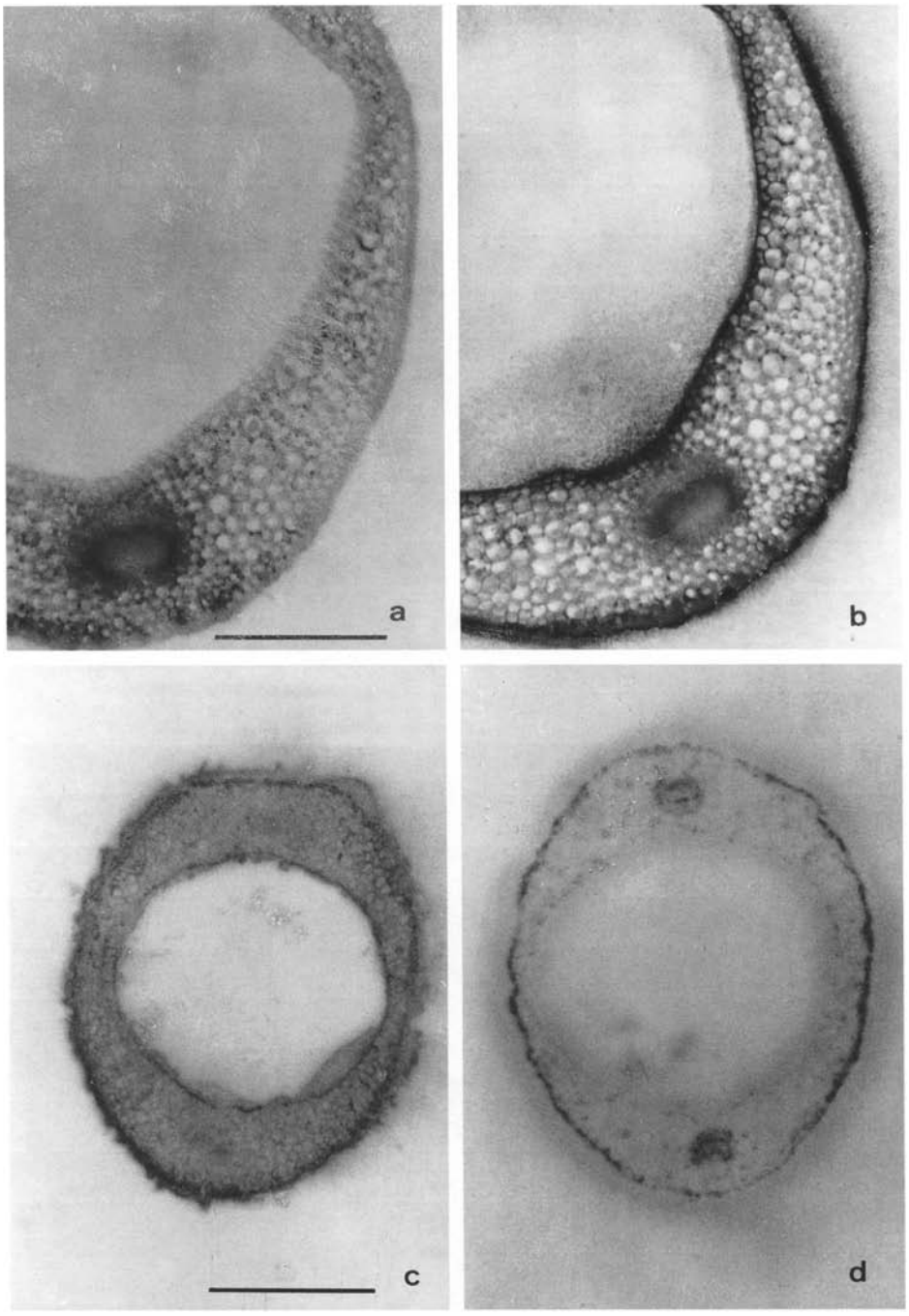

Fig. 7a–d. Histochemical localization of peroxidase activity in maize coleoptiles. Sections from the region about 10 mm below the organ tip were incubated for 5 min in buffer (control, **a**) or 3,3′-diaminobenzidine + H_2O_2 in buffer (**b**). Tissue prints were either obtained with 3,3′-diaminobenzidine (**c**) or *p*-phenylenediamine (**d**) as phenolic substrate. Bars: 500 µm (×47) in **a, b**; 1 mm (×23) in **c, d**

