



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

Jean-Marc Lacape<sup>a,\*</sup>, Gérard Gawrysiak<sup>b</sup>, Tuong-Vi Cao<sup>a</sup>, Christopher Viot<sup>a</sup>, Danny Llewellyn<sup>c</sup>, Shiming Liu<sup>c</sup>, John Jacobs<sup>d</sup>, David Becker<sup>e</sup>, Paulo Augusto Vianna Barroso<sup>f</sup>, José Henrique de Assunção<sup>g</sup>, Oumarou Palaï<sup>h</sup>, Sophie Georges<sup>b,h</sup>, Janine Jean<sup>b</sup>, Marc Giband<sup>a,f</sup>

<sup>a</sup> CIRAD, UMR AGAP, Avenue Agropolis, F-34398, Montpellier, France

<sup>b</sup> CIRAD, UPR SCA, Avenue Agropolis, F-34398, Montpellier, France

<sup>c</sup> CSIRO Plant Industry, P.O. Box 1600 Canberra, ACT, Australia

<sup>d</sup> Bayer CropScience NV, Technologiepark 38, Ghent, Belgium

<sup>e</sup> Bayer CropScience, BioScience Research, Lubbock, TX, USA

<sup>f</sup> Núcleo Cerrado da Embrapa Algodão, Rodovia GO-462, km 12, Zona Rural 75.375-000 Santo Antônio de Goiás, GO, Brazil

<sup>g</sup> EMBRAPA Algodão, Rua Osvaldo Cruz 1143, Centenario, 58.428-095 Campina Grande, PB, Brazil

<sup>h</sup> IRAD, Centre Régional de Recherche Agricole de Maroua, BP 33 Maroua, Cameroon

### ARTICLE INFO

#### Article history:

Received 28 August 2012

Received in revised form 2 January 2013

Accepted 2 January 2013

#### Keywords:

Cotton

Quantitative trait locus (QTL)

Yield component

### 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

\* Corresponding author. Tel.: +33 0467614471, fax: +33 0467615605.  
E-mail address: [marc.lacape@cirad.fr](mailto:marc.lacape@cirad.fr) (J.-M. Lacape).

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



**Table 2**

Descriptive statistics of traits in parents and RILs (mean, max, min, standard deviation) for 2 most informed data sets, Br7 and Ga7. Descriptions of variables are given in Table 1.

	NbRILs	MaxRIL	MinRIL	MeanRIL	SdRIL	VH8	GUA
Br7 experiment: within-boll yield components (means of 2 reps)							
ABW*	123	6.33	0.48	3.43	1.23	3.62	6.39
Lt%	123	18.47	0.86	8.08	3.76	0.93	5.17
PF	123	55.79	13.46	32.96	6.63	25.74	41.46
SN	123	33.0	2.7	20.2	6.2	19.6	36.4
LI	123	9.54	1.45	5.64	1.52	4.76	7.28
Sld	123	15.0	5.6	9.8	1.7	12.9	9.3
SSA	123	1.52	0.75	1.10	0.13	1.34	1.03
L.SSA	123	9.43	1.34	5.18	1.43	3.56	7.10
LN.S	123	30 705	4 977	15 325	3 795	10 673	18 169
LN.SSA	123	30 311	4 606	14 098	3 700	7 979	17 770
Br7 experiment: Other variables for morphology and phenology							
HairLeaf	128	4.0	1.0	2.3	0.8	2.0	2.8
HairStem	128	4.0	1.0	2.4	1.0	1.0	3.5
LeafShape	128	4.0	1.0	2.3	0.6	3.0	2.4
LeafSize	128	3.0	1.0	2.1	0.6	2.9	2.8
BollLocNo	128	5.0	3.0	3.9	0.3	3.5	4.0
BollSize	127	3.0	1.0	1.6	0.5	2.0	2.4
D1B	127	138.0	104.0	115.3	9.5	120.3	104.0
D1F	128	71.0	48.0	56.1	5.1	55.4	49.3
FuzzCol	127	3.0	1.0	2.5	0.6	2.8	3.0
OpenBud	112	2.0	1.0	1.8	0.4	1.0	1.0
PetCol	128	3.0	1.0	1.4	0.6	3.0	1.0
PetSpot	128	4.0	1.0	1.3	0.8	4.0	1.0
PolCol	128	3.0	1.0	1.9	0.8	3.0	2.2
Ga7 experiment (plant measurement made at 63 dae)							
HairLeaf	109	7.8	1.0	2.2	1.5	1.0	6.2
HairStem	109	8.0	1.0	2.1	1.6	1.0	5.0
LeafShape	109	6.0	1.0	4.3	1.2	6.0	3.5
LeafSize	109	8.0	1.0	5.7	1.2	8.0	5.6
StemNodeVeget	109	9.5	2.0	5.0	1.4	5.4	4.9
Height	109	95.6	9.3	50.4	17.6	71.2	66.0
StemNode	109	22.0	5.3	15.4	2.9	16.6	16.5
StemNodeLen	109	5.3	0.9	3.2	0.8	4.3	4.0
PetCol	108	1.0	-1.0	-0.5	0.6	1.0	-0.7
PolCol	107	3.0	2.0	2.8	0.4	3.0	3.0
PetSpot	108	5.2	1.0	1.3	0.8	5.0	1.0
BollLocNo	107	5.0	2.9	4.0	0.4	3.1	4.2
BollSize	105	5.2	1.8	2.9	0.7	3.9	4.8
D1B	79	138.0	97.0	117.1	10.5	120.0	105.5
D1F	108	97.0	51.0	66.0	9.6	69.5	55.5
Br8 experiment							
PF	126	43.30	11.92	32.08	6.20	25.30	45.30
Cs8 experiment							
HairLeaf	93	9.2	1.1	5.2	2.2	2.8	7.7
Height	93	168.1	57.0	117.2	22.1	146.3	104.6
StemNode	93	18.7	9.0	14.5	2.0	16.2	14.4
BollLocNo	93	4.5	3.5	4.0	0.2	3.6	4.0
ABW	70	5.90	1.01	3.06	1.26	3.60	6.30
SI	70	14.9	7.3	10.9	1.6	14.7	10.3
SN	70	34.5	6.5	18.7	6.9	19.3	39.0
PF	70	44.63	21.89	32.96	4.98	20.40	36.40
D1F	92	121.9	74.4	87.8	12.2	97.2	75.9
Cs9 experiment							
PF	66	44.69	24.38	33.79	4.15	23.30	36.80
Ge6 experiment							
Height	133	260.0	18.0	147.9	39.0	208.3	148.9
PetCol	124	2.0	0.0	0.5	1.9	2.0	0.0
PetSpot	124	6.0	1.0	1.5	1.2	4.0	1.0
PolCol	124	2.0	0.0	0.9	2.0	2.0	0.0
D1F	125	167.5	41.5	61.9	20.4	63.4	40.6
PF	93	53.40	14.96	31.50	7.92	20.92	37.88
Sif	94	20.5	5.9	12.2	2.7	14.8	11.5
Lu7 experiment							
Height	77	137.2	25.4	79.4	19.8	96.5	61.6
PF	71	45.16	22.94	33.81	4.75	25.21	40.69
Sif	70	12.9	6.7	9.9	1.5	13.4	10.1
Lu8 experiment							
PF	90	44.82	18.75	35.68	5.57	23.60	41.20
Mp7 experiment							
HairLeaf	136	4.0	0.0	1.7	0.9	1.0	2.8
FuzzCol	135	3.0	1.0	1.9	0.7	NA	1.0
Lt%	139	3.00	0.00	1.84	0.70	0.00	3.00
OpenBud	121	2.0	1.0	1.2	0.4	1.0	1.0
PetCol	108	2.0	1.0	1.3	0.4	2.0	1.0

