Protoplasma (2008)
DOI 10.1007/s00709-008-0303-1

PROTOPLASMA
Printed in Austria

Expansins and coleoptile elongation in wheat

Qiang Gao[1,2], Meirong Zhao[1], Feng Li[1], Qifang Guo[1], Shichao Xing[1], Wei Wang[1]

[1] State Key Laboratory of Crop Biology, College of Life Science, Shandong Agricultural University, Tai'an, Shandong
[2] Chemical Engineering Department, XuZhou College of Industrial Technology, Xuzhou, Jiangsu

Received 18 October 2007; Accepted 14 January 2008; Published online 26 August 2008
© Springer-Verlag 2008

Summary. Expansins are now generally accepted to be the key regulators of wall extension during plant growth. The aim of this study was to characterize expansins in wheat coleoptiles and determine their roles in regulating cell growth. Endogenous and reconstituted wall extension activities of wheat coleoptiles were measured. The identification of β-expansins was confirmed on the basis of expansin activity, immunoblot analysis, and β-expansin inhibition. Expansin activities of wheat coleoptiles were shown to be sensitive to pH and a number of exogenously applied factors, and their optimum pH range was found to be 4.0 to 4.5, close to that of α-expansins. They were induced by dithiothreitol, K^+, and Mg^{2+}, but inhibited by Zn^{2+}, Cu^{2+}, Al^{3+}, and Ca^{2+}, similar to those found in cucumber hypocotyls. An expansin antibody raised against TaEXPB23, a vegetative expansin of the β-expansin family, greatly inhibited acid-induced extension of native wheat coleoptiles and only one protein band was recognized in Western blot experiments, suggesting that β-expansins are the main members affecting cell wall extension of wheat coleoptiles. The growth of wheat coleoptiles was closely related to the activity and expression of expansins. In conclusion, our results suggest the presence of expansins in wheat coleoptiles, and it is possible that most of them are members of the β-expansin family, but are not group 1 grass pollen allergens. The growth of wheat coleoptiles is intimately correlated with expansin expression, in particularly that of β-expansins.

Keywords: Cell extension; Cell elongation growth; Cell wall; Expansin; Wheat coleoptile.

Abbreviations: DTT dithiothreitol; EDTA disodium ethylenediaminetetraacetate; PVDF polyvinylidene fluoride membrane.

Introduction

Expansins are cell wall proteins that induce cell wall extension in vitro and cell expansion in vivo by disrupting hydrogen bonds between cellulose microfibrils and matrix polymers (McQueen-Mason and Cosgrove 1994, Cosgrove 1999). Since they were first isolated from cucumber hypocotyls (McQueen-Mason et al. 1992), expansins have been identified in many plant species and organs (Cosgrove 1997, 2000; Cho and Cosgrove 2002; Li et al. 2003; Kwasniewski and Szarejko 2006). Previous research has shown that expansins participate in the process of plant growth. For example, Huang et al. (2000) reported a close relationship between the expression of expansin genes and rice coleoptile elongation in response to different oxygen concentrations. In another study, maize roots in low water potentials were able to continue growing by increasing the extensibility of the cell walls and this was correlated with an increase in expansin activity and transcript accumulation (Wu et al. 2001).

The expansin superfamily of plant proteins is made up of four families, designated α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA), and expansin-like B (EXLB) (Sampedro and Cosgrove 2005). α-Expansin and β-expansin proteins are known to have cell wall-loosening activity and to be involved in cell expansion and other developmental events during which cell wall modification occurs. α-Expansin proteins and some, but not all, β-expansin proteins are implicated as catalysts of "acid growth". While a role of α-expansins in cell extension has been reported for many plants (McQueen-Mason and Rochange 1999, Cosgrove 2000), β-expansins were originally represented by group 1 allergens of grass pollen (Cosgrove et al. 1997), which constitute a divergent group of β-expansin family expressed at high levels in the pollen of grasses but not of other plant groups, with additional research suggesting β-expansins are more abundant

Correspondence: Wei Wang, State Key Laboratory of Crop Biology, College of Life Science, Shandong Agricultural University, Tai'an, Shandong, 271018, People's Republic of China.
E-mail: wangw@sdau.edu.cn

in grasses than in dicots (Cosgrove et al. 1997, McQueen-Mason and Rochange 1999).

While expansins have been the subject of much research, more studies have focused on dicotyledons than monocotyledons. The characteristics of many α-expansins have been identified, but fewer of β-expansins. For example, it has been reported that all the α-expansin proteins that have been characterized so far have a pH optimum for cell wall extension of about 4 (McQueen-Mason et al. 1992, Li et al. 1993, Cho and Kende 1997). The pH optimum of only one class of β-expansin proteins has been characterized, namely, the group 1 grass pollen allergens (such as EXPB1 from maize), which has a broad pH optimum centered at about 5.5 (Li et al. 2003). The pH optimum of other β-expansin proteins in vegetative tissue is still unknown. Moreover, research into expansin expression and function in wheat, a typical monocotyledon, has been limited. Lin et al. (2005) isolated 18 expansin genes from winter wheat and demonstrated that the expression of wheat expansins may be involved in the heterosis in internode length and plant height. Jin et al. (2006) isolated two wheat expansin genes that are expressed during male gametophyte development. However, these few studies focused only on changes in mRNA levels and did not address protein accumulation or enzyme activity.

The aim of the present work was to evaluate the characteristics of wheat expansins, in particular those of β-expansins in vegetative tissues originally represented by group 1 grass pollen allergens, and find possible roles in the regulation of cell growth with wheat coleoptiles as ideal experimental units.