increase in irreversible extension of frozen-thawed coleoptile segments using the assay depicted in Fig. 4. Figure 8 shows that these peroxidase inhibitors produce a significant increase in irreversible extension even in the absence of H_2O_2. Based on these controls, azide and ascorbate inhibit the H_2O_2-dependent increase in irreversible extension by 60 and 100%, respectively.

Effect of H_2O_2 on cell-wall fluorescence. The deposition of polymeric material containing phenolic components such as cinnamic-acid derivatives in the cell wall should result in an increase of fluorescence under UV irradiation (Harris and Hartley 1976). When cross-sections of maize coleoptile segments incubated for 1 h in H_2O_2 were inspected in the fluorescence microscope using long-wavelength UV (390–420 nm) for excitation, they demonstrated a strong increase in fluorescence in the walls of the peripheral cell layers, particularly in the outer epidermal wall (Fig. 9). The fluorescence of the outer epidermal wall of the control is similarly observed in freshly cut sections and is thus caused by fluorescent material accumulated during the previous growth of the coleoptile. The colour of this fluorescence was bright greenish-yellow, in contrast to the more bluish fluorescence of the lignified tracheid walls

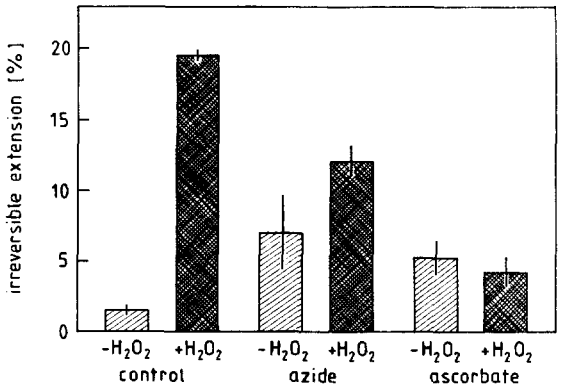

Fig. 8. Inhibition of the stiffening reaction elicited by H_2O_2 in isolated maize coleoptile cell walls by azide and ascorbate. The percent irreversible extension of frozen/thawed segments in the presence and absence of 1 mmol·l^{-1} H_2O_2 was measured as depicted in Fig. 4. Inhibitors (1 mmol·l^{-1} Na-azide, 10 mmol·l^{-1} ascorbic acid titrated to pH 6 with NaOH) were added at the time of thawing

in the vascular bundles. The difference in colour was more distinct with a shorter-wavelength filter combination (BP 365/FT 395/LP 397 nm). This observation provides direct evidence that H_2O_2 triggers the accumulation of an insoluble material in the cell wall with the fluorescence properties of phenolic compounds. Thus, there appears to be a net increase in bound phenolics in the presence of H_2O_2 rather than an increase in diphenolic coupling of existing polymers.

Conclusions. Taken together, the experiments described in this report provide in-vitro evidence for the existence of a H_2O_2-dependent wall-stiffening reaction in the primary cell walls of a growing plant organ. The results support the conclusion drawn from corresponding in-vivo experiments, namely that in the walls of growing cells a biochemical wall-stiffening mechanism operates which is necessary for fixing the extension generated by viscoelastic wall loosening (Hohl et al. 1995). Moreover, it is shown that the wall-stiffening reaction fulfills some criteria of a peroxidase-catalyzed phenolic cross-linking reaction although the phenolic substrate has not yet been identified. The decisive question raised by these data concerns the mode of control of cell-wall stiffening. The observation that this reaction can be elicited by adding a single factor, H_2O_2, indicates that the isolated wall contains all other ingredients for mechanical fortification of the wall structure. This suggests, but does not prove, that wall-stiffening in vivo is controlled by the secretion of H_2O_2 into the cell wall. Recently, it was shown that H_2O_2 is actually present in the walls of growing plant tissues (Schopfer 1994). However, quantitative information on apoplastic H_2O_2 levels will be necessary for confirming this attractive hypothesis.

