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cDNA Clones Encoding 1,3-β-Glucanase and a Fimbrin-Like Cytoskeletal Protein Are Induced by Al Toxicity in Wheat Roots[1]

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A cDNA library made from mRNA of Al-treated roots of an Al-sensitive wheat (*Triticum aestivum* cv Victory) cultivar was screened with a degenerate oligonucleotide probe derived from the partial amino acid sequence of the Al-induced protein TAl-18. Of seven clones that initially hybridized with the probe, one encoded a novel 1,3-β-glucanase having a calculated molecular weight of 46.3 and an isoelectric point of 6.0. Like the A6 1,3-β-glucanase gene products from *Brassica napus* and *Arabidopsis thaliana*, the predicted wheat protein had a C-terminal extension with three potential glycosylation sites. Northern analysis revealed that wheat 1,3-β-glucanase mRNA was up-regulated in Al-intoxicated roots, with highest expression after 12 h. The antibody to A6 1,3-β-glucanase from *B. napus* cross-reacted with a 56-kD protein that was induced after 24 h. A second partial cDNA clone showed similarity to genes encoding cytoskeletal fimbrin-like (actin-bundling) proteins. Although well studied in animals and fungi, fimbrins have not previously been described in plants. Fimbrin-like transcripts were up-regulated after 24 h of Al treatment in the Al-sensitive wheat cv Victory. In the Al-tolerant cv Atlas 66, fimbrin-like mRNA was up-regulated within 12 h by Al concentrations that did not inhibit root growth. Cellular stress associated with Al toxicity therefore causes up-regulation of a defense-related gene and a gene involved in the maintenance of cytoskeletal function.

Al toxicity is a major factor limiting plant growth in acid soils (Delhaize and Ryan, 1995; Kochian, 1995). Effects of Al toxicity include changes in the pattern of gene expression and protein synthesis. In a previous study we observed that growth-inhibiting amounts of Al induced an acidic, 18.2-kD protein in wheat (*Triticum aestivum*) roots called TAl-18 (Cruz-Ortega and Ownby, 1993). The partial amino acid sequence of TAl-18 showed similarity with the pathogenesis-related protein PR2 from parsley (Linthorst, 1991). TAl-18 is part of a growing number of proteins associated with Al stress. Snowden and Gardner (1993), Richards et al. (1994), and Snowden et al. (1995) identified seven Al-induced cDNAs in a cDNA library from an Al-sensitive wheat genotype. The proteins encoded by these wheat aluminum induced (*wali*) clones showed similarity to the metallothionein-like proteins (*wali1*), Phe ammonia-lyase (*wali4*), proteinase inhibitors (*wali3*, *wali5*, and *wali6*), and Asn synthetases (*wali7*). The degree of induction of *wali* genes was related to the degree of Al stress in both Al-sensitive and Al-tolerant cultivars.

Similarly, Ezaki et al. (1995) characterized two Al-induced cDNA clones from cultured tobacco (*Nicotiana tabacum*) cells. One cDNA encoded an auxin-related gene with a product that may regulate the plant cell cycle and the other encoded a glutathione *S*-transferase with antioxidant activity that may help resist Al-induced membrane lipid peroxidation. An anionic peroxidase that was induced by Al toxicity in the same system was also suggested to protect membrane lipids against Al-generated peroxides (Ezaki et al., 1996). Glutathione *S*-transferase and the anionic peroxidase thus represent gene products in which there is an easily identifiable mechanism by which up-regulation could confer Al tolerance. Basu et al. (1994) identified a wheat root protein, RMP51, that was rapidly induced in an Al-tolerant cultivar but not in an Al-sensitive cultivar. Recent work has shown that this protein, which is associated with the tonoplast membrane, co-segregates with the resistance phenotype in crosses between the Al-sensitive and the Al-tolerant cultivars (Taylor et al., 1997).

Usually, however, Al-induced proteins such as TAl-18 and the *wali* gene products do not correlate with defense against Al toxicity per se, but, rather, appear to be induced as a generalized response to various stresses including Al, Cu, and Cd toxicity and Ca deficiency. Researchers have long sought to characterize genes with products that confer Al tolerance. Whether or not gene activation is a key component of Al tolerance remains to be proven. On the other hand, identification of genes with expression correlated with toxicity ought to provide insight into the primary sites of Al damage in plant cells. In this study we have isolated two Al-stress-induced genes that play both a nonspecific role in response to Al stress and a specific role in cytoskeleton organization and functionality during Al toxicity.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L. cv Victory [Al-sensitive] and cv Atlas 66 [Al-tolerant]) seeds were obtained from Johnston Seed (Enid, OK). Seeds were germinated in Petri dishes on filter paper (no. 4, Whatman) wetted with deionized water. Two-day-old seedlings were transferred to ny-

