Plant Physiol. (1997) 113: 967–973

Osmotic Stress Suppresses Cell Wall Stiffening and the Increase in Cell Wall-Bound Ferulic and Diferulic Acids in Wheat Coleoptiles

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The relationship between the mechanical properties of cell walls and the levels of wall-bound ferulic (FA) and diferulic (DFA) acids was investigated in wheat (*Triticum aestivum* L.) coleoptiles grown under osmotic stress (60 mM polyethylene glycol [PEG] 4000) conditions. The cell walls of stressed coleoptiles remained extensible compared with those of the unstressed ones. The contents of wall-bound FA and DFA increased under unstressed conditions, but the increase was substantially reduced by osmotic stress. In response to PEG removal, these contents increased and reached almost the same levels as those of the unstressed coleoptiles. A close correlation was observed between the contents of FA and DFA and the mechanical properties of cell walls. The activities of phenylalanine ammonia-lyase and tyrosine ammonia-lyase increased rapidly under unstressed conditions. Osmotic stress substantially reduced the increases in enzyme activities. When PEG was removed, however, the enzyme activities increased rapidly. There was a close correlation between the FA levels and enzyme activities. These results suggest that in osmotically stressed wheat coleoptiles, reduced rates of increase in phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities suppress phenylpropanoid biosynthesis, resulting in the reduced level of wall-bound FA that, in turn, probably causes the reduced level of DFA and thereby maintains cell wall extensibility.

Osmotic stress has been shown to affect the mechanical properties of cell walls. In light-grown maize leaves the cell wall extensibility was increased under osmotic stress (Acevedo et al., 1971). Sakurai et al. (1987b), Sakurai and Kuraishi (1988), and Kutschera (1989) also reported that the cell walls of dark-grown squash and mung bean hypocotyls remained extensible under PEG-induced osmotic stress conditions for several days. On the other hand, the application of PEG to the roots of light-grown maize seedlings induced cell wall hardening of leaves within several minutes (Chazen and Neumann, 1994). In dark-grown soybean hypocotyls the extensibility, particularly plasticity, of cell walls decreased when growth was inhibited under water-deficit conditions (Nonami and Boyer, 1990). Thus, the effect of osmotic stress on cell wall extensibility is variable for plant species, organs, and/or growth conditions.

Cell walls from growing plant tissues are mainly composed of cellulose and matrix polysaccharides. The amount and structure of wall polysaccharides have been considered to be factors that determine the mechanical properties of cell walls (Taiz, 1984; Sakurai, 1991). The primary cell wall of gramineous plants also contains a significant amount of hydroxycinnamic acids such as FA and coumaric acid, which are ester-linked to wall matrix polysaccharides such as arabinoxylans (Hartley, 1973; Harris and Hartley, 1976; Shibuya, 1984; Kato and Nevins, 1985; Nishitani and Nevins, 1988; Yamamoto et al., 1989). FA bound to cell walls undergoes a peroxidase-catalyzed coupling reaction to produce DFA, which cross-links arabinoxylans (Fry, 1979; Ishii, 1991). Increases in the amounts of wall-bound FA and DFA in oat and rice coleoptiles were closely correlated with decreases in cell wall extensibility caused by aging (Kamisaka et al., 1990), light irradiation (Tan et al., 1992a; Miyamoto et al., 1994), and growth in air (Tan et al., 1991). This fact suggests that suppression of the increase in the amounts of FA and DFA may contribute to keeping the cell walls extensible. The present study deals with the changes in the mechanical properties of cell walls and the amounts of wall-bound FA and DFA in wheat (*Triticum aestivum* L.) coleoptiles in response to root-applied PEG. These measurements revealed that osmotic stress suppressed the cell wall stiffening in wheat coleoptiles and that this suppression could be interpreted in terms of the reduction of the increases in FA and DFA contents. We also measured the activities of PAL and TAL, the key enzymes in the phenylpropanoid pathway (Hahlbrock and Grisebach, 1979), as possible sites in the regulation of the formation of wall-bound FA and DFA under osmotic stress conditions.

