Plant Physiol. (1995) 108: 419–420

Plant Gene Register

Cloning of a Harvest-Induced β-Galactosidase from Tips of Harvested Asparagus Spears[1]

Graeme A. King* and Kevin M. Davies

New Zealand Institute for Crop & Food Research Limited, Levin Research Center, Private Bag 4005, Levin, New Zealand

We are investigating the early changes in physiology, biochemistry, and gene expression that follow harvest of asparagus (*Asparagus officinalis* L.) spears to identify factors regulating the postharvest senescence of horticultural crops harvested while physiologically immature. The tips of spears comprise immature developing tissues that appear particularly susceptible to harvest stress, and they are usually the first parts of the spear to show symptoms of postharvest senescence, including feathering and browning of bracts, tissue flaccidity, and cellular breakdown (King et al., 1990). These visual symptoms of senescence are preceded by rapid changes in carbon and nitrogen metabolism and gene expression. Within 48 h of harvest, the respiration rate of tips declines markedly, protein is lost, and both free amino acids (especially Asn) and ammonia accumulate (King et al., 1990). Major changes in gene expression occur within 6 h of harvest, including the de novo induction of specific genes (King and Davies, 1992).

We previously constructed cDNA libraries from mRNA extracted from tips of spears at harvest and from tips of spears held in the dark at 20°C for 12 h. Differential hybridization screening of these libraries isolated nine cDNA clones whose transcripts had altered expression in tips of harvested asparagus spears (King and Davies, 1992), including a cDNA clone for a harvest-induced Asn synthetase (King and Davies, 1992; Davies and King, 1993). We report here the cloning and characteristics of a full-length cDNA (pTIP31) encoding β-galactosidase using one of our original harvest-induced partial cDNA clones (pTIP11) as a hybridization probe (Table I).

The derived protein sequence of pTIP31 shares 71% amino acid identity with ABG1, a cDNA clone encoding a member of the apple β-galactosidase gene family (Ross et al., 1994), and 63% amino acid identity with DINCARSR12, an ethylene-regulated β-galactosidase cDNA clone isolated from a senescing carnation petal library (Ragothama et al., 1991; W.R. Woodson, personal communication). Transcripts homologous to pTIP31 increase in abundance in tips of harvested asparagus spears. Induction of β-galactosidase may explain the observation of Waldron and Selvendran (1990), who noted a 50% reduction in the levels of cell-wall-bound Gal throughout the length of asparagus spears held for several days after harvest.

Table I. *Characteristics of an asparagus cDNA clone encoding β-galactosidase*

Organism:	Asparagus (*Asparagus officinalis* L. cv Limbras 10).
Cloning and Sequencing:	The full-length cDNA (pTIP31) was isolated from a λgt10 cDNA library made from mRNA extracted from tips of harvested asparagus spears held in the dark at 20°C for 12 h. pTIP31 was isolated by screening the library with pTIP11, a 1.8-kb partial cDNA clone for a harvest-induced mRNA isolated from an asparagus tip cDNA library (King and Davies, 1992). The full-length cDNA was subcloned into pBlueScriptKS$^+$ (Stratagene), and both strands of the nucleotide sequence were determined using a commercial service (Lofstrand Laboratories, Gaithersburg, MD).
Characteristics of the cDNA:	pTIP31 encodes an mRNA of 3152 bp with 149 bp of untranslated 5′ and 504 bp of untranslated 3′ sequence, including a poly(A) extension of 41 bp. The derived protein sequence of 832 amino acids includes a strongly hydrophobic putative signal sequence at the amino terminus of 15 amino acids. Cleavage of the amino terminal signal sequence leaves a mature protein with a predicted molecular mass of 90.6 kD and a pI of 7.2.
Expression Characteristics:	Transcripts of approximately 3.1 kb are detected by northern blot analysis of total RNA. A high level of transcripts accumulates within 12 h of harvest in tips of asparagus spears (King and Davies, 1992).
Method of Identification:	Sequence homology to other β-galactosidase cDNA clones.
Antibodies:	Not available.

ACKNOWLEDGMENT

We thank Dr. Randy Woodson for helpful discussions concerning the identity of DINCARSR12.

Received October 3, 1994; accepted November 3, 1994.
Copyright Clearance Center: 0032-0889/95/108/0419/02.
The EMBL/GenBank/DDBJ data base accession number for the sequence reported in this article is X77319.

[1] Supported by a grant in aid from the Scientific Research Committee of the New Zealand Asparagus Council.

* Corresponding author; e-mail kingg@crop.cri.nz; fax 64–6368–3578.

LITERATURE CITED

Davies KM, King GA (1993) Isolation and characterization of a cDNA clone for a harvest-induced asparagine synthetase from *Asparagus officinalis* L. Plant Physiol **102:** 1337–1340

King GA, Davies KM (1992) Identification, cDNA cloning, and analysis of mRNAs having altered expression in tips of harvested asparagus spears. Plant Physiol **100:** 1661–1669

King GA, Woollard DC, Irving DE, Borst WM (1990) Physiological changes in asparagus spear tips after harvest. Physiol Plant **80:** 393–400

Ragothama KG, Lawton KA, Goldsbrough PB, Woodson WR (1991) Characterization of an ethylene-regulated flower senescence-related gene from carnation. Plant Mol Biol **17:** 61–71

Ross GS, Wegrzyn T, McRae EA, Redgwell RJ (1994) Apple β-galactosidase: activity against cell wall polysaccharides and characterisation of a related cDNA clone. Plant Physiol **106:** 521–528

Waldron KW, Selvendran RR (1990) Effect of maturation and storage on asparagus (*Asparagus officinalis* L.) cell wall composition. Physiol Plant **80:** 576–583