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lon screens and floated in 450 mL of macronutrient medium (Aniol, 1984). The pH of the nutrient medium was adjusted to 4.4 with either 0.1 M HCl or 0.1 M NaOH. All seedlings were grown in vigorously aerated nutrient solutions in a growth chamber with a 16-h photoperiod at 26°C (day) and 22°C (night) temperatures and illumination of 350 μmol m^{-2} s^{-1}. Four-day-old wheat seedlings were stressed by the addition of 80 or 400 μM $ALCl_3 \cdot H_2O$ to the nutrient solution. Leaves and roots were harvested at 6, 12, or 24 h of Al-stress treatment, immediately frozen under liquid N_2, and stored at −70°C until use. Estimation of Al toxicity was based on its effect on root growth. Root lengths were measured at time 0 and after 24 h of Al treatment. Relative growth was calculated with respect to the control root growth rate.

cDNA Library and Screening

Total RNA was extracted by the method of Kopeczynski et al. (1986) from root tips of intact cv Victory seedlings exposed to 80 μM Al for 12 h. Poly(A^+) RNA was prepared using an mRNA isolation kit (Fast Track, Invitrogen, San Diego, CA). A custom cDNA library was constructed in λ-ZAPII (Stratagene) according to the manufacturer's instructions. The cDNA was screened with a nonradioactively labeled (Lightsmith I, Promega) degenerate oligonucleotide probe (5′-GTC TGG GTT G/C GC GTC G/CAG/A G/A T-3′). This 21-mer probe was designed from the partial amino acid sequence YLDANPD of TAl-18 (Cruz-Ortega and Ownby, 1993).

Approximately 160,000 plaque-forming units were transferred onto nylon membranes. Hybridization and washing conditions were conducted according to the manufacturer's (Promega) specifications. The hybridization temperature was 50°C with 150 fmol mL^{-1} labeled probe. After three cycles of plating and rescreening, seven positive clones were selected. DNA from these selected plaques was rescued as Bluescript SK phagemids by co-infection with R408 helper phage (Stratagene) and was used to transfect *Escherichia coli* XL-1-Blue cells.

DNA Sequencing Analysis

The nucleotide sequence of the positive clones was determined on an automated DNA sequencer (model 373A, Applied Biosystems) using a terminator cycle sequencing kit (Prism Ready Reaction Dyedoxy kit, Applied Biosystems) with M13 reverse and T7 primers. The full-length cDNA (1,3-β-glucanase) and the partial fimbrin-like cDNA clones were sequenced by primer walking using six synthetic primers to complete the sequencing of both strands. Database searches were conducted using the National Center of Biotechnology Information's BLAST e-mail program (Altschul et al., 1990) and nonredundant protein sequence database from the FASTA program (Pearson and Lipman, 1988).

Northern Analysis

Total RNA was extracted from root tips exposed to 80 μM Al for 6, 12, or 24 h, and 20 μg of total RNA was electrophoresed on a 1.2% agarose gel containing 2.2 M formaldehyde. Equal loading of RNA was verified by ethidium bromide staining. The gels were blotted onto a nylon membrane (Magna, Micron Separations, Westboro, MA) by capillary transfer. Membranes were UV-cross-linked (254 nm) (Stratalinker, Stratagene) and prehybridized in hybridization buffer (50% formamide, 5× SSC, 2% blocking reagent [Boehringer Mannheim], 20 mM sodium maleate, 0.1% *N*-laurylsarcosine, and 0.2% SDS) for 2 h at 42°C. Membranes were hybridized at 42°C overnight with the full-length 1,3-β-glucanase and the partial fimbrin-like cDNA clones that were digoxygenin-labeled using the random-priming method according to the manufacturer's (Boehringer Mannheim) specifications.

After hybridization membranes were washed once for 15 min in 2× SSC and 0.1% SDS and twice for 15 min in 0.1× SSC and 0.1% SDS at 65°C. Membranes were then blocked with 2% blocking reagent in maleate buffer (100 mM maleic acid and 150 mM NaCl, pH 7.5) and exposed to antidigoxygenin IgG Fab conjugated to alkaline phosphatase. Membranes were washed in maleate buffer and exposed to Lumiphos 530 (Boehringer Mannheim) for chemiluminescent reaction. Hybrids were visualized after exposure to radiographic film at room temperature for 12 h.

Western Analysis

Total protein was extracted from roots and leaves of 4-d-old seedlings of the Al-sensitive wheat cv Victory treated for 6, 12, or 24 h with 80 μM Al. Roots of the Al-tolerant cv Atlas 66 were treated with 10, 20, 40, 80, 200, or 400 μM Al. Frozen tissue was homogenized in extraction buffer (1:4, w/v tissue) containing 50 mM KPO_4 (K_2HPO_4 and KH_2PO_4), pH 6.8, and 1 mM PMSF (Hird et al., 1993). This extract was centrifuged at 12,000*g* for 20 min at 4°C. The protein concentration in the supernatant (crude extract) was determined by the method of Bradford (1976). Total soluble proteins (50 μg of roots or 100 μg of leaves/lane) were denatured in preheated SDS sample buffer (100°C for 3 min) and resolved by 12% SDS-PAGE (Laemmli, 1970). After electrophoresis proteins were electroblotted to nitrocellulose. The blots were incubated with rabbit anti-*Brassica napus* A6 protein polyclonal antibody, which was kindly donated by Diane Hird and Rod Scott (University of Leicester, UK) (1:1000 dilution).

