SEQUENCE ANALYSIS OF *DET2* GENE IN DIFFERENT *GOSSYPIUM* SPECIES

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Abstract

Brassinosteroids (BRs) are a class of plant steroidal hormones that are involved in a wide variety of physiological and developmental programs. The first step in BR biosynthesis is catalyzed by a steroid 5a-reductase (*DET2*), and *DET2* gene controls the level of BR. In cotton, *DET2* gene (*GhDET2*) has been cloned in *G. hirsutum* (AADD tetraploid species). The genus *Gossypium* includes 45 diploid species and five tetraploid species. In the evolution process, many genes sequences changed. So some diploid and tetraploid *Gossypium* species were used as tested materials to study the change of *DET2* gene sequence in the cotton evolution process. Based on the full-length gene cDNA sequence of *DET2* in Genebank, we designed PCR primers to amplify *DET2* gene, and its sequences in different *Gossypium* species were analyzed. The results showed that *DET2* gene existed in all tested *Gossypium* species, and there were high similarity. DET2 gene expression level of D genomic species was significantly higher than that of the other cotton species in A, B, C genome. The results indicated that DET2 gene was not only in A, D genomic species, but also in B, C genomic species and there were minor change in the evolution process, this gene might be an indispensability gene during cotton growth and development, but its importance in different Gossypium species might be different. The research is valuable to understand the change of DET2 gene during the cotton evolution, and provides more information of DET2 gene for improving cotton cultivars.

Introduction

Brassinosteroids (BRs) are a class of plant steroidal hormones that are involved in a wide variety of physiological and developmental programs (Szekere and Koncz, 1998; Bajguz and Hayat, 2009; Wu et al., 2008; Divi and Krishna, 2009), including cell division and elongation, vascular differentiation, reproductive development, senescence, and biotic and abiotic tolerance (Ali et al., 2001; Cao and Zhao, 2007; Shu et al., 2011). More than 50 BRs have been isolated and identified from a wide variety of plant species (Bajguz and Tretyn, 2003); of these, brassinolide (BL) is the most biologically active. The first reaction towards BL formation is the conversion of campesterol (CR) to campestanol (CN). CN is converted to castasterone (CS) through either early or late C6 oxidation, and then CS is converted to BL (Fujioka and Yokota, 2003). The first step is catalyzed by a steroid 5a-reductase (Asami and Yoshida, 1999), *DET2*, which hydrogenates a (24R)-24 -methylcholest-4 -en-3-one intermediate to convert CR into CN (Li et al., 1996; Fujioka et al., 1997; Chory and Li, 1997; Li et al., 1997; Noguchi et al., 1999). *det2* (*deetiolated2*) mutant (Shu et al., 2011; Fujioka et al., 1997; Chory et al., 1991) had been identified originally in light-regulated development. When grown in the dark, *det2* exhibits inhibition of hypocotyl growth, expansion of cotyledons, development of primary leaf buds, and accumulation of anthocyanins (Chory et al., 1991). When grown in the light, *det2* has a short stature, dark-green leaves, reduced male fertility and apical dominance, and delayed senescence and flowering (Chory et al., 1991). In fact, *det2* mutant phenotypes can be rescued by application of BL, and the levels of endogenous BRs in *det2*

are below 10% of the wild type, indicating that endogenous BR levels are closely linked to the loss of activity of DET2 (Fujioka et al., 1997), *DET2* gene controls the rate-limiting step in BR biosynthesis .

DET2 gene has been identified in many plant species. In cotton, *DET2* gene (*GhDET2*) also has been cloned in upland cotton (*G. hirsutum* L., AADD tetraploid species). The original cultivation cotton (AADD complex Tetraploid species) is *G. herbaceum* L. (A1) and *G. raimondii* L. (D5) (Lui, 2007; Guo et al., 2004). In addition, the genus Gossypium includes 45 diploid species in eight genomic groups (2n = 2x = 26) and five tetraploid (2n = 4x = 52) species (Chen et al., 2007; Brubaker et al., 1999), and in the cotton evolution process, gene in the part of homologous chromosomes can change (Luo, 2007). Therefore, in order to know the genomes whether contain the *DET2* gene and analyse the difference of *DET2* gene among these genomic species, some *Gossypium* species from A, B, C, D diploid genomes and tetraploid (AADD) were selected as materials in this research. The results might be useful to understanding the change of *DET2* gene during the cotton evolution, and could provide more information of *DET2* gene for improving cotton cultivars.

Materials and Methods

Plant Materials and Growth Conditions

The selected *Gossypium* species and other information are shown in Table 1. Cotton plants were planted in a glasshouse.

