Molecular Genetic Mapping of the Major Effect Photoperiod Response Gene in Pima Cotton (*Gossypium barbadense* L.)

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Introduction

Cultivated Upland (*Gossypium hirsutum*) and Pima (*G. barbadense*) cotton has a narrow genetic base due to domestication and modern plant breeding practices. Tremendous genetic variability exists in wild and landrace accessions of tetraploid *Gossypium* species (Campbell et al., 2010; Wendel et al., 2009). The tropical gene pool could be an excellent source of genetic variability to reduce the genetic vulnerability in U.S. cotton. However, these tropical accessions flower only under short day lengths. This photoperiod sensitivity is a major barrier for the introgression of desirable traits from the tropical accessions in the cotton breeding programs in North America. Further, the lack of flowering of the tropical accessions prevents their effective evaluation for many important agronomic traits in the U.S. cotton growing regions. The linked molecular markers could help improve the speed and efficiency in the breeding and conversion of exotic germplasm to day-neutrality. Among the five tetraploid lineages of *Gossypium*, photoperiod response showed single gene inheritance in Pima cotton (Lewis & Richmond 1960). No marker-trait associations are available for photoperiod responses in tetraploid cotton. The objectives of the current study are to, 1. study the inheritance of the photoperiod response in tropical Pima cotton; 2. genetically map the photoperiod response locus in Pima cotton using SSR markers; and 3. establish the orthologous genomic region harboring photoperiod response locus in sequenced diploid D-genome, *G. raimondii*.

Material & Methods

The photoperiod sensitive *G. barbadense* line NC7018 (PI 326050) was crossed with photoperiod insensitive *G. barbadense* line Pima S-7 (Fig.1) to develop F_2 population. Line NC7018 was originally collected in Venezuela, can flower only in short photoperiod conditions, and Pima S-7 is a released cultivar that initiate flowering in both short and long photoperiod conditions (Fig. 1). A total of 211 F_2 plants were used for studying the inheritance and mapping of the photoperiod response trait in Pima cotton. Plants were grown in the greenhouse with 14 hr of continuous day-light. Phenotypic data was collected at two time points and plants were characterized as either flowering or non-flowering types depending upon flower initiation. SSR markers were used for basic genetic mapping of the photoperiod response locus. PCR products were resolved using capillary electrophoresis on an ABI 3730 sequencer. The JoinMap 4.1 was used to develop linkage map at LOD score of 6.0. A total of 110 genes involved in flowering time pathways in the model plants Arabidopsis, maize and rice were used for candidate gene mapping using *G. raimondii* genome sequence. Further, the flanking markers of the *Gb_Ppd1* was used to establish orthologous genomic region in *G. raimondii* and to search for putative genes with functions in flowering and find additional linked markers.

Results & Discussion

The F_2 population grown in long day photoperiod conditions showed 3:1 phenotypic ratio of sensitive : insensitive plants (χ^2 = 0.836, p = 0.3605) while photoperiod sensitivity in tropical Pima accession NC7018 is controlled by a single dominant gene *Gb_Ppd1*. Out of a total of 700 SSR primers, 61 (8.71%) were polymorphic between the two parents Pima S-7 and NC7018. Molecular mapping using polymorphic SSR markers showed that photoperiod response locus *XGb_Ppd1* was mapped on chromosome 25 of tetraploid cotton. The *Gb_Ppd1* gene was mapped near the centromere on chromosome 25 in a 3.3 cM interval flanked by co-dominant markers DPL 799 And DPL 377 (Fig. 2). Comparison of the current genetic map with *G. hirsutum* consensus genetic map of Blenda et al (2012) showed that marker order in the orthologous *Gb_Ppd1* region is generally conserved between pima and upland cotton (Fig. 2). Genomic targeting of *Gb_Ppd1* gene using *in silico* mapping of the linked flanking markers in the sequenced *G. raimondii* genome showed that *Gb_Ppd1* gene is mapped in a 5.8 Mb region flanked by SSR markers DPL 799 and DPL 377 on chromosome 10. Physical mapping further confirmed that *Gb_Ppd1* gene is mapped near the centromere. The order of the markers in the *G. raimondii* physical map is fairly consistent with current genetic map and with the Blenda et al.'s HDC map suggesting no major rearrangements present in the orthologous region of *Gb_Ppd1* gene between diploid D genome and polyploid D genome (Fig. 2). Annotation of the 5.8 Mb critical region showed that there were a total of 154 putative genes within this region. Two genes, *Gorai.010G161200* and *Gorai.010G157200*, showed significant homology to Arabidopsis genes involved in photoperiodism and flower development. A total of 110 candidate genes involved in flowering time in model plants were selected based on published papers for *in silico* mapping and to identify orthologous cotton genes in the *G. raimondii* genome. Only 13 genes showed homologs on chromosome 10, and none of them mapped in the 5.8 Mb region delineated by the flanking markers of the gene *Gb_Ppd1*. This indicates that none of the homologs of the major photoperiod response genes of model plants are the candidates for *Gb_Ppd1* gene in Pima cotton. Identification of linked molecular markers to the photoperiod response in the present study could help marker-assisted selection (MAS) for photoperiod sensitivity in the segregating populations early in the growing season. MAS can be used both during tropical desirable gene introgression as well as during the conversion of the exotic lines to day-neutrality which greatly improves the speed and efficiency of these processes. Expanded efforts are currently underway to genetically map the candidate genes, fine map the *Gb-Ppd1* gene and establish the genetic architecture of flowering time in tetraploid cotton.