MATERIALS AND METHODS

Plant Material and Coleoptile Growth Experiments

Caryopses of wheat (*Triticum aestivum* L. cv Daichino-Minori) were soaked in tap water for 5 h at 25°C, and then placed on two layers of moistened filter paper (type 2, Toyo Roshi, Tokyo, Japan) in a plastic box (33 × 25 × 11 cm^3). The box was covered with aluminum foil. Caryopses were allowed to germinate and grow for 2 d in the dark at 25°C.

On d 2, seedlings were transferred to two layers of filter paper containing 40 mL of deionized water or 60 mM PEG

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Abbreviations: CA, *trans*-cinnamic acid; CouA, *p*-coumaric acid; DFA, diferulic acid; FA, ferulic acid; PAL, Phe ammonia-lyase; *R*, relaxation rate; TAL, Tyr ammonia-lyase; T_0, minimum stress-relaxation time.

4000 (average molecular weight, 3000; Wako Pure Chemical, Osaka, Japan) solution in cylindrical plastic boxes (15 cm in diameter and 8 cm in height), which were covered with aluminum foil. For each treatment, several boxes, each containing 30 seedlings, were prepared. Seedlings were held vertically in the boxes. One day later, one-half of the PEG-treated seedlings were washed with deionized water and then transferred to boxes with two layers of filter paper containing 40 mL of deionized water. The total duration of the experiment was 6 d, and on each day the length of 20 coleoptiles per treatment was measured and the coleoptiles were excised.

All manipulations were done under dim-green light (approximately 0.02 W m^{-2} at handling level; Safe Light Filter No. 3, Kodak). The experiment was repeated four times with similar results.

The osmotic concentration of the 60 mM PEG solution was measured with a vapor pressure osmometer (model 5500, Wescor, Logan, UT), and the osmotic potential was calculated according to the formula $\pi = -CRT$, where π is the osmotic potential, R is the gas constant, T is the absolute temperature, and C is the molar concentration. The osmotic concentration was 250 mmol kg^{-1}, which is equivalent to an osmotic potential of −0.61 MPa.

Determination of Mechanical Properties of Cell Walls

We investigated the relationship between the physical properties and the chemical structure of cell walls of the wheat coleoptiles grown under osmotic stress. For this purpose, in vitro measurement of the mechanical properties of cell walls appears to have an advantage over in vivo measurement, because chemical changes of wall composition during measurement can be ignored in the former method. Also, we used methanol-killed wall specimens, which are easy to handle, since their physical properties are similar to those of frozen-thawed ones (Cleland, 1984). Coleoptiles were excised on each day of the experiments as described above, and the first leaf was removed using forceps. The excised coleoptiles were immediately boiled in methanol for 10 min and stored in fresh methanol at 4°C until use. Before measurement of the mechanical properties of cell walls, the methanol-killed coleoptiles were rehydrated for 1 h at room temperature with several changes of water. Mechanical properties of cell walls were measured by the stress-relaxation method reported by Yamamoto et al. (1970).

A preliminary experiment showed that in dark-grown wheat coleoptiles, the growth rate of the upper region was much higher than that of the lower region. Therefore, a 10-mm section of the subapical region of coleoptiles (from 5–15 mm below the tip) was fixed between the upper and lower movable clamps (the distance between the clamps was 5 mm) of a tensile tester (RTM-25, Toyo Baldwin Co., Tokyo, Japan) connected to a computer (PC-9801, NEC, Tokyo, Japan), and then stretched by lowering the lower clamp at 20 mm min^{-1} to produce a stress of 20 g. The decay of the stress at constant strain was determined for 60 s with a load cell of the tensile tester and recorded by the computer. Stress-relaxation parameters such as T_0, the time at the initial decay of stress, and R were calculated from the relaxation data recorded at appropriate time intervals (1 ms) on the basis of the equation reported by Fujihara et al. (1978). In addition to these parameters, the mechanical extensibility (strain/load, mm 20 g^{-1}) was also determined with the tensile tester by measuring the increase in distance between the clamps when the wall specimen registered 20 g of initial stress. The experiment to measure the mechanical properties of cell walls was repeated twice with similar results.