After incubation blots were washed three times in TBST (TBS plus 0.5% Tween 20) for 10 min and once in TBS for 10 min. The blots were then incubated in alkaline phosphatase-conjugated goat anti-rabbit IgG (1:20,000 dilution) for 2 h. Protein-antibody complexes were visualized by immersing the blots in a solution of 0.5 mg mL^{-1} 5-bromo-4-chloro-3-indolyl-phosphate and 0.3 mg mL^{-1} nitroblue tetrazolium in Tris-HCl, pH 9.5, and 1 mM $MgCl_2$. The reaction was stopped by washing with distilled water. All treatments were carried out at room temperature, except incubation with antibody, which was overnight at 4°C.

RESULTS

Isolation and Nucleotide Sequence Determination of a 1,3-β-Glucanase cDNA Clone

Seven positive clones were selected after three rounds of plaque hybridization between a degenerate oligonucleotide probe and a λ-ZAPII cDNA library constructed from mRNA of Al-stressed wheat roots. The nucleotide sequence of three clones, each of an insert size of approximately 2.1 kb, showed similarity to higher plant 1,3-β-glucanases. One clone was completely sequenced in both strands and was found to be a full-length clone of 2072 nucleotides (Cruz-Ortega et al., 1995). This wheat cDNA had a strong G + C bias, as do many genes encoding monocot 1,3-β-glucanases. The deduced polypeptide contained 461 amino acids (Fig. 1), the first 25 of which represent a putative signal peptide that is enriched in neutral and hydrophobic amino acids typical of eukaryotic signal sequences (Von Heijne, 1983). Similar to the *Arabidopsis thaliana* and *B. napus* glucanases, the wheat gene product contained a C-terminal extension with three conserved *N*-acetylglycosylation consensus sequences (N-X-S/T) at amino acids 336, 340, and 361. The deduced protein had a predicted molecular mass of 46 kD (without the signal peptide of 25 amino acids) and an estimated pI of 6.0.

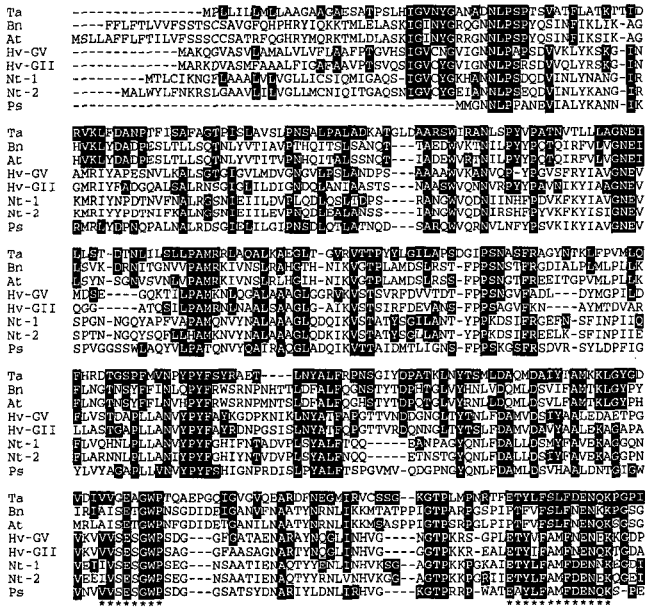

Figure 1. Comparison of the amino acid sequence of wheat 1,3-β-glucanase with other plant 1,3-β-glucanases. The predicted amino acid sequence of *T. aestivum* (Ta) 1,3-β-glucanases was aligned with the following 1,3-β-glucanases: *B. napus* (Bn), *A. thaliana* (At) (Hird et al., 1993), GV and GII from barley (*Hordeum vulgare*) (Hv-GV and Hv-GII) (Xu et al., 1992), tobacco (*Nicotiana tabacum*) (Nt-1, and Nt-2) (Linthorst, 1991), and pea (*Pisum sativum*) (Ps) (Chang et al., 1992). Amino acid residues denoted with an asterisk (*) are thought to participate in the enzyme active site. Identical amino acids are highlighted.

Amino Acid Sequence Alignment with Other 1,3-β-Glucanases

Figure 1 shows the alignment of the putative wheat 1,3-β-glucanase with other plant glucanases. It exhibits similarity to the *H. vulgare* neutral GV and the basic GII 1,3-β-glucanases (35 and 37% amino acid identity, respectively) (Xu et al., 1992; Malehorn et al., 1993), with two acidic tobacco 1,3-β-glucanases (32% amino acid identity) (Linthorst, 1991), and with the slightly basic pea 1,3-β-glucanase (32% identity) (Chang et al., 1992). The wheat protein is also similar to two basic 1,3-β-glucanases from *B. napus* and Arabidopsis (33% identity, Hird et al., 1993) in both size and the presence of the long amino acid extension at the C-terminal end.

