



Mapping QTLs for traits related to phenology, morphology and yield components in an inter-specific *Gossypium hirsutum* × *G. barbadense* cotton RIL population

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ABSTRACT

Two major cultivated cotton species, *Gossypium hirsutum* (*Gh*) and *G. barbadense* (*Gb*) contribute to the bulk of cotton fiber production worldwide (95%). These species are largely inter-fertile and each displays a series of distinctive characteristics in terms of numerous botanical features and, more importantly, in their agronomic performance, adaptability and overall fiber quality. A recombinant inbred line (RIL) population derived from an inter-specific cross between *Gh* and *Gb*, used previously for QTL mapping of fiber quality characteristics, has also been evaluated over 6 sites and 2 years for various plant morphological, phenological and yield component traits. A total of 27 traits were assessed across a varying number of locations (up to 6 locations, in Australia, USA, Brazil, Cameroon, Belgium and France) and years, representing up to 10 different combinations. Variability in many of these traits was observed among the RILs and they frequently showed transgression. One hundred and sixty six significant QTLs, covering the 27 traits, were detected by composite interval mapping when using individual datasets. Cases of confirmation of localizations of individual QTLs from different data sets were detected in 27 instances, indicating that the 166 individual QTLs in this study could be represented by a maximum of 121 chromosome positions. QTL were shared between traits related to hairiness (22 individual QTLs), plant morphology of vegetative (29 QTLs) and reproductive (37 QTLs) parts, phenology (17 QTLs), and yield-related traits (61 QTLs). This is the first report of QTL mapping in cotton for various within-boll yield-related traits assessed on a per-seed basis, including fiber mass per unit of seed surface area (5 QTLs), calculated number of fibers per seed (2 QTLs) or per unit of seed surface area (1 QTL). This report confirms the importance of considering such basic yield components in selection for better yielding cotton varieties.

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1. Introduction

Two allotetraploid cotton (*Gossypium*) species, *G. barbadense* L. (*Gb*) and *G. hirsutum* L. (*Gh*) contribute to 95% of the production of this natural fiber around the world (Percival and Kohel, 1990). Cotton production is a commodity of key economic importance in many developing and developed countries. Beyond their intra-species variability, the 2 cotton species, *Gh* and *Gb*, are well differentiated by numerous botanical descriptors, their overall

environmental adaptability and yield potential, and the quality of their lint fiber (lint is the seed fiber that can be spun into yarn, linters are short fibers, or fuzz, which adhere to the seeds seed after ginning). Most generic botanical descriptors that distinguish *Gb* from *Gh* relate to the yellow color of pollen and petals, presence of a petal spot on the flower, a larger leaf, and a lower boll locule number and seed number in *Gb* than *Gh*. When compared to *Gh*, *Gb* cultivars are generally more vegetative and are later in flowering and boll opening; their phenotypic plasticity is narrower and their overall better fiber characteristics are more prized in the international textile market.

Because of the complex nature of crop yield, and particularly in the case of cotton production of fiber per unit land area where

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heritability of yield (effect of environment) can be low, there is a need to decompose yield into its components at the plant and fruit/boll level, including number of bolls per plant, boll weight, and percent fiber (lint weight as a percentage of seedcotton (lint plus seed) weight). More specifically, at the within-boll or within-seed level, the weight of lint per boll can be accounted for by combinations of number of seeds per boll and weight of fiber per seed; or even at the single seed level by seed surface area, fiber surface density (number of individual fibers per unit of seed surface area) and weight per fiber, which in turn can be decomposed to the product of the mean fiber length by fiber linear density. From a crop physiologist's perspective, yield component analysis can provide significant insights into how differences in this complex trait, i.e. yield, come about (Yin et al., 2004). Ontogenic models for dissecting cotton yield into its basic determinants have been proposed (Worley et al., 1976; Coyle and Smith, 1997) and used to characterize contrasted cotton cultivars (Coyle and Smith, 1997; Smith and Coyle, 1997; Bednarz et al., 2006, 2007). Although the most basic components of fiber yield, fiber surface density and lint mass per unit of seed surface area were cited as early as 1920 (Hodson, 1920) as key contributors to yield potential (see also Groves and Bourland, 2008), the utilization of within-boll components in breeding programs has only been limited. Interestingly, some authors have shown that cultivar development by the different breeders of Upland (*G. hirsutum*) cotton in the USA aimed at maximizing yield and quality have apparently unconsciously followed different strategies in selection and this has resulted in different trait combinations, with some cultivars being selected for longer and heavier fibers, but with relatively fewer fibers/unit seed surface area; whereas others have selected for greater numbers of shorter and lower weight/unit length fibers (Coyle and Smith, 1997; Bednarz et al., 2006). Several studies within *Gh* reported significant general combining ability, GCA, of fiber surface density on the seed as a basic component of fiber yield, this trait being expected to respond favorably to selection (Lewis, 2001; Rahman, 2006; Groves and Bourland, 2008). However, comparison of the 2 species, *Gh* and *Gb*, for yield and within-boll yield components is only poorly documented (Basal et al., 2009). The reduced number of epidermal ovule cells elongating into fibers and the resulting lower fiber surface density reported for some *Gb* cultivars (Radin, 1986) possibly accounts for the lower yield potential in this species as compared to *Gh* (Basal et al., 2009).

