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# DNA Sequences of RAPD Fragments in the Egyptian cotton *Gossypium barbadense*

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**Random Amplified Polymorphic DNAs (RAPDs) is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence. Despite the fact that the RAPD technique has become a very powerful tool and has found use in numerous applications, yet, the nature of molecular variation(s) uncovered by the RAPD technique is still unclear. The aim of the following study, therefore, was to investigate the molecular nature of RAPD DNA fragments in four *Gossypium barbadense* cultivars. Five RAPD DNA fragments, generated by improved RAPD-PCR technique, and representing polymorphic and nonpolymorphic bands were analyzed at the molecular level using DNA sequence analysis. Nonpolymorphic RAPD DNA fragments showed homologies to previously characterized plant structural genes. Comparative nucleotide sequence analysis of two comigrating nonpolymorphic fragments revealed that these two DNA sequences are highly similar to each other, indicating that similarity of fragment size is a good predicator of homology. Polymorphic RAPD DNA fragments, on the other hand, showed homologies to middle and high-repetitive DNA sequences. These results promote the initiative to integrate these RAPD markers in cotton breeding applications, and DNA fingerprinting.**

**Key words:** *Gossypium*, MITEs, RAPD-PCR, repetitive DNA, sequence similarity, retrotransposons.

## INTRODUCTION

Random Amplified Polymorphic DNAs (RAPDs) is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence (Williams et al., 1990; Welsh and McClelland, 1990). RAPDs produce DNA profiles of varying complexity, depending on the primer and template used. Each amplification product is expected to result from the existence of two annealing sites in inverted orientations, 3` ends facing each other, within amplifiable distance (Williams et al., 1990). Polymorphisms could be caused by differences in nucleotide sequences at the priming sites (such as point mutations), or by structural rearrangements within the amplified sequence, (e.g., insertions, deletions, inversions) (Welsh and McClelland, 1990). RAPD analysis has been used in many applications and various organisms, especially in the plant sciences (Caetano-Anolles, 1994; Sharma and Mohapatra, 1996).

RAPDs has great advantages, since no prior knowledge of the target sequences is required for the design of primers, requires only nanogram amount of DNA, results are directly read from agarose gels, and the entire genome is screened (Williams et al., 1990). However, the nature of DNA sequences involved in RAPD fragments is little known, as many different kinds of sequences are probably involved (Schierwater, 1995). Indeed, in those studies where this question has been addressed, repetitive DNA has often been detected (Paran and Michelmore, 1993; Kostia et al., 1996), which may limit the applicability of RAPDs (Hopkins and Hilton, 2001). The purpose of this work is to report on the DNA sequences of RAPD fragments in the Egyptian cotton, *Gossypium barbadense*. Cotton (*Gossypium* spp.) is the world's leading fiber crop and the second most valuable oilseed. The economic impact of commercial cotton production is significant, and the value of the crop is roughly $20 billion dollars annually (Anonymous, 1997). While four *Gossypium* species are cultivated, *G. barbadense* cottons (Sea Island, Egyptian, and Pima cottons) are particularly prized for their superior fiber

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**Abbreviations:** MITEs, miniature inverted-repeat transposable elements; PCR, polymerase chain reaction; RAPDs, random amplified polymorphic DNAs.

quality and command a premium price that often compensates growers for the lower yields obtained relative to *G. hirsutum* cultivars (Wendel and Cronn, 2002) .

## MATERIALS AND METHODS

### DNA extraction

Total DNA was extracted from four *Gossypium barbadense* cultivars, Giza 45, 67, 70 and 75 using Qiagen DNeasy kit (Qiagen, Germany).

### RAPD-PCR

RAPD was carried out as described previously (Williams et al., 1990). Briefly, RAPD products were generated by two random primers A9B7, GGTGACGCAGGGGTAACGCC, and A1A13, CAGGCCCTTCCAGCACCCAC, from four *G. barbadense* cultivars. The RAPD-PCR was carried out in an ABI GeneAmp PCR system 9700 cycler and in 25 ul and using the following program: 94$^{o}$C for 5 min, and 45 cycles each with denaturing at 94$^{o}$C for 30s, annealing at 55$^{o}$C for 30s and extension at 72 $^{o}$C for 30s; and final extension at 72$^{o}$C for 10 min. Amplification products were then electrophoresed in 2% agarose gels and visualized by ultraviolet illumination after staining with ethidium bromide.

### Sequencing of RAPD fragments

RAPD fragments were excised from the agarose gel and purified using Qiagen Gel Extraction kit (Qiagen, Germany). Purified RAPD DNA fragments were then cloned in pCR 4-TOPO vector with TOPO TA cloning kit (Invitrogen, USA) in the competent *E. coli* strain TOPO 10. Plasmid DNA was isolated using QIA Spin mini-prep kit (Qiagen, Germany). Plasmid DNA was sequenced in both directions using BigDye Sequencing Kit and ABI 3700 DNA sequencer (ABI, USA). The multiple DNA sequence alignment was carried out using CLUSTALW (http://www2.ebi.ac.uk/clustalw; Thompson et al., 1994). Sequences from the *G. barbadense* RAPD fragments were compared with EMBL and EBML plant database information using FASTA algorithms (Pearson and Lipman, 1988).