Fractionation of Cell Wall Components

Whole coleoptiles excised on each day of the experiments as described above were killed by boiling in methanol and stored in fresh methanol until use. Before fractionation of the cell wall components, methanol-killed coleoptiles were rehydrated with water. Cell walls were fractionated by the method of Tan et al. (1991). Fifteen to 30 of the rehydrated coleoptiles were homogenized in water with a mortar and pestle; washed with water, acetone, and a methanol:chloroform mixture (1:1, v/v); and then treated with porcine pancreatic α-amylase (type I-A, Sigma) to remove starch. After the amylase treatment, a pectic substance (pectic fraction) was extracted three times with 20 mM ammonium oxalate (pH 4.0) at 70°C for 1 h. The remaining cell wall material was extracted with 0.1 M NaOH under N_2 gas at room temperature in the dark for 24 h. The residual wall material was extracted with 17.5% NaOH containing 0.02% $NaBH_4$ at room temperature for 18 h. The alkali-insoluble fraction was designated as the cellulose fraction. After the extraction of FA and DFA from the 0.1 M NaOH solution as described below, the remaining solution was combined with the 17.5% NaOH extracts and designated as the hemicellulose fraction (a mixture of noncellulosic polysaccharides extracted by alkali). The total sugar content of each fraction was determined by the phenol-sulfuric acid method (Dubois et al., 1956). The neutral sugar composition of the hemicellulose fraction was determined by GLC according to the method of Albersheim et al. (1967). The amounts of cell wall components were determined using three samples obtained from three replicate experiments.

Determination of Wall-Bound FA and DFA

FA and DFA were measured according to the method of Tan et al. (1991). Phenolic acids liberated from the pectin-depleted cell wall preparation by 0.1 M NaOH treatment were extracted three times with ethyl acetate after acidifying the alkali-extracted fraction to approximately pH 3.0 with HCl. The ethyl acetate extract was air-dried and stored in the dark. FA and DFA were determined using an HPLC system with a Unisil 5C18 column (4 × 250 mm, Gasukuro Kogyo, Tokyo, Japan) and a UV detector according to the method of Shibuya (1984) and Kamisaka et al. (1990). The amounts of FA and DFA were determined using *trans*-FA and *trans,trans*-DFA as the standards, respectively, with the latter being synthesized from dehydrovanillin acetate and malonic acid by Knoevenagel condensation (Richtzenhain, 1949). Recently, Grabber et al.

(1995) reported the presence of several DFA isomers in maize suspension cultures. In the present study, we determined only the amount of 5,5-coupled DFA, because other isomers were not detected in our samples. The amounts of FA and DFA were determined using three samples obtained from three replicate experiments.

Extraction and Assay of PAL and TAL

Whole coleoptiles excised on each day of the experiments as described above were immediately frozen with liquid N_2 and kept at −80°C until use. Extraction and assay of the enzymes were carried out essentially by the methods of Ward et al. (1989) and Sauter and Kende (1992). The frozen coleoptiles (0.2–0.5 g fresh weight) were homogenized in ice-cold 100 mM potassium-borate buffer (pH 8.8) containing 2 mM mercaptoethanol with a mortar and pestle and centrifuged at 16,000*g* for 10 min at 2°C. After centrifugation, the pellet was discarded and the supernatant (crude enzyme extract) was used for the PAL and TAL assays. The reaction mixture (total 2 mL) contained 0.5 mL of 4 mM L-Phe for the PAL assay or 4 mM L-Tyr for the TAL assay, 0.5 mL of extract, and 1 mL of 100 mM potassium-borate buffer (pH 8.8). The reaction mixture was incubated at 37°C for 60 min, and then 0.1 mL of 5 N HCl was added to stop the reaction. After the incubation, the mixture was extracted three times with ethyl acetate, and the ethyl acetate extract was air-dried. The amounts of CA and CouA that were produced by PAL and TAL, respectively, were determined using the HPLC system with a Unisil 5C18 column and a UV detector. The sample was eluted with a nonlinear gradient of 10 to 50% acetonitrile in 50 mM acetate buffer (pH 4.0) and monitored at 273 and 320 nm for CA and CouA, respectively. The enzyme activity was expressed as CA (for PAL) and CouA (for TAL) produced in 1 h. Protein content in crude enzyme extract was determined using the protein assay kit from Bio-Rad according to the method of Bradford (1976). Enzyme activities were determined using three samples obtained from three replicate experiments.