Table 2 (Continued)

	NbRILs	MaxRIL	MinRIL	MeanRIL	SdRIL	VH8	GUA
PetSpot	131	2.0	1.0	1.3	0.4	2.0	1.0
PolCol	110	2.0	1.0	1.5	0.5	2.0	1.0
Mp8 experiment							
HairLeaf	141	4.0	0.0	1.7	0.9	1.0	2.8
HairStem	141	3.4	0.0	1.6	0.9	0.0	2.4
Height	132	137.0	45.0	95.4	18.3	102.5	101.5
StemNode	131	18.5	8.5	13.9	1.7	14.0	12.5
StemNodeLen	131	10.0	3.1	7.0	1.2	14.0	12.5
StemNodeVeget	124	10.5	2.5	5.4	1.8	5.0	3.5
BollLocNo	131	5.0	2.3	3.9	0.5	3.0	4.0
D1F	131	135.0	58.0	75.7	15.0	72.0	64.0

there is a well established high correlation between delinted seed volume and seed surface area (Groves and Bourland, 2010), we used volume-to-surface conversion tables established earlier (Hodson, 1920) to deduce delinted seed surface area (SSA). Number of seeds (SN) per boll and the weight of fuzzy (not reported) or delinted seeds were calculated, and reported as weight of 100 seeds, or seed-index (Sif and Sid). Weight of the lint was also reported on a per seed (or per 100 seeds, as lint index, or LI), and per unit of seed-surface basis (L/SSA, in grams per cm<sup>2</sup>). An AFIS device was used in the laboratory of cotton fiber technology of CIRAD in Montpellier for measuring mean fiber length (mean length, Lw) from 5\*3000 individual fibers of the 250 fiber samples from the Brazil 2007 experiments. A maturimeter (FMTIII) was used to measure fiber fineness. The 2 parameters, mean length and fineness (equivalent to linear density) were used to estimate total fiber length (fiber weight divided by the fineness in unit of weight per unit of length) and total number of fibers per sample (total fiber length divided by mean fiber length). The estimate of fiber number was then reported on a per-seed basis as number of fibers per seed (LN/S) and on per unit of seed surface area basis (LN/SSA), knowing the surface area per seed.

### 2.3. Data analysis

#### 2.3.1. Analysis of variance and heritability

A separate analysis of variance was realized for the within-boll yield component data estimated from the Br7 experiment which comprised two replicates. In addition, a series of 11 variables measured on a minimum of 3 (maximum was 7) data sets were also subjected to an analysis of variance considering each data set as a replicate. The list of variables examined is presented in Table 1. Analyses used the GLM procedure of SAS software package (SAS Institute Inc., Cary, NC). The effect of genotypes (RILs) was tested against residual. Variance components were calculated using the VarComp procedure of SAS software package (SAS Institute Inc., Cary, NC) in the case of Br7 data, declaring the replicate effect as fixed and the genotype effect as random, and narrow sense individual heritabilities ( $h^2$ ) were calculated as the ratio between half the genotypic (RIL) and phenotypic variance (half genotypic variance + residual variance). The other data sets were analyzed using the Mixed procedure of SAS in order to take into account the unequal variance per data set. Heritabilities were thus not calculated. The frequency distribution for most traits fitted a normal distribution (not shown) and no data transformation was made prior to QTL analysis.

#### 2.3.2. QTL Analysis

A subset of 656 loci evenly distributed along the genetic map published previously (800 loci, 2044 cM) (Lacape et al., 2009) was used for QTL analysis. interval mapping (IM) and composite interval mapping (CIM) were conducted using WinQTL Cartographer 2.5 (Basten et al., 2003). For each variable, IM over the whole genome using multiple regression of phenotypic data on marker genotypic