Figure 1. Phenotypic differences in flowering (5 months after planting) for the two *G. barbadense* accessions grown under long day photoperiod conditions in North Carolina.

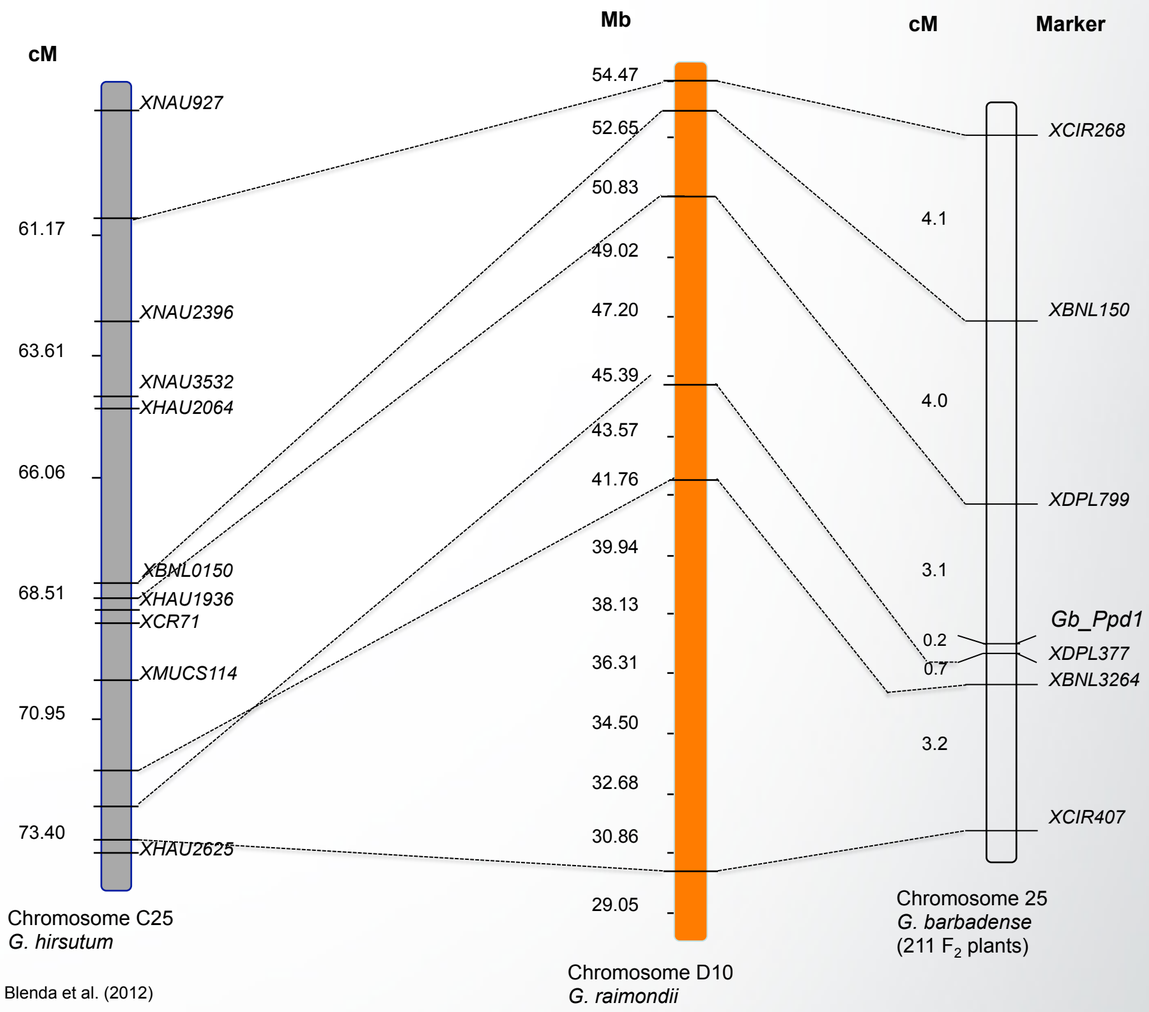

Figure 2. Molecular mapping of the *Gb_Ppd1* gene in *G. barbadense* and its genomic location in the *G. raimondii* genome, as compared to the existing HDC map (Blenda et al. 2012) in *G. hirsutum*.

References

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