Material and methods

Plant materials

Wheat seeds (*Triticum aestivum* L.) were allowed to germinate in the dark on moist filter paper at 26 °C for 1 day and then grown in the dark in moist vermiculite at 26 °C for 2.5 days. Apical sections of coleoptiles (1.5 cm) were excised and frozen at −20 °C for no more than 5 days. To measure the temporal pattern of wheat coleoptile growth, germinated seeds were grown in the dark for 1 day, and then the coleoptiles were measured and harvested every 6 h in green light.

Extraction of cell wall proteins

Cell wall proteins in the wheat coleoptiles were extracted according to the method of McQueen-Mason et al. (1992). Plant tissues were homogenized in a blender in cold buffer (20 mM sodium acetate, pH 4.5, 2 mM disodium ethylenediaminetetraacetate [EDTA]). Wall material was collected on a nylon screen (50 μm mesh) and thoroughly rinsed with cold distilled water. Proteins were extracted from wall material for 2 h on ice in extraction buffer (1 M NaCl, 25 mM HEPES, pH 7.0, 3 mM sodium metabisulfite, 2 mM EDTA, 5 mM dithiothreitol [DTT]) and were then squeezed through nylon mesh. The wall fragments were further extracted by suspension in the same extraction buffer for an additional 1 h. The proteins in the combined supernatants were slowly precipitated with 0.39 g of ammonium sulfate per ml for 12 h. Precipitated proteins were pelleted by centrifugation (20800 **g**, 15 min, 4 °C) and subsequently resuspended in sodium acetate (50 mM, pH 4.5). The solution was dialyzed for 12 h and the protein concentration was determined by the method of Bradford (1976). All samples were adjusted to 2 mg/ml.

Reconstituted wall extension of wheat coleoptiles

An extensometer was installed as described by Cosgrove (1989) with some modifications (Gao et al. 2007). Wheat coleoptiles were inserted between two clamps spaced 5 mm apart and subjected to a constant tension of 12 g. The plastic cuvette was filled with buffer solution or cell wall protein solution. Wall extension was detected by a linear variable displacement transducer (model WYTO-2; Hefei Keyu Transducer Co. Ltd., Hefei, China) as movement of the transducer core and recorded on a microcomputer.

Reconstituted wall extension was carried out according to the method of McQueen-Mason et al. (1992), with a little modification as described by Cosgrove and Li (1993). Coleoptiles were abraded with carborundum and then frozen at −20 °C. Wheat coleoptiles were thawed, boiled in water (20 s) to eliminate endogenous expansin activity, pressed, and clamped onto the extensometer. After pretreatment with sodium acetate buffer (50 mM, pH 4.5) for 30 min, the solution was exchanged with the same buffer containing cell wall proteins of wheat coleoptiles (2 mg/ml) and the extension was recorded for a further 60 min. Expansin activity was assayed by measuring the wall extension after addition of protein extracts.

Acid-induced extension of native wheat coleoptiles

Acid-induced cell wall extension was carried out according to the method of Cosgrove (1989). Coleoptiles were abraded with carborundum and then washed with water. The most apical 8 mm of the coleoptiles was cut and squeezed between two glass slides to remove cell sap. The segments were fixed on an extensometer between two clamps 5 mm apart, under a constant load of 12 g. The wall sections were first incubated in HEPES buffer (50 mM, pH 6.8) for 30 min and then in sodium acetate buffer (50 mM, pH 4.5) for 60 min to measure cell wall extension.

Antibody preparation

Expansin antibody was obtained from a recombinant expansin protein (TaEXPB23, GenBank accession number AY260547, from wheat coleoptile). The protein was extracted from host *Escherichia coli* cell and purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The band containing expansin protein was cut from the gel, dried, pulverized, and homogenized with Freund's adjuvants prior to immunization of the rabbit. Seven weeks after the initial injection, serum was harvested. Preimmune serum and nonimmune serum at the same dilution were used as controls.

Inhibition of acid-induced extension of native wheat coleoptiles by antibodies

Suppression of cell wall extension by antibodies was measured as reported previously (Inouhe and Nevins 1991). The outer surface of the coleoptile was abraded with moist carborundum, rinsed with distilled water, and placed in K-citrate phosphate buffer (10 mM, pH 6.5) containing TaEXPB23 antibody (1 : 50 dilution) for 1 h. After preincubation, the segments were rinsed with distilled water and fixed onto the clamps. Segments were submerged in sodium acetate buffer (50 mM, pH 4.5) for 60 min and the increase in section length was traced with the extensometer.

Immunoblot analysis

Cell wall proteins (20 μg for each sample) were separated on 12% (w/v) SDS-polyacrylamide gels and electro-transferred onto a polyvinylidene fluoride (PVDF) membrane (Immobilon, Millipore) according to the manufacturer's protocol. After blocking overnight with 3% bovine serum albumin in buffer (10 mM phosphate, 0.9% NaCl, pH 7.2), the membrane was incubated with expansin antibody (1 : 200 dilution, 3 h) and rinsed (3 times for 10 min each) in PBS. The membrane was then incubated with goat anti-rabbit immunoglobulin G-conjugated horseradish peroxidase in blocking solution (1 : 10000 dilution, 2 h) and rinsed once again. The signal was detected with 4-chloro-1-naphthol (Sigma) as the substrate.

Results

Activity of wheat coleoptile expansins

We measured expansin activity by the reconstituted wall extension of wheat coleoptiles (Fig. 1 A). The data suggested that extension of boiled cell walls was reconstituted by the addition of cell wall proteins in acidic, but not in neutral buffer. Little cell wall extension was observed in either acidic or neutral bovine serum albumin solutions (control). We also measured the endogenous expansin activity by the acid-induced extension of native wheat coleoptiles (Fig. 1 B). Native cell walls of wheat coleoptiles exhibited long-term irreversible extension when placed under tension in acidic solution. Our data are in agreement with previous studies of cucumber hypocotyls (Cosgrove 1989, McQueen-Mason et al. 1992) and oat coleoptiles (Li et al. 1993), implicating the presence of expansins in wheat coleoptiles.

