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Plant Gene Register

Arabidopsis Gene and cDNA Encoding Cell-Wall Invertase[1]

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Suc is the final product of photosynthesis, but before it can be utilized it must be cleaved into hexoses either by invertase (β-fructofuranosidase, EC 3.2.1.26) or by Suc synthase (EC 2.4.1.13). There are three recognized types of invertase present in plant cells: soluble neutral invertase, soluble acid invertase, and insoluble cell-wall acid invertase. Invertase genes encoding cell-wall and vacuolar (soluble) acid invertases have been characterized from *Daucus carota* (Ramloch-Lorenz et al., 1993) and from *Lycopersicon* (either *esculentum* or *pimpinellifollium*, Elliott et al., 1993), respectively. So far, no invertase gene from *Arabidopsis thaliana* has been isolated.

We report here the characterization of a cell-wall invertase gene from *A. thaliana* and its cognate cDNA. A genomic fragment containing *Atbfruct1* was identified by screening a genomic library (EMBL3, Clontech, Palo Alto, CA) with a 1-kb fragment from a cDNA encoding a cell-wall invertase in *D. carota* (Sturm and Chrispeels, 1990). The *Atbfruct1* cDNA clone was identified by screening an *A. thaliana* cDNA library with exon 3 of the *Atbfruct1* gene. The genomic clone *Atbfruct1* is 4237 bp in size (Table I). Alignment of the *Atbfruct1* gene sequence with that from its cognate cDNA showed the presence of seven exons. The organization of the gene is similar to that of invertase genes in *D. carota* (Ramloch-Lorenz et al., 1993) and *L. esculentum* (Elliott et al., 1993), but the size of the introns is significantly smaller in *A. thaliana*. In *Atbfruct1* and both tomato genes, exon 2 is only 9 bp long and encodes part of a highly conserved region found in all known invertase proteins (NDPNG). By way of contrast, in the *D. carota* gene this short nucleotide sequence is included in exon 1. There is one conflict between the gene sequence and the cDNA sequence: the third base in the codon coding for Arg^{510} is a T in the gene and a C in the cDNA.

The cDNA clone *Atbfruct1* is 1947 bp in size with an open reading frame of 1755 bp coding for a protein of 584 residues.

Table I. *Characteristics of Atbfruct1 from A. thaliana*

Organism:
Arabidopsis thaliana (L.) Heynh. ecotype Columbia.
Gene Product:
Cell-wall invertase (β-fructosidase, EC 3.2.1.26); hydrolysis of Suc.
Clone Types:
Genomic clone λ201 containing gene *Atbfruct1* in an *Eco*RI fragment of 4237 bp. cDNA clone *Atbfruct1*: full length, 1947 bp; pBluescript SK(−).
Techniques:
Heterologous screening of a genomic library with a 1-kb fragment from cell-wall invertase cDNA from *D. carota* (Sturm and Chrispeels, 1990). Homologous screening of a leaf cDNA library with exon 3 of the *Atbfruct1* gene.
Structure of the *Atbfruct1* Gene:
Number of exons/introns: seven exons/six introns. Exon positions: (1) 393–588; (2) 715–723; (3) 989–1860; (4) 1945–2106; (5) 2187–2434; (6) 2620–2707; (7) 2955–3307. Position of TATA box, 326–330. Position of ATG, 393–395. Position of STOP, 3132–3134.
Features of cDNA:
19-bp untranslated 5′ end; 176-bp untranslated 3′ end; 1755-bp open reading frame.
Characteristics of Deduced Protein:
Open reading frame encodes a polypeptide of 584 amino acids of M_r 66,280. Isoelectric point = 9.11. The deduced protein contains a putative peptide signal (M^1-A^{28}) and four potential glycosylation sites (N-X-S/T): N^{159}, N^{186}, N^{342}, N^{446}.

This protein has an amino-terminal peptide containing a hydrophobic region between polar N and C terminals with all the characteristics of a signal peptide and organization similar to the signal peptide of the cell-wall invertase from *D. carota* (Sturm and Chrispeels, 1990). The predicted excision site for the signal sequence using the rules of von Heijne (1986) is between A^{28} and S^{29}. When compared by pairwise similarity analysis (Clustal IV, Higgins and Sharp, 1988), the deduced ATBFRUCT1 amino acid sequence shows a high homology to other plant invertases (Sturm et al., 1990; Arai et al., 1992; Klann et al., 1992; Elliott et al., 1993; Hedley et al., 1993; C. Unger, M. Hardegger, S. Lienhard, and A. Sturm, EMBL m58362, unpublished data). Comparison of the five invertases so far published for which cellular localization has been demonstrated shows relative similarity scores of 24 to 30 between the invertases from different cell compartments

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(cell wall and vacuole), irrespective of species and even for invertases from the same plant (i.e. *D. carota*). Furthermore, invertases from the same cell compartment (the vacuole) have scores higher than 45 even though they come from different species. ATBFRUCT1 has similarity scores of 25 and 30 with the two characterized vacuolar invertases and a score of 45 with the cell-wall invertase from *D. carota*. These results strongly suggest that *Atbfruct1* codes for a cell-wall invertase.

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