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Isolation and characterization of 18 genes encoding α - and β -expansins in wheat (*Triticum aestivum* L.)

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Abstract Expansins are thought to be key regulators of cell wall extension during plant growth. In this study, we isolated 18 expansin genes from wheat, nine of which encode α -expansins while the other nine code for β -expansins. The cysteine-rich and tryptophan-rich regions of the deduced amino acid sequences of all 18 expansins were highly conserved. Genomic sequences were obtained for 17 of the genes, and their intron patterns were determined. Four (A, C, D, E) of the six intron positions known in expansin genes from other species were found to be occupied in these wheat expansin genes. Five wheat expansin genes were mapped to chromosomes 1L, 2L, 5L and 6L respectively, by in silico and comparative mapping. The 18 wheat expansin genes were expressed in leaf, root and the developing seed. Moreover, it was demonstrated that four β -expansin genes were up-regulated in the internode tissue in F_1 hybrids, suggesting that changes in the regulation of these genes in hybrid might contribute to the heterosis observed in internode length and plant height. We therefore conclude that expansins are encoded by a multigene family in wheat, and could play important roles in growth and development.

Keywords Expansin · Wheat · Gene expression · Heterosis

Introduction

Expansins are plant proteins that induce cell wall extension and stress relaxation at acidic pH condition (McQueen-Mason et al. 1992). Subsequent investigations indicated that expansins participate in a diverse range of developmental processes, including seed germination (Chen et al. 2001), meristem dynamics (Fleming et al. 1997), fruit softening (Brummell et al. 1999), root development (Zhang and Hasenstein 2000), leaf growth (Pien et al. 2001), stem elongation (Cho and Kende 1997), pollination (Cosgrove et al. 1997), floral opening and senescence (Gookin et al. 2003), as well as in adaptive responses to submergence, GA and wounding (Lee and Kende 2001). Although expansin genes are expressed in many plant organs and tissues, some expansins display organ- or tissue-specific expression patterns (Wu et al. 2001; Chen et al. 2001), while others are induced by treatment with GA or ethylene (Cho and Kende 1997; Trivedi and Nath 2004).

Expansins are encoded by multigene families (Wu et al. 2001; Li et al. 2002). Based on sequence similarity, expansins can be divided into at least four groups (Li et al. 2003). α -Expansins form the largest group, which contains 26 members in *Arabidopsis* and at least 32 members in rice. In *Arabidopsis* the second group, β -expansins, contains eight members that fall into three subgroups (β_1 – β_3). In rice the 17 β -expansins can also be divided into β_1 – β_3 subgroups. γ -expansins represent the smallest subgroup in *Arabidopsis* with only three members. The recently discovered δ -expansins are suspected to have arisen as the result of a recent duplication of β_1 -expansins (Li et al. 2003). Expansin-like and expansin-related genes show low similarity to the expansin genes and their functions have not been determined. They are designated EXLA and EXLB, and are assigned to subgroups β_2 and β_3 , respectively (Li et al. 2002).

Genes encoding expansins are scattered on different chromosomes in *Arabidopsis* and rice (Li et al. 2002). In addition, the members of a tandemly arranged cluster of

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Arabidopsis expansin genes show a high degree of similarity at both the nucleotide and amino acid sequence level (Li et al. 2002). The intron patterns in expansin genes in *Arabidopsis* and rice suggested that introns can occur at six positions, labeled A–F. Position A is located in the 5'UTR, and the others are in the coding region. In *Arabidopsis*, introns are found at only four of these positions (C, D, E and F), while all six positions are utilized in rice (Lee and Kende 2001; Li et al. 2002).

Expansin proteins contain a signal peptide at the N-terminus, which directs the nascent polypeptide into the endoplasmic reticulum/Golgi secretory pathway, and is removed as the protein enters the endoplasmic reticulum (<http://www.bio.psu.edu/expansins/>). The mature expansin proteins contain two conserved domains, a cysteine-rich region and a tryptophan-rich C-terminal segment that may function as a polysaccharide-binding domain. Moreover, β -expansins are glycosylated, but α -expansins are not, and this notable characteristic can be used to distinguish between the two groups (<http://www.bio.psu.edu/expansins/>).

Expansin genes have been investigated in *Arabidopsis* (Li et al. 2002), rice (Cho and Kende 1997; Lee and Kende 2001), maize (Wu et al. 2001), tomato (Reinhardt et al. 1998; Brummell et al. 1999; Rose et al. 2000; Chen et al. 2001), cotton (Orford and Timmis 1998), soybean (Downes et al. 2001; Lee et al. 2003), strawberry (Harrison et al. 2001) and peach (Obenland et al. 2003). However, no systematic identification of expansin genes has yet been reported in wheat. In this study, we isolated 18 wheat expansin genes, and analyzed their sequence characteristics, and their expression patterns.