Table 1. Materials used in this study.

Number.	*Gossypium* species	Chromosome No.	Genomes (Pan, 1998)
1	G. *herbaceum*	26	A1
2	G. *arboreum*	26	A2
3	G. *anomalum*	26	B1
4	G. *ausrale*	26	C3
5	G. *aridum*	26	D4
6	G. *davidsonii*	26	D3-d
7	G. *thurberi*	26	D1
8	*G. raimondii*	26	D5
9	G. *gossypioides*	26	D6
10	G. *hirsutum:yocatatum*	52	(AD)1
11	G. *tomentosum*	52	AD
12	G. *barbadense*	52	(AD)2
13	G. *hirsutum:sumian 12*	52	(AD)1

DNA and PCR

Total DNA was extracted from cotton leaf using CTAB method. PCR, using the DNA as a template, was performed using the following primers, *DET2*F: 5'-TCAGCCTTTACTTCCTCC-3' and *DET2*R: 5'-TCAGCCCTAACATTCACC-3' to amplify a 450-bp *DET2* (Luo et al., 2007; Shi et al., 2006) (GenBank No: DQ116446) fragment. PCR amplification was performed by initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturing at 94 °C for 45s, annealing at 54 °C for 45 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR product was detected by agarose gel electrophoresis.

The gene was cloned into the plant expression vector *pTG19-T*. The vector was transformed into *Escherichia coli* (*EH5α*) and then was high-throughput sequenced in Invitrogen. DNAMAN 6.0 software was used to analyze nucleotide sequence and amino acid sequence of *DET2* gene.

Isolation of RNA and Semi Quantitative RT-PCR

Total RNA was extracted from cotton leaf according the method of CTAB extraction. First-strand cDNA was synthesized by superscript II following the manufacturer's instructions (Invitrogen). PCR, using the cDNA as a template, was performed using the primers and process stated above.

A 412-bp fragment of *Histone* (GenBank No: AF024716), an internal control, was amplified by *Histone*F: 5'-GAAGCCTCATCGATACCGTC-3' and *Histone*R: 5'-CTACCACTACCATCATGGC-3'. PCR amplification was performed by initial denaturation at 94°C for 5 min followed by 28 cycles of denaturing at 94°C for 45s, annealing at 55°C for 45 s, extension at 72°C for 60 s, and a final extension at 72°C for 10 min. Each sample is repeated 3 times.

Results and Discussion

***DET2* Gene in Different *Gossypium* Species**

According to *DET2* gene sequences, a pair of specific primers (*DET2*F, *DET2*R) were designed and synthesized. The DNA from 13 tested cotton materials was used as the template to amplify the sequence respectively. The result showed that the sequence fragment existed in 13 tested materials (Fig. 1), indicating that the *DET2* gene not only existed in A and D genomic species which were the origin of tetraploid cotton, but also existed in B and C genomic species.

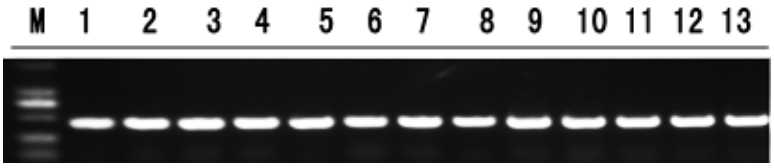

Figure 1. The result of PCR amplification of *DET2* gene.

Comparing *DET2* Gene Sequence in Different *Gossypium* Species

The amplification products were cloned into the vector *pTG19-T*. Comparing *DET2* gene sequence of the tested 13 *Gossypium* species by DNAMAN software, they had the same length fragment sequences of *DET2* gene of 430bp, and the consistency of nucleotide sequence and amino acid sequence of *DET2* gene was more than 98% (Fig. 2, Fig. 3 and Fig. 4).