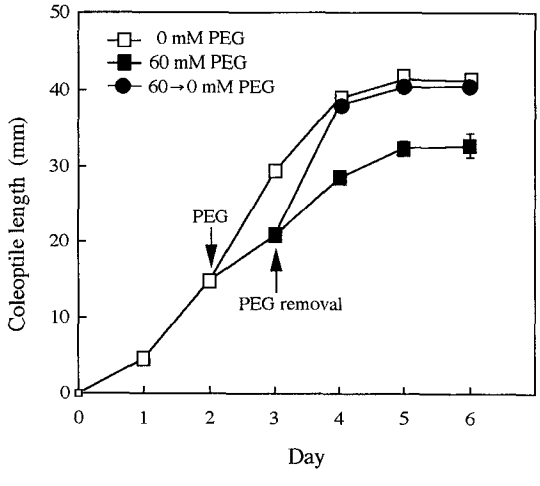

Figure 1. Effect of osmotic stress and its relief on growth of wheat coleoptiles. Wheat seedlings were grown on filter paper containing deionized water until d 2, and then either continuously treated with 60 mM PEG or transferred back to deionized water on d 3. Data are means ± SE (n = 20). Vertical bars represent SE. Error bars were smaller than the symbols for most points.

Table I. *Effect of osmotic stress on the mechanical properties of cell walls of wheat coleoptiles*

Treatments are as described in Figure 1. Data are means ± SE (n = 20). These measurements were repeated twice with similar results.

Day	0 mM PEG	60 mM PEG	60 → 0 mM PEG
T_0 (ms)			
2	63.6 ± 2.4		
3	87.4 ± 2.4	63.9 ± 1.8[a]	
4	98.8 ± 5.4	66.0 ± 1.3[a]	83.9 ± 2.3[b]
5	111.9 ± 7.2	68.6 ± 4.1[a]	107.5 ± 6.5
6	109.1 ± 7.6	73.3 ± 2.2[a]	111.0 ± 6.6
R (%)			
2	4.85 ± 0.07		
3	4.87 ± 0.07	4.67 ± 0.05[b]	
4	5.05 ± 0.09	4.44 ± 0.04[a]	4.62 ± 0.05[a]
5	5.42 ± 0.16	4.79 ± 0.07[a]	5.19 ± 0.15
6	5.30 ± 0.22	4.82 ± 0.08[b]	5.08 ± 0.10
Mechanical extensibility (mm 20 g^{-1})			
2	0.362 ± 0.015		
3	0.331 ± 0.009	0.360 ± 0.011[b]	
4	0.326 ± 0.008	0.375 ± 0.011[a]	0.405 ± 0.054
5	0.216 ± 0.013	0.364 ± 0.009[a]	0.189 ± 0.009
6	0.142 ± 0.006	0.289 ± 0.017[a]	0.132 ± 0.005

[a,b] Significant difference between 0 mM PEG (control) and each treatment at the 1 and 5% levels, respectively.

RESULTS

The length of unstressed coleoptiles increased up to d 5 and reached a final length of approximately 40 mm (Fig. 1). Application of 60 mM PEG to roots substantially reduced coleoptile growth. When PEG was removed after 1 d, the coleoptile growth rate substantially increased, and coleoptile lengths were the same as those of the unstressed coleoptiles by d 4.

Changes in the mechanical properties of cell walls of wheat coleoptiles grown under the conditions given in Figure 1 are shown in Table I. Stress-relaxation parameters T_0 and R of unstressed coleoptiles increased, whereas those of PEG-treated coleoptiles were relatively constant throughout the incubation. When PEG was removed after 1 d, T_0 and R increased and reached the same values as those of unstressed coleoptiles by d 5. These results indicate that the cell walls of the stressed coleoptiles remained loosened compared with those of unstressed ones. The mechanical extensibility of cell walls from both unstressed and recovered coleoptiles substantially decreased, whereas that of the stressed coleoptiles remained at a high value throughout the experiment.