Materials and methods

Plant materials

The wheat genotype BAU 3338 and 6554 were used in this study. For growth in the greenhouse, seeds were allowed to germinate on moist filter paper, and were then transplanted into pots. Young leaves, leaf sheath and the roots of seedlings at the trefoil stage were collected. Seeds were harvested at 2, 6, 8, and 12 days after pollination (DAP), respectively. The hybrid wheat AI9/JIA18 and its parents were also used for expression analysis. The internodal region, 0–1 cm above the uppermost node, was harvested at the booting stage; this region contains the intercalary meristem and most of the elongation zone. Harvested tissues were immediately frozen in liquid nitrogen.

RNA extraction

The frozen samples were ground to a fine powder under liquid nitrogen and transferred to 1.5-ml Eppendorf tubes; 800 μ l of prewarmed (90°C) extraction buffer

(containing 100 mM LiCl, 1% SDS, 10 mM EDTA, 100 mM TRIS-HCl pH 9.0) were added to each tube and mixed with an equal volume of phenol (pH 8.0). Tubes were then incubated in a water bath at 90°C for 2 min, and then cooled on ice. After adding 300 μ l of chloroform, the tubes were shaken for 10 min and then centrifuged at 12,000 rpm for 10 min at 4°C. The supernatants were transferred to fresh tubes and extracted twice with an equal volume of chloroform. The supernatant was collected and nucleic acids were precipitated with a one-third volume of 8 M LiCl for 16 to 18 h at 4°C. Tubes were then centrifuged at 12,000 rpm for 10 min at 4°C and the supernatants were removed. The precipitates were washed in 2 M LiCl and 80% ethanol, and redissolved in double-distilled water treated with diethylpyrocarbonate. Total RNA was digested with DNase I (Promega, Madison, Wis.) for 30 min and extracted twice with chloroform to eliminate residual DNA. Nucleic acids were precipitated with 0.3 M sodium acetate (pH 5.2) and 2 vols of ethanol, and collected by centrifugation (13,000 rpm for 15 min at 4°C).

DNA extraction

Total DNA was extracted from the leaves of seedlings by the cetyltrimethylammonium bromide (CTAB) method (Saghai-Marooof et al. 1984) with minor modifications.

Cloning and sequencing

cDNAs that contained putative ORFs were identified by comparing expansin cDNAs from rice with wheat ESTs in the GenBank database. The homologous ESTs were assembled into contigs with putative ORFs. Based on the contig sequences, expansin-specific primers were designed. Most primers anneal to the 3'UTR or 5'UTR, whereas several others anneal just beyond the start codon or stop codon (Table 1).

PCR amplifications were performed at 94°C for 3 min, followed by 40 cycles of 94°C for 1 min, 60–68°C (depending on the primer pair) for 1 min, and 72°C for 2 min, with a final extension at 72°C for 5 min. PCR products were separated by electrophoresis on a 1% (w/v) agarose gel and purified using a DNA purification kit (Sunbiotec, Beijing). The purified DNAs were cloned into the pGEM-T Easy vector (Promega) and sequenced by the Songon Company (Shanghai, China).

Sequence analysis

The deduced amino acid sequences of wheat expansin proteins were aligned using CLUSTALW (<http://clustalw.genome.ad.jp/>). The alignment was then used as the basis for the construction of the initial comprehensive tree. Distance analyses were performed using the