Immunoblot analysis of wheat coleoptile expansins

To correlate the expression of expansin proteins with acid-induced extension activity, extracted wheat coleoptile cell wall proteins were subjected to immunoblot analysis with the rabbit polyclonal antiserum raised against the recombinant wheat β-expansin protein TaEXPB23. Only one single band at a molecular mass of approximately 29 kDa was detected (Fig. 2), demonstrating the presence of expansin proteins in wheat coleoptiles. Preimmune serum and nonimmune serum at the same dilution did not detect any specific proteins in immunoblots.

Dependence of expansin activity on pH

Our data suggest that the optimal pH of wheat coleoptile expansins for reconstituted extension activity was between 4.0 and 4.5 (Fig. 3A). A similar optimal range was observed for the endogenous wall extension activity in wheat coleoptiles (Fig. 3B). Once the heat-inactivated cell walls were pretreated with exogenous cell wall proteins, the reconstituted walls required no further cell wall proteins for extension (Fig. 4 A). The expansins appeared to

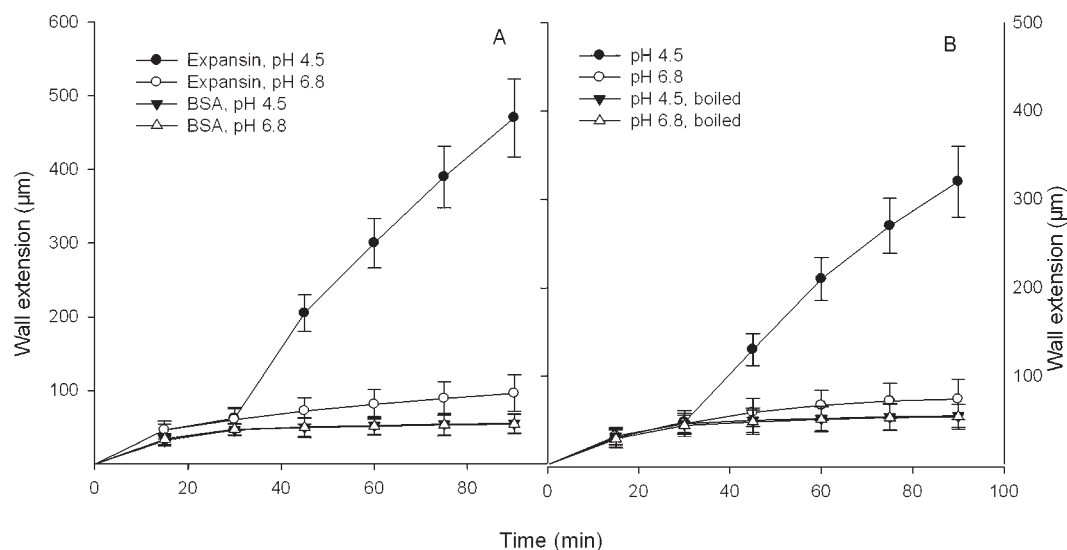

Fig. 1 A, B. Activity of wheat coleoptile expansin. **A** Reconstituted wall extension of wheat coleoptiles by cell wall proteins. After pretreatment of boiled cell walls with sodium acetate buffer (50 mM, pH 4.5) for 30 min, the solution was replaced with the same buffer containing cell wall proteins (2 mg/ml) for 60 min. Proteins in neutral buffer and bovine serum albumin in both acidic and neutral solutions were used as controls. **B** Acid-induced extension of native wheat coleoptiles. Native coleoptiles were suspended under tension in HEPES buffer (50 mM, pH 6.8) for 30 min, and the solution was then replaced with sodium acetate buffer (50 mM, pH 4.5) for 60 min. Native coleoptiles in neutral buffer and boiled wheat coleoptiles in both acidic and neutral solutions were used as controls. The wall extension was measured by the extensometer and all the experiments were repeated at least five times

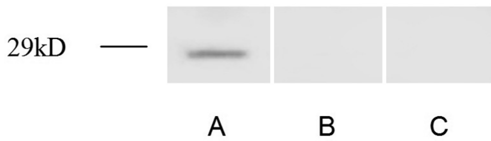

Fig. 2 A–C. Immunoblot analysis of cell wall proteins from wheat coleoptiles. After 12% SDS-PAGE, protein samples were electro-transferred onto a PVDF membrane and probed with the expansin antibody. **A** Immunoblot of proteins from wheat coleoptiles probed with polyclonal antibody against TaEXPB23 **B** Immunoblot of proteins from wheat coleoptiles probed with preimmune serum **C** Immunoblot of proteins from wheat coleoptiles probed with nonimmune serum

bind tightly to the coleoptile walls, as wall extension activity was not diminished by the exchange with expansin-free buffer. Native cell wall extension was observed in acidic solution and abolished in neutral solution (Fig. 4B). This phenomenon could be continuously repeated by alternating the pH of the incubation buffer (pH 4.5 and pH 6.8), suggesting that changes in the pH of the bathing solution only affect the conformation of expansins (perhaps not only one species) but not their affinity for the cell wall (Lee and Choi 2005).

