Concept of Graphical Genotypes for Analysing Introgressed Cotton Populations

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ABSTRACT

Cotton breeders have utilized genes from Gossypium barbadense to enhance fiber properties of Acala cotton G. hirsutum. This has been achieved only after many decades of careful selection and breeding. Extensive problems with interspecific hybrid population have frustrated breeders and prevented targeted introgression of genes for important fiber quality traits. Genomic maps, utilizing DNA markers, permit dissection of each linkage group into its parental pedigree origin. This is very interesting in G. hirsutum x G. barbadense segregating populations, since species-specific chromosome regions can be identified and followed in breeding. Some of these large regions correspond to locations of quantitative trait loci (QTL) for fiber span length, fiber strength and fineness. Two genetic mapping populations involving extensive G. hirsutum x G. barbadense introgression have been studied with Simple Sequence Repeat (SSR) and RAPD markers. Due to limited intra-specific DNA polymorphisms, species-specific DNA markers have been catalogued and scored on these two mapping populations. These markers have also been utilized to create graphical genotypes of genetically stable homozygous lines that demonstrate various levels of introgression. This graphical genotype can be associated with phenotype but more importantly compared with QTL analysis for fiber quality. These QTLs can provide a foundation for introgressive breeding to enhance fiber traits, as well as, improve genetic diversity in G. hirsutum.

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Introduction

Genetic diversity is essential for genetic improvement in both upland and *Pima* cotton. Directed introgression of *G. barbadense* into *G. hirsutum* can with time produce stable breeding lines. Genetic improvements in fiber strength and tolerance to Verticillium wilt (caused by *Verticillium dahliae* Kleb.) in the New Mexico Acala germplasm, and subsequent California Acala cultivars, were derived from *G. barbadense* introgression (Staten, 1971; Hyer and Bassett, 1985). Extensive diversity was observed among New Mexico breeding lines and cultivars, despite intense selection for Acala fiber quality (Bowman *et al.*, 1996). This may be partly a result of repeated introgression in the germplasm base from *G. barbadense* and Triple Hybrid germplasm.

Quantification of introgression at the genomic level is difficult and limited. Most research has focused on *G. hirsutum* introgression into *G. barbadense*. Percy and Wendel (1990) detected significant amounts of *G. hirsutum* allozyme alleles in cultivated and wild *G. barbadense*. Wang *et al.* (1995) screened *G. barbadense* germplasm with 106 RFLPs and reported ranges of 6-91 % *G. hirsutum* alleles. The non-random retention of *G. hirsutum* chromatin may be due to some selective advantage of some alleles. Quantification and tracking introgression is essential in managing interspecific germplasm in cotton.

The concept of graphical genotypes to display parental genome contributions to progeny was proposed by Young and Tanksley (1989). As linkage maps are created, a graphical genotype approach can be used to create a marker-based display of the entire genome or individual linkage groups.

Graphical genotypes have been used in various ways in tomatoes (Young and Tanksley, 1989), and soybeans (Lorenzen *et al.*, 1995). If DNA markers can be assigned species-specific designation then the graphical display can give a detailed presentation of the amount of interspecific introgression in progeny from wide crosses. Most of the DNA polymorphism is of interspecific origin permitting alleles to be assigned to *G. hirsutum* or *G. barbadense* when appropriate genetic standards are utilized.

Directed introgression between *G. hirsutum* and *G. barbadense* has been a component of parent breeding of the hybrid cotton project at New Mexico State University. A core set of these lines is genetically stable and exhibits a continuum of species-specific morphological and DNA markers from *G. hirsutum* to *G. barbadense* (Tatineni *et al.*, 1996). A graphical genotype display of NM24016 and progeny derived from hybridization with *G. hirsutum*, cv. TM1 would permit dissection of critical linkage groups into their parental pedigree origin.

Identification and tagging of classical quantitative trait loci (QTL) with molecular markers can be integrated

with graphical genotype displays to determine important regions for the QTL. This is especially informative if there is large interspecific variation for the QTL. One such trait is stomatal conductance, where *G. hirsutum* exhibits high stomatal conductance and typical *G. barbadense* is low (Lu *et al.*, 1994). Cantrell *et al.* (1998) developed an interspecific mapping population and demonstrated QTLs for reduced stomatal conductance derived from *G. barbadense*.

Materials and Methods

The breeding line, NM24016 was chosen for this study. NM24016 (H12156/2/77-505/Russian 5904)) is one genotype that displays combinations of species-specific morphological traits of typical *G. hirsutum*, cv. TM1 and *G. barbadense*, cv. 3-79. An F2 and F2.3 mapping population (n=118) was derived from the hybrid of NM24016 X TM1. A genetic map comprised of 28 linkage groups were created with Simple Sequence Repeat (SSR) and Random Amplified Polymorphic DNA (RAPD) markers. The total map distance was 1028 cM with an average distance between markers of 10.9 cM. All DNA markers were pre-screened against genetic standards TM1 (*G. hirsutum*) and 3-79 (*G. barbadense*) to identify species-specific alleles. The limited intra-specific polymorphism in Gossypium permits easy identification of polymorphisms of this type. Numerical marker and map data were analysed with QGENE ver. 2.29a (Clare Nelson, 1994) and SUPERGENE (Boutin *et al.*, 1995. Composite interval analysis of stomatal conductance (mmol m^{-2} s^{-1}) was computed with QTL Cartographer ver. 1.12 (Basten *et al.*, 1994).

