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REGULAR ARTICLE

Identification of a novel drought tolerance gene in *Gossypium hirsutum* L. cv KC3

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ABSTRACT

Development of drought resistant cotton cultivars has long been a major breeding objective, since water stress limits fiber production and productivity. Recent advances in functional genomics can increase the efficiency of conventional breeding for genetic improvement of crop plants for improved abiotic stress resistance. To this end, a novel drought resistance gene was identified by comparing the gene expression profile of *Gossypium hirsutum* cv KC3 and MCU12 by employing a cDNA-RAPD approach. Physiological and biochemical studies showed that KC3 has relatively better drought tolerance than MCU12. Among 25 random primers, OPA15 has identified differentially expressed cDNA in KC3. Results of BLASTP algorithm have shown that this cDNA has significant homology with P-Glycoprotein, ACC oxidase2 and ABC transporter which are involved either directly, or indirectly, in stress tolerance in animals and plants. Hence, the cDNA sequence identified in this study may be a novel gene that confers drought resistance in cotton as KC3 is well adapted and is widely cultivated in rain-fed tracts of Tamil Nadu, India. Further characterization of this gene may show it has potential application for development of cotton with improved drought resistance, through genetic engineering and/or marker aided selection (MAS).

Key Words: cotton; *Gossypium hirsutum*; drought; cDNA-RAPD.

INTRODUCTION

Cotton (*Gossypium* spp.) is the most preferred source of natural fiber and economically important for farmers globally. In India, cotton is an important cash crop and India has the largest cotton area in the world. However, cotton production is limited by biotic and abiotic factors. Among them, drought, or water deficit, is a major abiotic stress, which limits fiber development. Yield is severely affected when drought stress occurs during the reproductive phase (Selote and Chopra, 2004). More than 60% of cotton cultivated in India is grown under rain fed conditions where water stress frequently occurs in any phase of crop development. Drought during fiber elongation period results in decreased fiber length. Water stress after fiber elongation leads to fiber immaturity and low micronaire (a measure to denote fiber fineness and maturity). Hence, development of drought resistant cotton cultivars has long been a major cotton-breeding objective. However, limited knowledge of drought resistance mechanisms and the genes governing these mechanisms has slowed progress in this direction. Drought tolerance refers to the ability of the plant to with stand the water stress by better root system development etc.

Hence, the identification and characterization of drought resistance genes, employing functional genomics tools, and understanding the water relations of cotton is of the utmost importance and this may help increase the breeding efficiency towards cotton genetic improvement for higher production and superior lint quality under water stress (Hearn, 1994). The main objective of this study was, to identify gene(s) that are specifically expressed in drought tolerant cotton.

MATERIALS AND METHODS

PLANT MATERIAL

In a previous study, *Gossypium hirsutum* L. cv KC3 was shown to have relatively better drought tolerance than *Gossypium hirsutum* L. cv MCU12. It is widely grown in the rain fed regions of Tamil Nadu, India (Boopathi et al., 2008). The two lines, KC3 and MCU12, showed significant genetic dissimilarity and were grouped into two different clusters (Boopathi et al., 2008). To identify genes that are specifically expressed in a drought tolerant cotton cultivar, *G. hirsutum* L., KC3 and MCU12, which have contrasting drought resistance traits, were selected and plants were grown in the field of the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, India. Flowers were tagged on the day of anthesis and bolls at 10 d post anthesis (dpa) were collected and dipped in liquid nitrogen and then stored at -80°C. To confirm the experimental results, three biological replications were used.

DD-RT PCR USING cDNA-RAPD APPROACH

Total RNA was extracted from 10 dpa cotton bolls using a RaFlex™ isolation kit (GeNei™, Bangalore, India) essentially following the manufacturer's protocol. The quantity of RNA was measured by a NanoDrop™ 1000 (NanoDrop Technologies, USA). The extracted RNA quality RNA was checked using 0.8% agarose gel electrophoresis. To avoid DNA contamination during cDNA conversion total RNA was treated with DNase. The total RNA (DNase treated) was reverse transcribed to cDNA using a Revert Aid H-First Strand cDNA Synthesis kit (Fermentas, USA). The first strand cDNA was used as a template for second strand synthesis.

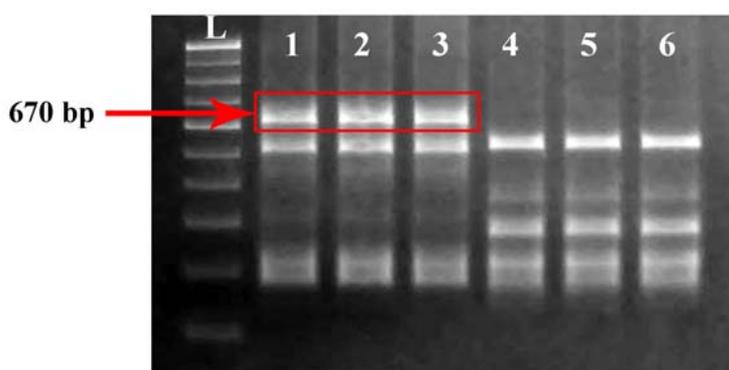
Differential Display Reverse Transcriptase Polymerase Chain Reaction (DDRT-PCR) using random primer (cDNA-RAPD) was performed in 20 µl volumes containing *Taq* DNA Polymerase 0.3 units, arbitrary 10-mer primer 0.5 µM, dNTPs 250 µM, and PCR buffer 1X, cDNA 1.0 µl in a thermocycler. After PCR amplification, products were separated by electrophoresis on a 1.5% agarose gel. The PCR fragments of the differentially expressed gene in KC3 were excised under a UV transilluminator (Fotodyne, USA) and purified using a Gel Elution Kit (GeNei™ Bangalore, India). The purified DNA product was cloned into T/A

cloning vector (InsTAclone™ PCR Cloning Kit Cat. no. #1214, Fermentas) according to the manufacturer's protocol.