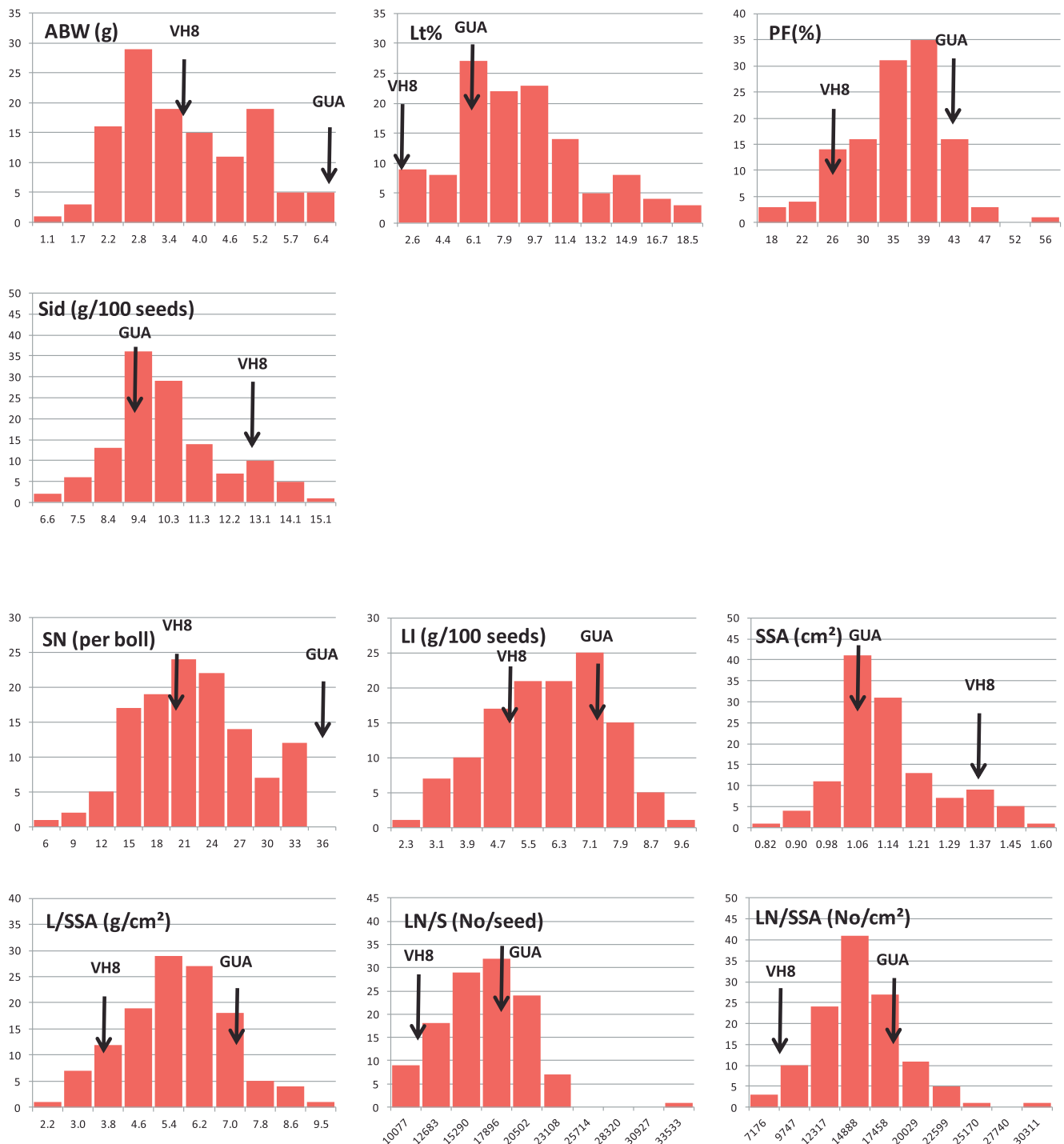
data was run with 1000 permutations to identify the minimum significant LOD (global risk of 5%) threshold score to be considered. Model 6 of CIM was then performed using 5 controlling markers pre-selected by forward-backward stepwise regression as cofactors. The results of the QTL position, proportion of phenotypic variance explained ( $R^2$ ), and effects that are reported are those derived from CIM. Chromosomes numbering followed the classical nomenclature system: c1–c13 and c14–c26 for the chromosomes of the  $A_t$  and  $D_t$  sub-genomes respectively, as this facilitated across-experiments comparisons. Only significant QTLs above the permutation threshold for each trait are reported. These thresholds varied between 3.15 and 4.95 (not shown). LOD peaks (LOD > threshold) were automatically localized using WinQTL Cartographer with the following parameters: – minimal space between peaks = 5 cM, and minimum LOD from top to valley = 1. The position of peaks and their one-LOD drop-off confidence intervals were recovered as outputs from WinQTL Cartographer. Graphical representations were generated with the MetaQTL package (Veyrieras et al., 2007). Cases of co-localizations of QTLs for the same trait and different data sets were inferred by their overlapping confidence intervals.

## 3. Results

### 3.1. Trait analysis

Most plant descriptors measured differed significantly between the two parents, Guazuncho 2 (*Gh*) and VH8 (*Gb*) (Table 2 and Supplementary Fig. S1). In contrast to the *Gh* parent, the *Gb* parent is characterized by its distinctive leaf features with large and lacinate leaves with reduced hairiness (Supplementary Fig. S1), flower features with large flowers with large yellow petals and pollen (both creamy for *Gh*) and presence of a red spot (absent in *Gh*) at the base of the petals (Supplementary Fig. S1). *Gb* has smaller bolls (3.6 g compared to 6.3 g for *Gh*) typically with 3 locules (4 in *Gh*), and its fibers are clearly longer (+10 mm) (Table 2, Supplementary Fig. S1), stronger (+11 to +16 g/tex, not reported here), and finer (–32 mtex) (Table 2) than those from the *Gh* parent. As compared to *Gh*, the *Gb* parent has fewer seeds per boll (20 versus 36) and those seeds are larger (weight 129 versus 93 mg per delinted seed, and surface area 1.34 versus 1.03 cm<sup>2</sup> per seed), with barely no fuzz (<1% o in weight as compared to 5.2% in *Gh*), have less lint mass per seed (47.6 versus 72.8 mg) and fewer fibers per seed (10,600 versus 18,200), or per unit of SSA (lint mass 35.6 versus 71.0 mg cm<sup>–2</sup>, and fiber number 8000 versus 17,800 per cm<sup>2</sup>).

RIL values (mean, maximum and minimum) for all of the traits examined are given in Table 2 and results of analysis of variance in Table 3. Variation among the RILs was very broad (see Table 2 and Fig. 1) and significant differences among the RILs were observed for all variables amenable to analysis of variance (Table 3). Distribution of phenotypic values in the RILs showed numerous cases of bidirectional transgressive segregants (Table 2). In some cases transgression was indicative of the poor yield ability of some RILs



**Fig. 1.** Frequency distribution of yield components in the RIL population from the experiment held in Brazil in 2007. The experiment was under field conditions and compared in a replicated trial (2 replicates) 123 RILs and the 2 parents. Values for the 2 parents (means of 2 replicates), Guazuncho 2 (Gua) and VH8 are indicated. Variables represent average boll weight (ABW), fuzz percentage (Lt%), percent of fiber (PF); weight of 100 delinted seeds (Sid), seed number per boll (SN), lint index (LI) or weight of fiber from 100 seeds, individual seed surface area (SSA), fiber mass per unit of seed surface area (L/SSA), calculated number of fibers per seed (LN/S) and per unit of seed surface area (LN/SSA).

