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Plant Gene Register

A Second Cell Wall Acid Invertase Gene in *Arabidopsis thaliana*[1]

Richard W. Mercier and Johann Peter Gogarten*

University of Connecticut, Department of Molecular and Cell Biology, 75 North Eagleville Road, Storrs, Connecticut 06269–3044

Invertases, or β-fructofuranosidases, hydrolyze Suc into Fru and Glc. Immobilized acid invertases in plants have been described as localized in the cell wall (Eschrich, 1980; Gogarten, 1986; Sturm and Chrispeels, 1990). Soluble acid invertases were ascribed to the vacuole (Leigh et al., 1979). At least in dicotyledonous plants these organelle-specific isoforms are encoded by separate genes (Sturm and Chrispeels, 1990; Unger et al., 1992). In maize a single mutation causes miniature seeds (Miller and Chourey, 1992) and these mutant seeds do not contain insoluble or soluble acid invertase. A characteristic of the known cell wall invertases in dicotyledonous plants is their acid pH optimum and their high pI (Eschrich, 1980; Gogarten, 1986; Hedley et al., 1993). The latter probably facilitates immobilization of the enzyme in the cell wall by ionic interactions; the former was interpreted to represent an acid-controlled switch for photosynthate partitioning (Eschrich, 1980).

We report here the nucleotide sequence of a gene encoding an acid invertase in *Arabidopsis thaliana* (Table I). The predicted pI of the encoded protein and the high sequence similarity to the carrot cell wall invertase (Table I), as well as our phylogenetic analysis (Fig. 1), indicate that this gene encodes a cell wall isoform. Recently another cell wall acid invertase from *A. thaliana* (isoform 1) was reported (Schwebel-Dugué et al., 1994). The deduced amino acid sequences of the two *Arabidopsis* acid invertases are only 54% identical; however, both invertases have a high predicted pI (isoform 1, 9.5; isoform 2, 9.7), and both are more similar to the carrot cell wall invertase (isoform 1, 54.3%; isoform 2, 66.5% identity) than to the soluble intracellular acid invertase from carrot (isoform 1, 46.4%; isoform 2, 43% identity) (see legend to Fig. 1 for accession numbers; see Unger et al. [1992], for the characterization of the soluble invertase from carrots).

To determine whether these two cell wall isoforms are restricted to *Arabidopsis* or whether they have a wider phylogenetic distribution, we reconstructed the evolutionary history of several cell wall invertases whose sequences had been submitted to GenBank (Fig. 1). As expected from the percentage of identity values, the two *Arabidopsis* isoforms do not group together: isoform 1 groups closer to the outgroup (soluble acid invertases) and isoform 2 groups closer to the cell wall invertase from *Daucus carota*. To assess the reliability of these groupings, we performed a bootstrap analysis. The bootstrap probabilities for the different groupings are indicated in Figure 1. According to Felsenstein and Kishino (1993) 100 minus the percentage of bootstrap probability gives an estimate of the probability that the grouping is assumed erroneously. In all bootstrapped samples the *Arabidopsis* cell wall isoform 1 branches off deeper and separate from the other cell wall invertases included in the analysis. We conclude that the gene duplication that gave rise to the two cell wall isoforms present in *Arabidopsis* occurred before the evolution of

Table I. *Characteristics of a second cell wall invertase encoding gene in Arabidopsis*

Organism:
Arabidopsis thaliana (L.) Heynh. ecotype Columbia.
Gene Product:
β-Fructofuranosidase (EC 3.2.1.26).
Function and Subcellular Localization:
Encodes an acid invertase targeted to the cell wall.
Source:
Genomic DNA library in λEMBL3 (Clontech, Palo Alto, CA).
Method of Identification:
PCR of genomic DNA using redundant primers targeted to conserved regions; subcloning and sequencing of PCR products; screening of genomic library with the PCR product.
(G+C) Content:
45% (G+C) in the coding region.
Chromosomal Localization:
Undetermined.
Structural Features of the Gene:
Two putative TATA boxes; two putative translation initiation sites; transit peptide; the coding sequence is interrupted by five introns of 144, 94, 95, 18, and 84 nucleotides.
Features of the Predicted Amino Acid Sequence:
584 amino acids; M_r = 66,243; theoretical pI 9.7; putative signal peptide cleavage site is similar to cleavage site in the cell wall invertase of *Daucus carota* (Sturm and Chrispeels, 1990).
Sequence Identity:
The encoded protein has 54.3% identity with the other *Arabidopsis* invertase isoform, 66.4% identity with the cell wall invertase from *D. carota*, and 43% identity with the soluble acid invertase from *D. carota*. (See legend to Fig. 1 for GenBank accession numbers.)

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* Corresponding author; e-mail gogarten@uconnvm.uconn.edu; fax 1-203-486-1784.

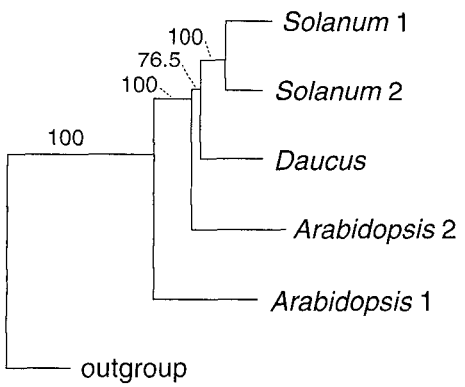

Figure 1. Phylogenetic comparison of cell wall acid invertases. Amino acid sequences were aligned using the program CLUSTAL V (Higgins and Sharp, 1988). The aligned sequences are available upon request from the authors. The amino- and carboxy-terminal ends that did not show a good alignment were deleted. The remaining 548 aligned amino acid positions were analyzed using PHYLIP 3.5c (available from J. Felsenstein, University of Washington, Seattle, WA). Topology and branch lengths of the depicted tree were calculated using protein distance matrix analysis. Numbers give the percentage of bootstrapped samples in which the group distal from the number was recovered by protein parsimony analysis. The sequences have the following accession numbers in GenBank: *Solanum tuberosum* 1, Z21486; *Solanum tuberosum* 2, Z22645; *D. carota*, X69321; *A. thaliana* 2, U11033; *A. thaliana* 1, X74515. The outgroup consisted of soluble acid invertases from *D. carota* (X75351), *Lycopersicon esculentum* (M81081), *S. tuberosum* (X70368), and *Vignia radiata* (D10265).

Dilleniidae (*Arabidopsis*), Rosidae (*Daucus*) and Lamiidae (*Solanum*). The two types of cell wall isoforms represented by *Arabidopsis* isoforms 1 and 2 should be present in most dicotyledonous plants. In contrast, the two cell wall invertase isoforms in *Solanum* are of a more recent origin.

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The GenBank accession number for the sequence reported in this article is U11033.

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