ISOLATION OF GENE TRANSCRIPTS WITH APPARENT SPECIFICITY TO CMS-D8 RESTORATION IN COTTON BY MRNA DIFFERENTIAL DISPLAY

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Abstract

Cytoplasmic male sterility (CMS), is a maternally inherited trait that inhibits production of viable pollen. It has been found in more than 150 plant species. CMS has been extensively utilized for hybrid production in major crops, e.g. maize, sorghum, rice, rapeseed, etc. However, even though the first CMS system, harknessii CMS (CMS-D2) was released in the early 1970s, no commercial cotton hybrids were produced by this CMS system in the US. Therefore, a new CMS system is needed.

Most CMS types have occurred naturally or in intraspecific crosses, while other CMS have also been induced by interspecific cytoplasm transfer. CMS-D8 was developed by introducing the cytoplasm from *G. trilobum* (D_8 genome) into cotton. D_8 was used as female to cross with cotton, and then the hybrid chromosome number doubled with colchicine. The resulting hexaploid line was recurrently backcrossed as female with cotton. From a segregating BC_5 population sterile plants were isolated as the CMS line and the restorer line was derived from fertile plants.

Compared with the fertile flowers of normal cotton, CMS-D8 sterile flowers are smaller with shorter filament, no pollen, and much smaller anther. When CMS-D8 is crossed with its D8 restorer line, F_1 plants are fertile with normal flower phenotype. But, the heterozygous F_1 plants produce two types of pollen grains in roughly equal numbers: one type stains with I_2-KI and the other does not stain, indicating that the latter has no starch accumulation, and are thus sterile. Therefore, the restoration of fertility is apparently a gametophytic system. The segregation ratio of the two pollen types also confirmed that the restoration to CMS-D8 by the D8 restorer is controlled by one gene (rf_2). In order to obtain insight into the mechanisms of CMS-D8 male sterility and its restoration, differential gene expression in anther tissues between a heterozygous D8 restorer line (8518R) and its isogenic non-restoring line (ARK 8518) were compared by using mRNA differential display techniques. Approximately 3000 cDNA fragments were assayed that represented ca. 10-20% of the genes expressed in the anther tissues. Among 100 differentially displayed cDNA bands, 38 were cloned, sequenced, and differential expression confirmed by reverse Northern blot analysis.

In the heterozygous D8 restored line, five up-regulated genes and 12 down-regulated genes were detected. The DNA sequences of the up-regulated genes did not show high homology to any known sequences in the GenBank. The down-regulated genes that were highly homologous to known sequences were: phosphoribosylanthranilate transferase for tryptophan synthesis, starch synthase for starch synthesis, calnexin for protein maturation, polyubiquitin for protein targeting for degradation, and ascorbate oxidase for pollen germination. Based on the above results, a picture regarding the D8 CMS and its restoration can be drawn as follows. In the heterozygous restored F_1 plants, the expression of the restorer gene (rf_2) suppresses the D8 cytoplasm effect, most likely its CMS-related gene expression, so that normal microsporogenesis and microspore development occurs. However, during microspore maturation, the microspores with the Rf_2 gene go through the first mitosis and starch accumulation, and develop into fertile pollen grains. On the contrary, the genes for amino acid, protein and starch synthesis, protein maturation and targeting for degradation, and pollen maturation are suppressed in the microspores with the non-restoring allele (rf_2). Consequently, the rf_2 pollen has no starch deposition and is sterile.

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