(for example, RILs with very low ABW or SN) that could sometimes be related at some sites to poor fertility (not shown), late flowering or poor fiber maturation (RILs with very low PF or fiber per seed). Among extreme examples of transgression, were some RILs that had very high linter percent (up to 18.5% in weight while high parent *Gh* was only 5.2% and *Gb* 0.9%), percent fiber (up to 55.8%, while high parent *Gh* was only 41.5% and *Gb* was 25.7%),

or seed mass (15.3 g per 100 delinted seeds, while high parent *Gb* was only 12.9 g and *Gh* was 9.3). Interestingly, transgression toward high values was also observed among the RILs for lint mass and lint number per seed (as high as 30,700 fibers per seed, as compared to 18,200 and 10,700 in *Gh* and *Gb*, respectively), or per SSA (as high as 30,300 fibers per cm<sup>2</sup>, as compared to 17,800 and 8000 in *Gh* and *Gb*, respectively).

**Table 3**

Analysis of variance A- for within-boll components and AFIS fiber parameters in Br7 experiment, B- for 11 other variables assessed over more than 3 data sets. Descriptions of variables are given in Table 1.

A	F value rep	Significance level	F value <sup>§</sup> RILs	h <sup>2</sup>
Within-boll yield components				
ABW	3.52	*	8.63	0.40
Lt%	0.44	NS	14.48	0.44
PF	0.01	NS	18.21	0.45
SN	5.2	*	7.68	0.39
LI	0.21	NS	10.63	0.41
Sid	0.24	NS	11.72	0.43
SSA	2.91	NS	9.09	0.41
L/SSA	0.41	NS	13.46	0.43
LN/S	4	*	9.86	0.41
LN/SSA	10	**	12.37	0.43
B				
	No. data sets		Variance RILs <sup>§</sup>	
Boll locule number	4		0.03	
D1F	5		26.22	
Leaf Hairiness	5		0.64	
Stem hairiness	3		0.78	
Plant height	5		217.10	
Main stem nodes	3		1.93	
Pollen color	4		0.25	
Petal color	4		0.18	
Petal spot	4		0.61	
Percent fiber	7		25.99	
Seed index	4		1.79	

<sup>§</sup> All significant at *P* 0.001.

Narrow sense individual heritability was estimated for traits measured in the Br7 experiment (Table 3). These were generally high for all yield components; lowest for ABW (0.40) and seed number (0.39) and highest for PF and fuzz percent (0.45 and 0.44 respectively). These *h*<sup>2</sup> values are similar to values reported earlier (Liu et al., 2011) for those traits in common (for example *h*<sup>2</sup> for ABW was 0.38).

### 3.2. Correlations among within-boll components and with fiber quality parameters

Trait correlations amongst the RILs (Table 4) were calculated between variables related to yield components and to fiber quality in the Br7 experiment. The highest correlation was between seed mass and seed surface area (*r*=0.91, Table 4) or seed mass and seed volume (*r*=0.97, not shown). Average boll weight correlated best with the number of seeds per boll (*r*=0.91), and with lint mass per seed (*r*=0.52) or per SSA (*r*=0.49). PF was more correlated with lint index, i.e. lint mass either per seed, (*r*=0.82) or per unit of SSA (*r*=0.96) than with seed number (*r*=0.33) or seed size (*r*=−0.39), as reported by (Basal et al., 2009) from other interspecific, *Gh/Gb*, comparisons. As expected, lint index, or mass of lint per seed, correlated best with lint mass per SSA (*r*=0.90) and with lint number per seed (*r*=0.85) or per SSA (*r*=0.73). The negative

**Table 4**

Phenotypic correlations between mean values for within-boll components and AFIS fiber parameters in Br7 experiment. Descriptions of variables are given in Table 1.

	ABW	Lt%	PF%	SN	LI	Sid	SSA	L/SSA	LN/S
Lt%	0.12								
PF%	0.39	0.07							
SN	0.91	−0.01	0.33						
LI	0.52	0.21	0.82	0.25					
Sid	0.19	−0.01	−0.39	−0.13	0.16				
SSA	0.03	−0.01	−0.35	−0.28	0.17	0.91			
L/SSA	0.49	0.21	0.96	0.36	0.90	−0.23	−0.26		
LN/S	0.25	0.35	0.69	0.00	0.85	0.10	0.15	0.78	
LN/SSA	0.22	0.33	0.82	0.12	0.73	−0.32	−0.32	0.86	0.89

relationship between fibers number per seed (or per SSA) and fiber length measurements (*r* in the range −0.30 to −0.40, not shown), also mentioned in (Smith and Coyle, 1997; Basal et al., 2009), can be related to the way the number of fibers was calculated (a fiber weight component divided by the product of the mean length by the fiber linear density estimate).

### 3.3. QTL analysis

A total of 27 traits were evaluated for QTL analyses. The summarized results of the 166 individual QTLs with LOD score superior to their permutation-based threshold are shown in Table S1 and graphical displays are shown in Fig. 2 for just 3 chromosomes (all chromosomes shown in Supplementary Fig. S2). Altogether, following the clustering of QTLs for the same trait within the same chromosome region (this was the case for 73 QTLs to-be-grouped in 28 metaQTLs), the 166 individual QTL probably corresponded to a total of 121 unique QTLs, 28 metaQTLs and 93 solitary QTLs (Supplementary Table S1 and Fig. S2).

