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HIGH RESOLUTION MAPPING OF FERTILITY RESTORER GENES FOR CYTOPLASMIC MALE STERILITY IN COTTON

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Abstract

In cytoplasmic male sterility (CMS)-based hybrid cotton breeding and genetics, the construction and utilization of a high resolution linkage map can facilitate marker-assisted selection for restorer lines and allow for greater precision in the isolation of the fertility restorer genes. The objectives of this research were to develop a high resolution linkage map for fertility restorer genes of cotton using various PCR-based DNA markers. B418 ($D_{2\text{-}2}$, Rf_1) possessing male sterile cytoplasm ($D_{2\text{-}2}$) and corresponding fertility restorer gene (Rf_1), was used as female to cross with D8R3 (D_8, Rf_2) possessing male sterile cytoplasm (D_8) and corresponding fertility restorer gene (Rf_2). The resulting F_1 was testcrossed as female with Suregrow 747 containing normal fertile cytoplasm and no fertility restorer gene. The testcross population (TC_1F_1) was grown in 2002/2003 winter in the greenhouse for male fertility evaluation and DNA marker analysis. The mature bolls from the heterozygous restored fertile plants were bulk harvested and grown as TC_1F_2 (equivalent to F_2) in the field in Las Cruces, NM in 2003. Segregation analysis of male fertility in the TC_1F_1 confirmed involvement of the Rf_1 gene in controlling fertility restoration in the CMS caused by $D_{2\text{-}2}$ cytoplasm, while the Rf_2 gene did not promote male fertility restoration in this system. No distortion of segregation in fertility (786 fertile vs. 246 sterile) was noted in the TC_1F_2 population. Bulked segregant analysis was used to identify additional molecular markers (SSR, RAPD, and STS) linked with the fertility restorer genes. Two of the five previously developed STS markers successfully discriminated fertile plants from sterile plants. So far, 28 SSR primer pairs were screened using two isogenic lines, which produced 5 potential polymorphic SSR markers.

Introduction

Cytoplasmic male sterility (CMS) is a maternally inherited condition in which the plant is unable to produce functional pollen grains. CMS is often associated with the expression of chimeric mitochondrial open reading frames (Schnable and wise, 1998). In many plant species male sterility caused by cytoplasmic genetic materials could be suppressed by the presence of fertility restorer genes resulting in fertility restoration. In cotton (*Gossypium hirsutum*) two cytoplasmic male sterility systems, known as $D_{2\text{-}2}$ and D_8 CMS systems, have been developed by transferring cytoplasms from wild species into the cultivated cotton (Meyer, 1975; Stewart, 1992). Fertility restoration by dominant genes is essential for hybrid seed production based on CMS to obtain high yield of hybrid seeds on a commercial scale. In cotton, two nuclear genes (Rf_1 and Rf_2) have been identified that are responsible in the restoration of fertility independently (Zhang and Stewart, 2001). In cotton, the Rf_1 fertility restorer gene is sporophytic and the Rf_2 is gametophytic in nature. The nuclear gene mediated restoration mechanism often involves the alteration of expression of CMS related mitochondrial genes (Schnable and Wise, 1998; Williams and Levings, 1992). However, the molecular mechanism leading to fertility restoration in cotton is not fully understood. This research aims to develop molecular markers using various molecular biology tools and to construct a high resolution map for the fertility restorer genes.

Materials and Methods

Plant Material

B418R (*Gossypium hirsutum*), carrying $D_{2\text{-}2}$ type of male sterile cytoplasm and its corresponding restorer gene (Rf_1) was used as female parent. Another line, D8R3 carrying D_8 type of male sterile cytoplasm and its corresponding restorer gene (Rf_2) was used as male parent. Hybridization was made between the two parents and the F_1 plants were testcrossed as female with Suregrow 747, which contains normal cytoplasm without any restorer genes. The progeny obtained from this testcross, which constituted TC_1F_1 population were grown in the greenhouse and scored for fertility, following the method described by Zhang and Stewart (2001). The male fertile plants from the TC_1F_1 population were harvested, and the seeds were planted in the field during 2003 to develop the TC_1F_2 population. Plants from both populations were scored for fertility to determine the segregation ratio for fertility restoration. The leaf tissue samples were collected from the segregating populations and DNA was extracted as described by Zhang and Stewart (2000).

Marker Analysis

Bulked segregant analysis was used to identify markers linked to the fertility restorer gene. The PCR protocol was standardized for University of British Columbia (UBC) primers, with some modification. The PCR reaction was in a 20 µl, consisting of template DNA 20 ng, 10 mM Tris-HCl, 10 mM KCl, 1.9 mM $MgCl_2$, 0.2 mM each of dNTPs, 0.5 units Taq polymerase, and 0.2 µM UBC primer. The PCR reaction was denatured at an initial step of 5 min at 94ºC, followed by 40 cycles of 30 s at 94ºC, 1 min at 35ºC and 2 min at 72ºC. A final extension for 7 min at 72ºC was followed after the last PCR cycle. The DNA from 10 fertile plants and 10 sterile plants was bulked separately and used as templates for screening RAPD primers. Altogether, 288 RAPD primers were screened for polymorphism. The RAPD primers that gave Polymorphic PCR fragments were re-screened using 8 fertile and 8 sterile individual plants. STS markers previously reported by Zhang and Stewart (2004) and Feng et al. (2004) linked with fertility restorer genes were also tested in this population. In order to obtain sufficient markers tightly linked with fertility restorer genes, we also screened SSR markers for polymorphism.