Table 1 Gene-specific primers

Target gene	Primer name	Sequence (5' to 3')
<i>TaEXPA1</i>	QCA ₁ 5'	GGCCTTCAATCTCTCATTTTCTTCTC
	QCA ₁ 3'	CCACCACCCCGTAGTACAAATTAGTAC
<i>TaEXPA2</i>	QCA ₂ 5'	ATGGAGACGAGACGTCCAGCGGTTTC
	QCA ₂ 3'	CTATCAGAATTGCCCTTTGCCCTCGAAGC
<i>TaEXPA3</i>	QCA ₃ 5'	GGTCGCTTTACCGAGCTAGAAAGGC
	QCA ₃ 3'	TCACACACCACTCCATGCATGTG
<i>TaEXPA4</i>	QCA ₄ 5'	TGAGGACTGGCCTGCCTGCTACATTAC
	QCA ₄ 3'	CAAACGGGCGACCCCATATTAACAG
<i>TaEXPA5</i>	QCA ₅ 5'	CGACGACATGGCGGTCTCAAGATG
	QCA ₅ 3'	AAGCACATTCCGGCGGCTGAACAT
<i>TaEXPA6</i>	QCA ₆ 5'	ATGGCAGCTGGGATGCGCTTCT
	QCA ₆ 3'	GGCGTCTGGAGAGTTTCATGCATGTTTCG
<i>TaEXPA7</i>	QCA ₇ 5'	TCTCCCTGCTTACTTACTGAGAAG
	QCA ₇ 3'	GAACAATCTCTGAGGCTCTGCAC
<i>TaEXPA8</i>	QCA ₈ 5'	CGAGCATCTCCGCACTTGCAAATTCAG
	QCA ₈ 3'	CCAACCGGCTAATGTGAGCACGCCTT
<i>TaEXPA9</i>	QCA ₉ 5'	TTCGCTCCCTCGCCTTCTTGCT
	QCA ₉ 3'	ATATGCATTACAGGCCACGCACCAAG
<i>TaEXPB1</i>	QCB ₁ 5'	GTGCTTTGACATGGCTCCTCTT
	QCB ₁ 3'	CAGCTTCAGCTGTACTGGACGAT
<i>TaEXPB2</i>	QCB ₂ 5'	CTCCCCACCGCCACTCGTCAGAT
	QCB ₂ 3'	ACTTGACCGTAGCAGCTAGCCGCGTAGT
<i>TaEXPB3</i>	QCB ₃ 5'	GCAGTGGTACTTTGACATGGCTCCTCTT
	QCB ₃ 3'	GAGTTCGGTTCAGTTCAGCAGCTTCAG
<i>TaEXPB4</i>	QCB ₄ 5'	GATGGCTTCTTCTTCCTCGTGGT
	QCB ₄ 3'	CATCATCATCTTCAGTACCCGCCCTT
<i>TaEXPB5</i>	QCB ₅ 5'	GTCGATATGGGTTCCGTCTCCTACGT
	QCB ₅ 3'	GCACGCCGTGCACATGAATCTAC
<i>TaEXPB7</i>	QCB ₇ 5'	CAGAGTCCACAGCAGAGCACCCTACT
	QCB ₇ 3'	ACGGCATCTCGAGCTCCTTGATC
<i>TaEXPB8</i>	QCB ₈ 5'	GGTTGTTTCATAGTCGACCAATATGG
	QCB ₈ 3'	TCCGATAACAGAGGTGTCTAAGTAAG
<i>TaEXPB9</i>	QCB ₉ 5'	GCTCCTAGTCCCAGTGCTTGCTAGCAAG
	QCB ₉ 3'	CAGAGACCAGGAGGGAACATCACAG
<i>TaEXPB10</i>	QCB ₁₀ 5'	GAAGACCTGTAGTGCCAATATGGCTG
	QCB ₁₀ 3'	ATCAGGCATGGAAGTCCGATAGTG

program ProtDist in the Phylip 3.5c package with a PAM250 substitution matrix. Phylogenetic trees were then constructed from the matrices using the neighbor-joining algorithm. Signal peptides were predicted with the program SignalP (<http://www.cbs.dtu.dk/services/SignalP/>), and ScanProsite and pI/MW (available on the ExPASy website at <http://www.expasy.org/>) were used to predict N-glycosylation sites and pI/MW.

Semi-quantitative RT-PCR analysis

Aliquots (2 µg) of total RNA were used for first-strand cDNA synthesis in 20-µl reactions containing 50 mM TRIS-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 50 µM dNTPs, 200 U of M-MLV reverse transcriptase (Promega) and 50 pmol of oligonucleotide primer (T₁₅). Reverse transcription was performed at 37°C for 60 min with a final denaturation at 95°C for 5 min. One-tenth of the first-strand cDNA was used as the template in a 20-µl RT-PCR. A 350-bp β-actin gene fragment was amplified as a positive control using the following primers 5'-CAGCAACTGGGATGAT-ATGG-3' and 5'-ATTTCGCTTTCAGCAGTGGT-3'.

Results and discussion

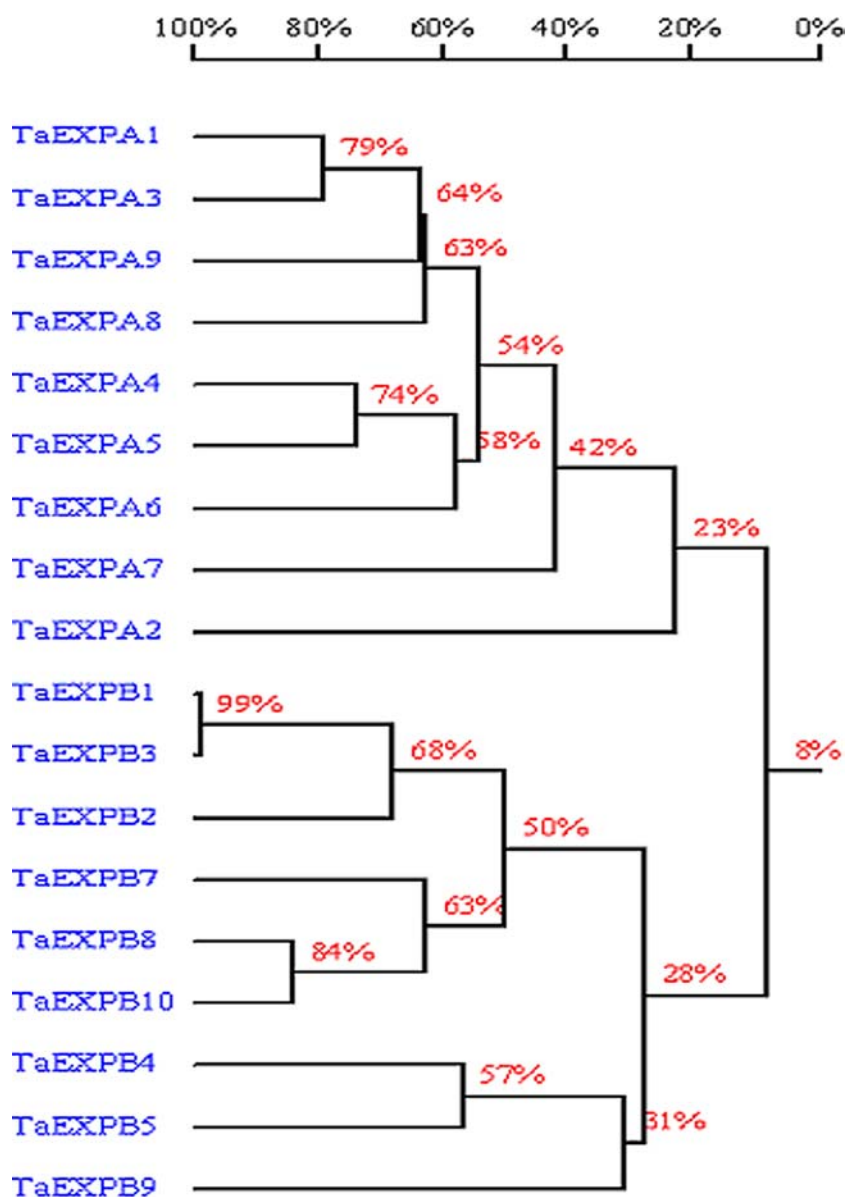
Cloning of eighteen wheat expansin cDNAs