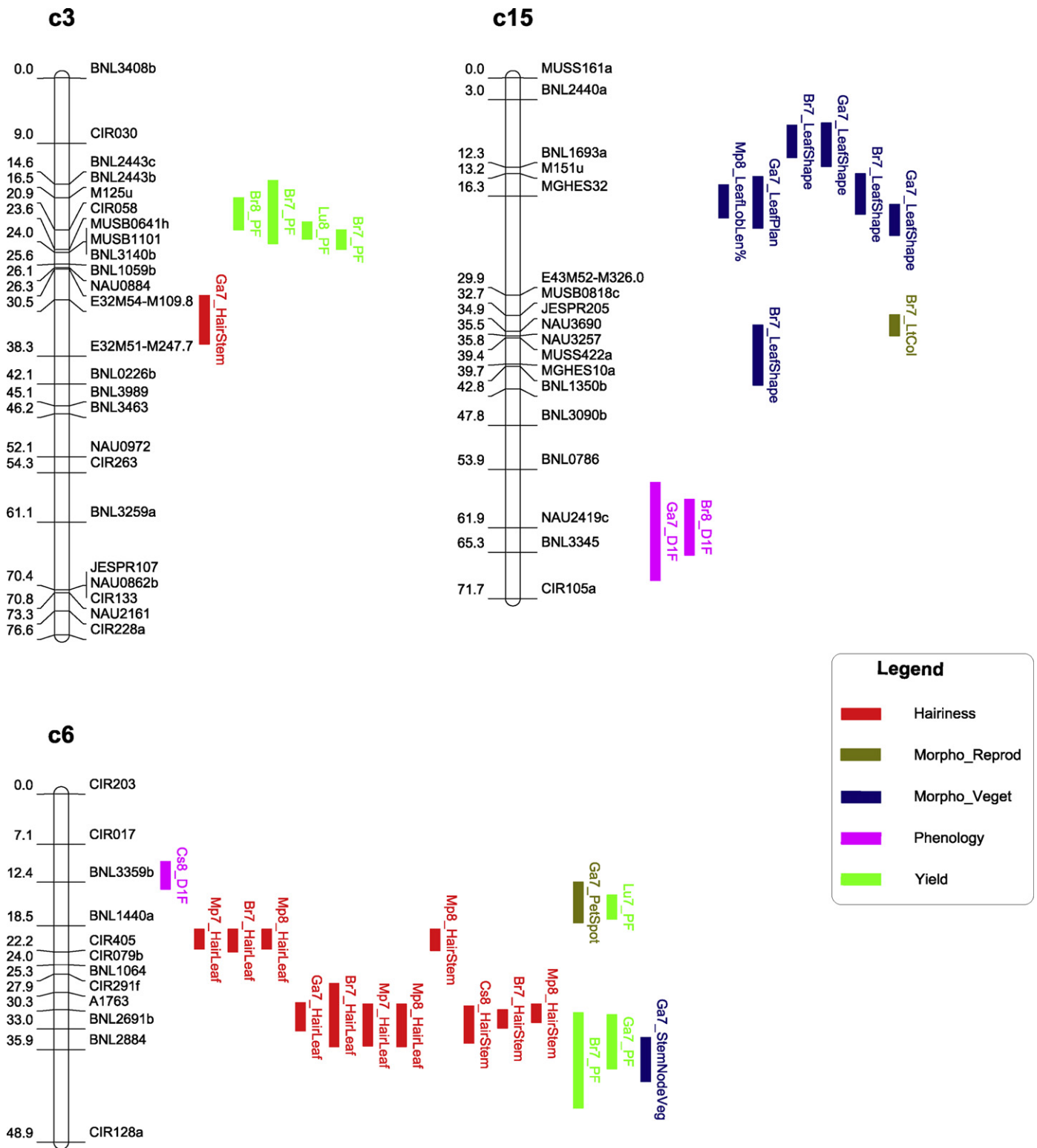
#### 3.3.1. Leaf and stem hairiness

Leaf and stem hairiness were assessed in 4 data sets and detected 22 significant QTLs. Half (11) of the 22 hairiness QTLs mapped along a central region of c6, all with high LOD scores and consistent parental effect with higher hairiness contributed by the *Gh* parent (Gua). These QTLs on c6 originated from all data sets and mapped within a fairly narrow distance (between 21 to 32 cM of the RIL map). The apparent clustering in 2 groups of 4 and 7 QTLs might be an artifact of mapping and possibly corresponded to a single locus. Our QTLs on c6 confirmed previous QTL data from backcross generations involving the same parents (Lacape and Nguyen, 2005), both analyses pointing toward the position of the known *t1* locus near the centromere of c6 (Endrizzzi et al., 1984). The two closest markers mapped on the RIL map are CIR291 and BNL4108, the latter being a bridge marker with another map were *t1* was also localized (Wan et al., 2007). Besides c6, other significant hairiness QTLs of lower LOD and *R*<sup>2</sup> values were detected on c2 (6 QTLs, 4 data sets), c3 (1 QTL), c13 (3 QTLs, the only chromosome for which VH8 contributed positively to leaf hairiness) and c25 (1 QTL); all except c3 confirming hairiness QTLs from the literature (Wright et al., 1999; Lacape and Nguyen, 2005).

#### 3.3.2. Morphology of vegetative parts

Apart from hairiness, the leaves of the 2 parents also differ in their size (smaller in Gua) and their degree of incision and planarity, with deeper lobes in VH8, close to the semi-okra phenotype (Supplementary Fig. S1). Leaf shape scored from 2 data sets mapped 6 QTLs. An upper region of c15 mapped 5 QTLs for leaf morphology (leaf shape), with high LOD scores (as high as LOD13.9 in Br7 set), of which 2 pairs of QTLs were reasonably co-localized (Fig. 2). The clustering on c15 near locus BNL1693 coincided with a cluster of QTLs related to leaf morphology in Jiang et al. (2000) using an *F*<sub>2</sub> population of a cross between 2 different *Gh* and *Gb* parents, with locus A1485 in common between the 2 maps. In Jiang et al. (2000) this region of c15 was inferred to encompass the *Okra-leaf* gene position on the classical map (Endrizzzi et al., 1984). Another QTL on c1 mapped a QTL for leaf shape at a position homoeologous to that on c15, as demonstrated by the fact that the same SSR marker BNL1693 mapped homoeologous positions. Our 2 QTLs for leaf size confirmed positions also reported in Jiang et al. (2000): on c5 (various leaf-related traits co-localising with a QTL with a high LOD, 6.13, on our map), and c20. In addition to leaf morphology, we also mapped 3 QTLs related to leaf color (c5, c23, c26).