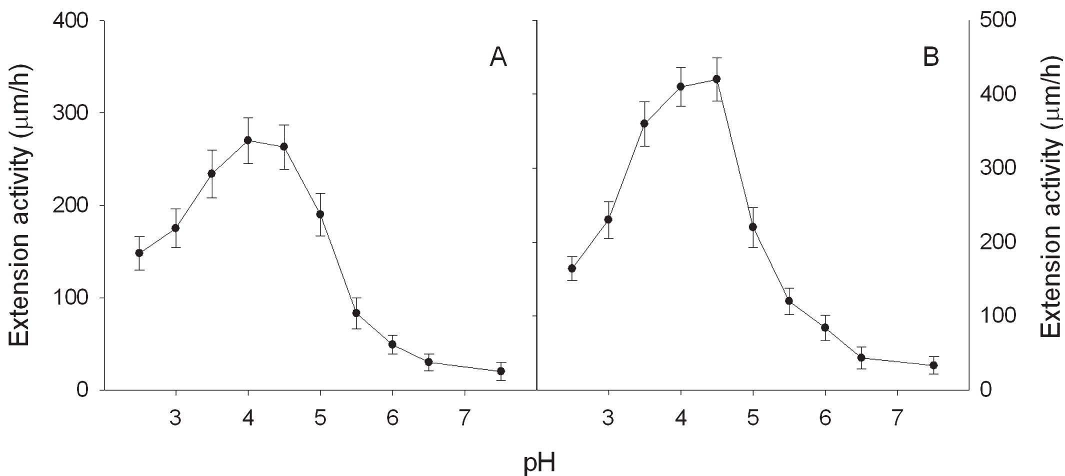

Fig. 3 A, B. Dependence of expansin activity on pH. **A** After pretreatment with sodium acetate buffer (50 mM, pH 4.5) for 30 min, the heat-inactivated cell walls were incubated in cell wall protein solution (2 mg/ml) at each pH value (2.5 to 7.5) for 60 min. **B** After pretreatment with HEPES buffer (50 mM, pH 6.8) for 30 min, native cell walls were incubated at each pH value (2.5 to 7.5) for 60 min. Expansin activity was assayed using an extensometer, by measuring the wall extension after addition of the second bathing buffer (50 mM citric acid for pH 2.5 to 4.0; 50 mM sodium acetate for pH 4.5 to 5.5; 50 mM HEPES for pH 6.0 to 7.5)

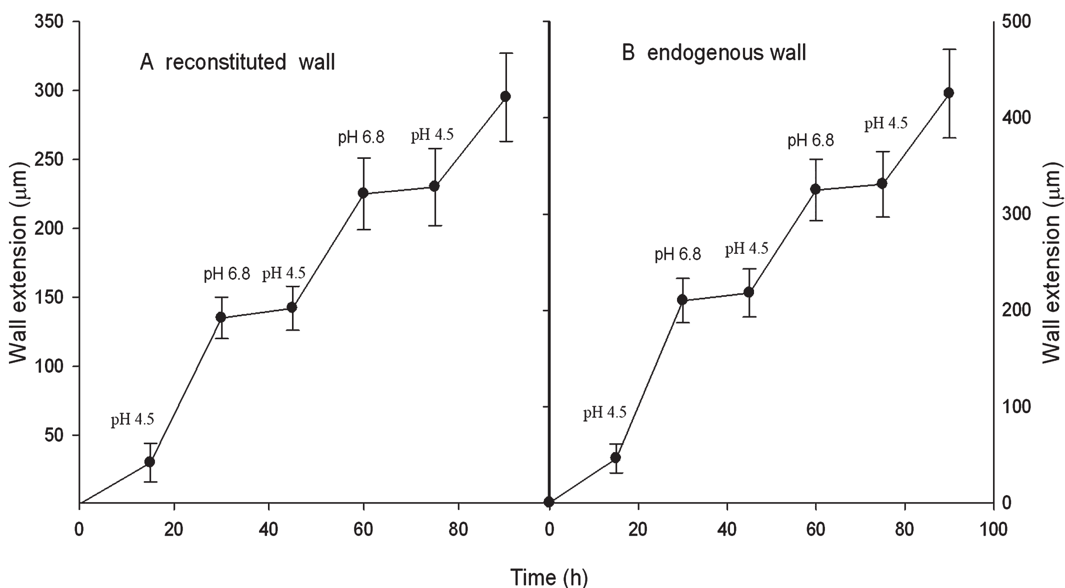

Fig. 4 A, B. Reversibility of reconstituted and endogenous wall extension of wheat coleoptiles. **A** Coleoptiles were thawed and boiled. After incubation in cell wall proteins (2 mg/ml) for 30 min, the heat-inactivated cell walls were clamped and submerged in HEPES buffer (50 mM, pH 6.8) for 15 min, and then placed in sodium acetate buffer (50 mM, pH 4.5) for another 15 min. **B** Coleoptiles were thawed and clamped on the extensometer. After pretreatment with HEPES buffer (50 mM, pH 6.8) for 15 min, native cell walls were incubated in sodium acetate buffer (50 mM, pH 4.5) for another 15 min

