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(54) Title: ISOLATION AND CHARACTERISATION OF AN ANTHR-SPECIFIC PROMOTER (COFS) IN COTTON

(57) Abstract: The present invention relates to an anther-specific cotton gene (CoFS), and active promoter fragments thereof. These
promoters show strong anther-specific activity.

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**ISOLATION AND CHARACTERISATION OF AN
ANTHER-SPECIFIC PROMOTER (COFS) IN COTTON**

BACKGROUND OF THE INVENTION

5 1. Technical Field.

The present invention relates to the field of plant molecular biology. In particular, the invention pertains to cotton promoters and their uses in creating transgenic plants, and more specifically to cotton anther-specific promoters.

10 2. Description of the Background Art.

Cotton is the most extensively used natural fiber in the textile industry. Annual production of cotton worldwide is over 100 million bales valued at 45 billion U.S. dollars. Although significant improvements have been made in quality and yield by means of classical breeding in the past decades, the potential for further improving the properties of cotton through classical breeding is limited due to requirements for species compatibility and available traits. Genetic engineering provides novel approaches for further improving cotton by introducing genes to create new germplasms with highly desirable characteristics, for example, insect pest resistance.

The anther is the male reproductive organ in flowering plants. Anther development can be divided into two general phases. During phase 1, most of specialized cells and tissues differentiate, microspore mother cells undergo meiosis and tetrads of microspores are formed. During phase 2, microspores are released from tetrads followed by pollen grain maturation, tissue degeneration, dehiscence and pollen release. Genes specifically expressed during anther and pollen development have been studied in a few plant species. Allen and Lonsdale, *Plant J.* 3:261-271, 1993; Bird, et al., *Plant Mol. Biol.* 11:651-662, 1988; Brown and Crouch, *Plant Cell* 2:263-274, 1990; Grierson et al., *Nucl. Acids Res.* 14:8595-8603, 1986; Hanson, et al., *Plant Cell* 1:173-179, 1989; Ursin et al., *Plant Cell* 1:727-736, 1989; John and Petersen, *Plant Mol. Biol.* 26(6):1989-1993, 1994; Atanassov et al., *Plant Mol. Biol.* 38:1169-1178, 1998; Liu et al., *Plant Mol. Biol.* 33:291-300, 1997; Treacy et al., *Plant Mol. Biol.* 34:603-611, 1997; Agnes et al., *Plant Mol. Biol.* 40:857-872, 1999. Among the 20,000 to 25,000 expressed genes in tobacco anther, only 10,000 genes are anther-specific. Kamalay and Goldberg, *Proc. Natl. Acad. Sci. USA* 81:2801-2805, 1984; Koltunow, et al., *Plant Cell* 2:1201-1224, 1990.

A promoter is a DNA fragment which determines the temporal and spatial specificity of gene expression during plant and animal development. Many tissue-specific genes and their promoters have been identified and isolated from a wide variety of plants and animals over the past decade, including cotton tissue-specific genes and promoters. Loguerico et al., *Mol. Gen. Genet.* 261(4/5):660-671, 1999; Kawai et al., *Plant Cell Physiol.* 39(12):1380-1383, 1998; Song and Allen, *Biochem. Biophys. Acta* 1351(1):305-312, 1997; Ma et al, *Biochim. Biophys. Acta* 1344(2):111-114, 1997; John, *Plant Mol. Biol.* 30(2):297-306, 1996; Rinehart et al., *Plant Physiol.* 112(3):1331-1341, 1996; Hasenfratz et al., *Plant Physiol.* 108(4):1395-1404, 1995; John and Peterson, *Plant Mol. Biol.* 26(6):1989-1993, 1994; John and Crow, *Proc. Natl. Acad. Sci. USA* 89(13):5769-5773, 1992. These plant tissue-specific promoters can be used to control the expression of foreign genes in transgenic plants in a tissue-specific manner that will dominate the majority of the second generation of transgenic crops. Some plant tissues do not express high levels of the transgene in all desired tissues or the particular desired tissue. In transgenic Bt cotton, for example, Bt gene expression level is extremely low in the flower, including in the anther, resulting in little protection from pest insects in these tissues. To achieve better control of pest insects of cotton, it would be highly advantageous to identify anther-specific promoters which can produce higher levels of gene expression in these tissues.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides a promoter that is cotton anther-specific, comprising the promoter of the cotton CoFS gene. The invention also provides a cotton anther-specific promoter comprising SEQ ID NO:2. Yet a further embodiment may provide a transgenic plant expressing a transgene under control of a cotton anther-specific promoter of the cotton CoFS gene.

In an aspect of the present invention, there is provided an isolated nucleic acid molecule encoding an anther-specific promoter comprising the promoter of the cotton CoFS gene, wherein said isolated nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID No:2.

In another aspect of the present invention, there is provided a transgenic plant expressing a transgene under control of an anther-specific promoter comprising the promoter of the cotton CoFS gene, wherein said promoter comprises the nucleotide sequence set forth in SEQ ID No:2.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the results of a CoFS cDNA differential display assay.

Fig. 2 provides a Northern blot showing CoFS gene expression.

Fig. 3 is a schematic diagram of constructs of CoFS promoter vector constructs and the cotton CoFS gene.

Fig. 4 provides the results of an assay of the expression of the *GUS* gene under the control of the CoFS gene promoter in transgenic tobacco plants.