Expression of the 1,3-β-Glucanase Transcript

The full-length cDNA clone was hybridized to total RNA isolated from roots of wheat cv Victory exposed to 80 μM Al for 6, 12, or 24 h. Northern analysis revealed two bands of about 2.0 and 2.2 kb. Transcript sizes corresponded to the cDNA clone, confirming that it was a full-length clone. An increase in 1,3-β-glucanase transcript was observed after 6 h of exposure to Al, which coincides with the onset of growth inhibition. Synthesis of both transcripts exhibited a transient increase, reaching a maximum after 12 h of Al exposure (Fig. 2). 1,3-β-glucanases thus represent proteins that are rapidly up-regulated in Al-intoxicated wheat roots. The second transcript is likely encoded by a closely related gene that has not yet been characterized. The 2.0-kb transcript appears to be more transiently up-regulated than the 2.2-kb transcript.

Cross-Reaction of a Wheat Root Protein with Antibody to *B. napus* 1,3-β-Glucanase

Extracts of total protein from roots and leaves of wheat seedlings treated for 6, 12, or 24 h with 80 μM Al were cross-reacted with rabbit anti-A6 1,3-β-glucanase from *B. napus*. Western analysis revealed a 56-kD protein that was particularly abundant in roots, but not leaves, after 6, 12, or 24 h of exposure to 80 μM Al (Fig. 3A). The large size of the protein cross-reacting with anti-A6 1,3-β-glucanase suggests that the putative wheat 1,3-β-glucanase retained all of the C-terminal extension and may also be heavily glycosylated. An enhanced amount of the same 56-kD protein was detected in roots of the Al-tolerant wheat cv Atlas 66 under growth-inhibiting conditions, which in this cultivar corresponded to 400 μM Al (Fig. 3B).

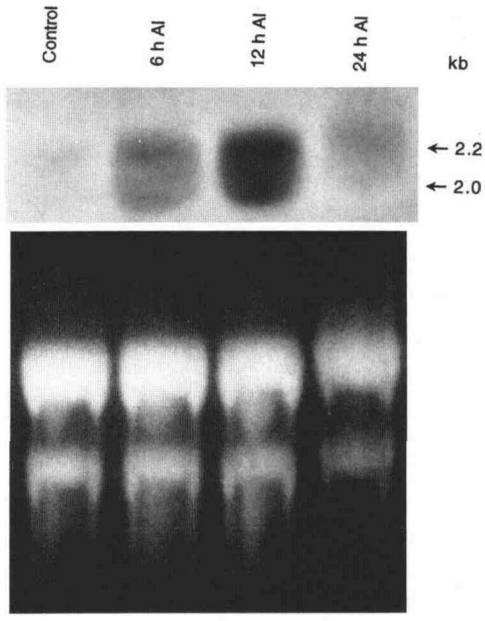

Figure 2. Expression of wheat 1,3-β-glucanase transcript during Al toxicity in roots of wheat cv Victory. Four-day-old wheat seedlings were subjected to 80 μM Al for 6, 12, or 24 h, and 20 μg of total root tissue RNA was loaded in each lane and resolved on a 1.2% agarose gel containing formaldehyde. RNA was transferred to a nylon membrane and probed with a gel-purified, digoxygenin-labeled full-length cDNA insert (top). Also shown is the corresponding ethidium bromide-stained gel (bottom).

Nucleotide Sequence Determination of Fimbrin-Like Protein from Wheat Roots

Another two of the seven positive clones selected from the screening of the cDNA library were similar to cytoskeleton actin-bundling proteins or fimbrins. These proteins are also called plastins in studies with human cells. Both strands of one clone 1.8 kb in length were sequenced. The nucleotide sequence exhibits 50% similarity with human I-plastin (Lin et al., 1994), T-plastin (Lin et al., 1993), and L-plastin (Lin and Leavitt, 1988), with slime mold (*Dyctiostelium discoideum*) fimbrin (J. Prassler, unpublished data; accession no. L36202), with the bakers' yeast (*Saccharomyces cerevisiae*) SAC6 gene encoding fimbrin (Adams et al., 1991), and with chicken (*Gallus gallus*) fimbrin (de Arruda et al., 1990). These proteins all belong to a group of actin cross-linking proteins that bundle actin filaments in vitro (Adams et al., 1991). All of these cDNA clones differ in size and have an open reading frame encoding proteins ranging from 570 to 630 amino acids. Consistent with related proteins in the databases and the observation that the wheat cDNA clone hybridized with a 2.4-kb transcript on northern analysis (Fig. 5A), the expected full-length size of the wheat-fimbrin-like cDNA clone would be about 2500 to 3000 bp. The 1800-bp cDNA clone has an open reading frame that extends from position 2 to 1345 and encodes a 448-amino acid polypeptide, in agreement with these predictions.