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References

Bolwell GP (1993) Dynamic aspects of the plant extracellular matrix. Int Rev Cytol 146: 261–324

Brisson LF, Tenhaken R, Lamb C (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. Plant Cell 6: 1703–1712

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J 3: 1–30

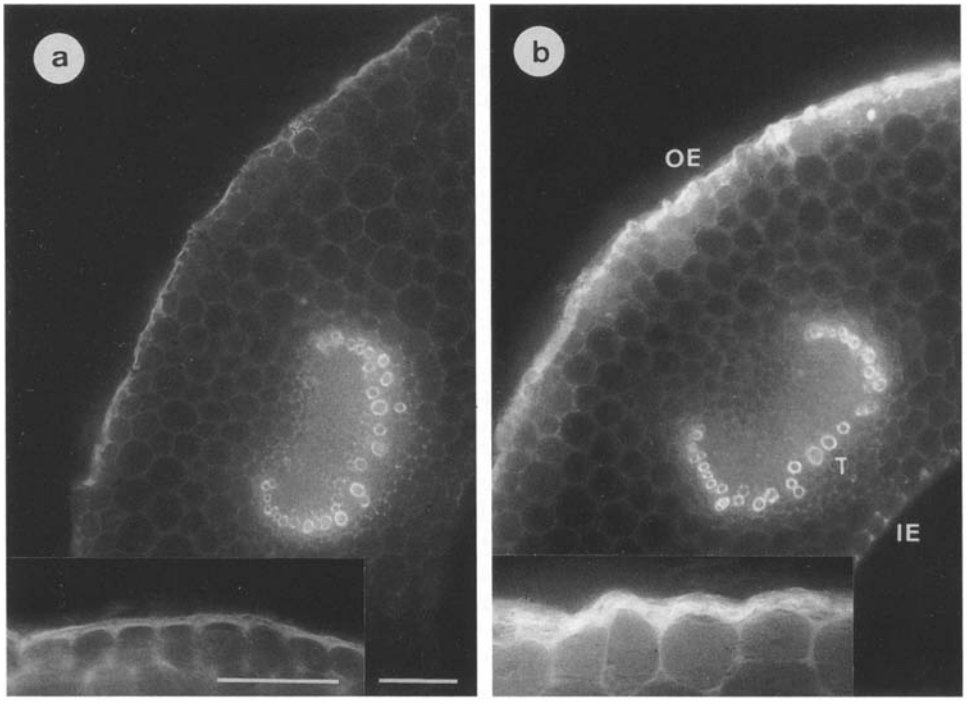

Fig. 9a, b. Effect of H_2O_2 on autofluorescence of maize coleoptile cell walls. Segments were incubated without preincubation for 1 h in water (**a**) or 1 mmol·l^{-1} H_2O_2 (b). After briefly washing in water, transverse sections were cut by hand and immediately examined under UV irradiation in the epifluorescence microscope. The *insets* show enlarged regions of the outer epidermis (*OE*, collapsed in **b**). *IE*, inner epidermis; *T*, tracheids at the periphery of vascular bundle. Bars: 100 µm (× 105); inset 50 µm (× 323)

Cassab GI, Lin L-S, Varner JE (1992) Ethylene effect on peroxidase distribution in pea. In: Reid PD, Pont-Lezica RF, del Campillo E, Taylor R (eds) Tissue printing. Tools for the study of anatomy, histochemistry, and gene expression. Academic Press, San Diego, Cal. pp 63–65