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Fig. 5 shows the results of an expression assay of the *GUS* gene under the control of the CoFS promoter in transgenic tobacco plants.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

5 An anther-specific gene (CoFS) and its corresponding promoter were isolated from cotton by differential display assay. The activity and tissue specificity of the isolated promoter was confirmed in transgenic tobacco plants using the CoFS promoter to control the expression of the *GUS* reporter gene.

10 Northern blot analysis of cDNAs from a variety of cotton tissues showed that a cDNA clone comprising the CoFS gene was strongly expressed in anthers, and also expressed in petal tissue, but less or not at all in other tissues. See Fig. 1.

) An anther-specific gene (named CoFS) was isolated from cotton. The isolated complete CoFS cDNA is 8.4 kb in length. See Table I. Based on the CoFS cDNA sequence, a CoFS promoter fragment (2.6 kb) was isolated. See Table II. Comparing the nucleotide and predicted
15 polypeptide sequences of the cotton CoFS gene with published sequences revealed that the gene was about 54-58% identical at the amino acid level with acyl-CoA synthetase (probable long-chain-acid cCoA ligase, EC 6.2.1.3) genes from some plants such as *Brassica napus* (X94624) and *Arabidopsis* (AL078468, AL161560). Less homology was found at the nucleotide level, indicating that CoFS is a novel gene found in cotton. Analyzing the CoFS gene sequence
20 revealed that it may contain 9-10 exons and 8-9 introns in its open reading frame, based on amino acid sequences of the known acyl-CoA synthetases.

) The CoFS promoter fragment was fused with the *GUS* gene to construct gene expression vectors for analyzing the function of the promoter. Transgenic tobacco plants with the CoFS promoter/*GUS* fusion genes were identified by Southern blot hybridization. In the transgenic
25 plants studied, *GUS* activity was detected in anther, and weakly in ovaries, styles and stigmas, but not in petals or other tissues. This result, together with Northern blot analysis, indicates that the CoFS promoter is anther-specific in cotton. The promoter controls specific gene expression at the transcriptional level in cotton anthers. The isolated promoter may be used in improving expression of desired genes in anther and related tissues of the plant sexual organs to create new
30 plant varieties, thereby enhancing quality and yield of the plant by gene manipulation.

The promoters of the present invention are useful in creating transgenic plants, especially including cotton, having improved expression of the transgene in anther tissue. Better expression of protective genes, such as the Bt gene, in anther tissue results in a plant with increased

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resistance to Bt-sensitive pests. Genes which may be expressed under the control of this promoter include any gene suitable for the purpose.

Table I. Sequence of the CoFS Gene From Cotton (SEQ ID NO:1).

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5 CCTCACATTTAAGCGGAAAAAATATTA ACTAATTACTAATTACTAAGGTCATGGGT
  TGC GCATTAAAGTTC ACTGACGATTGTGCAAATGATGTTCCATAGAGCTTAATTGAT
  GAAATGGGAACTCATGACCCGCTTGAAGTAACTCGACTTGTAGAACTCATGAAGAA
  GCTTATCTACTTGAAGTTTTGGTAGCCCAATGAAATACTCTCGTAAATCTAGAGTTAT
  TAGTGTA AACCCCTAAAGGGATCAAATTGTATAAATTTAAATCCCTTATGACTTTCAA
10 TTGTAGATAGACTCTAATCTCGATCATGGATGTA ACTCAATCTATTTGTTGGGTTTGG
  GGTGATTACTTCAATTCATTCCATT CATAGTTGTGAATATATTTGAGAGTATTTACGC
  AAACATTTGGTGTGTGCTATTTTTCCTTTGGTCTTTTGTTCCTTCGTTGCCCATTCGTTT
  GAGTTTGCTTTTCGCTATATTTTAATGCCTTAGAAAATTTTTCGAGAAATTCTCATTTT
  GTGAGAGTTAAGCGAACTTAGAATTATTTTTTTTAAATCGCTTAAGGCTGTATGGT
15 CTGTGAGACTAAAATTCTAGTCTCGTAACACTAATACAATCACAAGTAATTTACATT
  GTTCAAGTTCTTATTCACATAAGCGGTTGGATAAAGAAAATTA AAAAAAAAAACAATC
  GGATATAATTACAAAAAATAAATTGAAATGTGCAATAATACAAATAATAATTATT
  GCTAAAGGTAAATAAAAAATGTAAATAATTATCAATGAAGTTTGAAACCTTAAATG
  GTGAAGTTTGTGTCAACTAATAGAAGAAAAAATAAATTATTTATATAACTCTACTAA
20 TGTATTATTTTATTTTGTAAAATTGATTTATTTATATTATTTCTACTAAATTGATGTGG
  AATTAGTGATATCTACTTAATTA ACTATATATAATTATAATGAATCTCCGGGACTGT
  GACTGGTCAAAGATCATAAAGTGGTATCCAATAAATTTAAATGCACTTGTA AAAATA
  TTAGACTCATGATGGCACTGAGGCGGAGGTGAAGAGGCGGCAAAGCACATGGAGA
  AGCTATATAGAAAATTCTTTCACGAAAAAGGCAACTCTTGGCTTGTGTGTTGGGAAT
25 TGTGTTAAGAACTGGATTATATAAAAACAATTATATGGGGAAAGGAAATGGTCCAC
  TGTCAATAGTTTACTATAAGCAAGTTGGAGATATAAAATTA AATATATATTTCAGTAC
  ATATACGAGTTTGAGCAACAAAATTAGAGATCTTTTTTGTCAAGTTGATATCTTCAA
  TTTTATAACGTAAATGTTCTTTTGAAGGCAACAGTAATGATATATATATATGTAGAA
  GAAATTTAACTAAAAATAGATAATTAGGCTTAATTTAATTAATTCAAGTGCAATTGT
30 TTTATCATAATATATATTACATTACAAGGCTTGAATTATTCATATTTTAAATTTATTT
  ATTAGTTAACAAAGTAATTATTGGTGCAAAAATAAATAAATTATTACCCCATTTATCT
  ATTTTCTTAAATAAAAAAATATATTATATATGCCATTTACTCTCTTTAAAAAAATTTA
  ATTTACAAATAAACTAATAAATTTGTATATGATGATTTCTGAATGAGGGTTTTAATAC

