ANALYSIS OF SEMIGAMY EXPRESSION IN COTTON (*GOSSYPIUM BARBADENSE*)

Zhang Jinfa and James McD. Stewart
University of Arkansas
Fayetteville, AR
R. B. Turley
USDA-ARS, Cotton Physiology and Genetics
Stoneville, MS

Abstract

Semigamy is a type of facultative apomixis in which the male gamete does not fuse with the female nucleus following entry into the embryo sac, leading to production of paternal and maternal haploids. This phenomenon has been found in more than 10 plant species including cotton (*Gossypium barbadense*). Even through much research has been done since the semigametic line, 57-4, was isolated in Pima S-1 as a doubled haploid from a natural haploid mutant, some questions remain unanswered, such as the genetic and molecular mechanisms, stability of semigametic expression and early identification of haploids.

The genetic differences in photosynthesis and agronomic traits between the semigametic line, 57-4 and its putatively natural isoline, Pima S-1, were compared in replicated trials during 1996-1998. Compared with Pima S-1, 57-4 had a significantly lower chlorophyll a, b and total chlorophyll content in its functional leaves. 57-4 also had consistently lower stomatal conductance and lower transpiration rate, however, seedcotton yield did not differ significantly. But, 57-4 had significantly higher lint percentage, higher seed weight, higher micronaire (coarser fiber), lower boll weight, and shorter and stronger fiber. The genetic dissimilarity between 57-1 and Pima S-1 was also evaluated at the DNA level with molecular markers (RAPDs). Based on 171 RAPD markers generated from 20 informative primers, 57-4 was 93% identical to Pima S-1, while both of them shared only 57% of the markers with upland cotton (*G. hirsutum*). The polymorphic RAPD markers between 57-4 and Pima S-1 will be used as putative markers to tag the gene(s) controlling semigamy.

In 57-4 and a virescent semigametic line, Sev7, a wide range of seed weight with a substantial portion of unexpected small seed were observed. The seed weight was significantly and positively correlated with seed vigor as measured by germination percentage. Additionally, most of small seeds produced haploid plants, thus a significant and positive correlation between seed weight and ploidy level exists.

The stability of the semigametic lines in haploid production was evaluated by pedigree selection for three consecutive generations starting from base populations producing 46.3% and 43.2% haploid plants in 57-4 and Sev7, respectively, in 1996. In 1997, progeny rows of 57-4 produced an average of 69.9% haploids (range 14.3% to 75.5%), while progeny of Sev7 averaged 36.1% haploids (range 0% to 87.5%). In 1998, the progeny families of 57-4 produced 43.9% haploids (range 33.9% to 54.6%), while those of Sev7 produced 43.6% (range 35.7% to 50.4%). The data were subjected to analysis of variance to identify sources of variation in haploid production. Since the two lines produced the same haploid percentage in1998, no variation could be partitioned into genotypic differences. The majority of variation (68% in 57-4 and 64.6% in Sev7) was due to the variance among individual bolls within plant. In 57-4 and Sev7, 27.7% and 28.8% of the total variation, respectively, was explained by the variance among plants within a family. Variance among families explained only 4.0%, and 7.1% of the total variation in 57-4 and Sev7, respectively. Therefore, we conclude that semigametic expression in 57-4 and Sev7 is genetically stable. Environmental and developmental factors affect the variation in haploid production in the two lines.

A genetic study involving a cross between 57-4 and Pima S-1 confirmed the genetic stability of the semigametic trait. The (57-4 x Pima S-1)F_2, and BC_1F_1, i.e. (57-4 x Pima S-1)F_1 x both of the parents, were test crossed with Sev7. The F_2 population produced semigametic and non-semigametic F_3 progeny which fit to a 3:1 ratio. The (F_1 x Pima S-1) produced 50% semigametic lines and 50% non-semigametic lines, while the (F_1 x 57-4) produced 50% high haploid-producing lines and 50% low haploid-producing lines. The data confirmed that semigamy expression in cotton is conditioned by one incompletely dominant gene. When 57-4 was used as female parent, the F_1 gave 11.1% haploid, while the reciprocal F_1 produced no haploid. Surprisingly, the F_2 produced only 3.7% haploids. The F_1 as female backcrossed with 57-4 and Pima S-1 produced 13.0%, and 4.3% haploids, respectively, while the BC_1F_1 with 57-4 as female produced 15.2% haploids. The results could not be explained by the gametophytic control model in which haploid production is controlled by the genotype of the gamete. However, the data were consistent with expectations based on a sporophytic and gametophytic control model. Thus, we conclude that semigametic expression is sporophytically and gametophytically controlled by one gene.

Alloplasmic lines with cytoplasm from A_2, B_1, C_1, $D_{2\text{-}2}$, $D_{3\text{-}d}$, E_1, F_1, AD_1, AD_3, AD_4 and AD_5 were compared for haploid production in the 57-4 or Sev7 backgrounds. Haploid percentage was much lower in cytoplamic male cytoplasm (C_1 and $D_{2\text{-}2}$), indicating that the semigametic expression is suppressed in cytoplamic male cytoplasm.

The inheritance of chlorophyll content was investigated in a cross between 57-4 (lower chlorophyll concentration) and Pima S-1 (normal chlorophyll concentration). One gene

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:446-447 (1999)
National Cotton Council, Memphis TN

was estimated from generation mean analysis. Semigametic F_3 lines also had lower chlorophyll content than the non-semigametic lines. Significant correlation between haploid production and chlorophyll content was found. The results established that the genetic systems for controlling semigamy and chlorophyll content were associated, or were likely the same, indicating that the semigamy gene may have other functions. Further studies will provide an in-depth look for the mechanism underlying the relationship in reproductive biology.

More than 60 differentially expressed cDNAs had been cloned and sequenced by mRNA differential display, of which some sequences showed significant homology to genes coding for cell division-related proteins. The semigamy gene is likely related with cell division.