

Plant Gene Register

Nucleotide Sequence of a New Member of the Freezing Tolerance-Associated Protein Family in Wheat¹

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Exposure to low temperature induces and modulates the synthesis of specific proteins (Guy, 1990). We have previously identified a protein family specifically regulated by low temperature in wheat. Northern and western analyses revealed that this family is composed of at least four members (Houde et al., 1992b). Characterization of the cDNA encoding the 50-kD member, *Wcs120* (Houde et al., 1992a), showed the presence of two repeated domains that were also found in dehydrins and in late embryogenesis abundant and ABA-induced protein families (Dure, 1993). A second member of this family, *Wcs200*, corresponding to a protein of 200 kD, has been cloned, and Southern analysis along with protein microsequencing indicated that WCS200 contains the same repeats as WCS120 (Ouellet et al., 1993).

In this report, we describe the isolation and characterization of a third member of this family (Table I). The clone encodes a protein of 66 kD that cross-reacts with the anti-WCS120 antibody. The calculated molecular mass of the polypeptide is 46,797 D with an isoelectric point of 7.28. As is WCS120, the predicted protein is rich in Gly (26.7%), His (10.5%), and Thr (17.1%). DNA sequence comparison between *Wcs120* and *Wcs66* revealed that the coding portions of these transcripts were highly homologous, with an identity of 94%, when an "insertion" of 234 bases is excluded (from position 406). This insertion contains one additional repeat A (GEKKGVMENIKEKLPGGHGDHQQ) and three additional repeat B (TGTTYGQQGHTGTT). Thus, the WCS66 protein possesses 7 copies of repeat A and 14 copies of repeat B.

This high homology suggests that those proteins play similar roles during cold acclimation. It is likely that some of the genes from this family have been duplicated from a common ancestral gene. This gene duplication may help to allow a faster accumulation of proteins to protect cells against low-temperature stress. Expression of these genes in the sense

Table I. Characteristics of the *Wcs66* wheat freezing tolerance-associated protein

Organism:

Wheat (*Triticum aestivum* cv Norstar).

Source:

cDNA library in λ ZAP II, constructed from poly(A⁺) mRNA of 1-d cold-acclimated shoot.

Cloning Technique:

An antibody raised against the WCS120 protein (Houde et al., 1992b) was used to screen the cDNA library. Final identification of the 66-kD protein was accomplished by expressing the isolated clone in pBluescript after excision (Stratagene).

Sequencing Technique:

Dideoxy sequencing of both strands of miniprep plasmid DNA using the T7 sequencing kit (Pharmacia).

Features of the cDNA:

Contains 1629 nucleotides with a 5' nontranslated region of 76 nucleotides and an open reading frame of 1474 nucleotides. The 3' untranslated region is of 79 nucleotides.

mRNA Accumulation:

Accumulates preferentially during exposure to low temperature. Not induced by water stress, heat shock, or ABA. Run-on experiments suggest that the gene is mostly regulated at the transcriptional level.

Features of the Encoded Protein:

The molecular mass is 46,797 D with an isoelectric point of 7.28. It contains 7 Lys-rich repeats and 14 Gly-rich repeats. A homolog of the D-11 family of Lea proteins (Dure, 1993).

and antisense orientation in transgenic wheat will help us to demonstrate the importance of these genes in freezing tolerance.

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The nucleotide sequence data reported in this paper has been submitted to EMBL, GenBank, and DDBJ data bases under the accession number L27516.

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