GENE NOTE

Cloning of cDNA encoding COMT from wheat which is differentially expressed in lodging-sensitive and -resistant cultivars

Qing-Hu Ma1, Yang Xu, Zhan-Bing Lin and Ping He

Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Received 1 May 2002; Accepted 8 July 2002

Abstract

In the present study two cDNA fragments were cloned by nested-PCR using degenerate primers for COMT and found to be 93% identical at the nucleotide level. The deduced amino acid sequence of the two cDNAs showed a high degree of identity with COMT from other plants and were most similar to COMTs from monocots. RNA gel blot hybridization demonstrated that the wheat COMT gene W-cm5-1 was expressed in stem, root and leaf tissues. W-cm5-1 mRNA levels in the developing wheat stem were associated with the stem lodging character in two wheat cultivars. These results suggest that the action of the wheat COMT gene may be related to stem rigidity and lodging character in wheat.

Key words: Caffeic acid 3-O-methyltransferase, stem lodging, Triticum aestivum L.

Lignin is a phenolic cell wall polymer closely linked to cellulose and hemicelluloses, and is, second to cellulose, the most abundant biopolymer on earth. In plants, lignin mainly deposits in the walls of certain specialized cells such as tracheary elements, sclerenchyma and phloem fibres. This leads to a dramatic change in the cell wall properties, which imparts rigidity and structural support to the wall and assists in the transport of water and nutrients within the xylem tissue by decreasing the permeability of the cell wall. It has long been proposed that lignin synthesis might be related to stem strength. This issue is especially important in crop plants, where weak stem strength will lead to a lodging phenotype. To date, however, there is little information at the molecular level as to how the regulation of lignin synthesis affects crop lodging.

Reduced lignin levels have been observed in maize brown-midrib (bm) mutants and this has been associated with reduced stem strength. Recent work has confirmed that the bm3 mutant harboured a mutation in the gene for caffeic acid 3-O-methyltransferase (EC 2.1.1.68, COMT) (Vignols et al., 1995). COMT catalyses the multi-step methylation reactions of hydroxylated monomeric lignin precursors and is believed to occupy a pivotal position in the lignin biosynthetic pathway. In order to investigate whether COMT gene expression is associated with the stem lodging character of wheat, the COMT gene from wheat has been cloned and its expression in lodging-sensitive and -resistant wheat cultivars has been analysed.

Wheat plants (Triticum aestivum L. cvs H4564 and C6001) were grown in a naturally lit greenhouse with normal irrigation and fertilization. Total RNA was isolated from wheat tissues with the TRI reagent (Molecular Research Center, Inc, Cincinnati, USA) according to the manufacturer’s instructions. Poly(A)1 RNA was isolated using the PolyAT tract® mRNA Isolation Kit (Promega).

cDNA synthesis was based on the rapid amplification of the cDNA ends method (Frohman et al., 1988) using the oligonucleotide primer 5'-GACTCGAGTGCACATCGA(T)17-3'. To amplify COMT specific sequences, nested-PCR was performed using the following primers: 5'-primer as C21: 5'-TA(T/C/G)/GG(T/C/A/G)TGAC(T/C/A/G)GTG(A/G)TG(A/G)TG-3', and 3'-primers as C2: 5'-GACTGAGTCGACATCGA(T)17-3', and C25: 5'-(T/C/G)ATGAA-(T/C)CA(A/G)GA(T/C)AA(A/G)GT-3', and 3'-primers as C2: 5'-GACTGAGTCGACATCGA(T)17-3', and C25: 5'-(T/C/G)ATGAA-(T/C)CA(A/G)GA(T/C)AA(A/G)GT-3', and C26: 5'-(T/C/G)ATGAA-(T/C)CA(A/G)GA(T/C)AA(A/G)GT-3'. All primers except C2 were synthesized according to highly conserved amino acid sequences identified in COMT genes from other plants. The purified PCR fragments were cloned into the pGEM-T Easy vector (Promega) and DNA from several independent clones was sequenced. Sequence similarities were analysed using the SIM-Alignment Tool (Thompson et al., 1994) and data from the GenBank database.

In PCR experiments with primers C25 plus C2, and nested-PCR with primers C25 and C26, a 600 bp fragment was detected. A single fragment of about 500 bp in size was detected by nested-PCR with primers C21 plus C2. The sequencing results showed that they represented two cDNAs of different length, named W-cm5-1 and W-cm6-1. W-cm5-1 is 604 bp long, while W-cm6-1 is 517 bp long, both with a single open reading frame. W-cm5-1 and W-cm6-1 are 93% identical at the nucleotide level and 95% identical at the amino acid level, which suggested that they belong to the same kind of gene. Because of the high degree of identity between W-cm5-1 and W-cm6-1, W-cm5-1 was chosen for further analyses. The plant origin of W-cm5-1 was confirmed by Southern blot hybridization (data not shown).