Amino Acid Sequence Alignments with Fimbrin-Like Proteins from Other Organisms

A search of the protein sequence database also indicated that the predicted wheat cDNA-deduced amino acid sequence possesses similarity with fimbrin proteins. The wheat sequence was found to be 43% identical to Sac6p or fimbrin from bakers' yeast (Adams et al., 1991) and 41% identical to L-, T-, I-plastin (Lin and Leavitt, 1988; Lin et al., 1993, 1994), chicken fimbrin (de Arruda et al., 1990), and slime mold fimbrin (J. Prassler, unpublished data). The deduced amino acid sequence was aligned with other fimbrin amino acid sequences from I-, L-, and T-plastin from humans, slime mold, yeast, and chicken (Fig. 4). The wheat fimbrin-like amino acid sequence aligned from residues 182 of human plastins and chicken fimbrin and from 205 of yeast (Sac6p) fimbrin, suggesting that the partial wheat cDNA clone is missing approximately 182 to 205 amino acids from the N-terminal region. Like other fimbrin proteins, the putative wheat fimbrin-like protein possesses the two 27-amino acid domains that are very similar to actin-binding domains identified in other proteins such as chicken fimbrin (de Arruda et al., 1990), α-actinin (Blanchard et al., 1989), and I- and T-plastin (Lin et al.,

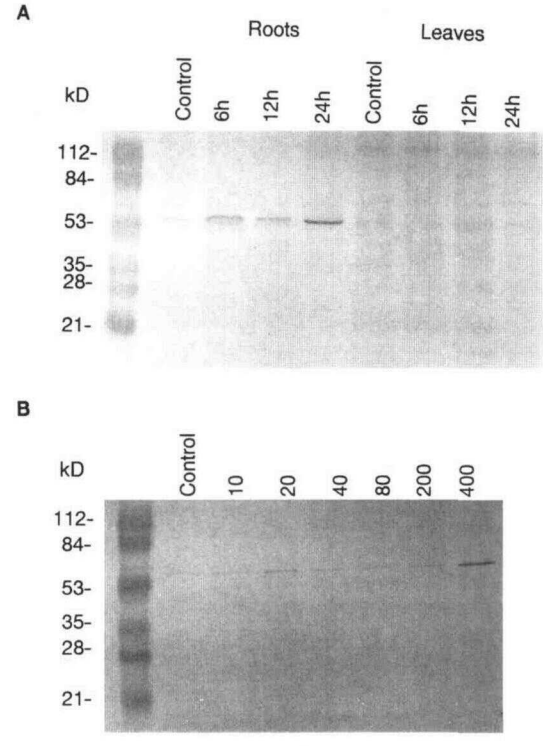

Figure 3. Immunoblot analysis of 1,3-β-glucanase in roots and leaves of the Al-sensitive wheat cv Victory (A) and the Al-tolerant wheat cv Atlas 66 (B). Four-day-old wheat seedlings were stressed with 80 μM Al in cv Victory and with 10, 20, 40, 80, 200, or 400 μM Al in cv Atlas 66. Total soluble protein was extracted from root tissue, resolved by SDS-PAGE, and electroblotted to a nitrocellulose membrane. Each lane contained 50 μg of soluble protein from roots and 100 μg of protein from leaves. 1,3-β-Glucanase was detected with anti-A6 protein from *B. napus*.