Overall plant morphology was assessed by 4 different traits: the number of vegetative nodes and 3 traits from mid- to end season growth period: number and average length of main stem nodes



**Fig. 2.** Positions of QTLs on the RIL map with 3 examples shown: – c3 with 4 QTLs for percent fiber as measured on 3 different sites and one QTL for stem hairiness, – c15 with 10 QTLs, including 1 QTL for linter color, 2 QTL for date of 1st flower, and 7 QTL for different leaf morphology traits, and – c6 with 19 QTLs, including 11 QTLs for hairiness, 1 for petal spot, 1 for D1F, 1 for number of vegetative nodes, 2 for fiber parameters and 3 for fiber percent. Details of QTLs are shown in Supplementary Table S1 and figures for all chromosomes are shown in Supplementary Fig. S2. Traits were grouped according to 6 different categories shown in different colors (see legend). The name of the QTL indicates data set (site and year), trait acronym, chromosome, LOD score and sign of additivity ('+' or '-' refers to the effect of the *Gh* (Guazuncho) and *Gb* (VH8) alleles).

and plant height, from 2 to 4 locations. These traits mapped 18 QTLs, often co-localized or nearly co-localized in accordance with the relationship between traits: on c7 (QTLs related to fewer and longer nodes with reduction by *Gua* parent), c8 (QTLs for higher

plant height contributed by *Gua*), c14, c21 and c25 (in each case, 2 QTLs related to an increased height and more stem nodes contributed by *Gua*). Solitary QTLs were mapped on c6, c16, c19 all from different trial locations. The only tentative confirmation of



a plant height-related QTLs from the literature was for the QTL for length of main stem nodes on c16 (or D7) that possibly corresponds with an earlier report from Song and Zhang (2009).

### 3.3.3. Morphology of reproductive parts

Confirmed locations were found for 3 flower traits of simple inheritance that neatly differentiate the 2 parents (Supplementary Fig. S1): petal spot (present in VH8, absent in Gua), petal color (creamy in Gua, bright yellow in VH8) and pollen color (creamy in Gua, bright yellow in VH8), all assessed from 4 data sets. The 3 traits were scored quantitatively on a 1–3 (petal and pollen color) and 1–5 (petal spot intensity) scale. The total QTLs detected were 10, 10 and 9, for petal spot, petal color and pollen color, respectively. Co-localized QTLs were detected with high LOD scores confirming the positions inferred from classical mutant mapping (Endrizzi et al., 1985) for the 3 loci, i.e. *R2* mapped along a central region on c7 (7 QTLs for petal spot trait mapped as 2 putative clusters, all within 13 cM centered on locus *R2*), *Y1* along upper half of c13 (10 QTLs for petal color mapped as 3 putative clusters within a large 20 cM window centered on locus ‘pet’ on our map), and *P1* on bottom of c5 (6 nearly co-localized QTLs for pollen color mapped within 6 cM in a region centered on locus CIR253), respectively (Supplementary Fig. S2). Despite their simple inheritance, the quantitative scoring of these traits allowed the detection of significant QTLs of lower LOD at other map locations, as for example petal spot QTLs on c6, c23 and c24, and pollen color QTLs on c7, c8 and c26. These supplementary QTLs may be indicative of modifier genes/factors also influencing trait expression, as this could be expected from the known quantitative segregation and typical co-dominant mode of inheritance of expression of these traits (intensity of the petal spot and degree of yellowness of the petal and pollen).

Four QTLs for fuzz color were also mapped. Among the 2 QTLs on c21, the QTL mapped on top of the chromosome has high LOD (8.2) and mapped at a similar location as where a qualitative locus named *LTCOL* was mapped in the BC1 genetic map from the same two parents (Lacape et al., 2003).

The open bud trait is characterized by a pistil protruding out from the flower bud, the normal phenotype being a closed bud. Although the 2 RIL parents display a normal (pistil enclosed inside the bud) phenotype, open bud phenotypes have been reported in progenies originating from other inter-specific *Gh* × *Gb* crosses (Rhyne, 1979; Qian et al., 2009). The trait was observed as segregating in data sets (Br7 and Mp7). Four QTLs were mapped on c13, c18 (position confirmed in 2 data sets) and c24. Interestingly, the positions of QTLs with the higher LOD scores on homoeologous chromosomes c13 (1 QTL with LOD7.4) and c18 (2 QTLs with high LOD of 16.0 and 11.1 of same directionality from Br7 and Mp7, respectively) confirmed the locations of the 2 allelic mutant loci for open bud on the classical cotton genetic map (Endrizzi et al., 1985) that had also been confirmed by Qian et al. (2009). The positions of these QTLs on homoeologous chromosomes 13 and 18 are syntenic in regions delineated by 2 duplicated SSRs, BNL2652 and MUSB1263, on our map.

### 3.3.4. Phenology

The 2 parents of the RIL population displayed a marked difference in terms of earliness, Gua being earlier in flowering (57 days after planting, DAP, against 71 as averaged over 5 sites) and boll opening (105 DAP against 120 as averaged over 2 sites) than VH8 (Table 2). Earliness, as assessed by the dates of flowering (D1F) and first boll opening (D1B) resulted in 17 QTLs (11 for D1F and 6 for D1B) on 11 different chromosomes. Although the 2 traits are correlated (coefficient of correlation of 0.56 and 0.70 in Br7 and Ga7, on 123 and 76 data respectively, not shown) the only case of co-localizations of QTL for D1F and D1B was on c14, while for D1F the only co-localizations of QTLs from 2 or more data sets

were observed on c7 (2 QTLs), c14 (4 QTLs) and c15 (2 QTLs). Co-localization of the 2 QTLs for D1F on c7 coincided with a QTL for number of vegetative main stem nodes (same directionality), consistent with the observation that the number of vegetative branches is an indirect indicator of earliness (Ray and Richmond, 1966). The grouping of 5 QTLs on c14 (4 for D1F and 1 for D1B, all of similar directionality, Gua alleles contributing negatively, i.e. favoring earliness) delineated 2 possibly separate regions (distant by 25 cM) with 3 and 2 QTLs respectively. Of the 3 QTLs for flowering date reported by (Song and Zhang, 2009) in an interspecific *Gh* × *Gb* BC<sub>1</sub> population, only the one on c22 (designated D4 in that study) possibly coincided with one detected in this population (for D1B in Ga7 data set).