Wheat EST sequences that showed high similarity to known expansin genes from rice were obtained by BLASTN searches in the NCBI database. Of these ESTs, 36 could be assembled into 18 cDNAs with putative ORFs, and gene-specific primers were designed to amplify sequences corresponding to these cDNAs from wheat. The 18 cDNAs ranged from 741 to 870 bp in length, all contained a ORF, and were designated *TaEXPA1* to *TaEXPA9* (GenBank Accession Nos. AY485121 and AY543528–AY543535), *TaEXPB1* to *TaEXPB5* (Accession Nos. AY543536–AY543540) and *TaEXPB7* to *TaEXPB10* (Accession Nos. AY543541–AY543544), respectively (Table 2). These results demonstrate that wheat expansins are encoded by a multigene family.

Based on the deduced amino acid sequences, a phylogenetic tree was constructed (Fig. 1). The 18 wheat expansin proteins were clearly separated into α- and β-subfamilies. Nine of them (*TaEXPA1*–*TaEXPA9*)

Table 2 Characterization of 18 wheat expansin genes

Expansin genes	Accession No.	Source of cDNA	ORF length (bp)	5'UTR (bp)	3'UTR (bp)
<i>TaEXPA1</i>	AY485121	Seedling leaf	756	264	394
<i>TaEXPA2</i>	AY543528	Developing seeds	780	—	—
<i>TaEXPA3</i>	AY543529	Young spikelets	756	53	297
<i>TaEXPA4</i>	AY543530	Seedling root	750	52	220
<i>TaEXPA5</i>	AY543531	Seedling root	756	—	241
<i>TaEXPA6</i>	AY543532	Seedling root	765	—	79
<i>TaEXPA7</i>	AY543533	Seedling root	777	60	195
<i>TaEXPA8</i>	AY543534	Seedling root	741	78	186
<i>TaEXPA9</i>	AY543535	Young spikelets	801	61	151
<i>TaEXPB1</i>	AY543536	Seedling leaf	795	—	—
<i>TaEXPB2</i>	AY543537	Seedling leaf	837	44	67
<i>TaEXPB3</i>	AY543538	Seedling leaf	795	—	—
<i>TaEXPB4</i>	AY543539	Spikelets at heading stage	813	—	—
<i>TaEXPB5</i>	AY543540	Spikelets at heading stage	798	—	156
<i>TaEXPB7</i>	AY543541	Young spikelets	813	78	165
<i>TaEXPB8</i>	AY543542	Seedling root	822	—	235
<i>TaEXPB9</i>	AY543543	Young spikelets	870	52	138
<i>TaEXPB10</i>	AY543544	Seedling root	816	—	297

Fig. 1 Phylogenetic analysis of 18 wheat expansin protein sequences. The numbers on the branches presented refer to the degree of shared sequence similarity

represent α -expansins, while TaEXPB1–TaEXPB5 and TaEXPB7–TaEXPB10 belong to the β -expansin class. The phylogenetic analysis also indicated that α -expansins are more divergent from each other than β -expansins. Pairwise sequence similarities between wheat expansin proteins ranged from 23 to 79% in the α -group and from 28 to 99% in the β -group, respectively; inter-group comparisons revealed only 8% similarity between the two groups.

Characteristics of wheat expansin proteins

Based on an analysis with the SignalP program, it was found that each of 18 wheat expansin proteins had a cleavable signal peptide at the N-terminus. The length of this peptide was variable, ranging from 17 to 29 residues. Predicted mature proteins of the 18 expansins contained from 226 to 264 amino acids. The pI of the α -expansins ranged from 6.53 to 9.79, and that of β -expansins ranged from 4.98 to 9.38. The molecular mass varied from 25.5 to 30.7 kDa.