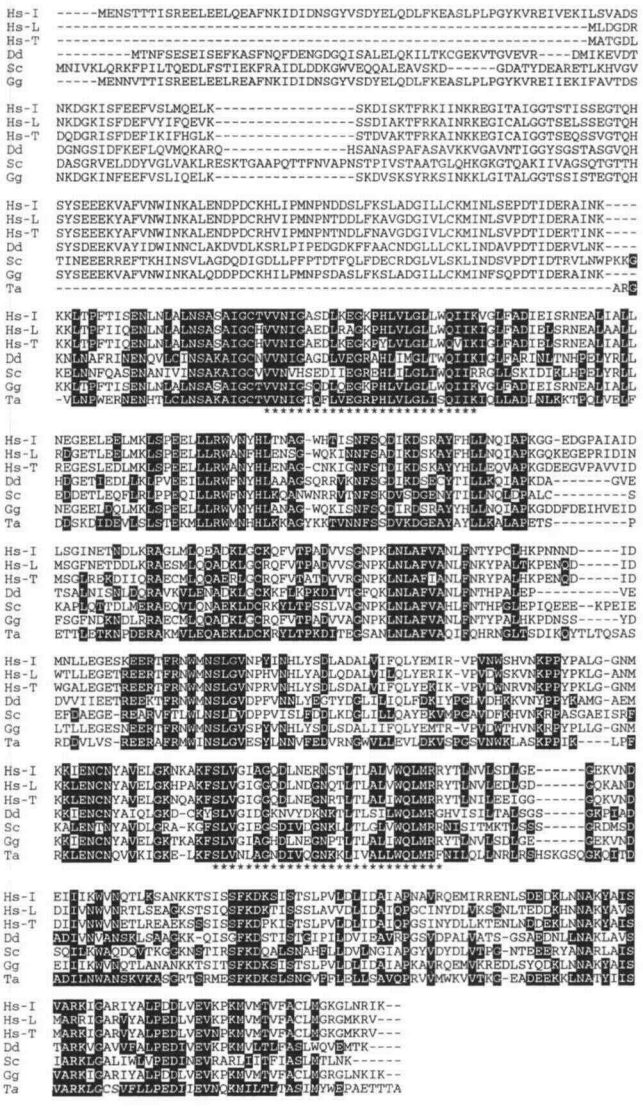

Figure 4. Amino acid alignment of fimbrin-like proteins from wheat and other organisms. Fimbrin/plastin sequences from *Homo sapiens* (Hs-I, Hs-L-, Hs-T-plastin, M22299; M34427; L05491) (Lin and Leavitt, 1988; Lin et al., 1993, 1994), *D. discoideum* (Dd, L36202) (J. Prassler, unpublished data), *S. cerevisiae* (Sc, X63867, X63918) (Adams et al., 1991), and *G. gallus* (Gg, X52562) (de Arruda et al., 1990) were aligned to the partial predicted amino acid sequence of a fimbrin-like protein of *T. aestivum* (Ta). Gaps to optimize alignments are designated by dashes (-). Asterisks (*) indicate residues that are thought to be involved in the actin-binding sites. Identical amino acids are highlighted.

1993). These two actin-binding domains are essential for the bundling activity of these proteins.

Expression of mRNA Hybridizing with Wheat Fimbrin-Like cDNA Clone

Northern analysis was used to examine the effect of Al toxicity on the expression of the fimbrin-like mRNA transcripts. Four-day-old wheat seedlings of the Al-sensitive cv Victory were exposed to 80 μM Al for 6, 12, or 24 h. The full-length cDNA probe hybridized in all treatments to a 2.4-kb transcript, which was more abundant in roots exposed to Al for 24 h than in control roots (Fig. 5A). This response was relatively slow considering that root elongation was completely inhibited after 6 to 12 h (Fig. 5B). To determine whether the same pattern occurred in the Al-

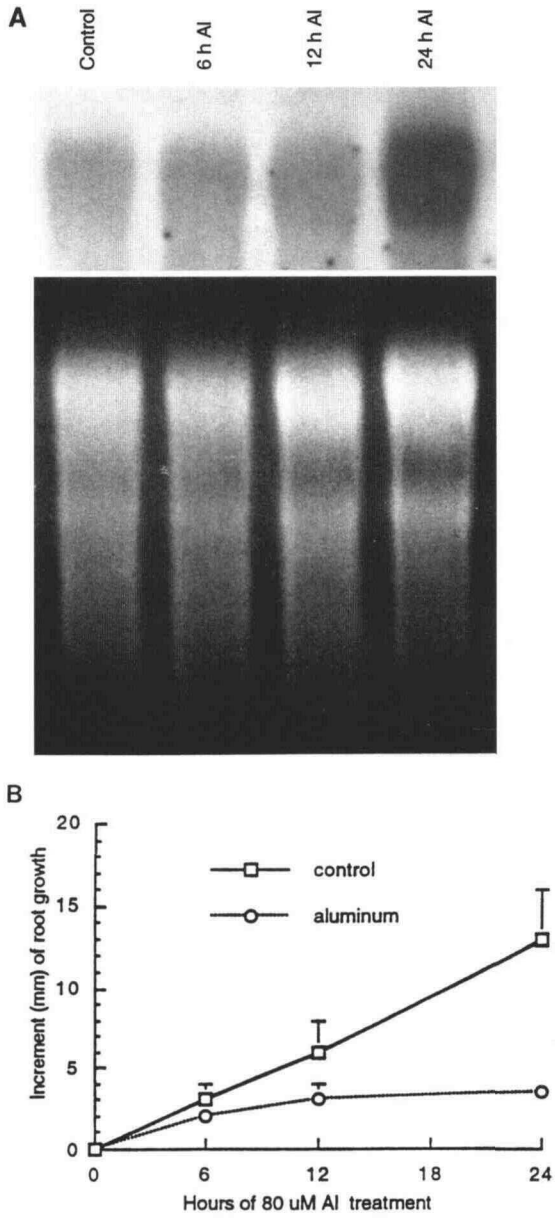

Figure 5. A, Expression of fimbrin-like mRNA in roots of the Al-sensitive wheat cv Victory. Twenty micrograms of total root tissue RNA was loaded in each lane and resolved on a 1.2% agarose gel containing formaldehyde. RNA was transferred to a nylon membrane and probed with a gel-purified, digoxygenin-labeled full-length fimbrin-like cDNA insert (top). Also shown is the corresponding ethidium bromide-stained gel (bottom). A repetition of this experiment revealed a similar pattern of fimbrin-like mRNA expression. B, Root growth of the Al-sensitive wheat cv Victory exposed to 80 μM AlCl for 6, 12, or 24 h. Values represent the means ± SE for eight measured seedlings.