Fry SC (1986) Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annu Rev Plant Physiol 37: 165–186

Goldberg R, Liberman M, Mathieu C, Pierron M, Catesson AM (1987) Development of epidermal cell wall peroxidases along the mung bean hypocotyl: possible involvement in the cell wall stiffening process. J Exp Bot 38: 1378–1390

Griffing LR, Fowke LC (1985) Cytochemical localization of peroxidase in soybean suspension culture cells and protoplasts: intracellular vacuole differentiation and presence of peroxidase in coated vesicles and multivesicular bodies. Protoplasma 128: 22–30

Harris PJ, Hartley RD (1976) Detection of bound ferulic acid in cell walls of the gramineae by ultraviolet fluorescence microscopy. Nature 259: 508–510

Hohl M, Schopfer P (1995) Rheological analysis of viscoelastic cell-wall changes in maize coleoptiles as affected by auxin and osmotic stress. Physiol Plant 94: 499–505

Hohl M, Greiner H, Schopfer P (1995) The cryptic-growth response of maize coleoptiles and its relationship to H_2O_2-dependent cell-wall stiffening. Physiol Plant 94: 491–498

Iiyama K, Lam TB-T, Stone BA (1994) Covalent cross-links in the cell wall. Plant Physiol 104: 315–320

Kamisaka S, Takeda S, Takahashi K, Shibata K (1990) Diferulic acid and ferulic acid in the cell wall of *Avena* coleoptiles – their relationship to mechanical properties of the cell wall. Physiol Plant 78: 1–7

Kim S-H, Shinkle JR, Roux SJ (1989) Phytochrome induces changes in the immunodetectable level of a wall peroxidase that precede growth changes in maize seedlings. Proc Natl Acad Sci USA 86: 9866–9870

Kutschera U, Schopfer P (1985) Evidence against the acid-growth theory of auxin action. Planta 163: 483–493

Kutschera U, Schopfer P (1986) Effect of auxin and abscisic acid on cell wall extensibility in maize coleoptiles. Planta 167: 527–535

MacAdam JW, Sharp RE, Nelson CJ (1992) Peroxidase activity in the leaf elongation zone of tall festuce. II. Spatial distribution of apoplastic peroxidase activity in genotypes differing in length of the elongation zone. Plant Physiol 99: 879–885

Ray PM (1987) Principles of plant cell expansion. In: Cosgrove DJ, Knievel DP (eds) Physiology of cell expansion during plant growth. Am Soc Plant Physiol Rockville, Md., pp 1–17

Roberts K (1994) The plant extracellular matrix: in a new expansive mood. Curr Opinion Cell Biol 6: 688–694

Schopfer P (1993) Determination of auxin-dependent pH changes in coleoptile cell walls by a null-point method. Plant Physiol 103: 351–357

Schopfer P (1994) Histochemical demonstration and localization of H_2O_2 in organs of higher plants by tissue printing on nitrocellulose paper. Plant Physiol 104: 1269–1275

Shedletzky E, Shmuel M, Trainin T, Kalman S, Delmer D (1992) Cell wall structure in cells adapted to growth on the cellulose-synthesis inhibitor 2,6-dichlorobenzonitrile. A comparison between two dicotyledonous plants and a graminaceous monocot. Plant Physiol 100: 120–130

Talbott LD, Ray PM (1992) Molecular size and separability features of pea cell wall polysaccharides. Plant Physiol 98: 357–368

Tan K-S, Hoson T, Masuda Y, Kamisaka S (1992) Involvement of cell wall-bound diferulic acid in light-induced decrease in growth rate and cell wall extensibility of *Oryza* coleoptiles. Plant Cell Physiol 33: 103–108

Zheng X, van Huystee RB (1992) Peroxidase-regulated elongation of segments from peanut hypocotyls. Plant Sci 81: 47–56