Two domains characteristic of expansin proteins, a cysteine-rich region and a tryptophan-rich C-terminal portion, were observed in all the wheat expansins

(Fig. 2). Indeed the wheat expansins possessed all the characteristics typical of the family, such as five conserved cysteines, the 'GACG' motif in the cysteine-rich region, and three tryptophans near the C terminus. However, some variations specific to wheat expansins were also observed in some conserved motifs. For example, in the motif 'HFD', the amino acid residue F is replaced by I in TaEXPB5 and by L in TaEXPB10, and the D was replaced by an L in TaEXPA7 and a G in TaEXPB5.

Alignment of α - and β -expansin protein sequences clearly showed that there were two characteristic insertions, α and β , in the central domain of the predicted proteins. The α -insertion was present in all α -expansins but absent in all β -expansins, while the β -insertion was present only in the β -expansins. The α -insertion is about 14 amino acids in length and contains four relatively well conserved residues (WCNP) at its C-terminal end. The β -insertion, however, usually consisted of seven residues, which were not conserved.

N-linked glycosylation sites were generally absent in α -expansins but present in β -expansins. Thus, six of the β -expansins (TaEXPB1, TaEXPB2, TaEXPB3, TaEXPB4, TaEXPB5 and TaEXPB9) had an N-linked glycosylation site near the N-terminus, while four

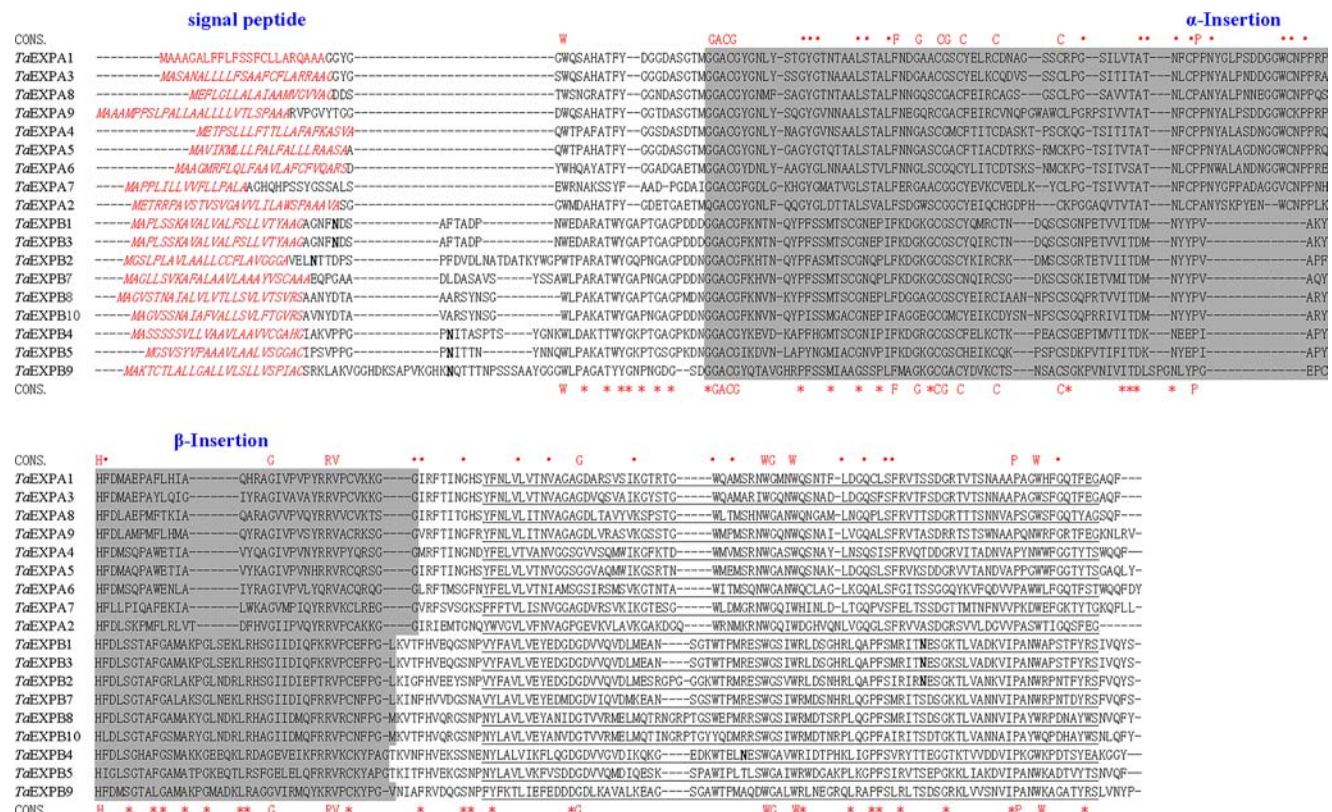


Fig. 2 Alignment of wheat expansin sequences. The sequences in *italics* represent the signal peptides predicted by the SignalP program (<http://www.cbs.dtu.dk/services/SignalP/>). Potential N-linked glycosylation sites are shown in **bold** and were predicted by using ScanProsite. *Capital letters* indicate amino acid residues that are conserved between α - and β -expansins, *dots* denote residues that

are conserved among *alpha*-expansins, and *asterisks* denote amino acids that are conserved among the *beta*-expansins. The *shaded sequences* represent the family-45 endoglucanase-like domain and the *underlined sequences* represent the 'cellulose-binding-like' domain. The α - and β -insertions within the centrose are also indicated