tolerant wheat cv Atlas 66, we exposed it to 80 μM Al, which inhibited growth only in the sensitive cv Victory, and to 400 μM Al, which inhibited growth of both cultivars (Fig. 6B). In cv Atlas 66, fimbrin expression was enhanced approximately 1.6-fold (densitometer scan, data not shown) after 12 h of exposure to 80 μM Al and remained above that of the control at 24 h (Fig. 6A). Thus, the Al-tolerant cv Atlas 66 exhibited up-regulation of fimbrin-like transcript more rapidly than did the Al-sensitive cv Victory and did so in response to an Al concentration that did not inhibit growth.

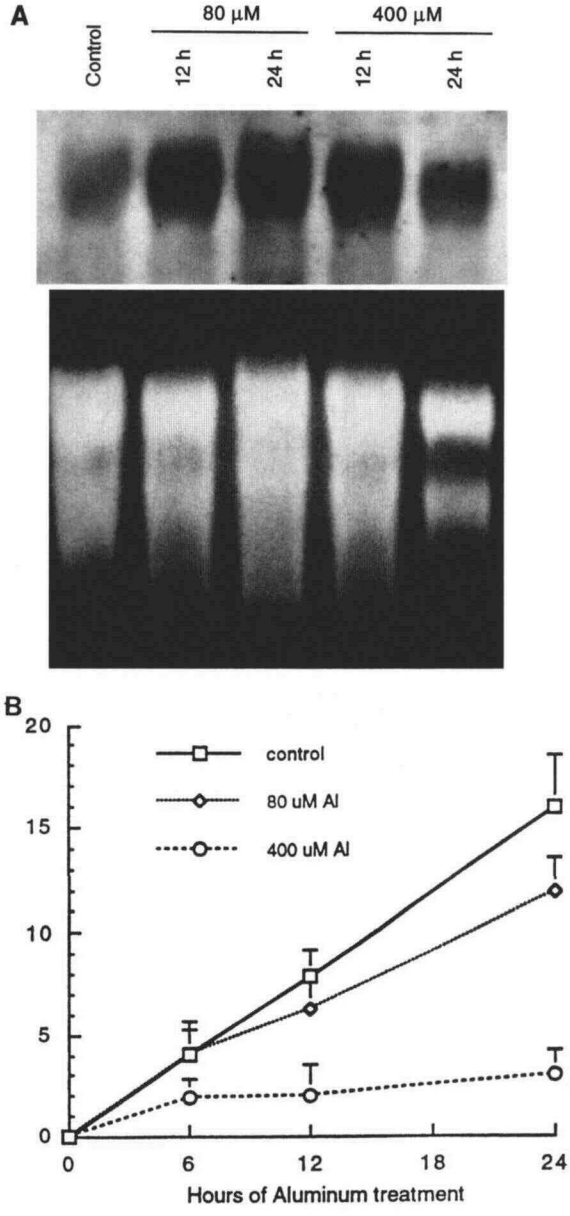

Figure 6. A, Expression of fimbrin-like mRNA in roots of the Al-tolerant wheat cv Atlas 66. Four-day-old wheat seedlings were subjected to 80 and 400 μM Al for 12 or 24 h. Twenty micrograms of total root RNA was loaded in each lane and resolved on a 1.2% agarose gel containing formaldehyde. RNA was transferred to a nylon membrane and probed with a gel-purified, digoxygenin-labeled full-length cDNA insert (top). Also shown is the corresponding ethidium bromide-stained gel (bottom). A repetition of this experiment revealed a similar pattern of fimbrin-like mRNA expression. B, Root growth of the Al-tolerant wheat cv Atlas 66 exposed to 80 and 400 μM Al for 6, 12, or 24 h. Values represent the means ± SE for eight measured seedlings.

DISCUSSION

In this study we screened a cDNA library made from Al-intoxicated wheat roots with a synthetic oligonucleotide derived from the partial amino acid sequence of TAl-18. None of the seven clones chosen for sequencing corresponded to the TAl-18 gene. Possibly because of its small size and strong GC bias, the probe hybridized with various abundant, GC-rich sequences in the library. Of the sequences identified, neither the 1,3-β-glucanase clone nor the fimbrin-like clone are strongly similar to TAl-18, and yet each was up-regulated during Al toxicity and each has a plausible role in the cellular response to Al.

