

Plant Gene Register

The *Ivr 1* Gene for Invertase in Maize¹

Jian Xu*, Gwendolyn H. Pemberton, Ernesto C. Almira, Donald R. McCarty, and Karen E. Koch

Plant Molecular and Cellular Biology Program (J.X., G.H.P., D.R.M., K.E.K.),
and DNA Sequencing Core Laboratory, 2301 Fifield Hall (E.C.A.),
University of Florida, Gainesville, Florida 32611

Invertases (β -fructofuranosidase, EC 3.2.1.26) catalyze the hydrolysis of Suc into Glc and Fru. Because of the central role of Suc in phloem translocation in higher plants, invertase is considered a key enzyme for carbohydrate metabolism and Suc import (Avigad, 1982). Several isoforms of invertase often can be present simultaneously in a given plant and/or organ. Acid and neutral types can be distinguished based on pH optima, or alternatively they can be classified as soluble or insoluble forms (Avigad, 1982). The latter are cell-wall-bound enzymes, whereas soluble invertase can be in vacuoles or cytoplasm (Avigad, 1982).

Soluble forms of this enzyme are especially important during cell expansion, such as occurs during leaf enlargement (Schaffer et al., 1987), early fruit/seed development (Sung et al., 1994), and an array of rapidly elongating tissues (J. Xu and K.E. Koch, unpublished data). Genes encoding soluble acid invertases have been characterized from carrot (Unger et al., 1994), tomato (Klann et al., 1992; Elliott et al., 1993), and mung bean (Arai et al., 1992). Insoluble cell-wall forms have also been isolated from carrot (Sturm and Chrispeels, 1990) and Arabidopsis (Schweibel-Dugué et al., 1994).

The maize (*Zea mays*) invertase sequence identified in the present study was isolated by screening a maize seedling genomic DNA library (EMBL 3; Clontech, Palo Alto, CA) with a 1.0-kb maize cDNA fragment, which in turn had been obtained by screening a maize root tip cDNA library (gt 10, Clontech) with a soluble acid invertase cDNA from tomato (Klann et al., 1992; Table I). One positive 8-kb genomic clone was isolated and characterized. *Bam*HI and *Kpn*I released three fragments, which were subcloned and sequenced.

The second exon is unusually small (9 bp) in the maize *Ivr 1* invertase and tomato soluble invertase genes (Elliott et al., 1993). This represents one of the smallest exons currently known to function in a plant genome (M. Schuler, personal communication). In addition, this exon encodes a highly conserved domain found in all invertase sequences identified to date (Asn-Asp-Pro-Asn-Gly, the β -fructosi-

Table I. Characteristics of *Ivr 1* from *Z. mays*

Organism:	<i>Zea mays</i> , B73.
Gene Product:	<i>Ivr 1</i> , soluble acid invertase (β -fructosidase, EC 3.2.1.26).
Techniques:	Heterologous screening of a root tip cDNA library with a 0.45-kb fragment from the soluble acid invertase cDNA from tomato (Klann et al., 1992). Homologous screening of a seedling genomic library with the 1-kb <i>Kpn</i> I- <i>Eco</i> RI fragment of the maize <i>Ivr 1</i> gene.
Number of Exons/Introns:	Seven exons/six introns. Exon positions: 1, 805–1220; 2, 1389–1397; 3, 1591–2484; 4, 3746–3908; 5, 4016–4252; 6, 4371–4459; 7, 4578–4788. Position of CAAT box, 487–490. Position of TATA box, 557–560. Position of start site (ATG), 805–807. Position of stop site (TGA), 4786–4788.
Characteristics of Deduced Protein:	Open reading frame encodes a polypeptide of 670 amino acids of <i>M</i> , 71,942. <i>pI</i> is 7.5. The deduced protein contains a putative peptide signal (Met ¹ -Ala ⁷³) (von Heijne, 1986) and five potential glycosylation sites (Asn-X-Ser/Thr): Asn ¹⁶⁵ , Asn ²⁷⁵ , Asn ⁵¹⁸ , Asn ⁵⁹⁵ , Asn ⁶³⁹ .

dase motif, Sturm and Chrispeels, 1990). In the maize gene, several introns were also found to contain one or more copies of the RY sequence motif (CATGCATG), which thus far has been implicated in seed-specific gene expression (Baumlein et al., 1992; Lelievre et al., 1992).