Results and Discussions

Abundant pollen grains could be observed in male fertile plants during anthesis, whereas the sterile plants produced only shrunken anthers without pollen grains. The male fertile plants produced comparatively larger flowers. Furthermore, the floral buds from fertile plants were also larger. When the immature anthers from a fertile plant were squeezed between fingers, a wet and coarse mass of viable pollen grains were released, which was absent in sterile anthers. All the F_1 plants were male fertile, indicating the dominant nature of fertility restoration. Fertility in the testcross TC_1F_1 population of 164 plants grown in the greenhouse fit a segregation ratio of 1:1 (P= 0.06). The TC_1F_2 population, derived from the fertile TC_1F_1 plants and comprising 1032 plants, was grown under field conditions in Las Cruces in 2003. The Chi-square test revealed that the TC_1F_2 population segregated in a 3:1 ratio (P= 0.33) for the male fertility phenotype. The results from both populations verified the involvement of one dominant restorer gene Rf_1 for fertility restoration in plants containing $D_{2\text{-}2}$ cytoplasm. This result is consistent with previous findings (Zhang and Stewart, 2001). Under field conditions, heterozygous restored F_1 plants may produce significantly fewer sterile plants than expected in the F_2 (Zhang and Stewart, 2001; Liu et al., 2003). Zhang and Stewart (2001) suggested that the male gametes without the dominant restorer gene allele Rf_1 are less competitive or completely unviable under some environmental conditions, resulting in distorted segregation ratios. This idea was reiterated by Liu et al. (2003). However, under winter greenhouse conditions in Las Cruces, the two types of male gametes having Rf_1 or rf_1 alleles seemed to have equal viability in fertilization.

Some RAPD primers amplified polymorphic bands from the bulked segregant analysis (Figure 1), but we failed to confirm the polymorphism in the individual fertile and sterile plants.

Among the five STS markers developed by Feng et al. (2004), only UBC 607-500STS and UBC147-1400STS were found to be reliable in this population (Figure 2). PCR primers and conditions will need to be optimized for other STS markers based on the sequence information provided.

We screened selected SSR primers for polymorphism between two pairs of isogenic lines with or without the Rf_2 allele. Out of 28 SSR primers which amplify SSR markers on D chromosomes, five appeared to be polymorphic between fertile and sterile plants (Figure 3). Some of the polymorphic SSR markers are known to be linked on the same D chromosome, while others are of unknown D chromosomes. Further investigation will help in identifying the chromosomal location of the two restorer genes.

References

Feng, C.D., J.F. Zhang, and J. McD. Stewart. 2004. Development of STS markers associated with cotton cytoplasmic male sterility restorer gene. Molecular Breeding (submitted).

Liu, L., W. Guo, X. Zhu, and T. Zhang. 2003. Inheritance of fertility restoration for cytoplasmic male sterility in *Gossypium hirsutum* L. Theor. Appl. Genet. 106:461-469.

Schnable P.S. and R.P. Wise 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. Trends in plant science. 3:175- 180.

Stewart, J. McD. 1992. A new cytoplasmic male sterility and restorer for cotton. Proc. Beltwide Cotton Res. Conf. p. 610.
Williams, M.E. and C.S. levings. 1992. Molecular biology of cytoplasmic male sterility. Plant Breeding Reviews. 10:22-47

Zhang, J.F. and J. McD Stewart. 2000. Economical and rapid method for extracting cotton genomic DNA. J. Cotton Sci. 4:193-201.

Zhang, J.F. and J. McD Stewart. 2001. Inheritance and genetic relationships of the D8 and D2-2 restorer genes for cotton cytoplasmic male sterility. Crop. Sci. 41:289-294.

Zhang, J.F. and J. McD. Stewart. 2004. Identification of molecular markers linked to the fertility restorer genes for CMS-D8 in cotton (*Gossypium hirsutum* L.). Crop Sci. 44: (in press).

Table 1. Results of fertility scoring in two mapping populations

Population	No. of fertile plants	No. of fertile plants	Expected ratio	X^2	p value
TC_1F_1	92	72	1:1	3.43	0.06
TC_1F_2	786	246	3:1	0.94	0.33