Results

The introgressed genotype NM24016 was verified to contain significant *G. hirsutum* and *G. barbadense* chromatin. This was already known by analysis of phenotype and RAPD markers (Tatineni *et al.*, 1996). However, the graphical genotype approach displays species-specific marker information by linkage group. This is best shown by viewing the graphical representation of Chromosome 2 (Fig. 1) which contains 8 markers and spans 91.4 cM. For this chromosome of NM24016, introgression from *G. barbadense* is noted for SSR loci G3800 and G3257. The remainder of the chromosome is mostly *G. hirsutum* chromatin.

One may then proceed to determine if this *G. barbadense* region on chromosome 2 has any effect on the phenotype of a segregating population derived from *G. hirsutum* and *G. barbadense*. The graphical genotype of chromosome 2 is most interesting when superimposed on a QTL composite interval analysis of the same chromosome (Fig.1). In this case, the QTL was for stomatal conductance. A significant QTL for this trait is detected near G3800. The average additive effect of the *G. barbadense* allele for G3800 was a decrease in stomatal conductance of 7.4 mmol m^{-2} s^{-1}. This QTL explained approximately 7% of the variability for this trait in the mapping population. This illustrates the importance of this region for stomatal conductance.

QTL analysis detected another significant region near RAPD marker S83L2 on linkage group 14 (graphic not shown). This linkage group contains 5 DNA markers and spans approximately 85 cM. S83L2 is a dominant marker and all F2.3 progeny with this fragment average 638 mmol m^{-2} s^{-1} while those progeny null for the fragment average 692 mmol m^{-2} s^{-1}. Display via graphical genotype of the progeny and NM24016 reveal clearly the fragment comes from *G. barbadense*.

Discussion

Graphical genotypes can be used to display amounts of *G. barbadense* introgression into *G. hirsutum*. The amount of introgression can be examined by linkage group and possibly chromosome (in the case of cotton chromosome 2). The utility of the concept is best demonstrated when combined with data from a QTL analysis. This way, the effect of important regions can be identified. Software (QGENE) is now available to employ selection in segregating progeny for various desired graphical genotypes. This is simply a refined marker-facilitated selection strategy and will prove to be useful in interspecific-derived cotton populations.

The dearth of intra-specific DNA polymorphism will limit the use of graphical genotypes in *G. hirsutum* and *G. barbadense*. One possible application maybe in backcross-derived populations where transgenes are being incorporated into proven genetic background. Selection for graphical genotypes would aid in recovering the recurrent parent while eliminating the donor parent genome.

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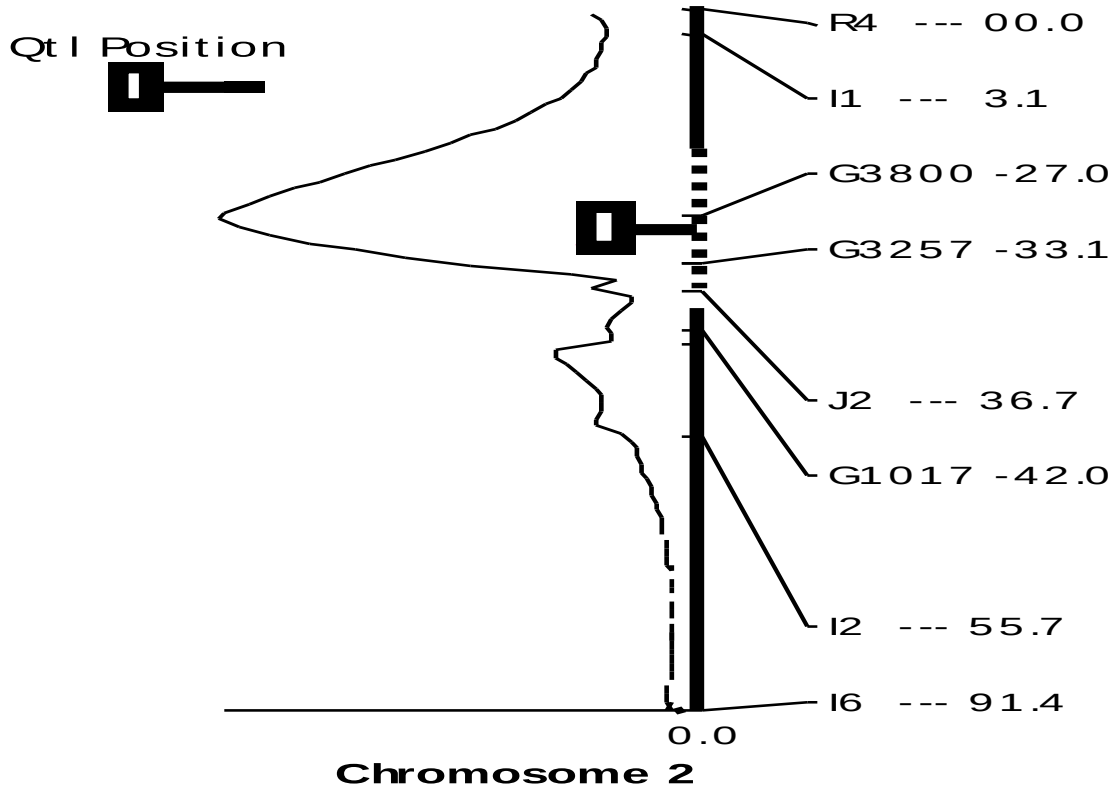
Figure 1. Graphical genotype of cotton chromosome 2 from NM24016 with *G. barbadense* ( ) and *G. hirsutum* ( ) regions displayed. A QTL for reduced stomatal conductance is located in *G. barbadense* region.



LOD = 2.0
R_2 = 7.0. The Additive effect = –7.39 mmol m^{-2} s^{-1} .