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AGTTATCATGATGATTTCAATACAATGGTTCCAAATGAATAAGGATTCCACTACAAC
ATTAAACTCACCATAATGGTGATTCCAATTGAGTGTTCTACATAATTATCATCATA
ATTCTTACTTGGCAGGATACAAAAATAATAATGGGTAAGGTAATTAATAATTA
ATAATTATCAATAAAGTTTTTATTATGGTGACAAAGTTTGATAATCATTATTAAATTA
5 TTAATTGAGTAAAATATTTAAATATAGTAATGTATATATAATGAATCTCCGGCGATG
TTGGCTGTTCAAAGATCATAAAATCCAATACATTTAAAATGCAGCTGTAAAATATGA
TGTAAGGGCGGATAAAGCACATGGAGAAGCCATATAGAAATTTCTTCCATGAAAA
AGGCGACTCTTAGCTTGTGTGTTGGGAATTGTGTTAAGTTATATGGGAAAGGAAATA
TTCCACTGTCAATAGTTTATTATAAGCAAGTGGGAGAAACAAAATTAAATATGTTAT
10 TTTGAAGGCAACAGTAATAATATAATTATAAATTAAAATAACATAATTTAGGCTTAA
TTTAATTAATGGTAAACTATAAAAAAAGTCATTTTTGTTTGCTTCAGATTACATTTT
AGTCACTTATGTTTGAAATGTTACGTTTTAGTCACTTACATTACCGTTTTGTACGAA
GTGGTCACTTTACCATTAAACTCTATTACCTCCCTAACGACAGTCCTACGTGGCAGT
CAAAATGAATTTTAAATGCTAACTTGGACGTCCAGTTGCTGGGACATTTTCCGGTTC
15 ACCTACAGCCACCTAATACTTAGGCGCTATATATTTCCCAAATTAATCTCCACATT
TCACTCCCAGAGCCCTCCATCTATGTAGAGAGCTGTAAAGAGAGAATATAAAAAAGG
GAAAGCTAGCTGAGGATCGTTTTGATTTTGGACATTTTGATGAAACGGCCTGGAAATA
ATTTTGTAATAGAGGTAGAGAAAGGTAAAGACCCCAGCGATGGACAGCCGTCGATC
GGTCCTGTTTATCGCAGTTCTTTTGCTGCTAATGGATTCCCTGCTCCGATTCTCTGGAA
20 TGGAGAGTTGCTGGGACATTTTCCGGTTCGTTTTTAGCTTTCTCTTTTTTACTTTACGC
TTCTTGCTTGGCTGCTAAGAAAATAAGGATACTAGGAGAAGATTTGACTTCTTCTAT
TCTTTGCTTTGATTTTCAAGATGGCTTTTCGCAATAGTTGCCATTTTTTTGAATTTTACT
TCCCCTTTTTTAAGTTGAGTAGATTTTTCTTCTAATTTGTTGGCTTTGTTATTTTTTAT
TTCGCGACGAGGTGGCGATGAAATCGAGAAACGTACTTTTAAGGATCCTATGAGAA
25 AGTTATATGCAAGATCGAAACGCCTAATATTTGAAACTATTGAATTTTAACGCTCAC
ACAGAGCAAGAATCGAGTTACTGGTATTTCCATTCTTATAGCTGAAAGATTGATGGC
TTTCATTCAACTCAATGTAAAACGTGAAATAAATTGTTTAATAGTAGTAATTATTTT
GGTTTTGATGCTTATGTGATGTGGAGATTAAAAATATTGCCTCCTTATAACTTAGCTGA
ACCGTAGATATGGGCTGATTGAACTTGCTAACTAACTGTATGACAGCTCCTATTAC
30 GAAAAAGTAATATTTTATTTGAGGATGATGATTTTCGATTTTCATTTTCTCCTTGCAGTA
TGTCAGTTGAGAAATATCCTGACAACCATATGCTTGGTCGCCGACAGATTGTGGATG
GGAAAGTATGTTGCTGCTGCAGTTTCCTTTTTTCTTGTTACTTTTGCCTTCTGTTTGTA
GTGCGGCCTTTGACTTTTAGTTTCATGTATCTAAATTGACATGCTTTGATTGCAGGCTG

