

Field evaluation of cotton near-isogenic lines introgressed with QTLs for productivity and drought related traits

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Abstract Quantitative trait loci (QTLs) for yield and drought related physiological traits, osmotic potential (OP), carbon isotope ratio ($\delta^{13}\text{C}$, an indicator of water use efficiency), and leaf chlorophyll content (Chl), were exchanged via marker-assisted selection (MAS) between elite cultivars of the two cotton species *Gossypium barbadense* cv. F-177 and *G. hirsutum* cv. Siv'on. The resulting near isogenic lines (NILs) were examined in two field trials, each with two irrigation regimes, in order to (1) evaluate the potential to improve cotton drought resistance by MAS and (2) test the role of physiological traits in plant productivity. NILs introgressed with QTLs for

high yield rarely exhibited an advantage in yield relative to the recipient parent, whereas a considerable number of NILs exhibited the expected phenotype in terms of lower OP (5 out of 9), higher $\delta^{13}\text{C}$ (4 out of 6) or high Chl (2 out of 3). Several NILs exhibited considerable modifications in non-targeted traits including leaf morphology, stomatal conductance and specific leaf weight (SLW). In *G. barbadense* genotypes, yield was correlated negatively with $\delta^{13}\text{C}$ and OP and positively with stomatal conductance, SLW and Chl, whereas in *G. hirsutum* yield was negatively correlated with $\delta^{13}\text{C}$, SLW and Chl. This dissimilarity suggests that each of the respective species has evolved different mechanisms underlying plant productivity. We conclude that the improvement of drought related traits in cotton NILs may lead to improved drought resistance via MAS, but that conventional breeding may be necessary to combine the introduced QTL(s) with high yield potential.

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Abbreviations

Chl Leaf chlorophyll content
GB *Gossypium barbadense*
GH *Gossypium hirsutum*
MAS Marker assisted selection
OA Osmotic adjustment

OP	Osmotic potential
QTL	Quantitative trait locus
SLW	Specific leaf weight
WUE	Water use efficiency
$\delta^{13}\text{C}$	Carbon isotope ratio

Introduction

Cotton (*Gossypium* spp.; *Malvaceae* family) is the world's leading fiber crop (<http://www.fao.org>) and among the most important oilseed crops. *G. hirsutum* L. and *G. barbadense* L. (noted hereafter as *GH* and *GB*, respectively), the two predominant elite cotton species, are both allotetraploids ($2n = 4x = 52$) that combine the 'A' and 'D' genomes of diploid species into a single 'AD' nucleus (Wendel 1989). Cotton is usually grown during the summer in arid and semiarid regions where water availability is often limited. Estimates indicate that cotton represents more than half of the irrigated agricultural land in the world (<http://www.panda.org>). Regardless of whether it is irrigated or not, cotton is often exposed to drought, which adversely affects both yield and lint quality (Pettigrew 2004).

Drought, induced by soil and/or atmospheric water deficit, poses the most important environmental constraint to plant survival and crop productivity (Boyer 1982). With increasing aridity and population growth, water is expected to become even scarcer in the near future (Chaves et al. 2003). Developing drought resistant crop plants is vital to meeting increased demand for agricultural products and mitigating the effects of an anticipated environmental shift towards greater aridity (Plucknett et al. 1987; Parry et al. 2005). This solution, however, requires comprehensive understanding of plant adaptive mechanisms and responses to water stress at their underlying physiological and genetic mechanisms.

Plants subjected to water stress show numerous physiological responses at the molecular, cellular, and whole-plant levels (Chaves et al. 2003; Shinozaki and Yamaguchi-Shinozaki 2007). Two drought related traits received special attention in the current study. Osmotic adjustment (OA), the active accumulation of solutes in response to water deficit resulting in reduced osmotic potential (Turner and Jones 1980; Blum 1988), has been suggested as an important trait in sustaining yield or biomass under water-limited conditions across different cultivars of crop plants

(reviewed by Blum 2005). OA helps to maintain cell turgor pressure and physiological processes as water deficits develop (Turner and Jones 1980; Morgan 1984). Selection for a single gene influencing OA was shown to increase yield under reduced water supply conditions (Morgan 2000). Water use efficiency (WUE), defined as dry matter or yield produced per unit of water consumed, is an important determinant of plant productivity under limited water supply (Endaie et al. 1991; Condon and Hall 1997). Carbon isotope ratio ($^{13}\text{C}/^{12}\text{C}$, expressed with a differential notation as $\delta^{13}\text{C}$) is commonly used as an indirect indicator of WUE (Farquhar and Richards 1984). However, high WUE (and $\delta^{13}\text{C}$) can be negatively, positively or not at all associated with yield (reviewed by Hall et al. 1994; Condon et al. 2004). Breeding *GB* cotton for high yield under irrigation in hot arid environments has substantially increased stomatal conductance in the absence of soil water stress (Cornish et al. 1991). In this respect, *GH* cultivars resemble those of a closely related species, *GB* (Radin 1992). As a result, modern cotton cultivars are characterized by extremely high stomatal conductance (Lu et al. 1994; Radin et al. 1994) and low WUE (Lu et al. 1996).