β -expansins (TaEXPB1, TaEXPB2, TaEXPB3 and TaEXPB4) had a site near the C-terminus. It was also noted that β -expansins had five more amino acid residues than α -expansins at the position between signal peptide and the first conserved tryptophans (Fig. 2).

As compared to other wheat expansin proteins, TaEXPB9 had 12 additional amino acid residues at its N-terminus. The function of this segment was still unknown. In maize, the predicted mature ExpB4 protein also contains an unusual extension of approximately 53 amino acids at its N-terminus (Wu et al. 2001).

Analysis of intron patterns

Intron patterns in expansin genes have previously been reported for *Arabidopsis* and rice (Li et al. 2002). In order to determine the intron patterns of wheat expansin genes, we used the gene specific primer pairs to amplify the genomic sequences. The genomic sequences of all genes except *TaEXPA7* were obtained (Table 3). Sequence comparisons indicated that only *TaEXPB4* lacked introns, while the intron patterns in the other genes varied. Previous studies had suggested that introns may be present at six different positions in expansin genes in *Arabidopsis* and rice, namely A, B, C, D, E and F (Lee et al. 2001; Li et al. 2002). In this study, four introns, at positions A, C, D and E, could be observed in wheat expansin genes (Table 3). Position A is located in the 5'UTR region, 56 bp upstream of start codon, and is utilized only in of *TaEXPA8*. Position C was occupied in 16 expansin genes, and the length of this intron varied from 81 to 121 bp. Intron D was observed in all eight β -expansin genes, and the length of this intron varied from 94 to 255 bp. Position E was utilized in seven wheat expansin genes (*TaEXPA1*, *TaEXPA3*, *TaEXPA8*, *TaE-*

XPB1–*TaEXPB3* and *TaEXPB9*) and the length of this intron varied from 95 to 498 bp.

It is noteworthy that TaEXPB1 and TaEXPB3 are 99% similar (Fig. 1) and both genes have introns at positions C and D, but these introns differ markedly from each other (Table 3).

Intron positions C and E are utilized in both α and β -expansin genes in *Arabidopsis*, rice and wheat, so it is presumed that these two introns were present in the common ancestor of the expansins (Li et al. 2002). Intron positions D and F are not used in α -expansins, suggesting that they might have arisen in β -expansins shortly after α/β -subgroup split. An intron is present at position F in 40% *Arabidopsis* expansins, and in 16.7% of rice expansin genes. However, we found that this intron was absent in the 17 wheat expansin genes examined in this study. Positions A and B were only occupied in rice and wheat, and are thus predicted to have emerged after the monocot/dicot split. Interestingly, intronless expansin genes have now been found in rice (*Os-EXPA11*, *Os-EXPA31*, *Os-EXPB1a*, *Os-EXPB8*, *Os-EXPB10* and *Os-EXPB13*) and wheat (*TaEXPB4*), suggesting that this group arose from a single recombination event with a reverse-transcribed DNA copy of a fully spliced mRNA (Frugoli et al. 1998).

Expression of wheat expansin genes

To determine the expression pattern of these expansin genes, we carried out semi-quantitative RT-PCR analysis using gene-specific primers with cDNA from various wheat tissues as templates. Since *TaEXPB1* and *TaEXPB3* are 99% similar, the expression pattern of *TaEXPB3* was not analysed.

Expression in leaf, sheath and root at the trefoil stage

It was found that five expansin genes—*TaEXPA3*, *TaEXPB2*, *TaEXPB4*, *TaEXPB7* and *TaEXPB9*—were expressed at comparable levels in leaf, root and leaf sheath (Fig. 3). Six genes—*TaEXPA5*, *TaEXPA6*, *TaEXPA8*, *TaEXPB5*, *TaEXPB8* and *TaEXPB10*—were expressed at high levels in root, but at low levels in leaf and sheath. Four genes (*TaEXPA1*, *TaEXPA2*, *TaEXPA7* and *TaEXPB1*) were expressed at higher levels in leaf than in other tissues. *TaEXPA9* was expressed at a higher level in leaf sheath than in leaf, but not in root. *TaEXPA4* transcripts were undetectable in all three tissues.

Based on the results of RT-PCR, we can conclude that several expansin genes can be expressed at similar levels in a single tissue. For example, *TaEXPA3*, *TaEXPB2*, *TaEXPB4*, *TaEXPB7* and *TaEXPB9* are expressed at the comparable levels in whole seedling, suggesting that some expansin genes may have overlapping biological functions. However, different genes show different expression patterns in different tissues.