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GAAAATACGTGTGGCAAACCTTACAGAGAAGTTTATGACATTGTAACAAAAGTTGGG
AATTCCATCCGAAGTTGTGATGTTGTGGAAGTAATGCTTTAACCTCCTTTTTTCCTTT
TAATTGTAAAATTATTGTCAATTTTTTTTATAACAAATATCCTATTTCTGGGGATCAA
TATCCACCCACAATTGATGCTAATAAAAAAAAAAATTAAGCTTTTTATTCTTGCTTA
5 CAGGGAGGAAAGTGTGGTATTTATGGTGCCAATTGCCCAGAATGGATAATTAGCAT
GGAGGTATGATCATTCTGGCATGTTTCATCTGATTTGCACAGTGGACATCCCAAGTT
ACTTAGATGTCGCTATAACTTGTTTCTTTGGATCATACTATTTGCTACCAAATTGCTT
GTTGCCCCGAAATGTTTACTAATGTTGCAAGATTGATACAGGCCTGCAACGCTCATGG
ACTCTATTGTGTTCCCTTTATATGACACTTTGGGTATTTCTTCTTGAGATCCAACAAAA
10 GCATTCTTTCAGTTTTTGGCAAACAACCTTTCTCTAACAACCATCTTATGTGTAT
TATGTACTCTTCATATAGTCTGTGATCATAATATCCTTAAACTCTTTAAATTATTCTT
GCATTCCAACGCGCCTGCTACTCTTTTTGAATGTTTGATACGTTGCTACATATTTGTA
GGTGCCGGTGCTGTGGAGTTTATCATATGCCATGCAGAAATTTCTATTGCTTTTGTAG
AGGAAAAGAAGATTAATGAGGTATGCCTGTTTACATCTATATTTGAAACCCTAGTAG
15 TGATATGGCAACTACTGGGAAAGATACTTCTAGATACTTGCAAAAATAAGATATCTA
TCTACAAAATAGATTCGATGTTTTATTTTATTATCAGCATTGCTTCTATGCTTGCTG
CTCACTTATGCATAGTTGATATTGATACAAGAATCTGTAATTTACAAATTTTCTGTT
CTTTTTTCTTTTGGGTTTCTCATGTAAGGTTTTCTTTTTCTTTTCAGCTGTTCAAAAC
ATTTCCAGCCTCAACAGAACACTTGAAAAGTAAGCTATCTGATTATTTAGGGGGGATT
20 CTTGAAATAGTACGTTACAAATTATTTATGTCCATGTATTTTGGCTATGCAGCAATTG
TTAGCTTCGGAAGGTAACACCTGAGCAGAAGGCAGAAGCTGAGAAGCATGGTTTG
AAGATATATCCTTGGGAGGAATTTTTGCAACTGGTAAGCTCTTTGCTCTGTTATTTT
CACTTCAATTTATCAGAAATAAAATTTATTCTGCTGAACTAATTGTGTCTATTTTAGC
AGACTGCCATCCTGTTAGTTACGAGTTCAGATGAAGCCATATGGCTGTAGAAAACAT
25 GCTTCTGGCAGTGCTTAATATGAACTAGGCTTCATTACATTTTCATGCATGCGCCTAT
ATCTTTTTTCTTAGCTAACCCTTATTGGATTGATGGATTTTAACCTGTAGATGACTT
ATGTTCTGATTTAAGCTCTTATGTCTGTTTCTTGCAGGGAGAAAATAAGAATTATGGT
CTTCCGGTGAAGAAGAAAACCTGATATCTGCACGATAATGTATACTAGTGGAACAAC
TGGTGATCCAAAGGGAGTATTGATTTCAAATGATAGTATTGTTACTCTTATAGCAGG
30 GGTGAAACGCCTGCTGGGGAGTGTAATGAACAGGTGACCTTTTCATTTTATTTTG
ACCATTTACCAGTCACTTGGTTTGATCTGTCGTTTCTTTTCTTTCTCCACCAAATTT
GACATGATTGTTCCCTTGTTTTCCCTTTCTCATTGTTTGTTTCTTGTAATTTACAG
TTGACTATGAAGGATGTATATATTTCTTATCTTCCTCTTGCTCATATCTTTGACCGGG