The amount of cellulose per unit length of coleoptile increased by about 3-fold from d 2 to 6 under unstressed conditions (Fig. 2A). The increase in the cellulose content was reduced by PEG treatment from d 4 to 6. After PEG was removed on d 3, the content rapidly increased from d 4 to 5 and reached almost the same value as that of the unstressed coleoptiles by d 5. The amount of hemicellulose

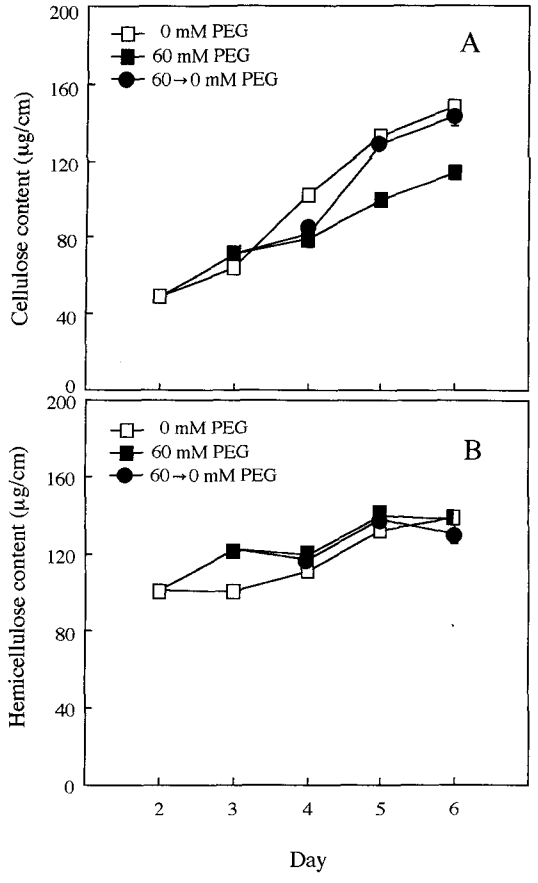

Figure 2. Changes in sugar contents of cellulose (A) and hemicellulose (B) in cell walls of wheat coleoptiles. Treatments are as described in Figure 1. Sugar content in each fraction was determined by the phenol-sulfuric acid method. Data are means ± SE from three replicate experiments. Error bars were smaller than the symbols for most points.

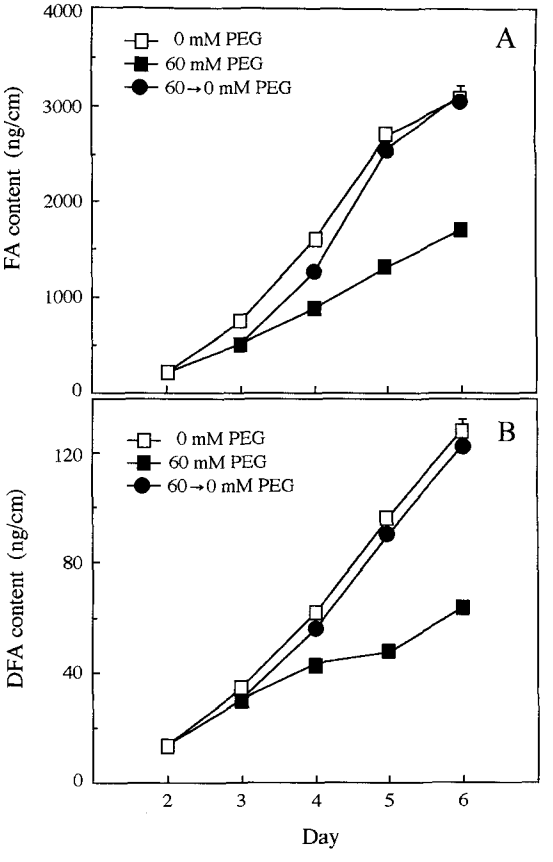

Figure 3. Changes in the amounts of wall-bound FA (A) and DFA (B) in wheat coleoptiles. Treatments are as described in Figure 1. FA and DFA contents are expressed per unit length of coleoptile. Data are means ± SE from three replicate experiments. Error bars were smaller than the symbols for most points.

(polysaccharides extracted with 0.1 M NaOH and 17.5% NaOH) per unit length of unstressed coleoptiles slightly increased during the experiment (Fig. 2B). Although PEG treatment increased the amount of hemicellulose by d 3, it had no effect from d 4 to 6. The removal of PEG also did not affect the hemicellulose content. The neutral sugar composition of the hemicellulose fraction under unstressed conditions was: Ara, 20 to 23%; Xyl, 37 to 45%; Glc, 23 to 30%; Gal, 3 to 5%; Man, 1.5 to 4.5%; and rhamnose, 0.7 to 1.1%. PEG hardly affected these values (data not shown). The amount of the pectic fraction was very small (approximately 1% of the total wall content) and was not affected by PEG (data not shown).