Breeding efforts to improve crop adaptation to water-limited conditions through direct selection have been hindered by the complex genetic basis of plant productivity and drought responses, characterized by low heritability and large 'genotype \times environment' (G \times E) interaction (Blum 1988; Ceccarelli and Grando 1996; Mittler 2005). Genomic approaches offer unprecedented opportunities to dissect quantitative traits into their single genetic determinants, quantitative trait loci (QTLs), thus enabling transfer of specific genomic regions between different genetic backgrounds through marker assisted selection (MAS) (Tuberosa and Salvi 2006). The application of QTL analysis provided opportunities to identify chromosomal regions controlling productivity under drought and related physiological traits, such as OA, canopy temperature, stay green, rooting depth, carbon isotope discrimination, and reduced leaf area (reviewed by Tuberosa and Salvi 2006; Cattivelli et al. 2008). However, only a few examples of MAS for traits associated with drought resistance have been reported (Cattivelli et al. 2008), and none have been reported in cotton.

In a previous study, QTLs associated with productivity and related physiological traits have been identified in inter-specific cotton (*GB* \times *GH*) populations under

water-limited and well-watered environments (Saranga et al. 2001, 2004). Productivity of cotton in well-watered versus water-limited conditions was largely accounted for by different QTLs, indicating that adaptation to both conditions can be combined into the same genotype (Saranga et al. 2001). QTL likelihood intervals for high seed cotton yield and low leaf osmotic potential corresponded in three genomic regions, implicating OA as a major component of improved cotton productivity under arid conditions (Saranga et al. 2004). QTLs for $\delta^{13}\text{C}$ showed only incidental association with productivity, indicating that high WUE can be associated with either high or low productivity. The finding that the *GH* allele was favorable at some loci and the *GB* allele at other loci illustrates how recombination of favorable alleles from different species may form novel genotypes that are better adapted to arid conditions than either of the parental species.

In this study, near isogenic lines (NILs) introgressed with selected genomic regions associated with productivity and physiological traits were developed and field tested in order to (1) evaluate the potential to improve cotton drought resistance by marker assisted selection and (2) test the role of physiological traits in plant productivity.

Materials and methods

Development of NILs

Seven genomic regions containing QTLs conferring productivity and drought related traits, osmotic

potential (OP), $\delta^{13}\text{C}$ and leaf chlorophyll content (Chl), were selected for the development of NILs (Table 1). A marker-assisted backcross program was conducted for the introgression of targeted regions using *GH* cv. Siv'on and *GB* cv. F-177 as donor and recipient genotypes. The procedure used well established protocols for genomic DNA extraction (Paterson et al. 1993) and RFLP marker analysis (Reinisch et al. 1994).

Donor genotypes were drawn from the original F_3 mapping population genotyped with 279 DNA markers. Plants containing the favorable allele at the targeted region, carrying a minimum of chromatin from the donor species (an average of about 40%), were backcrossed three times to the recipient parent to produce BC_3F_1 progenies. In BC_2F_1 as well as in BC_3F_1 progenies, 25 plants per target region were genotyped with the appropriate markers, flanking and within-target regions, to identify plants heterozygous at the target markers. For each target region, about 10 BC_3F_1 were selfed to produce 40 BC_3F_2 progenies, which were genotyped to identify plants homozygous at the target markers. Subsequently, BC_3F_3 progenies genotyped with a total of 105 microsatellite markers revealed an average 6.6% of the donor genome (including the target region).