Table 3 Intron positions of wheat expansin genes

Gene	Length (bp)	Intron position/length (bp)			
		Position A	Position C	Position D	Position E
<i>TaEXPA1</i>	1188		109	—	338
<i>TaEXPA2</i>	872		98	—	—
<i>TaEXPA3</i>	1137		117	—	267
<i>TaEXPA4</i>	844		97	—	—
<i>TaEXPA5</i>	860		104	—	—
<i>TaEXPA6</i>	883		121	—	—
<i>TaEXPA8</i>	937	115	104	—	95
<i>TaEXPA9</i>	2399		88	—	1513
<i>TaEXPB1</i>	1283		100	121	271
<i>TaEXPB2</i>	1698		111	255	498
<i>TaEXPB3</i>	1330		94	160	271
<i>TaEXPB4</i>	810		—	—	—
<i>TaEXPB5</i>	970		81	94	—
<i>TaEXPB7</i>	1026		97	125	—
<i>TaEXPB8</i>	1330		98	153	—
<i>TaEXPB9</i>	1176		113	91	105
<i>TaEXPB10</i>	1166		120	233	—

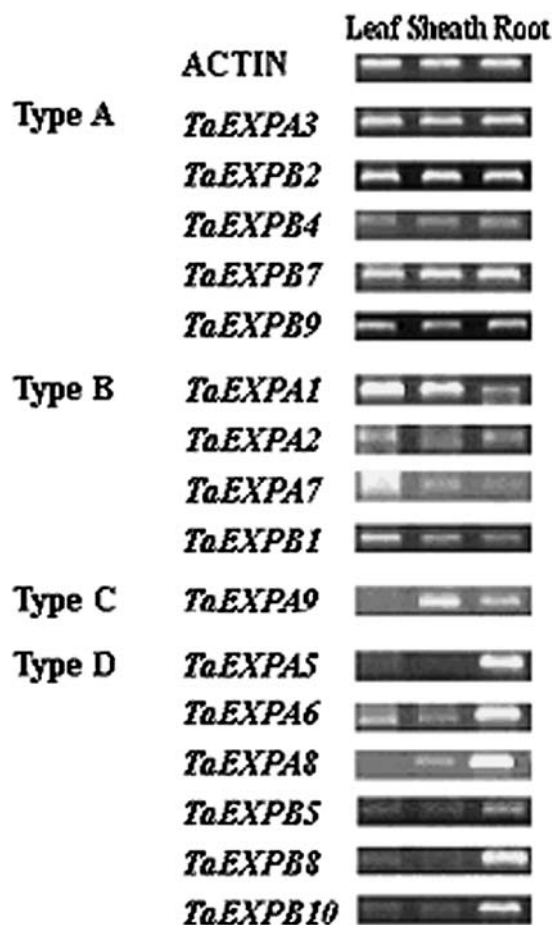


Fig. 3 Expression patterns of wheat expansin genes in young seedlings. Type A: equal expression in leaf, sheath and root. Type B: expression higher in leaf than in sheath and root. Type C: expression higher level in sheath than in leaf and root. Type D: expression higher level in root than in leaf and sheath. L, Leaf; S, Sheath; R, Root

It is difficult to determine the exact functions of each gene since the expression patterns are complex, although the profiles do provide some clues as to possible functions.

Expression in developing seed

Sixteen wheat expansin genes are expressed in the developing seed (the exception is *TaEXPA5*). However, the expression patterns of different genes in developing seeds from 2 to 12 days after pollination (DAP) were highly variable, and could be classified into six types (Fig. 4). Two genes, *TaEXPB2* and *TaEXPB4*, were constitutively expressed in the developing seeds throughout this period (Fig. 4, Type A). *TaEXPB5* shows a gradual increase in expression during seed development (Fig. 4, Type B). Five genes (*TaEXPA1*, *TaEXPA3*, *TaEXPB1*, *TaEXPB7* and *TaEXPB9*) reached a peak of expression at 6 DAP (Fig. 4, Type C₁) and then transcript amounts declined, while two genes,

