MOLECULAR MAPPING OF CMS-D8 RESTORATION AND GENE EXPRESSION SPECIFIC TO D8 RESTORATION

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Abstract

CMS-D8 was developed by transferring the D8 cytoplasm from the American diploid wild species, *Gossypium trilobum* (D_8 genome) into cultivated tetraploid cottons (*G. hirsutum* and *G. barbadense*). Male fertility can be recovered by two independent dominant restorer genes; Rf_1 from the D2 restorer and Rf_2 from the D8 restorer. Identification of molecular markers closely linked to the restorer genes could facilitate the development of hybrid cotton.

Bulked segregant analysis was used to tag RAPD markers that were linked to the restorer genes. Three test crosses were used for mapping Rf_2 and one for Rf_1. Two RAPD markers, UBC 111_{3000} and UBC 188_{500}, were found to be associated with Rf_2 in coupling phase. UBC 188_{500} was closely linked to Rf_2 with an average genetic distance of 2.9 cM, although the distance varied from 0 to 5.1%, depending on the segregating population. A survey for UBC 188_{500} in several *Gossypium* species and cotton cultivars revealed that this RAPD marker was absent in normal cotton cultivars. However, it was present in the D8 restorer line of *G. hirsutum*, and in *G. raimondii* (D_5), *G. trilobum* (D_8) and several wild species of Australia. This indicates that Rf_2 and the DNA corresponding to the RAPD marker were both introgressed into the tetraploid cotton nuclear background from the D_8 genome. A RAPD marker, UBC 169_{Rf}, was found to cosegregate with Rf_1. The two RAPD markers, UBC 188_{500} and UBC $169_{Rf,}$ were population specific. Since Rf_1 and Rf_2 were tightly linked (in repulsion) with a genetic distance of 1 cM, a linkage group comprising Rf_1, Rf_2 and three RAPD markers was established. Construction of a high-resolution linkage map containing Rf_1 and Rf_2 is underway.

Differential display of mRNA was employed to compare transcript patterns between 8518, a normal upland cotton, and isogenic line 8518R that had D8 cytoplasm and the D8 restorer gene, Rf_2. 8518R with D8 cytoplasm was developed by repeatedly backcrossing the original D8 restorer, D8R, with 8518 as the recurrent parent. For comparison, two *G. barbadense* lines, 57-4 and Pima S-1, and D8R were included in the study. Anthers from one day preanthesis buds of the five genotypes were harvested and stored in liquid nitrogen prior to RNA extraction. Differential display of cDNA transcripts was performed using 12 anchor primers (9 dT plus 3'MN) and six decamer primers. Bands differential displayed following electrophoresis were excised from the gels, reamplified, cloned and sequenced. Thus far, 26 DNA sequences have been analyzed and compared with sequence databases using the BLAST program. Ten sequenced cDNA fragments were present only in the two restorer lines, 8518R and D8R, and absent in the three normal cottons, 8518, 57-4 and Pima S-1, while 8 sequenced fragments were present only in the three normal cottons. Four fragments were absent in D8R but present in the other four lines and 2 fragments were present only in D8R. One fragment was present and another absent only in 8518. Seventeen cDNA fragments were highly homologous to known sequences in GenBank, for example, cysteine proteinase, phosphoribosylanthranilate transferase, pectinesterase, starch synthase, nuclear transport factor, 4-coumarate-CoA ligase, aminopeptidase, ankyrin-like protein, eIF-4, and calnexin. The functions of these genes as they relate to male fertility restoration in cms-D8 system require further investigation.

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