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TGATTGAGGAATTATTTATTTTCGCATGGTGCTTCAATAGGATTTTGGCGTGGGGTAA
GCATGATTAGTTAGTACTCTGACAACAAATACGGGTTCAATCAAATCAGCAAGTGCT
TATTTGTTTCATCTTCAGGATGTGAAACTATTGGTTCGAAGATATTGGAGAGCTAAAG
CCAAGTATCTTCTGTGCTGTTCTCGTGTCTTAGATAGAATTTATTCAGGTAAACTTT
5 CTTATATTCGTGGATTAGGCAATGTCATTTTTGGGTTGTTTGTGGAGGTTATACTAAG
CAACCTGGAACATGTACTAGCTGGAAAACCTGTCTTAATTTACTTATTTTTAGTATTT
ATAAATGAAACAAAACCTAGATTGATTCACTTTTCTGTAAATAACAATGAATATATAC
TCAGCTTTTTTTCAGAAGATGCATGTTCTCAGCTGTGAGATTGTCATAACCTTTGTACA
TTATCAGGTTTACTACAGAAGATTTCTTGCGGGCGGCTTATTGAAAAAGAAGATGTT
10 TGATTTAGCATACACATAGTAAGTTACTCTCATATTTTCAGTTTCTTATGTGAAGCTG
TTCATTTTATCTGCTGGCCGCCCCAAAAATATTGATTGGAAATAGAGTTAAATTGCTCT
ATTAGTTCTGCCACTGCAGACTCACCGGAGTAAAGGAAAATAAAAGATATTTGGGC
ATTCTCTAACAAGCAACAGGGTCAAAGCATATTTTTCCTTGTAGACAAATATAGAA
TTTGTTAGAGTTGTGTGACCCAAATTCTAGTTAAAAAAAAGTGGCAAGATAGGGGG
15 ATTTGTGGGGGCATCGGAGGCCCCACGGTACGGTACAGACTGCACAAGTGGAATT
CGTATAAAAGTACACTTCTTCTATTTGATATTGATTTGAATAAGGTGTTTCAACCTTA
TTGCATTGCTTCTATTAGGTTTTGATTAGAATAAGGTTTATAGGTCGTCGTCTCTCTC
TGCCCGTGGTTTTGTGTGTTATATTTTACCCTCTTTCTTTACGATTCATTGTCATTAT
CGAGGTTTGTTTTTTACAGAATTGTCTCAATCCCTTTGGGTTTATGAGCTTTTGCATT
20 AGTAGAGATCCATTTGCAGTCTGTGATTGCACTTTTCGTGAATATGTTTAACAGAGTT
ACTGAATCAGGATTACGGTTTCTTGGCTTTGATTTTACTAATATCTGACATCTGTGAT
AACCTACAGCAAATACTACAACATGAAGAAGGGCCGCAAACATGGAGAAGCATCTC
CAATTTGTGACAAAATTGTATTTAGTAAGGTGATGAAAGTCTTCATTGATACATTAT
ATGCACGAGGCTCCTTGAATATTGGCCAAAAGCCCATTAATCACATTTACCTGCAA
25 CATAATCCTCTTGACTACTCAAATCTCATGTTGAGTTGTAATTTTTCTCAGGTAAAGC
AAGGATTGGGAGGGAATGTGCGGCTTATTCTATCTGGTGCAGCACCTCTTTCAGCTC
ATGTGGAAGAGTTCTTGCGAGTTGTGGCATGTTGTCATGTTCTGCAAGGATATGGTA
TAGTTGAAGTCAGCCTTTGTGCTTGTGATAAGTTCTTTTTTCCCTTTACCAGCTGTG
CACACTGGCTGCAACATGAACATTTATTATTATGTTGATCCAAATGTAGGTCTGACG
30 GAGAGTTGTGCGGGGAGTTTTGTCTCTTTACCTAATGAATTGTCAATGCTTGGTACTG
TGGGGCCTCCCAGTACCAAACATAGATGTACGCCTGGAATCTGTTCCCGAAATGAAT
TATGATGCCCTTGCTAGCACACCACGGGGGGAAATTTGTATCAAAGGAAATACATTA
TTCTCAGGATACTACAAACGTGAAGACCTCACCCGTGAAGTATTGATTGATGGATGG

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TTCCACACAGGTCTTCCAACCTTTTGTTTCTTTTAAGGTTCTATGCATTATTAGTTTTTA
 TCTATAAGTTGAAGACCTTGAATCTTTGTGCATTAGGGGATATTGGAGAGTGGCAAC
 CTAATGGAAGCATGAAGATTATTGATCGAAAGAAGAACATTTTTAAGCTTTCACAAG
 GTGAATATGTTGCTGTTGAGAACCTGGAGAACATTTACGGTCTCGTGTGCTAGCTATTG
 5 ATTCGGTACATCTCTTATGCTCTCTTTGATACATTAACATACTGCTTCTCGGATAT
 GTAGCCATGCACTGAATGTTGGTCAAACGTAAAATTGATTTTGAAATGATTGGCAAA
 TTAACATTTTCTTTCTTATGTTACCTTATGATTGCATTCCTTTTTTAGCACTAGGTTT
 CAACCCATTGCCATTGATGGTTGCTTGATTGAACAAAAATAAACATAATAATCGAAA
 TATGCATGTCATGTTACAGTGTTTTTATCGTATCAGTTGTGTAAACATGTGTCAAAAT
 10 CCTTAAACAGAAATATGACAAATGTACTAAATATGTTAAATCATGCTTAAGCGCATC
 ATATGGTATCTAAATTTGTCATACATATATGTCATGGAAGTGATGTAAAATAAACTA
 TAGTTTATGTCAGATTTGTAATTTACTTGCTGGAGATTGGCATTCTTTTAACTTTTC
 AGTTTCATGTCCTTTATCAATTCAGATATGGATTTACGGAAACAGCTTTGAGTCGTTT
 CTTGTTGCGGTTGTAAACCCCAATAAGGAAGCACTTGAAAGCTGGGGTGCCGACAAT
 15 AACGTAAGTGGTGAAGTTCGAGTCCCTCTGTCAAACCCCAAGGCCAAAGAGTTCATA
 CTTGGGGAGCTCGCAAAGACTGGCAAAGAGAAAAAGGTTAGTTATTCATGCTTTTTG
 CCCCTTTTTTATTTTTCAAATTTATGAAATATGGGTTTATCAATTCATACTGAAAT
 ATTATAATCTTTACTCAGCTAAAAGGTTTTGAATTCTTTGGATCC