Molecular dissection of traits through mapping of quantitative trait loci, QTL, in cotton has concentrated mostly on fiber quality parameters (Shen et al., 2006; Rong et al., 2007; Qin et al., 2008; Luan et al., 2009; Lacape et al., 2010), including a recent QTL meta-analysis integrating QTL data from the same interspecific *Gh* × *Gb* RIL population reported here with QTL data from the literature (Lacape et al., 2010). Reports for QTLs for yield or yield-related traits have been scarce, mostly based upon intra-*hirsutum* segregating populations and were only poorly documented in terms of consistency of QTL detection between different studies (Ulloa and Meredith, 2000; Shen et al., 2007; Wang et al., 2007; Wu et al., 2009; Liu et al., 2011). He et al. (2005) mapped QTLs for yield traits in an inter-specific *Gh* × *Gb* F_2 population and detected QTLs for seed index (5 QTLs), lint index (1), number of seeds per boll (6) and lint yield (7), while a few other reports have detected QTLs for seed number, seed weight, and boll weight as part of larger sets of traits (Saranga et al., 2001; Mei et al., 2004; He et al., 2007).

This report complements our 2 earlier publications reporting (i) a genetic map from the inter-specific *Gh* × *Gb* RIL population (Lacape et al., 2009) and (ii) a meta-analysis of fiber quality QTLs (Lacape et al., 2010). The same experimental sites used for fiber measurements were used for the different measurements related to phenology and plant morphology, as well as for various

yield-related traits, including within-boll components contributing to fiber yield.

2. Materials and methods

2.1. Plant material and experimental conditions

The population under study was a RIL population, at F_8 as the average stage of single seed descent, deriving from an inter-specific *Gh* × *Gb* cross involving Guazuncho 2 (*Gh*), further referred as 'Gua', and VH8-4602 (*Gb*), further referred as 'VH8', as parents (Lacape et al., 2009). Subsets of the initial 140 RILs that were used to build an SSR-AFLP genetic map (Lacape et al., 2009) were planted in 6 locations (France, Belgium, Brazil, Cameroon, Australia, and USA) and different years (between 2006 and 2009) as detailed in (Lacape et al., 2010), altogether representing 10 different combinations of data sets. Experiments were glasshouse experiments in Ghent (Belgium) in 2006 (Ge6) and in Montpellier (France) in 2007 (Mp7) and 2008 (Mp8), and field experiments in Brazil in 2007 (Br7) and 2008 (Br8), in Garoua (Cameroon) in 2007 (Ga7), in Narrabri (Australia) in 2008 (Cs8) and 2009 (Cs9), and in Lubbock (USA) in 2007 (Lu7) and 2008 (Lu8).

Among all experiments, the one in Brazil from 2007 (Br7) was the most comprehensive comprising 123 RILs and parents tested in the field under a 2 complete randomized block design (250 elementary plots), and individual plots of 1 row of 8 meters. Among other sites, the number of RILs was usually smaller due to seed availability and the experimental design was not always replicated (see Table 1 in Lacape et al., 2010).

2.2. Phenotype analysis

The RILs were evaluated for up to 27 different traits at some, but not all sites and on varying numbers of the RIL population, depending on resource and seed availability at each of the different sites or years. This varied for example, from 1 data set, for within-boll components assessed in the 2 replicates of the Brazil experiment of 2007 (Br7) to up to 7 data sets, for lint percentage. The traits, detailed in Table 1, were grouped into 6 categories, representing leaf and stem hairiness as well as other morphological descriptors for vegetative and reproductive parts, flowering and fruiting phenology, and yield-components. Plant vegetative descriptors were related to leaf and stem hairiness, leaf color and shape; plant height and stem node number as measured at mid- to end of season. Plant reproductive descriptors included occurrence of the open bud trait (pistil protruding from corolla), flower color (petal and pollen) and petal spot color intensity, boll locule number and color of the fuzz. Many of these descriptors were scored through a qualitative scale as described in Table 1. Plant phenology was characterized through earliness indicators, including days from planting to first flower (measured on 5 data sets), D1F, and days to first boll split (2 data sets), D1B (Table 1).

Yield-related traits comprised a series plant-based and of within-boll components. Average boll weight (ABW), percent fiber after ginning (PF), seed number per boll (SN) and seed weight (as grams of 100 fuzzy and/or delinted seeds) were assessed in between 2 and 7 experiments. In Brazil in 2007, a more detailed series of characterizations of within-boll yield components was undertaken from the harvest of 5 bolls from the complete set of 250 plots (123 RILs, 2 parents and 2 replicates). The seed-cotton harvest of five bolls chosen from well-open bolls on the median fruiting branches was pooled across several plants. Following the ginning of the seed-cotton, fiber weight and PF were calculated, and after acid delinting, percent of fuzz or linters, Lt% was calculated and the volume index of the delinted seeds was measured by alcohol displacement. As

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