Figure 3 shows the changes in the amounts of FA and DFA extracted with 0.1 M NaOH from cell walls during the experimental period. FA and DFA contents of cell walls of unstressed coleoptiles increased rapidly as the coleoptiles grew. By contrast, under osmotic stress conditions the increase was substantially reduced. In response to PEG removal, the amounts of FA and DFA increased and reached almost the same value as those of the unstressed coleoptiles by d 5. A close correlation was observed between the mechanical extensibility or T_0 and the FA or DFA contents of cell walls (Table II). The ratio of DFA to FA contents decreased from d 2 to 5 under both unstressed and stressed conditions; the PEG treatment did not affect the values (Fig. 4).

Figure 5 shows the changes in the activities of PAL and TAL per unit total protein. PAL and TAL activities of unstressed coleoptiles increased progressively until d 4 (PAL) or d 5 (TAL), by 11- (PAL) or 7-fold (TAL) of the initial levels, then decreased on d 6. PEG treatment greatly suppressed the increase in both enzyme activities. When PEG was removed after 1 d, PAL and TAL activities substantially increased, reaching the same values as those of

Table II. *Correlation coefficients between the mechanical properties of cell walls and the wall constituents in wheat coleoptiles*

Cell Wall Constituent	T_0	Mechanical Extensibility
	ms	*mm 20 g*$^{-1}$
FA (ng/cm)	0.88[a]	−0.91[a]
DFA (ng/cm)	0.87[a]	−0.91[a]
Cellulose (μg/cm)	0.80[b]	−0.88[a]
Hemicellulose (μg/cm)	0.39	−0.58

[a,b] Statistically significant at the 0.1 and 1% levels, respectively.

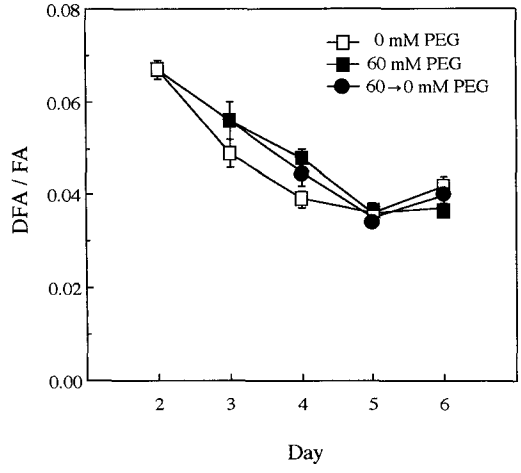

Figure 4. Ratio of DFA to FA in wheat coleoptile cell walls. The ratio was calculated from data of Figure 3. Treatments are as described in Figure 1. Data are means ± SE from three replicate experiments.

the unstressed coleoptiles on d 5 and then decreasing in parallel with the unstressed values on d 6. A close correlation was observed between the changes in activities of PAL or TAL and the rate of increase in the amount of wall-bound FA (Fig. 6). The activities of PAL and TAL per coleoptile showed similar changes (data not shown), and

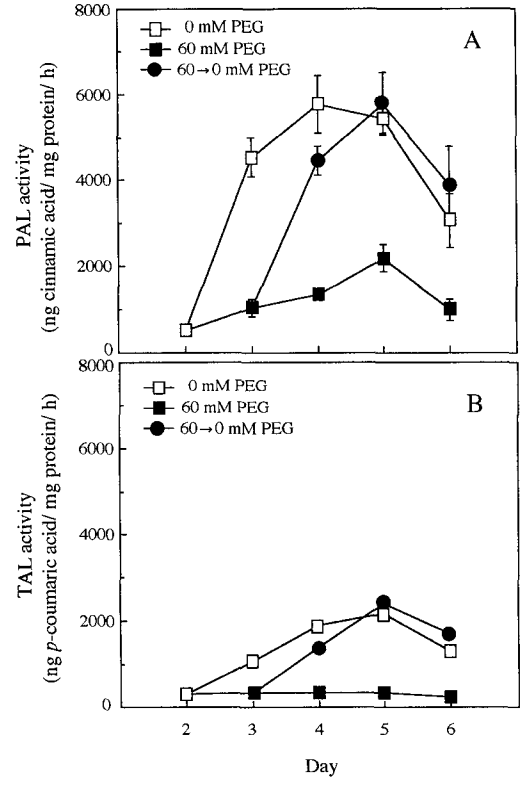

Figure 5. Changes in PAL (A) and TAL (B) activities in wheat coleoptiles. PAL and TAL activities are expressed as CA and CouA produced during 1 h of incubation per mg of protein, respectively. Treatments are as described in Figure 1. Data are means ± SE from three replicate experiments. Error bars for TAL activity were smaller than the symbols.