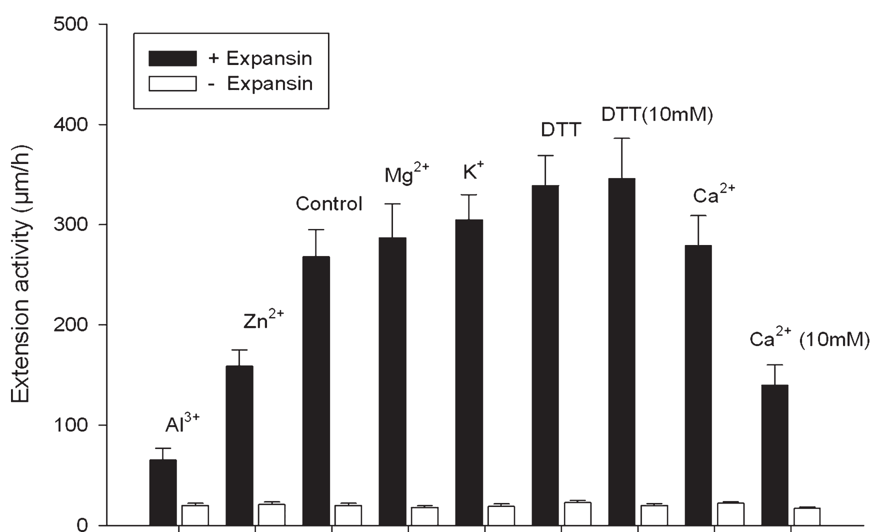

Fig. 5. Effects of various ions and DTT on reconstituted wall extension of wheat coleoptiles. Following pretreatment of boiled cell walls with sodium acetate buffer (50 mM, pH 4.5) for 30 min, the solution was replaced with cell wall proteins (2 mg/ml) containing 1 mM $ZnCl_2$, $AlCl_3$, $MgCl_2$, KCl, $CaCl_2$ (1 mM and 10 mM), or DTT (1 mM and 10 mM). Expansin activity was assayed by measuring the wall extension rate after addition of the extracts. Solutions without cell wall proteins were used as controls

Effects of various ions and DTT on reconstituted extension activity of wheat coleoptile walls

The effects of various ions and DTT on the creep rate of wheat coleoptiles are summarized in Fig. 5. Our data suggested that DTT, a thiol-reducing agent, enhanced the wall extension activity of wheat coleoptiles reconstituted with cell wall proteins. The data are in agreement with the results reported by Cosgrove (1989), who demonstrated that DTT stabilized or even enhanced the endogenous extension of native cucumber cell walls under acidic conditions. Endogenous acid-induced extension in cucumber hypocotyl walls was previously shown to be sensitive to a number of exogenously applied factors (Cosgrove 1989). In this study, the reconstituted wall extension was induced by K^+ and Mg^{2+}, but inhibited by Al^{3+}, Ca^{2+} (10 mM), and Zn^{2+} (Fig. 5). These effects were not observed when the same pretreated cell walls were incubated in solutions containing ions and DDT but lacking cell wall proteins, indicating that they were mediated by cell wall proteins.

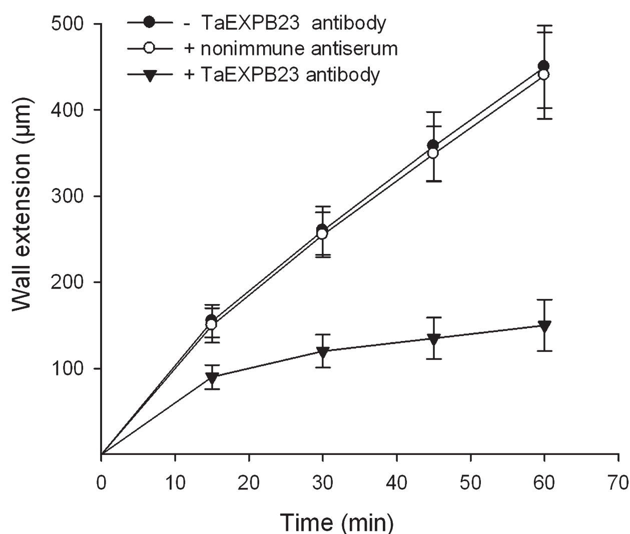

Fig. 6. Inhibition of acid-induced extension of native wheat coleoptiles by expansin antibody. Coleoptiles were abraded, rinsed, and placed in K-citrate phosphate buffer (10 mM, pH 6.5) containing expansin antibody (1 : 50 dilution) for 1 h. After preincubation, the segments were rinsed and fixed onto the clamps. Segments were submerged in sodium acetate buffer (50 mM, pH 4.5) for 60 min and the increase in section length was traced with an extensometer. K-citrate phosphate buffer without expansin antibody or with nonimmune antiserum were used as controls

Inhibition of acid-induced extension of native wheat coleoptiles by expansin antibody

Native cell walls of wheat coleoptiles were incubated with expansin antibody, which resulted in great inhibition of the acid-induced extension of wheat coleoptiles (Fig. 6), but no suppression of cell wall elongation was observed with nonimmune antiserum, suggesting that cell wall extension is closely related to expansin activity, especially that of the β-expansins recognized by the TaEXPB23 antibody.

Expansins and temporal growth of wheat coleoptiles

Figure 7 shows the temporal growth and native expansin activity of wheat coleoptiles. The growth rate was faster at earlier phases in the course of coleoptile growth, with the greatest rate at about 36 h, and then it slowed down after about 48 h; little growth was observed at about 72 h (Fig. 7A). The temporal trend of expansin activities in native cell walls was also measured (Fig. 7B), which corresponded to the temporal trend of coleoptile growth.

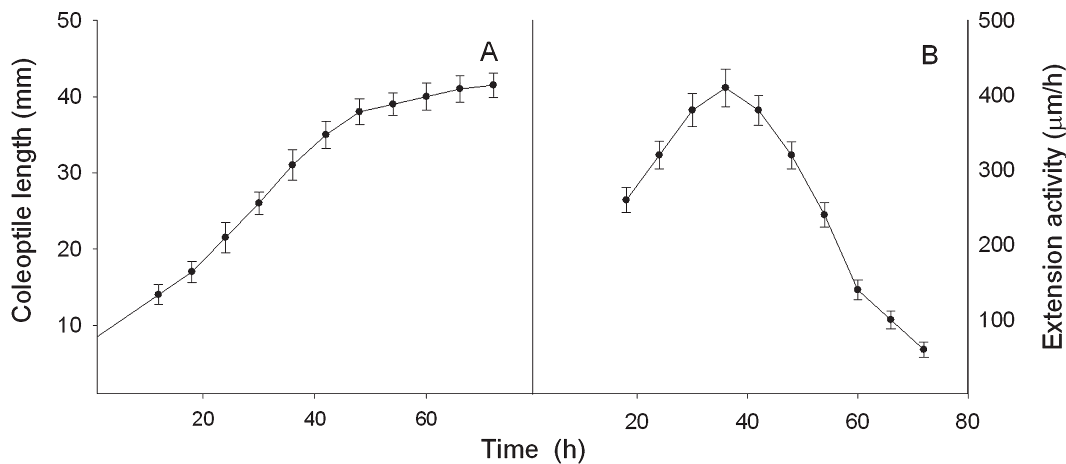

Fig. 7 A, B. Wall extensibility and temporal growth of wheat coleoptiles. Wheat seeds were sown and soaked in distilled water for 1 day and then grown in the dark. The coleoptiles were measured and harvested every 6 h in green light. **A** Growth curve of wheat coleoptiles. Data represent the means of 20 coleoptiles. **B** Wall extensibility of wheat coleoptiles over time