The maize *Ivr 1* gene described here shows a 42% sequence similarity at the amino acid level to insoluble invertase (Sturm and Chrispeels, 1990) compared to a shared identity of approximately 60% with soluble invertases (Arai et al., 1992; Klann et al., 1992; Elliott et al., 1993; Unger et al., 1994). Extensive sequence similarity is also evident in several key domains. In addition, the sequence at the C terminus of the *Ivr 1* protein is markedly more similar to that of soluble versus insoluble invertases. This region is known to encode C-terminal propeptides that may function as vacuolar targeting signals (Chrispeels, 1991; Bednarek and Raikhel, 1992). These results are consistent with a soluble invertase designation for the *Ivr 1* genomic clone described here from maize.

ACKNOWLEDGMENTS

We thank Drs. E. Klann and A.B. Bennett for the generous gift of the tomato soluble invertase cDNA fragment.

¹ This research was supported by a grant from the National Science Foundation (Cellular Biochemistry) and by the University of Florida Agricultural Experiment Station (journal series No. R-04501).

* Corresponding author; e-mail kek@gvn.ifas.ufl.edu; fax 1-904-392-5653.

Received November 21, 1994; accepted December 21, 1994.

Copyright Clearance Center: 0032-0889/95/108/1293/02.

The EMBL accession number for the sequence reported in this article is U16123.

LITERATURE CITED

- Arai M, Mori H, Imaseki H** (1992) Cloning and sequence of cDNAs for an intracellular acid invertase from etiolated hypocotyls of mung bean and expression of the gene during growth of seedlings. *Plant Cell Physiol* **33**: 245–252
- Avigad G** (1982) Sucrose and other disaccharides. In FA Loewus, W Tanner, eds, *Encyclopedia of Plant Physiology, New Series*. Springer-Verlag, New York, pp 216–347
- Baumlein H, Nagy I, Villarroel R, Inze D, Wobus U** (1992) cis-Analysis of a seed protein gene promoter: the conservative RY repeat CATGCATG within the legumin box is essential for tissue-specific expression of legumin gene. *Plant J* **2**: 233–239
- Bednarek SY, Raikhel NV** (1992) Intracellular trafficking of secretory proteins. *Plant Mol Biol* **20**: 133–150
- Chrispeels MJ** (1991) Sorting of proteins in the secretory system. *Annu Rev Plant Physiol Plant Mol Biol* **42**: 21–53
- Elliott K, Butler WO, Dickinson CD, Konno Y, Vedvick TS, Fitzmaurice L, Mirkov TE** (1993) Isolation and characterization of fruit vacuolar invertase genes from two tomato species and temporal differences in mRNA levels during fruit ripening. *Plant Mol Biol* **21**: 515–524
- Klann E, Yelle S, Bennett AB** (1992) Tomato fruit acid invertase complementary DNA. *Plant Physiol* **99**: 351–353
- Lelievre JM, Oliveira LO, Nielsen NC** (1992) 5'-CATGCAT-3' elements modulate the expression of glycinin genes. *Plant Physiol* **98**: 387–391
- Schaffer AA, Sage O, Goldschridt EE, Goren R** (1987) Invertase and sucrose synthase activity, carbohydrate status and endogenous IAA levels during *Citrus* leaf development. *Physiol Plant* **69**: 151–155
- Schwebel-Dugué N, Mtili NE, Krivitzky M, Jean-Jacques I, Williams JHH, Thomas M, Kreis M, Lecharny A** (1994) *Arabidopsis* gene and cDNA encoding cell-wall invertase. *Plant Physiol* **104**: 809–810
- Sturm A, Chrispeels, MJ** (1990) cDNA cloning of carrot extracellular β -fructosidase and its expression in response to wounding and bacterial infection. *Plant Cell* **2**: 1107–1119
- Sung SS, Sheih WJ, Geiger DR, Black CC** (1994) Growth, sucrose synthase, and invertase activities of developing *Phaseolus vulgaris* L. fruits. *Plant Cell Environ* **17**: 419–426
- Unger C, Hardegger M, Lienhard S, Sturm A** (1994) cDNA cloning of carrot (*Daucus carota*) soluble acid β -fructofuranosidases and comparing with the cell wall isoenzyme. *Plant Physiol* **104**: 1351–1357
- von Heijne G** (1986) A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res* **14**: 4683–4690