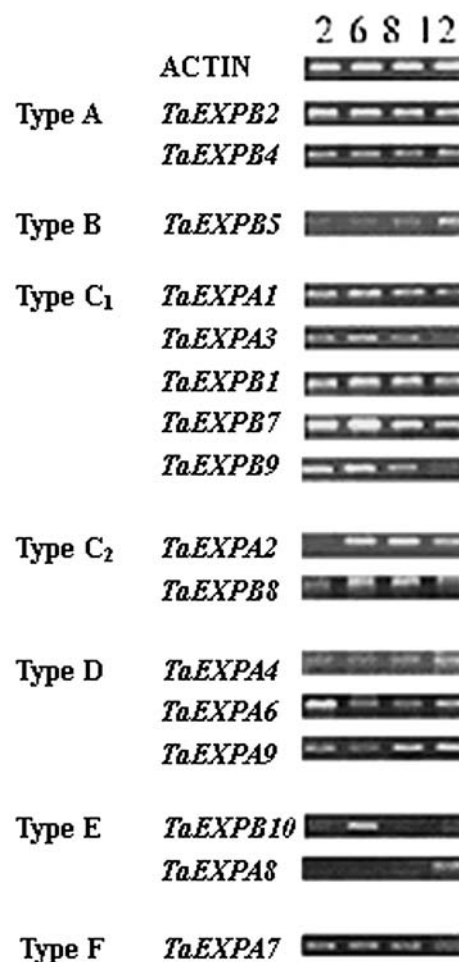


Fig. 4 Expression patterns of wheat expansins in developing seeds. Type A: constitutive expression in the developing seeds from 2 to 12 DAP (days after pollination). Type B: expression increases gradually during seed development. Type C: expression increases in early stages and then decreases as seed development progresses. Peak expression occurs at 6 DAP (C₁) or at 8 DAP (C₂). Type D: Expression is high at 2 DAP, decreases at 6 or 8 DAP and then increases again. Type E expression is detected only at 6 DAP or 12 DAP. Type F: expression remains stable at 2, 6 and 8 DAP, but decreases at 12 DAP

TaEXPA2 and *TaEXPB8*, reached their peak expression at 8 DAP (Fig. 4, Type C₂). In the case of *TaEXPA4*, *TaEXPA6* and *TaEXPA9*, expression was high at 2 DAP, decreased at 6 or 8 DAP and then increased again at 12 DAP (Fig. 4, Type D). *TaEXPB10* and *TaEXPA8* were only expressed at 6 and 12 DAP, respectively (Fig. 4, Type E). Finally *TaEXPA7* was expressed at similar levels at 2, 6 and 8 DAP, but transcript levels decreased at 12 DAP (Fig. 4, Type F).

Differential expression in internode tissue between an F₁ hybrid and its parents

It was previously suggested that differential gene expression might contribute to heterosis in plant hybrids



Fig. 5 Expression patterns in hybrids and their parents. Type A: the expression level in the hybrid is higher than in either parent (up-regulated in hybrid, URH). Type B: the expression level in the hybrid is similar to that in the parent with the higher expression (high-dominant in hybrid, HDH). Type C: the expression level in the hybrid is similar to that in the parent with the lower expression (low-dominant in hybrid, LDH). Type D: the expression level in the hybrid is lower than in either parent (down-regulated in hybrid, DRH). F, female parent; H, hybrid; M, male parent

(Sun et al. 2004). Since expansins play important roles in plant development, we wished to determine if expansin genes might be also differentially expressed between hybrids and their parents. For this study, the winter wheat hybrid AI 9/JIA18 and its parental lines were used. The adult hybrid reaches a height that is 13 cm higher than that of either parent, and the uppermost

internode of the hybrid is 6 cm longer than those of the parents. Since the length of the uppermost internode length showed obvious heterosis in the hybrid, we carried out RT-PCR analysis on the internode tissues including the intercalary meristem and most of the elongation zone (Fig. 5). *TaEXPB8* is not expressed in this tissue, and the other 16 expansin genes were all differentially expressed between the hybrid and its parents. The expression patterns could be classified into four types. 1. Up-regulated in hybrid (URH): four β -expansin genes (*TaEXPB1*, *TaEXPB2*, *TaEXPB7* and *TaEXPB9*) were expressed at higher levels in hybrid than in either parent (Fig. 5, Type A). 2. High-dominant in hybrid (HDH): the expression level of *TaEXPA5* in the hybrid was similar to the higher in the parental lines (Fig. 5, Type B). 3. Low-dominant in hybrid (LDH): *TaEXPA3*, *TaEXPB4* and *TaEXPB5* were expressed in the hybrid at levels to the lower in the parental-lines (Fig. 5, Type C). 4. Down-regulated in hybrid (DRH); the other eight genes (*TaEXPA1*, *TaEXPA2*, *TaEXPA4*, *TaEXPA6*, *TaEXPA7*, *TaEXPA9*, *TaEXPB8* and *TaEXPB10*) were expressed at lower levels in the hybrid than in either of the parents (Fig. 5, Type D).

It is interesting to note that the expression levels of all 16 wheat expansin genes in the hybrid internode differed from those in the parental lines. In particular, four β -expansin genes were expressed at higher levels in hybrid than in either parent. We propose that the differential expression of wheat expansin genes might be related to the heterosis in plant height seen in wheat hybrids, and possibly in other cereal crops, for the following reasons. Firstly, the stem length shows notable heterosis in this hybrid, and elongation of the internodes is the major determinant of the increase in plant height or heterosis in stem length; Secondly, differences in gene expression between hybrid and parental lines was previously suggested to be responsible for the observed heterosis in hybrid performance (Sun et al. 2004). Finally, expansins have been shown to be involved in the elongation of internode in rice (Cho and Kende 1997). The link between increased expansin gene expression and heterosis in plant height and root development is certainly worth investigating further.

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