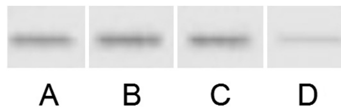

Fig. 8 A–D. Immunoblot analysis of expansin protein expression in wheat coleoptiles over time. Cell wall proteins were separated by SDS-PAGE and blotted onto a PVDF membrane. The presence of expansin was detected with the TaEXPB23 antibody. Wheat coleoptiles after 24 (**A**), 36 (**B**), 48 (**C**), and 72 h (**D**) growth. Preimmune serum and non-immune serum at the same dilution did not detect any specific proteins in immunoblots (data not shown)

The temporal expression of wheat coleoptile expansin proteins was also evaluated with our β-expansin antibody (Fig. 8). Protein immunoblot analysis confirmed the expression of expansin proteins in wheat coleoptiles, which was consistent with their activity. Higher levels were detected at 24, 36, and 48 h, but lower levels at 72 h, suggesting that the growth rate of wheat coleoptiles is related to the expression of expansins, more specifically, those β-expansins recognized by the TaEXPB23 antibody.

Discussion

Expansins, cell wall-loosening proteins, are important elements of many physiological processes in plants (Cosgrove 2000, Cho and Cosgrove 2002, Li et al. 2003, Kwasniewski and Szarejko 2006). In this study, we found that cell wall proteins extracted from wheat coleoptiles induced creep extension of heat-inactivated walls in acidic buffer (Fig. 1A); similar results were also noted in acid-induced extension of native wheat coleoptiles (Fig. 1B). Western blot analysis of proteins extracted from cell walls of wheat coleoptiles identified only one protein band at about 29 kDa (Fig. 2). These results demonstrate the presence of expansin(s) in wheat coleoptiles. But what are the biochemical characteristics of the expansins in wheat coleoptiles and the relationship between expansin expression and coleoptile growth?

Using the proteins extracted from cell walls of wheat coleoptiles, we determined the characteristics of the expansins. Previous research has shown expansin activity to be dependent on pH (McQueen-Mason et al. 1992), but the optimum pH varies among different expansins (Sampedro and Cosgrove 2005). All the α-expansin proteins that have been characterized so far have a pH optimum for cell wall extension of about 4 (McQueen-Mason et al. 1992, Li et al. 1993, Cho and Kende 1997). For example, the cucumber wall-extension-inducing proteins CsEXPA1 (or Ex29, a 29 kDa protein) and CsEXPA2 (or Ex30, a 30 kDa protein) display an optimum pH range of 3.5 to 4.5 (McQueen-Mason et al. 1992, Catalá et al. 2000). The optimum pH of oat coleoptile expansin, which can be recognized by the antibody raised against CsEXPA1 (Ex29), is 4.5 to 5.0 (Li et al. 1993). The pH optimum of only one class of β-expansin proteins has been characterized, namely, the group 1 grass pollen allergens (such as EXPB1 from maize), and this is centered at about 5.5 (Li et al. 2003). However, the pH optimum of other β-expansin proteins in vegetative tissue is still unknown. In this study, endogenous and reconstituted wall extension data suggest that the optimum pH for wheat coleoptiles is 4.0 to 4.5 (Fig. 3), which is consistent with the pH optimum of α-expansin. Meanwhile, as shown in Fig. 2, only one protein band of about 29 kDa was observed by Western blot

with antibody raised against TaEXPB23, and the cell wall extension of native wheat coleoptiles was greatly inhibited by this antibody (Fig. 8). All of these results suggest that most of the expansins in wheat coleoptiles can be recognized by the antibody against TaEXPB23, a member of the EXPB family from wheat coleoptile vegetative tissue. However, these expansins do not belong to the group 1 grass pollen allergens, because the pH optimum of expansins in wheat coleoptiles, about 4.5, is different from that of the group 1 grass pollen allergens (about 5.5). It was expected that the β-expansin proteins in somatic tissues would have a pH dependence closer to that of α-expansins, but this expectation remains to be tested experimentally (Sampedro and Cosgrove 2005). We have also found that expansin proteins extracted from wheat coleoptiles and cucumber hypocotyls can induce extension of cell walls in each other (Gao et al. 2007), albeit at a different level of activity. This implies that dicotyledons and grasses have a common biochemical mechanism for at least part of acid-induced wall extension, despite the significant differences in wall composition between these two classes of plants (Carpita 1996, Cosgrove 1997).

Our data (Fig. 5) demonstrate that DTT enhanced the wall extension activity of wheat coleoptiles. In addition, expansin activities were induced by K^{+} and Mg^{2+}, but inhibited by Zn^{2+}, Al^{3+}, and Ca^{2+} (10 mM) (Fig. 5). These results are similar to those previously obtained from cucumber hypocotyls (Cosgrove 1989, McQueen-Mason et al. 1992). However, our attention was drawn to the different effects of 1 and 10 mM Ca^{2+} concentrations.