W-cm5-1 (Accession No. AF502287) showed a high degree of similarity to published COMT sequences both at the DNA and amino acid levels. The amino acid identity of W-cm5-1 with COMT from other plants ranges from 64% to 83% in the compared region (Table 1). In addition, the amino acid sequence of W-cm5-1 has more similarity to COMT sequences from monocots than sequences from dicots. W-cm5-1 also shares the five most conserved motifs found in

1 To whom correspondence should be addressed. Fax: +86 010 6259 0839. E-mail: mgh@ns.ibcas.ac.cn

© Society for Experimental Biology 2002
Lodging phenotype in wheat, the expression of W-cm5-1 developmental stages and similar genetic background. Stem tissues were collected at different examined. These two cultivars were chosen because they share a sensitive (C6001) and lodging-resistant (H4564) cultivars was included to confirm that the RNA preparations are not degraded and to serve as an internal control for variations in gel loading and blotting.

Fig. 1. RNA gel blot analysis of W-cm5-1 gene expression in wheat tissues. RNA blots were hybridized with the W-cm5-1 cDNA fragment: L, leaf; S, stem; R, root. Hybridization with a 18S rDNA probe has been included to confirm that the RNA preparations are not degraded and to serve as an internal control for variations in gel loading and blotting.

Acknowledgements

This work was supported by grants from the Chinese National Special Foundation for Transgenic Plant Research and Commercialization (999-A-031), the National Natural Science Foundation of China (No. 30070067) and the Innovation Project of the Chinese Academy of Sciences. We wish sincerely to thank Dr Charles H Hocart (Australian National University, Australia) and Dr Bettina Deavours (Plant Biology Division, The Samuel Roberts Noble Foundation, USA) for critical reading of the manuscript.

References

Bugos RC, Chiang VL, Campbell WH. 1991. cDNA cloning, sequence analysis and seasonal expression of lignin-bispecific caffeic acid/5-hydroxyferulic acid O-methyltransferase of aspen. Plant Molecular Biology 17, 1203–1215.


Table 1. The percentage of amino acid identity between wheat COMT W-cm5-1 and COMT sequences present in the GenBank database

<table>
<thead>
<tr>
<th>Species</th>
<th>% Identity</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loliurn perenne</td>
<td>83</td>
<td>AF010291</td>
</tr>
<tr>
<td>Festuca arundinacea</td>
<td>83</td>
<td>AF153825</td>
</tr>
<tr>
<td>Saccharum officinarum</td>
<td>80</td>
<td>AJ231133</td>
</tr>
<tr>
<td>Zea mays</td>
<td>78</td>
<td>M73235</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>76</td>
<td>AF387900</td>
</tr>
<tr>
<td>Medicago sativa</td>
<td>67</td>
<td>M63853</td>
</tr>
<tr>
<td>Populus tremuloides</td>
<td>67</td>
<td>U13176</td>
</tr>
<tr>
<td>Eucalyptus gunnii</td>
<td>66</td>
<td>X74814</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>66</td>
<td>AB013837</td>
</tr>
<tr>
<td>Zinnia elegans</td>
<td>64</td>
<td>U19911</td>
</tr>
</tbody>
</table>

Table 2. W-cm5-1 mRNA levels in the different developmental stages of wheat stem

<table>
<thead>
<tr>
<th>Stages</th>
<th>C6001</th>
<th>H4565</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongation</td>
<td>100</td>
<td>84.2</td>
</tr>
<tr>
<td>Heading</td>
<td>26.4</td>
<td>84.8</td>
</tr>
<tr>
<td>Milky</td>
<td>29.5</td>
<td>91.2</td>
</tr>
</tbody>
</table>

Northern blots were quantified using a Phosphor Image and mRNA levels were normalized by comparison to 18S rRNA. C6001 elongation stage was arbitrarily set at 100 and relative arbitrary units are given by Northern hybridization with Phosphor Image and 18S rRNA normalization (Table 2). While W-cm5-1 mRNA levels remained fairly constant at each developmental stage in H4564, W-cm5-1 mRNA levels declined markedly at the heading and milky stages in C6001, relative to the level present at the elongation stage.

It is known that wheat requires intensive lignin synthesis to impart stem strength and rigidity at the heading and milky stages (data not shown) and the decrease in COMT gene expression in C6001 during these stages may affect stem development and contribute to the lodging phenotype of this cultivar. Cloning of W-cm5-1 allows this hypothesis to be tested further by down-regulating expression of W-cm5-1 in wheat using the antisense or gene silencing approach.