20 Table II. Sequence of the Cotton CoFS Promoter (SEQ ID NO:2).

GGGAAGCTTATCTACTTGAAGTTTTGGTAGCCCAATGAAATACTCTCGTAAATCTAG
 AGTTATTAGTGTAACCCCTAAAGGGATCAAATTGTATAAATTTAAATCCCTTATGAC
 TTTCAATTGTAGATAGACTCTAATCTCGATCATGGATGTAAGTCAATCTATTTGTTGG
 GTTTGGGGTGATTACTTCAATTCATTCCATTCATAGTTGTGAATATATTTGAGAGTAT
 25 TTACGCAAACATTTGGTGTGTGCTATTTTTCTTTGGTCTTTTGTCTTCGTTGCCCAT
 TCGTTTCGAGTTTGCTTTTCGCTATATTTTAATGCCTTAGAAAATTTTTGCGAGAATTCT
 CATTTTGTGAGAGTTAAGCGAACTTAGAATTATTTTTTTTAAAATCGCTTAAGGCTGT
 ATGGTCTGTGAGACTAAAATTCTAGTCTCGTAACACTAATACAATCACAAGTAATTT
 ACATTGTTCAAGTTCTTATTCACATAAGCGGTTGGATAAAGAAAATTAACAAAAAAC
 30 AATCGGATATAATTACAAAAAATAAATTGAAATGTGCAATAATACAAATAATAAT
 TATTGCTAAAGGTAAATAAAAAATGTAAATAATTATCAATGAAGTTTGAAACCTTAA
 ATGGTGAAGTTTGTGTCAACTAATAGAAGAAAAAATAAATTATTTATATACTCTAC
 TAATGTATTATTTATTTTGTAAAATTGATTTATTTATATTATTTCTACTAAATTGATG

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TGGAATTAGTGATATCTACTTAATTAAGTATATATAATTATAATGAATCTCCGGGAC
TGTGACTGGTCAAAGATCATAAAGTGGTATCCAATAAATTTAAAATGCACTTGTA
ATATTAGACTCATGATGGCACTGAGGCGGAGGTGAAGAGGCGGCAAAGCACATGGA
GAAGCTATATAGAAAATTCTTTCACGAAAAAGGCAACTCTTGGCTTGTGTGTTGGGA
5 ATTGTGTTAAGAACTGGATTATATAAAAAACAATTATATGGGGAAAGGAAATGGTCC
ACTGTCAATAGTTTACTATAAGCAAGTTGGAGATATAAAATTAATATATATTCAGT
ACATATACGAGTTTGAGCAACAAAATTAGAGATCTTTTTTGTCAAGTTGATATCTTC
AATTTTATAACGTAAATGTTCTTTTGAAGGCAACAGTAATGATATATATATATGTAG
AAGAAATTTAACTAAAAATAGATAATTAGGCTTAATTTAATTAATTCAAGTGCAATT
10 GTTTTATCATAATATATATTACATTACAAGGCTTGAATTATTCATATTTTAAATTTAT
TTATTAGTTAACAAAGTAATTATTGGTGCAAAAATAAATAAATTATTACCCCATAT
CTATTTTCTTAAATAAAAAAATATATTATATATGCCATTTACTCTCTTTAAAAAAATT
TAATTTACAAATAAACTAATAAATTTGTATATGATGATTTTGAATGAGGGTTTTAAT
ACAGTTATCATGATGATTTCAATACAATGGTTCCAAATGAATAAGGATTCCACTACA
15 ACATTAACTCACCATAATGGTGATTCCAATTGAGTGTTCCTACATAATTATCATCAT
AATTCTTACTTGGCAGGATACAAAAATAAATAATGGGTAAGGTAATTAATAATTA
AATAATTATCAATAAAGTTTTTATTATGGTGACAAAGTTTGATAATCATTATTAAATT
ATTAATTGAGTAAAATATTTAAATATAGTAATGTATATATAATGAATCTCCGGCGAT
GTTGGCTGTTCAAAGATCATAAAATCCAATACATTTAAAATGCAGCTGTAAAATATG
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CGGTTACCTACAGCCACCTAATACTTAGGCGCTATATATTTCCCAAATTATTCTC
CACATTTCACTCCCAGAGCCCTCCATCTATGTAGAGAGCTGTAAGAGAGAATATAAA
30 AAAGGGAAAGCTAGCTGAGGATCGTTTGATTTTGGACATTTTGGGATCC

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Table III. Sequence of a CoFS 275 bp cDNA Fragment (SEQ ID NO:3).

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 CGTGGCTACTGTGAACGAGCCGTGGCTACTGTGAACGTGCCGTGGCTACTGTGAACG
 TGCCGTGGCTACTGTGAACGAGCCGTGGTCACTGTGATACGTGCCGGGAGTTTTGTC
 5 TCTTTACCTAATGAATTGTCAATGCTTGGTACTGTGGGGCCCCATACCAAACATAGA
 TGTACGCCTGGAATCTGTTCGAAAAAAAAAAAAAACTGAATTCCGAGT

The following non-limiting examples are included to illustrate the invention.

Example 1. Isolation of an Anther-specific cDNA Fragment Encoding a CoFS Sequence

10 Expressed in Cotton Anthers

Cotton seeds were surface-sterilised with 70% ethanol for 30-60 seconds and 10% H₂O₂ for 30-60 minutes, followed by washing with sterile water. The seeds were germinated on ½ MS medium at 28 C with 16 hr lighting. Cotyledons and hypocotyls were cut from sterile seedlings as transformation explant material. Cotton plants were grown in pots for DNA and RNA
 15 extraction. Total RNA was extracted from young fibres, ovaries, anthers, petals, sepals, leaves and roots of cotton using the guanidinium thiocyanate method or SV Total RNA Isolation System (Promega). Poly (A)⁺ RNA was purified using oligo(dT)-cellulose spin columns from an mRNA purification kit (Pharmacia Biotech). Total RNAs from different tissues of cotton were used to reverse-transcribe first-strand cDNAs. These cDNAs were used as templates in
 20 differential display PCR.