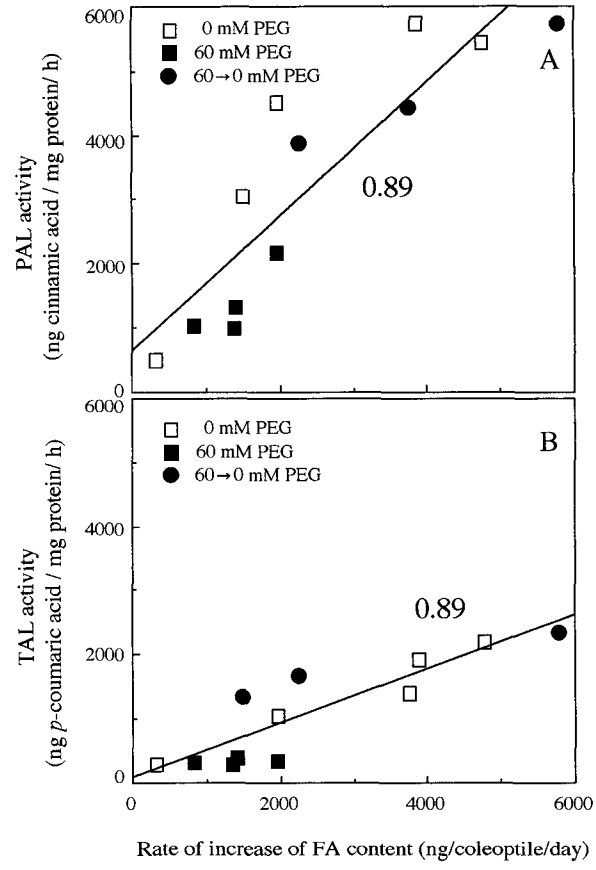

Figure 6. Relationship between PAL activity (A) and TAL activity (B) and the rate of increase in the amount of wall-bound FA in wheat coleoptiles. Treatments are as described in Figure 1. The rate of increase in the amount of wall-bound FA (increment of FA per day) was calculated using the data of Figures 1 and 3. The PAL and TAL activities are from Figure 5.

the changes in PAL and TAL activities per coleoptile were also highly correlated with the rate of increase in the amount of wall-bound FA (r = 0.85 and 0.89 for PAL and TAL, respectively).

DISCUSSION

PEG-induced osmotic stress applied to roots of dark-grown wheat seedlings reduced coleoptile growth. In response to PEG removal, the coleoptile growth rate increased and coleoptile length was the same as that of the unstressed coleoptiles (Fig. 1). Rapid growth recovery upon relief of PEG-induced osmotic stress was also observed with squash hypocotyl (Sakurai et al., 1987a), mung bean hypocotyl (Kutschera, 1989), and chick-pea epicotyl (Muñoz et al., 1993). Thus, the application of 60 mM PEG for 1 d to roots of wheat seedlings may represent osmotic stress without physical damage.

Stress-relaxation analysis for measuring the mechanical properties of cell walls is based on the Maxwell viscoelastic model (Yamamoto et al., 1970). Of the stress-relaxation parameters, T_0 corresponds to a Maxwell component with the lowest viscosity, and the reciprocal of R

($1/R$) corresponds to the number of components per unit volume (Masuda and Yamamoto, 1985; Sakurai, 1991). Therefore, T_0 has been considered to represent the viscous nature of cell walls; for instance, an increase in T_0 indicates that the cell walls become more viscous or stiff, whereas a decrease indicates that the cell walls become less viscous or loosened. An increase in T_0 and R has been reported to correlate negatively with the capacity of cell walls to extend in intact growing barley (Sakurai et al., 1984), rice (Hoson and Masuda, 1991), and oat (Kamisaka et al., 1990; Miyamoto et al., 1994) coleoptiles that had been fixed by boiling with methanol. The data on mechanical properties (Table I) suggest that the cell walls of unstressed coleoptiles became stiff as the growth rate slowed, and that osmotic stress prevented the cell wall stiffening as previously observed with PEG-treated squash (Sakurai and Kuraishi, 1988) and mung bean (Kutschera, 1989) hypocotyls.

