FERTILITY RESTORATION OF CMS-D8 IN COTTON: ALLELISM AND MOLECULAR MECHANISM

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RESEARCH PROBLEM

Cytoplasmic male sterility (CMS) systems have been considered as one of the most efficient means for production of hybrid seed. CMS eliminates the need for hand emasculation and ensures F_1 seed production by insect pollinators or wind. The University of Arkansas has developed a new CMS system (CMS-D8) for cotton based on an American wild species (*Gossypium trilobum*, D_8) cytoplasm (Stewart, 1992). Its specific restorer (D8 restorer) was also developed by introducing a nuclear restorer gene (Rf_2) from the D_8 genome into cotton that can restore fertility to CMS-D8. Another restorer gene (Rf_1) in the D2 restorer for CMS-D2 can also restore its fertility. However, the genetic relation between the two restorer genes needs to be determined. The mechanisms through which male sterility and its restoration in CMS are realized are unclear. Understanding the genetic and molecular basis of CMS and restoration will help explore new sources for the CMS-D8 restorer and facilitate the development of new CMS and restorer lines.

BACKGROUND INFORMATION

Previously, the two restores, D8 restorer (D8R) and D2 restorer (D2R), were found to independently restore fertility to CMS-D8. In an allelic test, only one sterile plant out of 191 was isolated in the test-cross (D8R x D2R)F_1 x TM-1, indicating a tight linkage between Rf_1 and Rf_2 (Zhang and Stewart, 1999; Zhang and Stewart, 2000a). However, if by chance the sterile plant were from seed contamination, the two genes could be allelic, since both were transferred into cotton from two closely related species, *G. harknessii* (D_2) and *G. trilobum*. Genetically and cytologically, these two *Rf* genes have different restoring mechanisms in that Rf_1 is sporophytic and Rf_2 is gametophytic. In the restored heterozygous F_1 plants of the latter (CMS-D8 x D8R), half of the pollen grains did not stain with I_2-KI, indicating no starch deposition. Thus, all fertile F_2 plants were produced (Stewart *et al.*, 2000; Zhang and Stewart, 2000b). It was speculated that expression of the essential genes for microspore development and maturation, including those responsible for starch synthesis, were suppressed in the male gametes without the Rf_2 gene.

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RESEARCH DESCRIPTION

To confirm that there is one restorer gene (Rf_1) in the D2 restorer, B418R, a D2R line was used as female to cross with ARK8518 in the 1997 growing season at the University of Arkansas in Fayetteville. The resulting F_1 was selfed and test-crossed as female with ARK8518 in 1998. To verify the genetic relation between Rf_1 and Rf_2, D8R was crossed as female with D2R to produce F_1 hybrids in 1997. The F_1 plants were grown and crossed again as female with ARK8518 in 1998. The (D2R x ARK8518)F_2 and two test-crosses, i.e., (D2R x ARK8518)F_1 x ARK8518 and (D8R x D2R)F_1 x ARK8518 were grown and evaluated for fertility in the field in the 1999 growing season.

In previously studies we isolated and sequenced approximately 40 cDNAs that were differentially expressed between ARK8518 and its isogenic line, 8518R (Zhang *et al.*, 1999). 8518R was heterozygous for Rf_2 gene (Rf_2rf_2). A reverse Northern blot analysis was employed to confirm the differential gene expression.

RESULTS AND DISCUSSION

Inheritance of Restoration in the D2 Restorer

In past studies, one, two, or three restorer genes were suggested to be responsible for fertility restoration to CMS-D2 (Meyer, 1975; Weaver and Weaver, 1977; da Silva *et al.*, 1981; Kohel *et al.*, 1984). To verify the number of restorer genes required for restoration, male fertility in the F_2 of D2R x ARK8518 and testcross (D2R x ARK8518)F_1 x ARK8518 was evaluated. If there is only one restorer gene and it is segregated normally in the pollen, a 3 fertile to 1 sterile ratio would be expected in the F_2 population. However, out of 492 F_2 plants, only 36 sterile plants were observed. The chi-square test indicated that two duplicate restorer genes existed in the D2 restorer (3:1, χ^2=0.31). On the other hand, when the F_1 of D2R x ARK8518 with the D2 cytoplasm was backcrossed as female to ARK8518, 62 fertile and 56 sterile plants were obtained, as expected for a one restorer allele model (1:1, χ^2=0.95). The result confirmed that there is only one restorer gene (Rf_1) in the D2 restorer for CMS-D2 restoration. It also demonstrated that the non-restorer allele (rf_1) from tetraploid cotton is not transmitted normally through pollen when it is in the D_2 cytoplasm. A similar result was observed when Rf_1 was in the D_8 cytoplasm (Zhang and Stewart, 2000b). Therefore, in the D_2 cytoplasm, rf_1 pollen is much less competitive than the Rf_1 pollen, resulting in fewer sterile plants than expected in the F_2 population. The D_2 cytoplasmic effect might explain the conflicting segregation data reported in other studies of CMS-D2 restoration.

Genetic Relation between Rf_1 and Rf_2

In the test-cross population of (D8R x D2R)F_1 x ARK8518, out of a total of 1,730 plants, only seven sterile plants were observed, which is highly significantly deviated from an expected one-fourth sterile plants if Rf_1 and Rf_2 were independently assorted (χ^2 =558.1). Statistical analysis confirmed that Rf_1 and Rf_2 are tightly linked with a recombination frequency of 0.81. It is in an agreement with the data obtained from another test-cross, i.e. (D8R x D2R)F_1 x TM – 1 (Zhang and Stewart, 1999; 2000b).

In this same cross, two types of fiber, fluffed and non-fluffed, were observed during harvesting. Individual plants were evaluated on the basis of a scale of 0-4 (0= normal fluffed, 4= typical non-fluffed; 1-3= intermediate). Out of 908 plants scored, 153 plants were rated normal (0), and 285 plants were rated 1, while 24, 206, and 240 plants had ratings 2, 3, and 4, respectively. Obviously, rating 2 was the clearcut between the normal fluffed and non-fluffed types. Therefore, ratings 0 and 1 were considered normal fluffed, and ratings 2 to 4 non-fluffed. The non-fluffed fiber trait is not associated with the D_8 cytoplasm, and the chi-square test indicated that one nuclear gene conditions the segregation of the fiber trait.

Mechanism of CMS-D8 Sterility and Restoration

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 (ARK8518) were compared by using mRNA differential display techniques. Approximately 3,000 cDNA fragments were assayed that represented approximately 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 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 CMS-D8 and its restoration can be drawn as follows. In the heterozygous restored F_1 plants, expression of the restorer gene (rf_2) suppresses the D_8 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.

PRACTICAL APPLICATION

The present genetic study showed that gamete selection occurs in the D_2 cytoplasm in that pollen with the non-restoring gene (rf_1) is much less competitive than its counterpart (Rf_1 pollen). The implication is that other genes on the same linkage group as rf_1 will also be abnormally transmitted into next generation through pollen, and thus distorted ratios will be obtained. The results present evidence in cotton for the first time that exotic cytoplasm affects genetic segregation. Therefore, precaution should be taken in interpreting genetic data when an exotic cytoplasm is involved.

The reconfirmation of the tight linkage between Rf_1 and Rf_2 indicated that a large population will be required to isolate a recombinant carrying both *Rf* alleles that presumably would have enhanced restoration ability.

The comparison for differential gene expression between the heterozygous D8 restorer and the normal isogenic lines represented the first attempt in using the differential display techniques to study the molecular basis of CMS and restoration. The genes isolated provide useful information for understanding the mechanism of CMS and restoration in cotton.

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