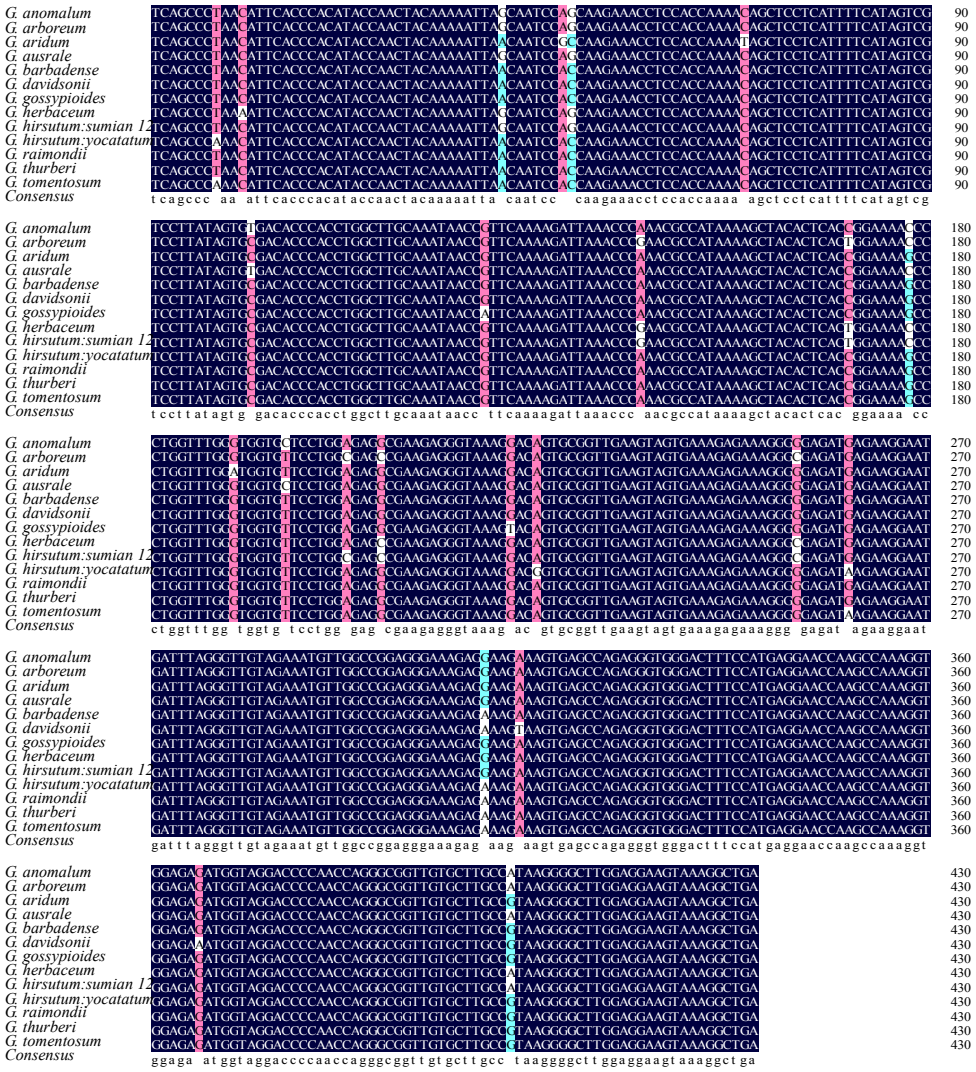

Figure 2. Sequences contrast of *DET2* gene segment from different *Gossypium* species.

DET2 gene sequence of *G. hirsutum:sumian 12* (AADD tetraploid cotton specie) was similar with A genomic species (*G. herbaceum* and *G. arboretum*), but the consistency between *G. hirsutum:sumian 12* and the other *Gossypium* species respectively were 98%. The consistency of *DET2* gene sequence among some *Gossypium* species was 100%, such as: (1) AADD tetraploid species *G. hirsutum:sumian 12* and A genomic species *G. arboreum*; (2) *G. anomalum* in B genome and *G. ausralee* in C genome; (3) D genomic species *G. davidsonii*, *G. thurberi*, *G. raimondii* and AADD tetraploid species *G. barbadense*; (4) AADD tetraploid species *G. tomentosum* and *G. hirsutum*:*yocatatum*.

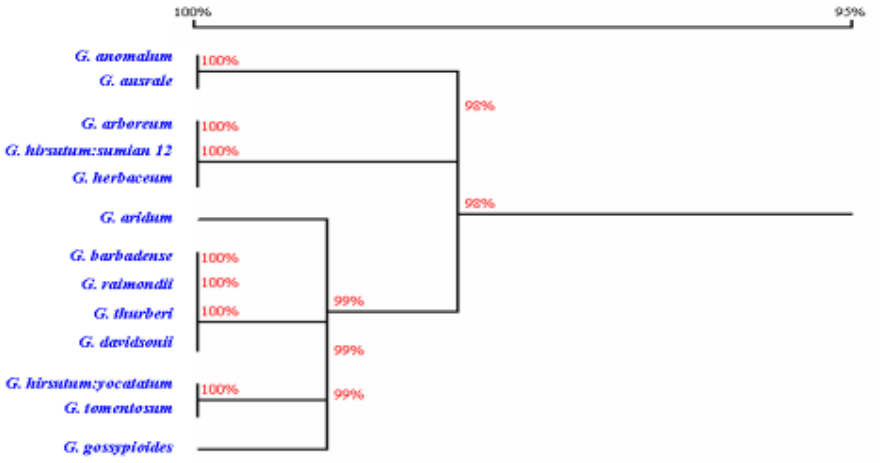

Figure 3. Phylogenetic analysis of *DET2* gene segment from different *Gossypium* species.