## RESULTS AND DISCUSSION

Despite the fact that the RAPD technique has become a very powerful tool and has found use in numerous applications, yet, the nature of molecular variation(s) uncovered by the RAPD technique is still unclear (Black, 1993; Perez et al., 1998). In this regard, we have investigated the molecular nature of RAPD DNA fragments in four *G. barbadense* cultivars using improved RAPD-PCR technique. The technique involved the use of 20-mer random primer and higher annealing temperature, as these two parameters have been previously reported to ensure RAPD reproducibility, reduce nonspecific amplification products, and maximize signal detection (Benter et al., 1995; Kristensen et al., 2001). The cotton RAPD amplification products, generated by primer A9B7 and A1A13, can be classified into two groups: variable (polymorphic) and constant (nonpolymorphic) (Figures 1 and 2). Constant fragments are diagnostic for a genus, and operationally identify members of a certain genus exclusively if the fragment is a unique polymorphism in a comparison of genera, genus-specific band or character (Williams et al., 1990). Similarly, fragments polymorphic at the species level will operationally identify members of a given species if the fragment is constant among all members of the species, species-specific band or character (Welsh and McClelland, 1990). This is evident in Figure 1, as the specificity of the RAPD amplification products range from the cultivar variation level to higher taxonomic levels.

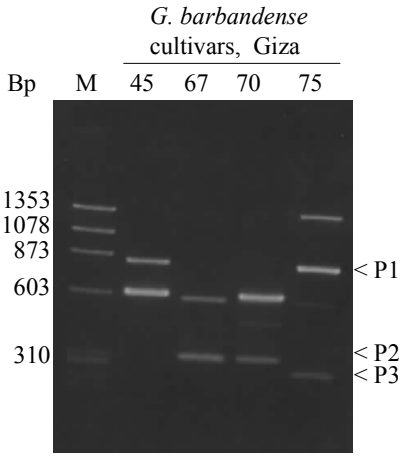


**Figure 1.** RAPD products from four *G. barbadense* cultivars generated by random primer A9B7. Sequenced fragments are marked by arrows.

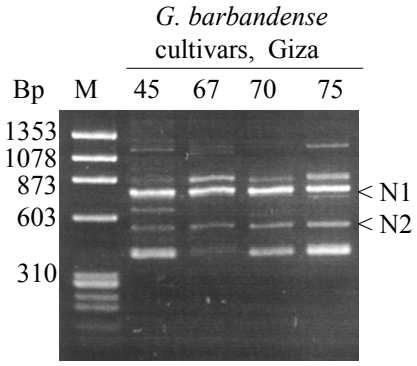


**Figure 2.** RAPD products from four *G. barbadense* cultivars generated by random primer A1A13. Sequenced fragments are marked by arrows.

Five RAPD DNA fragments representing polymorphic and nonpolymorphic bands were further investigated at the molecular level using DNA sequence analysis. These bands were designated according to their molecular

nature as detected on the agarose gel, either polymorphic (P) or nonpolymorphic (N). Complete DNA sequence of the aforementioned RAPD fragments was determined and deposited in the NCBI nucleotide sequence database, GenBank (Table 1).

**Table 1.** Molecular characterization of *G. barbadense* RAPD DNA fragments and their GenBank accession numbers. Clones N1 and N2 were generated by primers A1/A13, while P1, P2 and P3 by primers A9/B7.

| Clone | Molecular weight (bp) | Accession number |
|---|---|---|
| N1 | 710 | AY082383 |
| N2 | 552 | AY082384 |
| P1 | 742 | AY082385 |
| P2 | 316 | AY082386 |
| P3 | 220 | AY082387 |

Analysis with the FASTA software using EMBL and EMBL plant database information revealed that the nonpolymorphic RAPD DNA fragment (N1) produced high DNA sequence homology of 74.2% identity with upland cotton, *G. hirsutum,* clones associated with fiber development (GenBank accession number BH021873 ).

DNA sequence of N2 has 73.6% DNA sequence identity to rice (*Oryza sativa*) putative serine/threonine kinase-like protein, (GenBank accession number AP003334).

Polymorphic RAPD DNA fragment (P1) showed a 79.1% identity to rice low-complexity GC-rich region (accession AC090482). This result suggests that the DNA sequence of (P1) represents a novel class of repetitive DNA, not previously characterized nor sequenced in *Gossypium* (Zhao et al., 1998).

Similarly, polymorphic fragment (P2) showed sequence identity to another class of repetitive DNA known as T*y*1-*copia* retrotransposons (Malik et al., 2000). P2 shared a 97% identity to sugar beet, *Beta nana*, T*y*1-*copia* retrotransposons (AJ48201). It is noteworthy that T*y*1/*copia*-like retrotransposons were previously identified in Upland cotton, *G. hirsutum* (Vanderwiel et al., 1993), and the Egyptian cotton (Zaki, 1996).