Experimental design and growth conditions

The NILs and their parental genotypes were examined under two irrigation regimes (well-watered control and water-limited treatment) in two field trials conducted at two different locations and years,

Table 1 QTLs targeted for making near-isogenic lines in the two cultivated cotton species *G. barbadense* (*GB*) and *G. hirsutum* (*GH*)

Target region	Chromosome and flanking markers	Length of interval (cM)	Associated traits	Recipient parent	# of tested NILs	
					Trial 1	Trial 2
#1	LGA02 (pAR792, M1688, pGH232a)	68.6	SC ^w , OP ^d , Chl ^w	<i>GB</i>	4	3
#2	Chr. 06 (pAR936, G1099)	43.4	SC ^d , OP	<i>GB</i>	5	3
#5	Chr. 02 (A1325, pAR451)	48.5	SC ^d	<i>GB</i>	4	3
#9	LGD05 (pAR3-42, A1210, pAR239)	46.8	$\delta^{13}\text{C}^d$, Chl	<i>GB</i>	3	3
#3	Chr. 25 (pAR969, pGH309, pAR839)	49.5	SC, OP ^d	<i>GH</i>	4	3
#8	Chr. 22 (pAR243, pAR757)	25.9	$\delta^{13}\text{C}^d$, Chl	<i>GH</i>	4	3
#10	LGA06 (a1474, pGH550, PXP3-89)	38.8	$\delta^{13}\text{C}$	<i>GH</i>	4	0

Traits are abbreviated as: Chl, leaf chlorophyll content; OP, osmotic potential; SC, seed cotton yield; $\delta^{13}\text{C}$, carbon isotope ratio

^{d, w} Indicate QTLs detected only under water-limited or well-watered environment, respectively

(four environments in total). A total of 28 NILs introgressed with seven target regions were tested in trial 1. Based on phenotypic evaluation, 18 NILs carrying six genomic regions were tested in trial 2 (Table 1). Trial 1 was sown on April 9, 2006, at the experimental farm of the Hebrew University of Jerusalem in Rehovot (31°54' N, 34°47' E). At this location the soil is brown-red degrading sandy soil (Rhodoxeralf) composed of 76% sand, 8% silt and 16% clay. Trial 2 was sown on March 30, 2007 at Bnei-Darom farm (31°50' N 34°41' E). The soil at this location is sandy clay composed of 51.3% sand, 6.9% silt and 41.8% clay. Both Rehovot and Bnei-Darom are located in the coastal plain of Israel. In both trials, temperature during the field experiment varied between a minimum of 8–18°C to a maximum 27–35°C, in April and July, respectively. A split-plot factorial (line \times irrigation regime) block design with six replicates was employed, with irrigation treatment in main plots and genotypes in sub-plots. Each sub-plot consisted of a single row 2.25 \times 1.5 m (length \times row spacing) or 4 \times 0.96 m in trial 1 and 2, respectively, with \sim 9 plants per m of row.

The amounts of water applied to the well-watered treatment were calculated according to “Class A” pan evaporation multiplied by crop coefficients, consistently with commercial cotton practices. Fine adjustments of water application were made to maintain the recommended daily growth rates of the main stem (reflecting plant water status). The water-limited treatment was irrigated with the amount of water required to maintain half of the recommended daily growth rates. Irrigation was applied via a drip system. In trial 1, irrigation was applied twice a day (due to low stored moisture of the sandy soil), with total amounts of 653 and 357 mm for the respective treatment. In trial 2, irrigation was applied twice a week (as acceptable in commercial cultivation), with total amounts of 572 and 292 mm, respectively.

Phenotypic measurements

Physiological measurements were conducted during flowering, unless indicated differently, using the youngest fully expanded leaf. Leaf chlorophyll content (Chl) was measured four (trial 1) or two (trial 2) times per season using a chlorophyll meter (SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan). Samples

for measurements of turgid OP were collected in early morning. A whole leaf was sampled from each plot, placed in a test tube with its petiole immersed in distilled water and rehydrated for 5 h at 4°C (to obtain full turgor). Subsequently, leaves were frozen in liquid N and kept at -18°C until measured. Samples were defrosted, and OP of the leaf sap was assessed using a vapor-pressure osmometer (Model 5520; Wescor Inc., Logan, UT, USA). Osmotic adjustment (OA) was calculated as the difference in OP between well-watered and water-limited plants (Blum 1989; Turner et al. 2007). Carbon isotope ratio ($\delta^{13}\text{C}$) and specific leaf weight (SLW) analysis were conducted using the same 11-mm diameter leaf discs. Three leaf discs were sampled from the youngest fully expanded leaf and from each of the two leaves below it at \sim 10% boll ripening stage. Leaf discs were oven-dried, weighed (for SLW), powdered, and $\delta^{13}\text{C}$ was determined as previously described (Saranga et al. 1998). Stomatal conductance was measured twice in trial 1 at early afternoon between 13:00 and 15:00 using a steady-state porometer (LI-1600; LI-COR, Lincoln, NE USA). Stomatal density was assessed for the well-watered treatment of trial 1, using a single leaf per plot. Three nail polish copies were taken from the abaxial side of each leaf and analyzed under a light microscope (Axiovert 200M; Zeiss, Germany). Digital photographs were taken and the number of stomata per mm^2 was determined. Leaf size measurements were conducted in trial 2 using the youngest fully expanded leaf and two leaves below it, sampled from one plant in each plot and measured with a leaf area meter (LI-3100, Li-Cor, Lincoln, NE, USA). Yield was harvested manually at full boll opening, from 1 or 2 m of plants row in trials 1 and 2, respectively. The harvested seed cotton was ginned with a miniature gin and lint yield was determined. Since seed cotton yield and lint yield were closely related, only the lint yield is presented throughout this paper.