Comparing amino acids sequence of *DET2* in all tested *Gossypium* species found that (Fig. 4), there were 5 amino acids difference among tested *Gossypium* species, the amino acids sequence consistency of them was 99.2%. Comparing Figure 2 and 4 found that the variability of *DET2* amino acids sequence was lower than nucleotide sequence, this indicated that some nucleotide were synonymous change, they did not cause amino acid change.

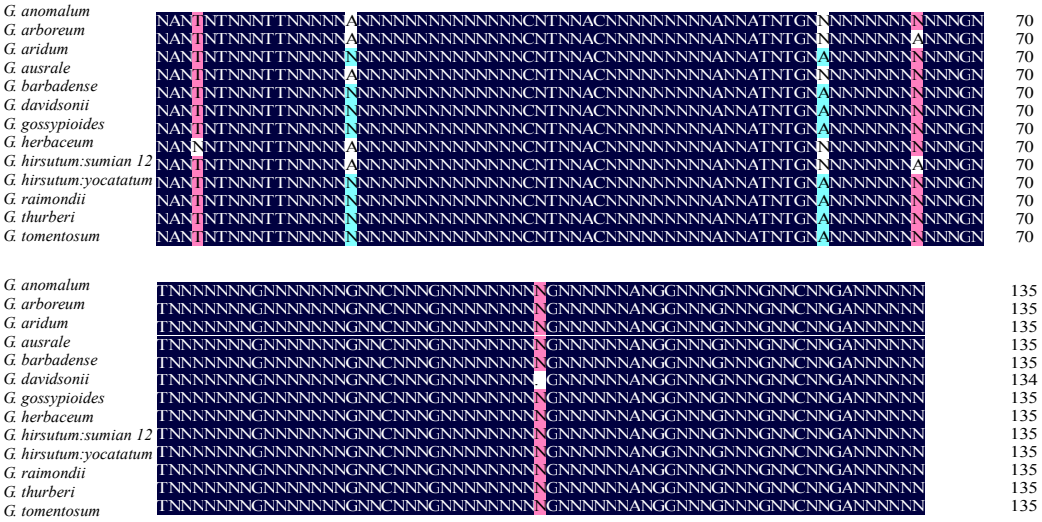

Figure 4. Amino acid sequences contrast of *DET2* from different *Gossypium* species.

There are many viewpoints about the cotton genome evolution. Some people considered that cotton originated from B genome, and some people considered that E genome was the primitive ancestors, and there were different points about the relationship near or far among these genomes (Zhou et al., 1995; Wang and Li, 1990). In this study, *DET2* gene sequences in A, B, C, D genomic species and tetraploid species were more consistent, indicating that *DET2* gene in cotton in the evolution process had minor change, the gene might be an indispensability gene during cotton growth and development. But it is difficult to find out the evolution relationship of these genomes from the *DET2* gene sequence fragments.

***DET2* Gene Expression in Different *Gossypium* Species**

Figure 5 showed the expression level of *DET2* gene in all tested *Gossypium* species, there were differences of *DET2* gene expression level among *Gossypium* species. This indicated that although all tested *Gossypium* species had *DET2* gene, but its importance in different species might be different. The *DET2* expression levels of *Gossypium* species in A, B and C genome were significantly lower than that of D genome and tetraploid species, but there were no difference among D genome and tetraploid *Gossypium* species, indicating that D genomic species which were the origin of AADD tetraploid cotton might be the donor of *DET2* to the AADD tetraploid (Luo, 2007).

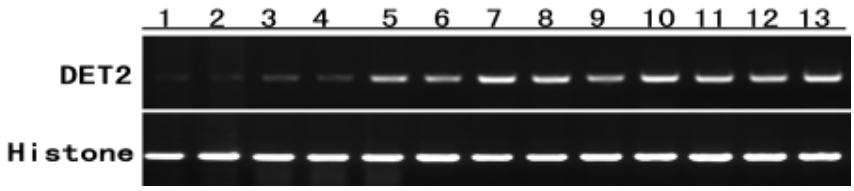

Figure 5. The expression of *DET2* gene in different *Gossypium* species.

Acknowledgments

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