### 3.3.5. Yield components

Average boll weight (ABW) is an important component of yield potential that clearly differentiated the 2 parents (VH8 having smaller bolls, 3.6 g, against 6.4 g for Gua). Among the 10 QTLs related to ABW (including actual weight and qualitative size score), 3 were fairly co-localized on c10 (higher weight by Gua). Two other parameters also differentiated the 2 parents, boll locule number (typically 3 for VH8 and more generally in the species *Gb* and 4 for Gua and more generally in the species *Gh*) and PF (lower for VH8). Boll locule number, assessed in 4 data sets, mapped 7 solitary QTLs on 7 different chromosomes. Percent fiber (PF), as assessed in 7 data sets, mapped 24 QTLs on 11 different chromosomes, with 4 nearly co-localized QTLs (positive contribution by Gua in each case) on c3, three on c21, and two on c6, c9, c11 (the only case with co-localized QTLs with reverse additive effects), and c20. Notably, the 2 QTLs for PF in a central region of c6 (from Ga7 and Br7) were reasonably co-localized with leaf and stem hairiness QTLs (Fig. 2) and with the localization of the *t1* locus as was already reported in intra-specific populations (Guo et al., 2006; Wan et al., 2007). In 3 cases, QTLs for PF corroborated other reports from the literature: – in the bottom of c9 with the association of PF with the SSR marker BNL1317<sub>191</sub> (Zeng et al., 2009), – in a top region of c18 with 2 QTLs for PF mapped in the interval JESPR204-CIR221 (Wang et al., 2011), with positive effects by *Gh* alleles in both cases, and – in a top region of c20 near marker MUSS467 (Wang et al., 2011). Percent fuzz (Lt%) mapped 5 QTLs on different chromosomes, the most significant (LOD5.8) on c26 possibly corresponding to the known recessive naked seed gene *n2* described in *Gb* (Endrizzi et al., 1984), in accordance with our observations that F<sub>1</sub> hybrids of Gua (fuzzy seed) × VH8 (naked seed) and their progenies after backcross to the *Gh* parent all have fuzzy seeds.

### 3.3.6. Within-boll yield components

Traits in this category (Table 1) consisted of 7 within-boll traits that have an impact on the elaboration of overall fiber yield in the field. Two of these traits were measured over 2 or more sites, while 5 were measured on one experiment (Br7). Data from this experiment, the most comprehensive among the 10 with 123 RILs evaluated in a 2 replicates augmented bloc design, were averages from 2 measures. A total of 15 significant QTLs (14 from Br7 and 1 from Cs8 data sets) were detected for yield components, including seed number per boll (3 QTLs), seed index (1 QTL) and lint index (2), seed surface area (1), weight of fiber per seed surface area (5), and number of fibers per seed (2) or per unit of seed surface area (1). Individual traits revealed only a few QTLs, their LOD and effects were often high (max LOD is 7.1, max R<sup>2</sup> is 0.16), and positive parental effects always derived from the higher yielding Gua parent (Supplementary Table S1). The QTL for seed-index on c8 co-localized with a QTL for ABW (larger seeds and larger bolls contributed by Gua). Similarly on c10, QTLs for ABW and for seed number per boll (SN) co-localized. Chromosomes 12 and 19 represented other examples of co-localization of QTL for correlated

traits, involving in each case LI, LN/SSA and L/SSA (Supplementary Table S1 and Supplementary Fig. S1).

#### 4. Discussion

In this study we characterized an inter-specific RIL population for phenological and morphological traits, as well as for traits participating to overall fiber yield over several locations and years. The 2 parents of the RIL population were purposely selected to be highly contrasting, as the *Gh* parent, Guazuncho 2, is a cultivar of high fiber yield potential in South America while the *Gb* parent, VH8, has very low yield and was chosen essentially because of its unique fiber quality characteristics. The superior agronomic performance of Guazuncho may be related to a series of earlier known intrinsic characteristics encompassing an early flowering habit, the ability to produce a high number of fruiting bodies of larger size and a higher fiber percentage. However, the estimates we made of within-boll, within-seed, yield components, done for the first time in cotton over a segregating population, allow a more precise characterization of these differences and give an insight of the genetic bases of these characters. On a per-seed basis the *Gh* parent had more fiber mass and more fiber number per seed as well as per unit of seed surface area (Table 2): 73 vs. 48 mg/seed, 18,200 vs. 10,600 fibers per seed, 71 vs. 36 mg cm<sup>-2</sup>, and 17,800 vs. 8000 fibers per cm<sup>2</sup>. These values are extending the range of variation reported by Basal et al. (2009) who also compared a number of *Gh* and *Gb* accessions, and reported ranges of 11–13,000 and 12–15,000 fibers per cm<sup>2</sup> in the 2 species, respectively. Conversely, variation in fiber initials density at 0 dpa as reported in Romano et al. (2011) among cultivars of the 2 species were essentially overlapping, within ranges 29–33,000 and 32–40,000 in *Gb* and *Gh*, respectively. Within *Gh*, the variety Guazuncho 2 is intermediate between the 2 genotypes, DPL458 BR and FM966 studied in Bednarz et al. (2006) for fiber number per seed and per SSA (with similar ways of calculations), although its mass of fiber per SSA, 71 mg cm<sup>-2</sup> is higher than either of these varieties (53–59 and 63–67 mg cm<sup>-2</sup>, respectively, depending on plant density). These parameters are key contributors to overall fiber yield.

Most QTL reports in cotton have related to fiber quality characteristics (Shen et al., 2006; Rong et al., 2007; Qin et al., 2008; Luan et al., 2009; Wu et al., 2009; Lacape et al., 2010), while only a few reports have considered other traits, such as yield-related traits (Ulloa and Meredith, 2000; Shen et al., 2007; Wang et al., 2007; Liu et al., 2011). We located 166 QTLs for 27 different traits related to plant morphology, flowering phenology, and yield components. Although the relatively low number of individuals analyzed in this report has probably hampered the precision and accuracy in QTL detection (Beavis, 1994; Schön et al., 2004), it is likely that QTLs with highest effect (significance based on permutation test thresholds) had been detected, while environmental effect had been accounted-for in a number of cases due to replicate measurements at the same or different locations as evidenced by the co-localisation of many QTLs over sites and years. Some yield-related traits were measured over a single experiment (Br7), composed of 250 elementary plots under a replicated augmented blocs design. The results obtained from this set of data were congruent with each other, and also with data from other experiments when available. Furthermore, the LOD scores of the QTLs detected were all significant and usually high (Table S1), thus allowing an insight into the genetic nature of some important yield-related traits.

