Plant Physiol. (1995) 108: 439–440

Plant Gene Register

Complete cDNA and Genomic Sequence Encoding a Flooding-Responsive Gene from Maize (*Zea mays* L.) Homologous to Xyloglucan *Endo*transglycosylase[1]

Imad N. Saab* and Martin M. Sachs

Department of Agronomy, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 (I.N.S., M.M.S.); and United States Department of Agriculture/Agriculture Research Service, Plant Physiology and Genetics Unit, S108 Turner Hall, 1102 South Goodwin Avenue, Urbana, Illinois 61801 (M.M.S.)

The vast majority of identified flooding-responsive genes encode enzymes of Glc-P metabolism, which function to allow limited energy production in the face of limited oxygen supply (Sachs, 1994). However, in addition to the induction of these genes, flooded plants also undergo structural changes, such as formation of intercellular air spaces, which may help in survival by facilitating gas exchange between submerged and aerated plant parts (Drew et al., 1979). These structural modifications have not been addressed at the molecular level.

Here we report the isolation of a complete cDNA (MZEXETHOM) and genomic sequence (*g1005*) encoding a flooding-responsive gene that is homologous to XET and related glucanases (Table I). XET is a recently discovered enzyme that is proposed to play a role in wall loosening during cell expansion and fruit softening (e.g. Nishitani and Tominaga, 1992; Redgwell and Fry, 1993). The activity of XET is that of transglycosylation: the enzyme catalyzes cutting and rejoining of xyloglucans, and thus allows molecular grafting between polysaccharide chains that cross-link cellulose microfibrils (Fry et al., 1992; Nishitani and Tominaga, 1992). In accordance with its proposed role, increased XET activity was correlated with growth of cells in culture (Hetherington and Fry, 1993) and with altered wall rheology during water deficit (Spollen et al., 1993). In addition, increased XET activity was also correlated with fruit ripening, which results from enzymatic degradation of the cell wall, leading to loss of structural integrity. In this case, XET is proposed to loosen the cell wall prior to degradation by other enzymes (Redgwell and Fry, 1993). Thus, *g1005* is likely to encode a flooding-responsive form of XET or a related glucanase, which may be involved in cell wall metabolism during processes leading to structural modifications.

The deduced amino acid sequence of MZEXETHOM exhibits homology to XET sequences from a range of species including soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.), Vigna (*Vigna angularis*), tomato (*Lycopersicon esculentum* L.), and *Arabidopsis thaliana* (Okazawa et al., 1993). Stretches of homology are present along the entire length of the sequence, and the homology is particularly high in the middle and carboxy-terminal regions. For example, the

Table I. *Characteristics of cDNA and genomic sequences encoding a xyloglucan* endo*transglycosylase homolog from maize*

Organism:	
	Zea mays B73Ht.
Gene:	
	wusl1005(gfu) (Peschke and Sachs, 1994).
Gene Product:	
	Homologous to XET; actual function unknown.
Method of Identification:	
	Comparison with XET sequences in GenBank.
Source:	
	cDNA library in λZAPII (Stratagene) constructed from mRNA isolated from roots of preemergent seedlings (2 d old) that were treated anaerobically for 6 h; genomic library in λGEM11 (Promega).
Cloning Techniques:	
	For cDNA cloning, cDNA library was screened with a partial clone of MZEXETHOM (Peschke and Sachs, 1994) and six clones were selected for sequence analysis. For genomic cloning, genomic library was screened with the partial clone and then with a gene-specific 3′-end fragment. Two genomic clones were selected for restriction mapping, Southern analysis, subcloning, and sequencing.
Features of cDNA Sequence:	
	1240 bp long, containing a complete open reading frame of 843 nucleotides. The first ATG is 120 nucleotides downstream from the 5′ end. Contains 257 nucleotides in the 3′ untranslated region.
Features of Genomic Sequence:	
	Sequence is 1827 bp long covering the complete cDNA open reading frame. Gene contains one intron, 125 nucleotides long, starting 261 nucleotides downstream of the initiation codon.
Features of Deduced Protein:	
	The open reading frame encodes 280 amino acids with a predicted mass of 30.77 kD and a pI of 6.23.

[1] This work was supported by a grant from the U.S. Public Health Service (National Institutes of Health: 5 R01 GM34740) and U.S. Department of Agriculture/Agricultural Research Service funds to M.M.S.

* Corresponding author; e-mail i-saab@uiuc.edu; fax 1-217-333-6064.

Abbreviation: XET, xyloglucan *endo*transglycosylase.

deduced sequence shares 67% identity and 81% positives with the wheat sequence over a 90-amino acid stretch. *g1005* is also homologous to an *Arabidopsis* gene specific to meristems (*meri-5*; Medford et al., 1991) and to a brassinosteroid-regulated gene from soybean (Zurek and Clouse, 1994). Furthermore, MZEXETHOM and all homologous sequences contain several identical stretches, including a nine-amino acid stretch proposed to be the catalytic domain of *Bacillus subtilis* lichenases (a related set of glucanases; Borriss et al., 1990). Thus, analysis of MZEXETHOM and the encoding gene will likely offer molecular insights into flooding-induced structural modifications. Current and future studies will address the function of the protein and characterize the regulatory elements required for gene induction.

Received October 24, 1994; accepted November 17, 1994.

The GenBank accession numbers for the sequences reported in this article are U15781 (MZEXETHOM) and U15964 (*g1005*).

LITERATURE CITED

Borriss R, Buettner K, Maentsaelae P (1990) Structure of the beta-1,3–1,4-glucanase gene of *Bacillus macerans:* homologies to other beta-glucanases. Mol Gen Genet **222:** 278–283

Drew MC, Jackson MB, Giffard S (1979) Eythlene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. Planta **147:** 83–88

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

Hetherington PR, Fry SC (1993) Xyloglucan endotransglycosylase activity in carrot cell suspension during cell elongation and somatic embryogenesis. Plant Physiol **103:** 987–992

Medford JI, Elmer S, Klee HJ (1991) Molecular cloning and characterization of genes expressed in shoot apical meristems. Plant Cell **3:** 359–370

Nishitani K, Tominaga R (1992) Endo-xyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. J Biol Chem **267:** 21058–21064

Okazawa K, Sato Y, Nakagawa T, Asada K, Kato I, Tomita E, Nishitani K (1993) Molecular cloning and cDNA sequencing of endoxyloglucan transferase, a novel class of glycotransferase that mediates molecular grafting between matrix polysaccharides in plant cell walls. J Biol Chem **268:** 25364–25368

Peschke VM, Sachs MM (1994) Characterization and expression of anaerobically induced maize transcripts. Plant Physiol **104:** 387–394

Redgwell RJ, Fry SC (1993) Xyloglucan endotransglycosylase activity increases during kiwifruit (*Actinidia deliciosa*) ripening. Plant Physiol **103:** 1399–1406

Sachs MM (1994) Gene expression in maize during anoxia. *In* AS Basra, ed, Stress Induced Gene Expression in Plants. Harwood Academic Publishers, Chur, Switzerland, pp 87–102

Spollen WG, Wu Y, Sharp RE, Saab IN (1993) Regulation of cell expansion in roots and shoots at low water potentials. *In* JAC Smith, H Griffith, eds, Water Deficits: Plant Responses from Cell to Community. Bios Scientific Publishers, Oxford, UK, pp 37–52

Zurek DM, Clouse SD (1994) Molecular cloning and characterization of brassinosteroid-regulated gene from elongating soybean (*Glycine max* L.) epicotyls. Plant Physiol **104:** 161–170