



US007098324B2

(12) **United States Patent**  
**Haigler et al.**

(10) **Patent No.:** **US 7,098,324 B2**  
(45) **Date of Patent:** **Aug. 29, 2006**

(54) **CHITINASE ENCODING DNA MOLECULES FROM COTTON EXPRESSED PREFERENTIALLY IN SECONDARY WALLED CELLS DURING SECONDARY WALL DEPOSITION AND A CORRESPONDING PROMOTER**

(75) Inventors: **Candace H. Haigler**, Lubbock, TX (US); **Hong Zhang**, Lubbock, TX (US); **Chunfa Wu**, Lubbock, TX (US); **Chun-Hua Wan**, Gaithersburg, MD (US); **Deshui Zhang**, Lubbock, TX (US)

(73) Assignee: **Texas Tech University**, Lubbock, TX (US)

(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 169 days.

(21) Appl. No.: **10/350,696**

(22) Filed: **Jan. 23, 2003**

(65) **Prior Publication Data**  
US 2004/0049808 A1 Mar. 11, 2004

**Related U.S. Application Data**

(63) Continuation-in-part of application No. 09/918,083, filed on Jul. 30, 2001, now abandoned.

(51) **Int. Cl.**  
**C12N 15/29** (2006.01)  
**C12N 15/82** (2006.01)

(52) **U.S. Cl.** ..... **536/24.1**; 435/320.1; 800/287; 800/314

(58) **Field of Classification Search** ..... 536/24.1; 435/320.1, 419, 468; 800/287, 278, 298, 800/290, 314

See application file for complete search history.

(56) **References Cited**

**U.S. PATENT DOCUMENTS**

5,004,863	A	4/1991	Umbeck	
5,474,925	A	12/1995	Maliyakal et al.	
5,495,070	A	2/1996	John	800/287
5,521,078	A	5/1996	John	
5,530,187	A	6/1996	Lamb et al.	
5,597,718	A	1/1997	John et al.	
5,620,882	A	4/1997	John	
5,981,834	A	11/1999	John et al.	
6,096,950	A	8/2000	John	
6,169,174	B1	1/2001	Hasegawa et al.	
6,211,430	B1	4/2001	John	
6,225,536	B1	5/2001	Kasukabe et al.	
6,259,003	B1	7/2001	Fujisawa et al.	
6,329,570	B1	12/2001	Martineau	

**FOREIGN PATENT DOCUMENTS**

EP 0 834 566 A2 4/1996

**OTHER PUBLICATIONS**

Rinehart et al. *Plant Physiol* (1996) 112:1331-1341.\*

Chlan et al., "Class I Chitinases in Cotton (*Gossypium hirsutum*): Characterization, Expression and Purification," *Plant Science* 161:143-154 (2001).

John et al., "Gene Expression in Cotton (*Gossypium hirsutum* L.) Fiber: Cloning of the mRNAs," *Proc. Natl. Acad. Sci. USA*, 89(13): 5769-5773 (1992).

John, "Characterization of a Cotton (*Gossypium hirsutum* L.) Fiber mRNA (Fb-B6)," *Plant Physiol.*, 107(4): 1477-1478 (1995).

Ma et al., "Differential Expression of a Lipid Transfer Protein Gene in Cotton Fiber," *Biochim. Biophysica Acta*, 1257(1): 81-84 (1995).

John et al., "Characterization of mRNA for a Proline-Rich Protein of Cotton Fiber," *Plant Physiol.*, 108(2): 669-676 (1995).

John et al., "Structural Characterization of Genes Corresponding to Cotton Fiber mRNA, E6: Reduced E6 Protein in Transgenic Plants by Antisense Gene," *Plant Mol. Biol.*, 30(2): 297-306 (1996).

Rinehart et al., "Tissue-Specific and Developmental Regulation of Cotton Gene FbL2A. Demonstration of Promoter Activity in Transgenic Plants," *Plant Physiol.*, 112(3): 1331-1341 (1996).

Orford et al., "Abundant mRNAs Specific to the Developing Cotton Fibre," *Theor. Appl. Genet.*, 94:909-918 (1997).

Orford et al., "Characterization of a Cotton Gene Expressed Late in Fibre Cell Elongation," *Theor. Appl. Genet.*, 98: 757-764 (1999).

Orford et al., "Expression of a Lipid Transfer Protein Gene Family During Cotton Fibre Development," *Biochim. Biophysica Acta*, 1483(2): 275-284 (2000).

Ihara et al., "Cloning and Sequencing of Cotton Homologs of besA Gene Encoding Cellulose 4-β-glucosyltransferase," *Wood Res.* 84:1-6 (1997) (abstract).

Yamamoto, "Isolation and Characterization of cDNAs that are Preferentially Expressed in Cotton Fibre (*Gossypium hirsutum*)," Texas Tech Univ., Univ. Microfilms Int., DA 9522349, Diss. Abstr. Int. 56 (3), 1263 (1995) (abstract).

Pear et al., "Higher Plants Contain Homologs of the Bacterial celA Genes Encoding the Catalytic Subunit of Cellulose Synthase," *Proc. Natl. Acad. Sci. USA* 93:12637-12642 (1996).

Levorson et al., GenBank Accession No. Q39799 (Jul. 15, 1998).

Levorson et al., GenBank Accession No. Q39785 (Jul. 15, 1999).

Levorson et al., GenBank Accession No. AAD11255 (Feb. 1, 1999).

Hudspeth et al., GenBank Accession No. S72528 (Jun. 22, 1999).

Hamel et al., GenBank Accession No. Q09023 (Oct. 1, 1994).

(Continued)

*Primary Examiner*—David T. Fox

*Assistant Examiner*—Stuart F. Baum

(74) *Attorney, Agent, or Firm*—Nixon Peabody LLP

(57) **ABSTRACT**

The present invention relates to isolated nucleic acid molecules encoding endogenous cotton chitinases and corresponding promoters, which are preferentially expressed in secondary walled cells during secondary wall deposition. The polypeptide encoded by the nucleic acid molecule, a DNA construct linking the isolated nucleic acid molecule with a promoter, the DNA construct incorporated in an expression system, a host cell, a plant, or a plant seed are also disclosed. The present invention also relates to a DNA construct linking the isolated promoters with a second DNA as well as expression systems, host cells, plants, or plant seeds containing the DNA construct. Methods of imparting resistance to insects and fungi, regulating the fiber cellulose content, and methods of expressing a gene preferentially in secondary walled cells during secondary wall deposition are also disclosed.