Osmotic stress reduced the increase in cellulose content per unit length from d 4 to 6 (Fig. 2), suggesting that osmotic stress prevented the thickening of cell walls in the later period of coleoptile growth. A significant correlation between the cellulose content and the T_0 or the mechanical extensibility (Table II) suggests that, at least in part, the reduction of the increase in cellulose content is involved in maintaining cell wall extensibility under osmotic stress conditions, since young extensible walls contain less cellulose than mature nonextensible walls (Nishitani and Masuda, 1979; Wakabayashi et al., 1993). A potent inhibition of the synthesis of cellulose under osmotic stress conditions has also been reported in cultured tobacco cells (Iraki et al., 1989), in cotton roots (Zhong and Läuchli, 1988), and in expanding grape leaves (Sweet et al., 1990).

The cross-linkage of arabinoxylans by DFA bridges is considered to be involved in a decrease in cell wall extensibility (Fry, 1986). Indeed, an increase in wall-bound DFA was associated with a decrease in cell wall extensibility in rice and oat coleoptiles (Kamisaka et al., 1990; Tan et al., 1991,1992a; Miyamoto et al., 1994). The exogenous application of FA to water-grown rice seedlings increased the FA content in coleoptile cell walls but not the DFA content, whereas it decreased the cell wall extensibility of coleoptiles (Tan et al., 1992b). These findings suggest that, like DFA, the increase in FA content also decreased the cell wall extensibility, possibly by interfering with the enzymatic degradation processes of arabinoxylans (Fry, 1984). In the present study osmotic stress substantially reduced the increase in the amount of wall-bound FA and DFA (Fig. 3). A close correlation between the mechanical extensibility or T_0 and the FA or DFA contents (Table II) suggests that FA and DFA are important cell wall constituents for determining the mechanical properties of cell walls of intact growing wheat coleoptiles and that osmotic stress maintains cell wall extensibility by suppressing the increase in wall-bound FA and DFA.

Although the ratio of DFA to FA in cell walls of wheat coleoptiles decreased during growth, osmotic stress did not affect these values (Fig. 4). This result suggests that osmotic stress did not affect the step of the formation of DFA. Therefore, the suppression of the increase in wall-bound DFA in stressed coleoptiles was mainly due to the suppression of the increase in FA content. To investigate the mechanism by which osmotic stress inhibits the increase in wall-bound FA content, the enzyme activities involved in the biosynthesis of FA were examined. PAL and TAL activities (Fig. 5) changed in parallel with the increase in wall-bound FA (Fig. 3). Osmotic stress substantially suppressed the increases in enzyme activities, but when it was relieved the activities increased rapidly. A close correlation between the changes in activities of PAL or TAL and the rate of increase in the amount of wall-bound FA (Fig. 6) suggests that PAL and TAL are involved in determining the level of wall-bound FA in wheat coleoptiles and that osmotic stress suppresses the enzyme activities, thereby reducing the feruloylation of arabinoxylans.

The mechanism by which osmotic stress inhibits the activities of PAL and TAL is not known. Ward et al. (1989) reported that exogenously applied ABA inhibited the fungal pathogen-induced increase in PAL activity by suppressing the induction of PAL mRNA in soybean hypocotyls. In general the levels of endogenous ABA in plant tissues increase rapidly under osmotic stress conditions (Walton, 1980; Davies and Mansfield, 1983). Therefore, it is possible that osmotic stress increases the level of endogenous ABA in wheat coleoptiles, which, in turn, suppresses PAL and TAL activities by inhibiting the induction of mRNAs for the enzymes. This possibility is now under investigation.

ACKNOWLEDGMENTS

We wish to thank Prof. Y. Masuda (Tezukayama College, Nara, Japan) for his careful reading of the manuscript and invaluable criticism. We also thank T. Kotani for her technical assistance.

Received July 1, 1996; accepted December 4, 1996.

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