The recombinant bacterial clone was sent for automated sequencing with M13 forward and reverse primers (Inst Model/ABI 13100-1699-014 at GeNei™ Bangalore, India) and the vector sequence was removed by matching the primer sequence. Removal of the vector sequence was reconfirmed by VecScreen (<http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html>). Nucleotide sequences were BLAST searched using BLASTN and BLASTP algorithms (<http://www.ncbi.nlm.nih.gov>)

RESULTS AND DISCUSSION

Among 25 arbitrary primers used in this study, the OPA15 primer produced a differential banding pattern between the two cotton lines (Figure 1)



L: 100 bp Ladder

Lanes 1,2 and 3: cDNA synthesized from KC3 RNA

Lanes 4,5 and 6: cDNA synthesized from MCU12 RNA

Figure 1. Profile of DDRT-PCR:cDNA-RAPD with OPA15 showing differential expression of a gene (670 bp) in KC3.

The differentially expressed bands found in cv KC3, which is widely cultivated in rain-fed regions of Tamil Nadu, was eluted and purified using a Gel Elution Kit. The purified DNA was cloned into a T/A cloning vector. Transformed colonies were identified based on blue/white colony selection and further confirmed by colony PCR with OPA15 primer (data not shown).

The recombinant bacterial clone was subjected to automated sequencing with M13 forward and reverse primers. Sequencing resulted in a length of 836 bp. The gene sequence was screened and confirmed by matching forward and reverse primer. Further, the contaminating vector sequence was removed using VecScreen (<http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html>)

The 670 bp processed gene sequence was BLAST searched using the BLASTN algorithm (Altschul et al., 1997). Since the results of BLASTN did not show any similarity with publicly available sequences, the identity search was also made using the BLASTP algorithm (Altschul et al., 1997). The results revealed significant matches with seven protein classes viz., P-glycoprotein (PGP), ABC transporter, ACC oxidase2, katanin, 3'(2'), 5'-bisphosphate nucleotidase, putative caffeic acid methyltransferase and class III peroxidase. The expected (E) value was taken into consideration for the appropriateness of the match. Three protein sequences viz., P-glycoprotein (PGP), ABC transporter and ACC oxidase2 had the lowest E values of 3e-05, 0.004 and 0.39, respectively with the query sequence.

The PGP is a subgroup of ATP binding cassette (ABC) family, which exists in mammals. It is composed of 1280 amino acids with 170–180 kDa and is glycosylated in human cell. Recently PGP was also discovered in plants. Twenty-one kinds of PGP (AtPGP1-AtPGP21) are distributed in different tissues of *Arabidopsis thaliana* (Blakeslee et al., 2005) and it acts as an auxin transporter. Indole-3-acetic acid was transported from shoot tip to root tip by PGP (Blakeslee et al., 2005). Recent studies have indicated that PGP1 and PGP19 mediate auxin efflux, but PGP4 has mediated auxin influx (Noh et al., 2001; Geisler et al., 2005). Fang et al. (2007) found that PGP exists in rice and was related to salt tolerance. The ABC transporter family is very large (79 members in *Escherichia coli*, 29 in *Saccharomyces cerevisiae*, and 131 in *Arabidopsis*) and its members are found in all organisms. Encoded enzymes couple ATP hydrolysis to the transport, across various membranes, of a great variety of substrates, including lipids, heavy metal ions, inorganic acids, glutathione conjugates, sugars, amino acids, peptides, secondary metabolites and xenomolecules (Rea et al., 1998). ACC oxidase catalyzes one of the rate-limiting steps for ethylene biosynthesis in plants (Wang et al., 2003). The hormone ethylene is not only responsible for initiation of fruit ripening, senescence and dormancy but also for the regulation of many other plant developmental processes such as seed germination, root initiation, growth, floral differentiation, sex differentiation and response to environment stresses. ACC oxidase expression is reproductive tissue specific and its activity was found to have a 10-15 fold greater level in immature reproductive tissues than in vegetative sepals and petals (Pogson et al., 1995). Interestingly, the cDNA, identified in this study, was isolated from 10 dpa cotton flowers. Hence, it can be speculated that the cDNA identified in this study may have a role in cotton drought tolerance as it has significant homology with the genes described above

CONCLUSION

The cDNA (GenBank Id: FJ409476) generated from this experiment may be a potential candidate gene for drought resistance improvement in cotton through genetic engineering and/or MAS. However, further study is required to completely characterize the gene (such as full length identification, influence of water stress on gene expression etc) before arriving a valid conclusion. Studies, in this direction are in progress.

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