Previous studies have reported the importance of Ca^{2+} as a second messenger in plant cells and its roles in plant cell growth and development (Hepler 2005). Increases in [Ca^{2+}] lead to an inhibition in shoot or coleoptile growth, whereas reduction promotes cell and tissue elongation (Tagawa and Bonner 1957). Additionally, research has also implicated changes in [Ca^{2+}] in the control of plant cell growth, largely based on the premise that Ca^{2+} produces a pronounced effect in determining the structural rigidity of the cell wall (Jones and Lunt 1967). Low [Ca^{2+}] makes cell walls more pliable and easily ruptured, whereas high [Ca^{2+}] rigidifies the wall and makes it less plastic (Hepler 2005). Reports have also shown that calcium affects expansin gene expression during fruit softening. Wang et al. (2005) infiltrated tomato fruits with 4% calcium chloride and found that expansin expression was inhibited at late stages of fruit softening. In the current study, we applied different Ca^{2+} concentrations in reconstituted wall assays and noted that, while 1 mM Ca^{2+} had a small effect on expansin activity, extension was inhibited by 10 mM Ca^{2+} (Fig. 5). Our data and that of others suggest that calcium might, at least in part, affect expansin activity directly, in addition to modifying the structural rigidity of the cell wall directly. Future research is needed to clearly elucidate the effects of Ca^{2+} on the mechanisms underling wall creep as well as the link between Ca^{2+} and expansin gene expression in the growth and development of the plant cell.

While there are no known specific inhibitors of expansins, their location on cell walls makes them accessible for antibodies which can be used to inhibit expansin activity. lnouhe and Nevins (1991) found that antibodies raised against wall proteins inhibited both auxin-induced growth and wall autolysis of corn coleoptiles. Research by Su Chen (China Agricultural University, Beijing, unpubl. data) demonstrated that the AtEXP1 antibody is an inhibitor of stomatal movement. In this study, acid-induced extension of wheat coleoptiles was greatly inhibited by the vegetative β-expansin protein antibody raised against TaEXPB23, which revealed that expansin expression, in particular that of vegetative β-expansin protein, is intimately correlative with cell wall extension of wheat coleoptiles (Fig. 6). However, other factors may also be involved in coleoptile cell wall extension, since wall extension was still observed in the presence of superfluous antibody. These factors may be potential expansins not recognized by the antibody, or other proteins. For example, xyloglucan endotransglucosylase/hydrolases and glucanases are also involved in cell wall extension (Fry et al. 1992, Li et al. 2003).

What then is the relationship between expansins and wheat coleoptile growth? Cell division rarely occurs in wheat coleoptile growth, its elongation is primarily attributed to cell expansion (Philippar et al. 1999), and consequently, this makes the coleoptile an ideal unit with which to investigate the mechanisms of cell elongation. Cell extension growth in turgid plant organs is brought about by chemically loosening the structure of growth-limiting cell walls, resulting in the relaxation of wall tension and concomitant osmotic water uptake. The rate of cell expansion depends on cell wall-yielding properties and turgor pressure inside the cell (Cosgrove 1993, Proseus et al. 2000). In addition, the susceptibility of walls to expansin action as well as other enzymes in the cell wall is also correlated with cell growth (Cosgrove and Li 1993). Previous studies of cell extension focused mainly on turgor pressure. While the plant cell wall behaves as a tough polymeric network to restrain cell expansion, the yielding of it to turgor pressure is generally recognized as a key control point for plant cell enlargement (Cosgrove 1999). To enlarge, cells selec-

tively loosen this network, enabling it to yield to the expansive forces generated by cell turgor pressure. The enzymes that act on this network control the process of cell growth (Li et al. 2003). The biochemical mechanism of this wall-loosening reaction has not yet been elucidated, although numerous potential wall-loosening proteins have been investigated (Hoson 1993; Cosgrove 1999, 2000). Expansins play an important role in the enlargement of living cells (Cosgrove and Li 1993, Muller et al. 2007). Our results (Figs. 7 and 8) suggest that the extension rate of wheat coleoptiles is associated with cell wall extensibility, and that this is closely related to expansin expression (Fig. 8). Integrating the data in Fig. 6 into Figs. 7 and 8, we propose that the growth of wheat coleoptiles is intimately correlated with the expression of expansins, in particular those recognized by antibody raised against TaEXPB23, a member of the β-expansin family.

However, it must be noted that, because the expansin antibody used in this study was raised against wheat TaEXPB23, we cannot conclude that the detected protein expression (Fig. 8) represents all the expansins involved in wheat coleoptile growth, particularly in light of the fact that there is a large gene family of expansins in plants and several expansin homologues are usually expressed in different tissues and organs at the same time (Lin et al. 2005, Sampedro and Cosgrove 2005). A rigorous separation and purification of the cell wall proteins and (or) possession of an expansin antibody that can distinguish a single expansin member from others in an expansin family will be necessary for the functional analysis of all expansin members.

In conclusion, our results indicate the presence of expansins in wheat coleoptiles, most of which can be recognized by the antibody raised against TaEXPB23, a member of β-expansin family, but which do not belong to the group 1 grass pollen allergens. The growth of wheat coleoptiles is intimately correlated with expansin expression, in particular that of β-expansins.

Acknowledgments

We are grateful to D. J. Cosgrove (Pennsylvania State University, U.S.A.) for kind technical assistance with the experiments. This research was supported by the National Natural Science Foundation of China (30671259) and by the Institute of Science and Technology of Shandong Province in China (nr. 2006JY06-5).