Differential display analysis was carried out with the Differential Display Kit (Clontech). First-strand cDNA was synthesised with 2 µg total RNA as starting materials of reverse transcription and oligo(dT) as primers at 42 C for 1 hour. Reactions of differential display PCR were carried out with an initial cycle consisting of 94 C for 5 minutes, 40 C for 5 minutes and 68
 25 C for 5 minutes, followed by two cycles consisting of 94 C for 2 minutes and 40 C for 5 minutes and 68 C for 5 minutes, and then 25 cycles consisting of 94 C for 1 minute, 60 C for 1 minute and 68 C for 2 minutes, and a final extension at 68 C for 7 minutes. Target differential display bands were excised and re-amplified for further analysis. PCR fragments, DNA and cDNA fragments were subcloned into vectors, and plasmid DNA and phagemid DNA prepared with a
 30 Qiagen Plasmid Kit were used as templates in PCR reactions. The PCR products were sequenced by autosequencer.

Cotton cDNA was synthesised using a cDNA synthesis kit (Stratagene). Cotton cDNA libraries were constructed by inserting the cDNA fragments into the ZAP express vector

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(Stratagene). Reproducible anther- and petal-specific differential display products (see Fig. 1) were targeted for further analysis. The cDNAs in each target band were harvested and regenerated by PCR amplification. The isolated cDNAs were subsequently subcloned into a vector and sequenced.

5 To confirm which cDNA transcripts specifically accumulated in cotton anthers, cDNA expression patterns were analyzed by Northern blot hybridization with total RNA isolated from cotton fibers, ovules, anthers, petals, sepals, stems, leaves and roots, using probes from the cDNA clones. For Northern blot analysis, RNA samples from different cotton tissues were separated on agarose-formaldehyde gels, and transferred onto Hybond-N nylon membranes by
10 capillary blotting. RNA Northern blots were hybridised in ExpressHyb solution (Clontech) at 68 C with ³²P-cDNA probes prepared by random labelling (Prime-a-Gene Labelling System, Promega). After hybridisation, the blots were washed at 68 C in 0.1× SSC, 0.5% SDS for 30-60 minutes.

One clone was identified as a 275 bp CoFS cDNA fragment (see Table III). The cDNA
15 fragment was found to share 73% homology with the acyl-CoA synthetase gene (X94624) of *Brassica napus* in a region of 33 amino acids of the open reading frame. Northern blot hybridization revealed the CoFS cDNA transcripts accumulated largely in cotton anthers, and also accumulated more or less in petals, but these transcripts were not detected in RNA from fibers, ovules, stems, leaves and roots (see Fig. 2). This result shows that CoFS cDNA
20 expression is anther-specific in cotton.

Example 2. Isolation and Structure Analysis of CoFS Gene

Plant materials from cotton were prepared as in Example 1. Tobacco seeds were surface-sterilized with 70% ethanol for 30-60 seconds and 0.1% HgCl₂ for 15 minutes, followed by
25 washing with sterile water. The seeds were germinated on ½ MS medium in light at 28 C, and leaves cut from sterile seedlings were used as experimental materials.

Total DNA was extracted and purified from leaves of cotton and tobacco plants according to the following method. Leaf tissues (2-4g) were thoroughly homogenised in liquid N₂. The homogenized tissues were placed in a 50 ml tube with 20 ml ice-cold extraction buffer and
30 sedimented at 2500 rpm for 15 minutes. After removing the supernatant, each pellet was resuspended in 10 ml lysis buffer and incubated at 65 C for 30 minutes. Ten milliliters chloroform was added to each tube and mixed with the samples. The samples then were sedimented at 3500 rpm for 10 minutes. The supernatant was transferred to a clean tube, and

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chloroform extraction was repeated once more. The supernatant was transferred to a clean tube, and 0.6 volume isopropanol was added to each tube for DNA precipitation. After centrifuging at 3500 rpm for 30 minutes, the DNA was washed with 70% ethanol. The isolated genomic DNA was then dissolved in sterile water for use.

5 Cotton genomic DNA libraries were constructed from leaves of cotton plants. DNA was partially digested with *Bam*HI, and the DNA fragments were cloned in the *Bam*HI site of ZAP express vector (Stratagene). The cotton genomic DNA libraries were screened using CoFS gene fragments isolated by Genome Walk PCR as probes.

10 Genome Walker libraries were constructed using the Universal Genome Walker Kit (Clontech). Genomic DNA from leaves of cotton plants was digested with five restriction enzymes respectively, purified by phenol/chloroform extraction and precipitated in ethanol. The digested DNA was ligated to Genome Walker adaptors.

Two Genome Walker polymerase chain reactions were carried out successively: 1 µl of each Genome Walker DNA library was used as the templates in the primary PCR, and the 15 primary PCR products were used as templates in the secondary PCR. The PCR was started at 95 C for 1 minute, followed by 35 cycles of 95 C for 15 seconds and 68 C for 4 minutes and a final extension at 68 C for 6 minutes. Target PCR bands were purified using a GeneClean Kit (Bio 101).

