STS MARKERS CO-SEGREGATE WITH COTTON CYTOPLASMIC MALE STERILITY RESTORER GENE RF1

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RESEARCH PROBLEM

Marker-assisted selection (MAS) can be used with high efficiency for indirect selection of both qualitative and quantitative traits by the selection of molecular markers that are tightly linked with the genes controlling the aim traits (Mohan et al., 1997). However, such markers as RFLP, AFLP, and RAPD have disadvantages in MAS because of expense or accuracy. A reliable but inexpensive molecular marking system is needed to aid in the breeding of restorer parental lines for hybrid seed production in cotton.

BACKGROUND INFORMATION

In several crops, restorer-of-fertility genes have been tagged with different kinds of molecular markers such as RFLP, AFLP, and RAPD. RFLP and AFLP techniques are expensive and time- and labor-consuming, thus they not suitable for marker-assisted selection in plant breeding programs. The RAPD method is quick and simple but prone to errors. Sequence-tagged site (STS) markers avoid the disadvantages of other markers in that they allow the use of the Polymerase Chain Reaction (PCR) with a specific primer that yields a single marker associated with the trait in question. Because of this, RFLP, AFLP, and RAPD markers often have been converted into STS markers for MAS. We have found RAPD markers associated with a cotton cytoplasmic male sterility restorer gene, Rf_1, located 4.5cM and 2.7 cM away from Rf_1, and three markers that co-segregate with Rf_1. Conversion of the latter three RAPD markers to STS markers will increase their usefulness in development of male parental lines for hybrid production. Also, they should allow detection of other potential resources of restorer genes from different cotton species.

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RESEARCH DESCRIPTION

PCR bands (RAPDs) that co-segregated with the cotton Rf_1 gene in a testcross population were retrieved from an agarose gel, cloned in a pGEM-T vector, and then transformed into *E. coli* strain JM109. Cloned DNA fragments were sequenced with a Perkin Elmer ABI Prism 377 DNA sequencer in the Core Molecular Biology Laboratory, Dale Bumpers College of Agricultural, Food and Life Sciences, University of Arkansas. STS primers 18 to 21 nucleotide bases in length were designed from the RAPD fragments using Stanford Web Primer software, and the oligonucleotides were synthesized commercially. The primers were tested in PCR using DNA from the original segregating testcross population (B416R X Ark8518) X Ark8518 to determine the genetic distance between the STS markers and the Rf_1 gene. PCR products were electrophoresed on 1% agarose gel to confirm the uniqueness and size of the STS markers.

The specific primers were also used in PCR reactions using DNA from different *Gossypium* species or hybrids (Table 1) to explore for other potential restorer-gene sources.

RESULTS

Sequence analysis and development of sequence tagged site (STS) markers

The 3 RAPD fragments linked with cotton CMS restorer gene Rf_1, following cloning and sequencing, were found to be 1346bp, 726bp, and 500bp in length. Forward and reverse STS primers were designed that gave fragment lengths of 1343bp, 717bp, and 475bp, respectively.

These STS primer pairs were used to test the original segregating testcross population. STS_{1346} primers amplified a PCR fragment from each fertile plant, but no product from sterile plants. STS_{475} primers amplified one fragment from fertile plants, but no band from sterile plants. The STS_{717} primer pair amplified one fragment from each sterile plant but two fragments from fertile plants, one of which was the specific Rf_1-associated fragment. The two fragments had slight differences in length (Fig. 1). Subsequent sequence analysis showed that these two bands were homologues from the D and A subgenomes. All specific STS markers were located at the same chromosomal positions as the original RAPD markers.

STS Markers in Cotton Species and Some Interspecies Progeny

When PCR was used to amplify DNA from other *Gossypium* species using the STS primer pairs, the STS_{1346} primer pair amplified the same size fragment from ($D_{2\text{-}1}xAD_4$), $D_{2\text{-}2}$, D_4, (D_5xAD_4), D_9, and ($D_{10}xAD_1$) as the restorer line, a larger fragment from $2(A_2xD_1)$ and D_8, but no fragment from other species. The STS_{475} primers amplified the specific fragment from $2(A_2xD_1)$, $D_{2\text{-}2}$, (D_5xAD_4), D_8, D_9, and ($D_{10}xAD_1$), a

larger fragment from D_4, and no fragment from other species. The STS_{726} primers amplified the specific band from C_1, $D_{2\text{-}2}$, D_8 and a smaller fragment from other species.

Among the species or hybrids examined, *G. harknessii* contained all three STS markers found in the fertile plants of the population. *G. trilobum* also contained three STS fragments, two that corresponded to the fragment in fertile, restored plants, but the fragment amplified by STS_{1346} was larger than that from fertile plants. (D_5xAD_4), D_9, (D_{10}xAD_1) and (A_2xD_1) each contained two specific fragments, whereas ($D_{2\text{-}1}$xAD_4) and D_4 contained only one specific band (Fig. 2).

PRACTICAL APPLICATION

For breeding restorer lines, the fertility restoration ability of each plant must be tested by hybridizing these plants as pollen source with CMS lines, a process that is expensive, laborious, and time-consuming. The STS markers developed in this study can be used to select fertile plants from a segregating population in the seedling stage. Plants with Rf_1-associated molecular markers can then be used for backcross or forward breeding without testing for restoration in each generation. These three markers will be useful to accelerate the transfer of the restorer gene to elite male parental lines. Zhang and Stewart (2001) found a RAPD marker that was 1.8cM from the male fertility restorer gene Rf_2. Thus, it is possible to pyramid two restorer genes, Rf_1 and Rf_2, into a single elite parental line through the indirect selection with these molecular markers. Moreover, these STS fragments may be used as landmarks to screen a *G. harknessii* genomic library for clones that may contain the specific Rf_1 gene. The full length of the Rf_1 gene could then be obtained through the technique of "chromosome walking."