1,3-β-glucanases are classified as pathogenesis- or defense-related proteins because they are often associated with pathogen infection, particularly by fungi. Our observation that Al toxicity induces a 1,3-β-glucanase is consistent with previous reports that these enzymes are induced by other metal ions including Co, Ba, Mn, Hg, and Ag (Fink et al., 1990). Generally, 1,3-β-glucanases play an important role in defense against fungal attack in both dicotyledonous and monocotyledonous plants (Simmons, 1994); however, their role in metal toxicity is not known. Considering that Al toxicity may render plant root cells more leaky (Zhao et al., 1987; Ownby, 1993), we suggest that during Al stress root cells are more susceptible to pathogen attack. In this vulnerable state root cells may synthesize 1,3-β-glucanases, TAl-18, and other stress proteins as a protective response against further attack by fungi or other plant pathogens.

The wheat 1,3-β-glucanase described here is unique, having characteristics of several dissimilar glucanases previously described. For example, it is relatively acidic (with a pI of 6.0), which is the hallmark of glucanases generally secreted to the intercellular spaces (Simmons, 1994). It has a putative N-terminal 25-amino acid signal peptide that is likely to mediate transport into the ER secretory pathway, leading to extracellular secretion. However, the wheat enzyme also possesses a C-terminal extension with three potential *N*-glycosylation sites. In this respect it is more like the basic 1,3-β-glucanases found in dicots such as *B. napus* and Arabidopsis than those found in monocots such as barley. Since glycosylation of 1,3-β-glucanase during processing is thought to be involved in transport to the vacuole (Shinshi et al., 1988), we believe that our wheat 1,3-β-glucanase is probably localized in this organelle.

Western analysis of wheat root proteins using anti-*B. napus* A6 antibody (Fig. 3) revealed an immunoreactive protein of 56 kD with an induction by Al that paralleled that expected of our cDNA product. The latter, however, has a predicted molecular mass of only 46.3 kD even if the amino acid C-terminal extension is retained at maturity. This 10-kD discrepancy could be due to the presence of carbohydrates at the eight potential glycosylation sites

throughout the protein. However, it seems unlikely that the 1,3-β-glucanase would be approximately 18% carbohydrate by weight. More likely, the carbohydrate side chains interfere with the binding of SDS to the protein, retarding its movement through the polyacrylamide matrix during electrophoresis (Dunbar, 1987).

Our wheat cDNA library also contained a partial 1800-bp cDNA clone with strong similarity to fimbrin genes from various fungi and vertebrates. This report provides the first evidence, to our knowledge, for fimbrins in higher plants. Fimbrins, a highly conserved family of actin filament-bundling proteins, are composed of two structural domains, a headpiece and a core domain. The headpiece contains an EF-hand Ca^{2+}-binding domain close to the N-terminal end of the protein. Unfortunately, no comparison of this domain could be made with the putative wheat fimbrin gene product because the cDNA is missing the region encoding the N terminus of the protein. However, the core domain contains two actin-binding domains formed by a region of 26 to 28 well-conserved amino acids. These adjacent actin-binding domains are thought to enable these proteins to cross-link actin filaments into bundles or to bind two actin microfilaments (de Arruda et al., 1990). Yeast mutants lacking the SAC6p fimbrin gene do not form normal actin structures, lose asymmetry of cortical actin distribution, and are defective in morphogenesis (Adams et al., 1991). It is likely that plants also utilize fimbrins to maintain the integrity and functional array of actin filaments in the cell cytoskeleton.

In wheat roots Al toxicity causes changes in cell size and shape, along with disruption of cytoskeletal dynamics (Grabski and Schindler, 1995). It is possible that this response involves disruption of cellular Ca^{2+} homeostasis by Al. Although vigorously debated (Kochian, 1995), some studies point to an increase in cytoplasmic Ca^{2+} during Al stress (Delhaize and Ryan, 1995; Rengel et al., 1995); however, this response usually occurs after the primary Al effect. Studies with soybean (*Glycine max*) cells in culture have shown that both elevated cytoplasmic Ca^{2+} and treatment with Al (Grabski and Schindler, 1995) cause a rapid, reversible increase in the rigidity of the actin network. The increased tension of the cytoskeletal actin associated with Al toxicity may involve extensive cross-linking of actin filaments by fimbrins, leading to up-regulation of fimbrin gene expression to replenish cellular fimbrin pools. Further work is clearly required to determine how Al affects the expression and function of actin-binding proteins such as fimbrin in plant cells.

NOTE ADDED IN PROOF

Recent screening of the Arabidopsis genome has revealed two genes that encode fimbrin. One of these, fimbrin 1, is expressed in actively dividing cells of the root primordia, and the other, fimbrin 2, is expressed only in the root cap. Fimbrin 1 lacks the EF-hand Ca^{2+}-binding domain and does not complement a fimbrinless mutant of *S. cerevisiae* (N.-H. Chua, personal communication).

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