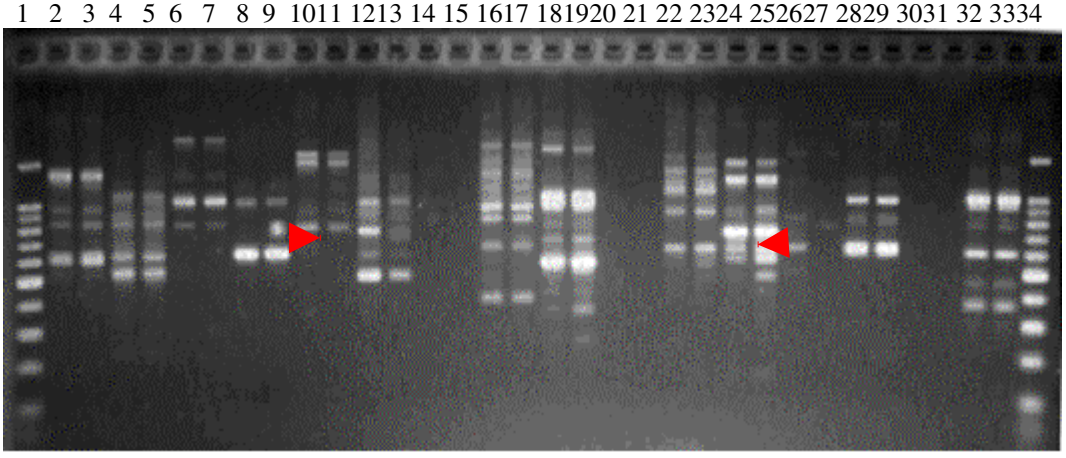
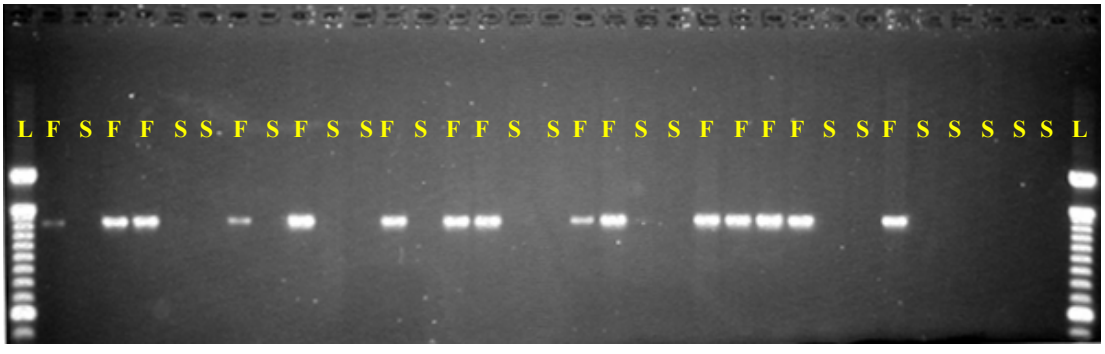


Figure 1. Gel image showing results of UBC RAPD primer screening using bulked segregant analysis (BSA) method to identify RAPD marker associated with fertility restorer gene. Red arrow indicated polymorphism. Lane 1 and 34 are100 bp DNA ladders. Even lanes represent fertile bulk odd lanes represent sterile bulk.



L= 100 ladder; F= Fertile plant; S= sterile plant

Figure 2. Gel image showing amplification of a 1400 bp fragment by fertile plants in a testcross population. The STS primer UBC 147-1400 was used prime the PCR reaction.

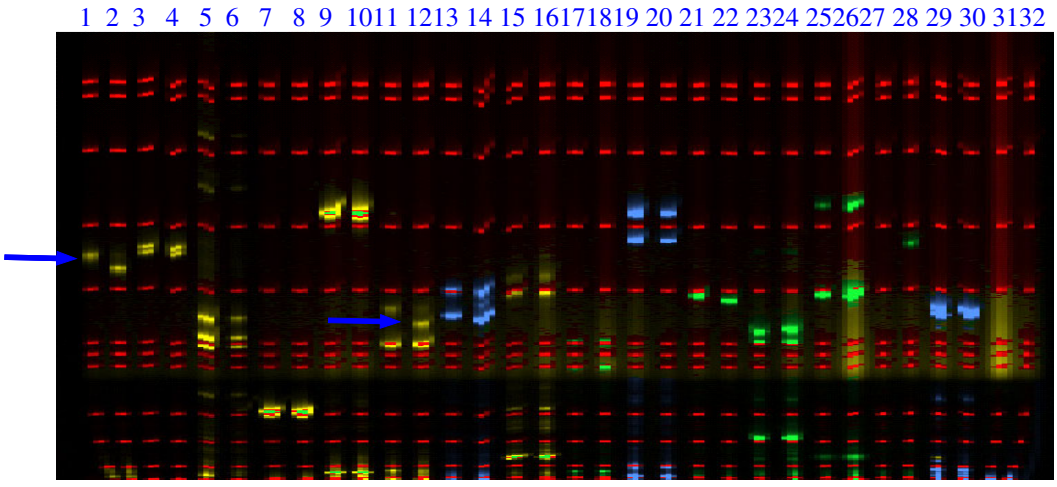


Figure 3. Polyacrylamide gel image showing the results of SSR primers screened for polymorphic markers for fertility restorer gene (Rf_2). Sixteen SSR markers were used to amplify DNA from two isogenic lines. The odd lanes represent DNA from male fertile line and the even number lanes represent DNA from male sterile isogenic line. The polymorphic markers are indicated by an arrow.