Some of the QTLs reported here represented confirmations of earlier reports with different germplasm, such as many of the QTL for morphological traits and for various descriptors of leaves and flowers that have relatively simple inheritance. For other traits, QTL

consistency was only partial with reports in the literature from *Gh* × *Gb* populations. Coincident QTL localizations were noted with those of Song and Zhang (2009) for QTL for earliness (c22-D4) and leaf size (c5) and with Wang et al. (2011) for QTLs for PF on c18 and c20. A QTL was reported for lint index in He et al. (2005) on the same chromosome, along the middle of c19 (named qLI17), but at a different location (here at the bottom) and of reverse additivity, while none of the five QTLs for seed index and 6 QTLs for number of seed per boll detected by He et al. (2005) corresponded to those in this study (1 for SI and 2 for SN). In another report, He et al. (2007) analyzed 69 F<sub>2:3</sub> families of a *Gh* × *Gb* cross and reported 8 QTLs for seed index, including one on c8 at the same location, but of reverse additivity, 4 QTLs for lint index, including one on c19 in common, but at a different location, and QTLs for number of seeds per boll that did not correspond with any detected here. Similarly none of the QTLs for different yield-related traits reported (Saranga et al., 2001; Mei et al., 2004) corresponded with ours. Lastly, (Zeng et al., 2009) identified associations of some SSRs marker alleles and phenotypic traits (PF, ABW, fiber quality) among multi-cross offspring, including PF on homoeologous c12 and c26 (not detected in this study) and c9 and c23 associated with BNL1317 as a linked marker. This marker was mapped on the RIL map on c9 where 2 QTLs for PF were also located.

Possible reasons why the various QTLs reports only partly agreed may be linked to the fact that species and genotypes differed (the *Gb* parent used in this study is relatively unique as it is an obsolete cultivar from the Caribbean Islands), but may also be related to the fact that most cotton QTL mapping populations are usually too small in size with a lower power detection (Beavis, 1998), including in this report, and finally also highlight some of the difficulties that cotton researchers' face in measuring particularly yield traits where extraneous factors – pests, diseases, soil type, plant nutrition etc., can have a significant impact on different yield components. This lack of congruency had been already noted in the case of QTL for fiber characteristics (Lacape et al., 2010), even though they are known to be of higher heritability than agro-morphological traits and yield components.

An association between a number of the traits measured was observed as significant phenotypic correlations (Table 4) and resulting QTL co-localizations. The most prominent cases can often be explained by the direct trait-to-trait dependences as, for example, between seed number (SN) and boll weight (ABW), as co-localized QTLs on c10 (2 and 3 QTLs respectively) and c4 (1 QTL of each), between lint per seed surface area (L/SSA) and percent fiber (PF), as co-localized QTLs on c5 or c21, and between fiber weight per seed (F/S), number of fibers per seed (FN/S), and fiber weight per seed surface area (F/SSA), as co-localized QTLs on c12 or c18.

Our study compared progenies from a cross between 2 highly contrasting genotypes of the species *Gh* and *Gb*. Direct utility of our QTL results in guiding selection within *Gh*, the most cultivated species, remains to be demonstrated as genetic determination of traits may differ between the intra- and the inter-specific breeding context. Globally, the number of bolls per unit of land has been described as the major determinant of fiber yield (Worley et al., 1974). However, in this study, we have tried to consider more basic yield components, as assessed at the level of a single boll or of a single seed. A predominance of GCA for yield-related traits such as LN/S and LN/SSA, among *Gh* cultivars has been found (Rahman, 2006), that enhances their responsiveness to selection. Bednarz et al. (2007) showed that breeding of Upland *Gh* cotton in the USA has led to modern cultivars with higher ginning out-turn, smaller seed and boll mass, with fewer seeds per boll and more bolls per square meter; and they concluded that “selection for increased seed lint mass per unit seed surface area may be the next reasonable selection criterion”. Similar lines of evidence were exemplified in

Bowman et al. (2001) who reported a predominance of additivity for the number of ovule fiber cells within *G. hirsutum*, suggesting a possibility for progress through combining diverse alleles in breeding. Coyle and Smith (1997) emphasized that maximization of lint yield among the most recent Upland US cultivars has resulted from 2 different directions, cultivar DP90, for example, combining high fiber weight per SSA with longer and heavier fibers, while Tamcot CAMD-E had a greater number of shorter fibers with a lower weight of fiber per unit of length. The variables under study all contribute to some extent to an ontogenic model for cotton yield as proposed in (Worley et al., 1976; Coyle and Smith, 1997). The understanding of the genetics of some of the basic traits determining yield, and the study of the correlations between these traits, may help breeders optimize each one of the yield components while minimizing negative correlations, and focus on those traits more amenable to selection (i.e. those with a less complex genetic basis).

## 5. Conclusion

This study has enriched a series of QTL reports related to cotton plant morphology as well as phenology. Simply inherited traits, like hairiness and other plant morphological descriptors were confirmed in their chromosomal localizations. Some of the QTLs are novel, and this study, for example, constitutes the first report of mapping of QTLs for earliness and percent fiber after ginning, two important traits for commercial cotton production, as well as QTLs related to within-boll yield components assessed at the seed level. Dissecting yield into its components, combined with component analysis, is expected to provide better insights on how the genetics of plant development affect crop performance, with key role for modern crop physiology in future breeding progress (Yin et al., 2004). General objectives of cotton breeding programmes should be, apart from breeding for better fiber quality, to select ideal genotypes (ideotype) which produce more lint per unit of land area from an optimal combination between a high number of seeds per unit of land area (the combined effect of the number of plants per unit of land area, of bolls per plant, and of seeds per boll), and the quantity of lint per seed or per seed surface area (could be through an increased number of individual fibers per SSA, or through combined changes in average length and fineness).

## Authors' contributions

Jje, CV, JML participated in the experiment conducted in Montpellier. Jja co-ordinated all Bayer CS contributions and conducted the experiment in Belgium. DB conducted the experiments in Lubbock (USA). DL and SL co-ordinated all CSIRO contributions. PO and SG conducted the RIL experiment in Cameroon. MG, PAVB and JHdA conducted the field experiments in Brazil. GG conducted fiber analyses, GG and JML conducted seed and fiber analyses for Br7 samples, and CV, TVC and JML conducted data analysis. JML conceived and coordinated the project, MG and JML drafted the manuscript. All authors contributed to the interpretation of the results, read and approved the final manuscript.

## Acknowledgements

Data on the various locations were collected by many colleagues that are all thanked by the authors. Experiments in Brazil and Cameroon were conducted thanks to the collaborative links of CIRAD with EMBRAPA and with IRAD, respectively. The French National Research Agency, ANR, through its program specialized in plant genomics, Génoplante, has sponsored this research (project nr ANR-06-GPLA-018).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fcr.2013.01.001>.

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