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Research Article

Cloning of a MADS Box Gene (*GhMADS3*) from Cotton and Analysis of Its Homeotic Role in Transgenic Tobacco

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Abstract: A MADS box gene (*GhMADS3*) was cloned from cotton (*Gossypium hirsutum* L.) based on EST sequences. The predicted protein sequence of *GhMADS3* showed 85%, 73%, and 62% identity with *Theobroma cacao TcAG*, *Antirrhinum majus FAR*, and *Arabidopsis thaliana AG*, respectively, and was grouped with *AG* homologues when the full length sequences excluding N-extensions were compared. *GhMADS3* expressed in the wild type cotton flower primarily in stamens and carpels, which was comparable to *AG* in *Arabidopsis*. However, it was not expressed in floral buds of a homeotic cotton variant *chv1*. Ectopic expression of *GhMADS3* in tobacco (*Nicotiana tabacum* L.) resulted in flowers with sepal-to-carpel and petal-to-stamen transformation. The carpelloid first whorl organs, with stigmatic tissue on their upper edges, had a white appearance when compared with the dark green color of the wild type sepals. At times, long filaments were observed at the fusion site of the first carpelloid oranges. The second whorl organs in staminoid were usually smaller than the wild type and the color was changed from pink to white. These results suggest that GhMADS3 has a homeotic role in flower development.

Keywords: MADS-box; *AG* subfamily; homeotic role; cotton; flower development

MADS box genes are defined by the highly conserved 56-amino-acids-long motif known as the MADS (*MCM1-AGAMOUS-DEFICIENS-SRF*) box and are present in animals, fungi, and plants [1]. Plant MADS box genes form a large family for transcription factors and are involved in various aspects of developmental processes, including flowering time control, floral meristem identity, floral organogenesis, fruit formation, seed pigmentation and endothelium development [2], control of root structure [3], and fruit ripening [4]. During flower development, MADS box genes exert pivotal roles: all *Arabidopsis* floral organ identity genes, except *AP2*, are MADS box genes. Mutations of these genes usually result in homeotic transformation of floral organs. Extensive researches on MADS box genes revealed that the angiosperms employ homologous genes in floral organ identity but the regulation of the floral organ identity is complex.

Cotton (*Gossypium hirsutum* L.) is the world's most important fiber crop, and its flower development is quite different from the model plant *Arabidopsis thaliana*. Cotton plants simultaneously develop both vegetative and reproductive organs. The branches on a cotton plant can be classified as either vegetative

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branches or fruiting branches. Flowers arise from fruiting branches, and once a flower forms, the initial growth of a fruiting branch is terminated[5]. The arrangement of a wild cotton flower is generally similar to *Arabidopsis*, comprising of sepals, petals, stamens, and carpels. However, at the outmost of a cotton flower, there is an extra 'bract whorl', which does not exist in *Arabidopsis*. It is certain that the study on cotton flower development is important from either theoretical or practical point of view. However, homeotic abnormalities and floral MADS box genes have rarely been reported in cotton. A stable homeotic variant, *chv1*, has been identified from somatic embryo regenerated cotton plants. Morphological analyses suggest that all floral organs of *chv1* plants are converted into bract-leaf-like organs[6]. For the purpose of studying the expression of homeotic genes in the *chv1* flowers, a number of floral MADS-box genes were cloned. The isolation and expression pattern of the *GhMADS1* have been reported previously[7]. In this article, the characteristics of the *GhMADS3* are reported.

1 Materials and Methods

1.1 Plant materials

Cotton and tobacco (*Nicotiana tabacum* L.) plants were grown under natural conditions during the growing seasons in Chongqing, China, and were transferred to a green house in winter. Two cotton cultivars, Xuzhou 142 and Chuanmian 239, and a cotton homeotic variant, *chv1*, were used for mRNA isolation preparation. Variant *chv1* was regenerated from the cell culture of Chuanmian 239, and all floral organs of this variant were transferred into bract-leaf-like organs [6]. The tobacco cultivar K326 was used for transgenic research.

1.2 Cloning and sequence analysis of the coding region of *GhMADS3*

The MADS-box region of *Arabidopsis AG* was used as the probe sequence to search the GenBank cotton EST database (database before January 2002) using the tBLASTn procedure. All the hit sequences were used for contig analysis by the Seqman II procedure (DNASTAR Inc., Madison, WI, USA). The 3′-end common sequence excluding MADS-box of each contig were used for further search with the purpose of extending 3′-end sequence. PCR was carried out for the interested contig sequences to amplify the complete ORF. For the *GhMADS3*, PCR primers were designed as: GME-up 5′-TCAAGTTAG-GAAGCATGGTG-3′ and GME-dn 5′-CCCATAAC ATTAGACTAGTGA-3′. The floral buds cDNA of Xuzhou 142 was used as the template. PCR products were cloned into the pUCm-T vector and the determination of DNA sequences was performed on the Abi Prism 3700 sequencer. Sequences analyses were carried out using the LASERGENE sequence analysis software (DNASTAR Inc., Madison, WI, USA).

1.3 Phylogenetic analysis

Full-length amino acid alignment of 21 published *AG* homologues and *GhMADS3* was performed using the ClustalX version 1.83 package [8]. ClustalX multiple alignment parameters were gap opening 8 and gap extension 2, using the Gonnet series protein weight matrix. The N-extensions present in several *AG* subfamily genes were excluded from the alignments. The phylogenetic tree was obtained by the Protdist (Dayhoff PAM model) and the Neighbor (neighbor joining method) programs of the PHYLIP package (version 3.65, provided by Felsenstein J, Department of Genome Sciences, University of Washington, Seattle. WA, USA). The tree was visualized using the TreeView package [9]. The protein sequences used in this study were retrieved from the GenBank, and their accession numbers are listed below: AG (CAA37642), CUM1 (AAC08528), CUM10 (AAC08529), FAR (CAB42988), FBP6 (CAA48635), FBP7 (CAA57311), FBP11 (CAA57445), GGM3 (CAB44449), GhMADS3 (AAL92522), MASAKO C1 (BAA90744), MASAKO D1 (BAA90743), NAG1 (AAA17033), PLE (AAB25101), PMADS3 (CAA51 417), PTAG1 (AAC06237), PATG2 (AAC06238),

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