RAPD DNA fragment (P3) showed a 68.6% sequence identity, in 83 bp overlap, to rice putative miniature inverted-repeat transposable elements MITE-adh, type D-like (accession AC113338). MITEs represent another class of plant repeated DNA sequences (Freschotte et al., 2002). Based on our current knowledge, this is the first reported MITEs-like sequence in *Gossypium*.

The use of RAPDs for comparative purposes relies on the assumption that similarity of fragment size is a dependable indicator of homology (Rieseberg, 1996). To test the validity of this assumption, homology of the nonpolymorphic RAPD DNA fragment (N1) between two different *G. barbadense* cultivars, Giza 45 and 67, was determined and further investigated by DNA sequence alignment. These clones were designated G45 and G67 respectively. Comparative nucleotide sequence analysis of G45 and G67 using the ClustalW program revealed that these two DNA sequences are highly similar to each other, producing an alignment score of 98.5 (Figure 3). The high degree of similarity indicates that similarity of fragment size is a good predicator of homology, at least among closely related populations or species. It is noteworthy that aforementioned degree of homology reported in this study is higher than the previously reported study by Rieseberg (1996), who scored 91% homology of comigrating RAPD fragments in sun flower plants. The higher percentage of homology reported in our study could be due to two imperative reasons. First, the modified improved RAPD-PCR with the use of 20-mer random primer and higher annealing temperature, significantly increase RAPD reproducibility, and thus higher homology. Secondly, in our study, homology was investigated by DNA sequence analysis, while Rieseberg (1996) employed Southern blot and restriction analysis. It is known that DNA sequence analysis provides the most specific and sensitive method for detecting homology (Franca et al., 2002).

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G45 CCGCGAGGGCATAAGTTCTCTCTTGCCTTATCTATAGATAAGAACGAATCTCCTCGACTC 60
G67 CCGCGAGGGCATGAGTTCTCTCTTGCCTTATCTATAGATAAGAACGAATCGCCTCGACTC 60
    ************ ************************************* *********

G45 GACGGATATGATGGATGGAATGGGTAGAAGGTTTAGGTTATTGTGTGTTGATTTAGTTAG 120
G67 GACGGATATGATGGATGGAATGGGTAGAAGGTTTAGGTTAGTGTGTGTTGATTTAGTTAG 120
    ************************************* ******************

G45 GACTTTTTGTCTCCCTTTCGTTATCTTCCGCCCCGGGGGGATGACCTGTGGGAAGCGAAA 180
G67 GACTTTTTGTCTCCCTTTCGTTATCTTCCGCGCCGGGGGGATGACCTGTGGGAAGCGAAA 180
    ******************************* ***************************

G45 ATTAAGGGCGAATTCGTTTAAACCTGCAGGACTAGTCCCTTTAGTGAGGGTTAATTCTGA 240
G67 ATTAAGGGCGAATTCGTTTAAAGCTGCAGGACTAGTCCCTTTAGTGAGGGTTAATTCTGA 240
    ********************** ************************************

G45 GCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTC 300
G67 GCTTGGCGTAATCTTGGTCATAGCTGTTTTCTGTGTGAAAATTGTTATCCGCTCACAATTC 300
    ************* *************** ****************************

G45 CACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCT 360
G67 CACACAACATACGAGCCGGGAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCT 360
    ******************* ****************************************

G45 AACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCC 420
G67 AACTCACATAAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCG 420
    ********* **************************************************

G45 AGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTT 480
G67 AGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTT 480
    ************************************************************

G45 CCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAG 540
G67 CCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAG 540
    ************************************************************

G45 CTCACTCAAAGG 552
G67 CTCACTCAAAGG 552
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**Figure 3.** Comparative DNA sequence analysis of nonpolymorphic RAPD (N1) for *G. barbandense* G45 and G67 using CLUSTALW.

Repetitive DNA has often been detected in RAPD fragments (Paran and Michelmore, 1993; Kostia et al., 1996). In this regard, our results showed that two of the three (66%) cotton polymorphic RAPD DNA fragments represent highly repetitive DNA sequences, while the remaining one represents middle-repetitive DNA sequences. This is in accordance with the result of Williams et al. (1990), who used 11 RAPD fragments as hybridization probes on Southern blots of digested genomic DNA from soybean and found that seven of them (63.6%) hybridized to highly repetitive, and the remaining four hybridized to middle-repetitive DNA.

RAPD markers are well suited for plant breeding applications and DNA fingerprinting (Williams et al., 1990). The current report clearly demonstrates the powerful potential for using RAPD markers in cotton improvement. RAPD has been successfully used in identification and differentiation between different cotton cultivars. In addition, molecular characterization of one of cotton's RAPD DNA fragments revealed that this clone is similar to clones responsible for fiber development in cotton. These results have promoted the initiative to integrate these RAPD markers and DNA fingerprinting in cotton breeding applications. Currently, specific primers are being designed for these RAPD markers to employ them for cotton improvement, through direct manipulation of genes, which can produce improvements not possible through classical breeding programs.

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