The screens revealed two CoFS gene positive clones. One clone contained a 4.801 kb 20 cotton CoFS gene region, and the other contained a 3.913 kb cotton DNA fragment covering part of CoFS promoter region. Three CoFS promoter fragments (0.7, 1.4 and 2.6 kb, respectively) were isolated from the cotton Genome Walker libraries. The complete CoFS gene isolated from cotton was 8.4 kb in length, including a 2.6 kb promoter region. The sequences are provided in 25 Tables I and II.

Example 3. Functional analysis of CoFS promoters

To characterize the function of the CoFS promoter in anther-specific expression of the CoFS gene, a 0.7 kb fragment, a 1.4 kb fragment and a 2.5 kb fragment of the CoFS promoter 30 were fused with the *GUS* coding sequence in the gene expression vector pBI121 (replacing the CaMV35S promoter), respectively. See Fig. 3.

Vectors were constructed as follows. A *Hind*III site and a *Bam*HI site were created by PCR at the 5'-end and 3'-end of the 0.7, 1.4 and 2.4 kb CoFS promoter fragments respectively. The *Hind*III/*Bam*HI fragment was initially subcloned into pGEM-T vector (Promega). Plasmid

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DNA containing the CoFS promoter fragments was digested with *Hind* III and *Bam*HI, and the digested fragment was isolated by agarose gel electrophoresis. Three chimeric CoFS promoter/*GUS* constructs were generated by insertion of the 0.7, 1.4 or 2.4 kb fragment, respectively, replacing the CaMV 35S promoter, into the *Hind* III/*Bam*HI sites of pBI121 vector.

5 The CoFS promoter/*GUS* fusion gene constructs were used to transform tobacco by *Agrobacterium*-mediated gene transfer, using the pBI121 vector containing a CaMV35S promoter/*GUS* fusion protein as a positive control. The CaMV35S promoter is a constitutive promoter, active in all plant tissues. Odell et al., *Nature* 313:810-812, 1985; Ow et al., *Proc. Natl. Acad. Sci. USA* 84:4870-4874, 1987; McCabe and Martinell, *Biotechnol.* 11:596-598, 1993.

10 The binary vectors containing CoFS promoter/*GUS* fusion genes were transferred into *Agrobacterium tumefaciens* strain LBA 4404. Tobacco transformations were carried out using the leaf-disc method (Horsch, et al., 1985). Tobacco leaves were cut into pieces about 2×2 cm, and immersed in the *Agrobacterium* suspension for five minutes. The infected tobacco explants were cultivated on MS medium with 1 mg/L 6-BA for 48 hours at 28 C and transferred onto
15 selection MS medium containing 100 mg/L kanamycin and 1 mg/L 6-BA for 20-30 days. Kanamycin-resistant (transformed) shoots were selected. The transformed shoots were cut from the calli and rooted on MS medium with 50-100 mg/L kanamycin. The transformed tobacco plants were transplanted to soil for growing to maturity.

Transgenic tobacco plants possessing the chimeric CoFS promoter/*GUS* gene (or
20 35S/*GUS* gene), and negative control, non-transformed plants were analyzed by DNA Southern blot hybridization and by *GUS* histochemical assay. For Southern blot analysis, total genomic DNA from the transgenic tobacco leaves was digested with restriction enzymes, separated on agarose gels, and transferred onto Hybond-N nylon membranes by capillary blotting. DNA Southern blots were hybridized in ExpressHyb solution (Clontech) at 68 C with ³²P-DNA probes
25 prepared by random labelling (Promega Prime-a-Gene Labelling System). After hybridization, the blots were washed at 68 C in 0.1× SSC, 0.5% SDS for 30-60 minutes. The ³²P-labelled nylon membranes were exposed to X-ray film at -70 C for autoradiograph. See Fig. 4 for the results.

Histochemical assays for *GUS* activity in transgenic tobacco plants were conducted according to a protocol described previously, Jefferson, *Plant Mol. Biol. Rep.* 5:387-405, 1987,
30 with some modifications. Fresh tissues from the plants were incubated in X-gluc (5-bromo-4-chloro-3-indolylglucuronide) solution consisting of 0.1 M sodium phosphate (pH 7.0), 10 mM ethylene diaminetetraacetic acid (EDTA), 0.5 mM potassium ferrocyanide and 0.1% X-gluc (Clontech chemical) overnight. The stained plant materials were then cleared and fixed by

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rinsing with 100% and 70% ethanol successively, and the samples were examined and photographed directly or under a microscope. See Fig. 5.

The results of Southern blot analysis demonstrated that CoFS promoter/*GUS* gene was integrated into the tobacco genome. More than 50 tobacco transgenic plants were obtained and transplanted in soil to grow to maturation. Consistent with the results from Northern blot analysis of cotton, the *GUS* gene driven by the CoFS promoter was specifically and strongly expressed in tobacco anthers. Weak activity of *GUS* gene under CoFS promoter was also detected in ovaries, styles and stigmas, but no *GUS* activity was detected in petals or other tissues in all the transgenic tobacco plants studied. This result, together with the above Northern blot analysis, indicates that the CoFS promoter is able to control specific gene expression at the transcriptional level in plant anthers.

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CLAIMS

1. An isolated nucleic acid molecule encoding an anther-specific promoter comprising the promoter of the cotton CoFS gene, wherein said isolated nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID No:2.
2. A transgenic plant expressing a transgene under control of an anther-specific promoter comprising the promoter of the cotton CoFS gene, wherein said promoter comprises the nucleotide sequence set forth in SEQ ID No:2.

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SEQUENCE LISTING

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 Cheng, Ning H

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 in Cotton

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<213> Arabidopsis sp.

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