ACKNOWLEDGMENTS

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LITERATURE CITED

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Table 1. *Gossypium* species or hybrids used to screen for potential cotton CMS restorer genes with STS primers.

Species or crosses	Genome	Species or crosses	Genome
G. herbaceum	A_2	(*G. tuneri* x *G. hirsutum*)	D_{10}X AD_1
2(*G. arboreum* x *G. thurberi*)	A_2X D_1	*G. stocksii*	E_1
G. anomalum	B_1	*G. longicalyx*	F_1
G. capitis-viridis	B_3	*G. nelsonii*	G
G. sturtianum	C_1	*G. bickii*	G_1
(*G. armourianum* x *G. hirsutium*)	$D_{2\text{-}1}$x AD_1	*G. nobile*	K
G. harknessii	$D_{2\text{-}2}$	*G. pulchellum*	K
G. davidsonii	$D_{3\text{-}d}$	*G. hirsutum* (TM-1)	AD_1
G. aridum	D_4	*G. barbadense* (57-4)	AD_2
(*G. raimondii* x *G. mustelinum*)	D5xAD4	*G. tomentosum*	AD_3
G. gossypioides	D_6	*G. mustelinum*	AD_4
G. trilobum	D_8	*G. darwinii*	AD_5
G. laxum	D_9		

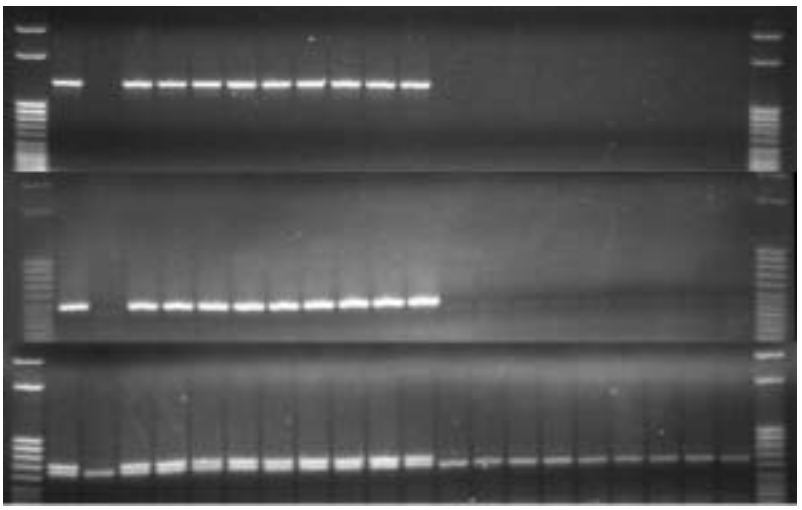

Fig. 1. Profiles of STS markers associated with cotton CMS restorer gene *Rf_1*. a. STS_{1343}; b. STS_{475}; c. STS_{717}. Lanes 1 and 22: 100 bp molecular weight marker (Promega); Lane 2: *Rf_1* bulked; Lane3: *rf_1* bulked; Lane 4-12: fertile plants; Lane 13-21: sterile plants.

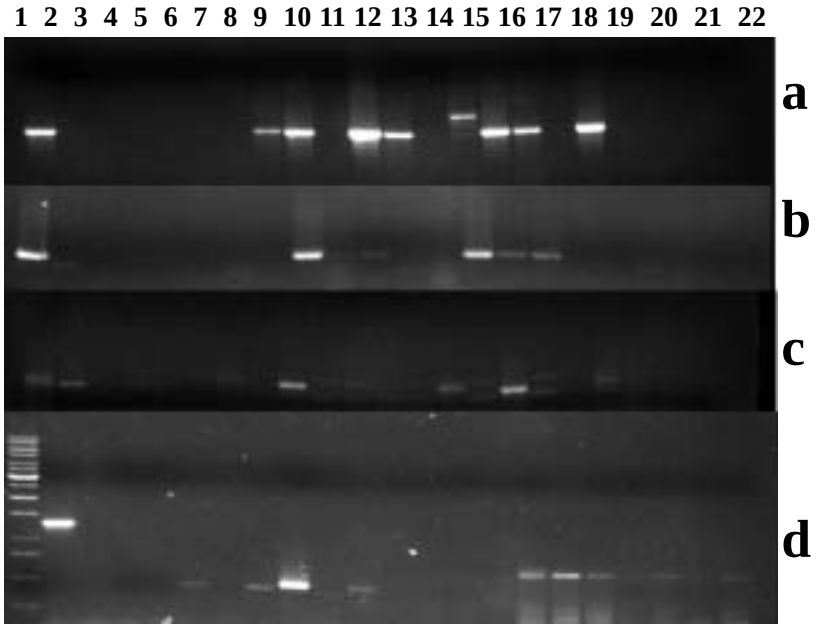

Fig. 2. Profile of fragments from different species or hybrids following PCR with STS primers. a. STS_{1375}; b. STS_{475}; c. STS_{717}. Lane1 to 22 (left to right): Rf_1, rf_1, A_1, $2(A_2XD_1)$, (B_1XA_1), B_3, C_1, $(D_{2\text{-}1}XAD_4)$, $D_{2\text{-}2}$, $D_{3\text{-}d}$, D_4, $(D_5x\ AD_4)$, D_6, D_8, D_9, $(D_{10}XAD_1)$, E_1, F_1, G, G_1, K, K. d. Lane1 Marker, then profiles of STS amplified by 3 pairs of STS primers each in turn on fertile bulked DNA (lanes 2, 9 and 16), sterile bulked DNA, AD_1 through AD_5.