References

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Carpita NC (1996) Structure and biogenesis of the cell walls of grasses. Annu Rev Plant Physiol Plant Mol Biol 47: 445–476

Catalá C, Rose JKC, Bennett AB (2000) Auxin-regulated genes encoding cell wall-modifying proteins are expressed during early tomato fruit growth. Plant Physiol 122: 527–534

Cho HT, Cosgrove DJ (2002) Regulation of root hair initiation and expansin gene expression in *Arabidopsis*. Plant Cell 14: 3237–3253

Cho HT, Kende H (1997) Expansins in deepwater rice internodes. Plant Physiol 113: 1137–1143

Cosgrove DJ (1989) Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls. Planta 177: 121–130

Cosgrove DJ (1993) Water uptake by growing cells: an assessment of the controlling roles of wall relaxation, solute uptake and hydraulic conductance. Int J Plant Sci 154: 10–21

Cosgrove DJ (1997) Creeping walls, softening fruit, and penetrating pollen tubes: The growing roles of expansins. Proc Natl Acad Sci USA 94: 5504–5505

Cosgrove DJ (1999) Enzymes and other agents that enhance cell wall extensibility. Annu Rev Plant Physiol Plant Mol Biol 50: 391–417

Cosgrove DJ (2000) Loosening of plant cell walls by expansins. Nature 407: 321–326

Cosgrove DJ, Li ZC (1993) Role of expansin in cell enlargement of oat coleoptiles. Plant Physiol 103: 1321–1328

Cosgrove DJ, Bedinger P, Durachko DM (1997) Group-I allergens of grass pollen as cell wall-loosening agents. Proc Natl Acad Sci USA 94: 6559–6564

Fry SC, Smith RC, Renwick KF, Martin DJ, Hodge SK, Matthews KJ (1992) Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochem J 282: 821–828

Gao Q, Guo QF, Xing SC, Zhao MR, Li F, Wang W (2007) The characteristics of expansins in wheat coleoptiles and their responses to water stress. J Plant Physiol Mol Biol 33: 402–410

Hepler PK (2005) Calcium: a central regulator of plant growth and development. Plant Cell 17: 2142–2155

Hoson T (1993) Regulation of polysaccharide breakdown during auxin-induced cell wall loosening. J Plant Res 106: 369–381

Huang J, Takano T, Akita S (2000) Expression of α-expansin genes in young seedlings of rice (*Oryza sativa* L.). Planta 211: 467–473

Inouhe M, Nevins DJ (1991) Inhibition of auxin-induced cell elongation of maize coleoptiles by antibodies specific for cell wall glucanases. Plant Physiol 96: 426–431

Jin Y, Tashpulatov AS, Katholnigg H, Heberle-Bors E, Touraev A (2006) Isolation and characterisation of two wheat β-expansin genes expressed during male gametophyte development. Protoplasma 228: 13–19

Jones RGW, Lunt OR (1967) The function of calcium in plants. Bot Rev 33: 407–426

Kwasniewski M, Szarejko I (2006) Molecular cloning and characterization of β-expansin gene related to root hair formation in barley. Plant Physiol 141: 1149–1158

Lee Y, Choi D (2005) Biochemical properties and localization of the β-expansin OsEXPB3 in rice (*Oryza sativa* L.). Mol Cells 20: 119–126

Li LC, Bedinger PA, Volk C, Jones AD, Cosgrove DJ (2003) Purification and characterization of four β-expansins (*Zea* m1 isoforms) from maize pollen. Plant Physiol 132: 2073–2085

Li Y, Jones L, McQueen-Mason S (2003) Expansins and cell growth. Curr Opin Plant Biol 6: 603–610

Li ZC, Durachko DM, Cosgrove DJ (1993) An oat coleoptile wall protein that induces wall extension in vitro and that is antigenically related to a similar protein from cucumber hypocotyls. Planta 191: 349–356

Lin Z, Ni ZF, Zhang Y, Yao YY, Wu HY, Sun QX (2005) Isolation and characterization of 18 genes encoding α- and β-expansins in wheat (*Triticum aestivum* L.). Mol Genet Genomics 274: 548–556

McQueen-Mason S, Cosgrove DJ (1994) Disruption of hydrogen bonding between cell wall polymers by proteins that induce plant wall extension. Proc Natl Acad Sci USA 91: 6574–6578

McQueen-Mason SJ, Rochange F (1999) Expansins in plant growth and development: an update in an emerging topic. Plant Biol 1: 19–25

McQueen-Mason S, Durachko DM, Cosgrove DJ (1992) Two endogenous proteins that induce cell wall extension in plants. Plant Cell 4: 1425–1433

Muller P, Bourdais G, Reidy B, Bencivenni C, Massonneau A, Condamine P, Rolland P, Conéjéro G, Rogowsky P, Tardieu F (2007) Association of specific expansins with growth in maize leaves is maintained under environmental, genetic, and developmental sources of variation. Plant Physiol 143: 278–290

Philippar K, Fuchs I, Luthen H, Hoth S, Bauer CS, Haga K, Thiel G, Ljung K, Sandberg G, Bottger M, Becker D, Hedrich R (1999) Auxin-induced K^+ channel expression represents an essential step in coleoptile growth and gravitropism. Proc Natl Acad Sci USA 96: 12186–12191

Proseus TE, Zhu GL, Boyer JS (2000) Turgor, temperature and the growth of plant cells: using *Chara corallina* as a model system. J Exp Bot 51: 1481–1494

Sampedro J, Cosgrove DJ (2005) The expansin superfamily. Genome Biol 6: 242

Tagawa T, Bonner J (1957) Mechanical properties of the Avena coleoptile as related to auxin and to ionic interactions. Plant Physiol 32: 207–212

Wang WY, Zhu BZ, Luo YB, Zhai BQ, Lü J (2005) Effect of calcium on polygalacturonase, lipoxygenase and expansin during tomato fruit softening. J Agric Univ Hebei 28: 12–15

Wu Y, Thorne ET, Sharp RE, Cosgrove DJ (2001) Modification of expansin transcript levels in the maize primary root at low water potentials. Plant Physiol 126: 1471–1479