Statistical analysis

The JMP[®] 5.0 statistical package (SAS Institute 2005) was used for statistical analysis. The 18 NILs tested in both trials as well as the parental genotypes were subjected to analysis of variance. A factorial split-plot model was employed separately for each trial and genotype group (recipient parent and its

derivative NILs). Analysis of variance was applied with genotype (G) and irrigation (I) as fixed effects, and block (B) and B \times I as random effects. The effect of irrigation was tested against B \times I (error a), whereas genotype and G \times I interaction were tested against the residual (error b). Student's *t*-test was used to compare between each NIL and its recipient parent separately under each environment.

Linear correlation analyses with data of all the tested genotypes (30 and 20 in trials 1 and 2, respectively) were employed to study the association between lint yield and the various physiological variables. Separate correlation analysis was applied for each group of genotypes under each trial and irrigation treatment.

Results

Phenotypic differences between NILs and their recipient parents

Analysis of variance, carried out separately for each group of the cotton genotypes (*GB* and *GH*), usually

revealed highly significant effects of genotype and irrigation (Tables 2–3). The G \times I interaction was also significant in most cases, hence, comparisons between the NILs and their recipient parents were done separately for each of the irrigation regimes in each trial.

Lint yield of the recipient parents, Siv'on and F-177, under the well-watered treatments was within the acceptable range obtained in Israel by commercially grown irrigated cotton (Table 4). In trial 1, the average lint yield (across all genotypes) under water limitation was 40% lower in *GB* and *GH* genotypes as compared with the well-watered treatment. In trial 2, however, average lint yield was increased by 9% in *GB* and reduced by 9% in *GH* under the water-limited treatment.

Four sets of NILs (#s 1, 2, 3 and 5) were introgressed with QTLs associated with improved productivity (Table 1). Two NILs carrying target region #1 (1-2 and 1-4) exhibited in the trial 1 well-watered treatment significantly or nearly significant higher yield as compared with the recipient parent. However, this trend was not consistent in the other

Table 2 Analysis of variance for variables examined in trial 1 for two groups of cotton (*G. babadense* and *G. hirsutum*) NILs and their recipient parents

Source of variation	df ^a	F Ratio					
		Lint	OP	$\delta^{13}\text{C}$	Chl	SLW	Stomatal conductivity
<i>G. babadense</i> genotypes							
Genotype (G)	12 (11)	9.98***	7.81***	16.03***	10.73***	10.33***	9.36***
Irrigation (I)	1 (1)	112.55***	278.66***	24.84**	122.85***	5.49	31.08*
G \times I	12 (11)	3.07***	2.66**	2.85**	1.45	1.32	3.98***
Block (B)	5 (3)	6.71*	6.13*	4.86*	0.51	0.24	0.03
Error a (B \times I)	5 (3)	1.63	1.46	1.79	2.60*	6.10***	2.61
Error b (residual)	119 (65)						
<i>G. hirsutum</i> genotypes							
Genotype (G)	6 (6)	40.54***	12.12***	7.95***	11.06***	36.41***	6.97***
Irrigation (I)	1 (1)	9.26*	410.34***	10.36*	368.34***	21.73**	56.04***
G \times I	6 (6)	5.27***	2.83*	0.51	2.54*	1.63	3.74**
Block (B)	5 (3)	0.99	10.24*	1.49	2.66	10.68**	3.54
Error a (B \times I)	5 (3)	5.46***	1.05	2.44*	0.65	1.35	1.13
Error b (residual)	60 (36)						

Traits are abbreviated as: Lint, lint yield; OP, osmotic potential; $\delta^{13}\text{C}$, carbon isotope ratio; Chl, leaf chlorophyll content; SLW, specific leaf weight

^a df number for stomatal conductance are indicated in parenthesis

*, **, *** Indicate significance at $p \leq 0.05$, 0.01 and 0.001, respectively

Table 3 Analysis of variance for variables examined in trial 2 for two groups of cotton (*G. barbadense* and *G. hirsutum*) NILs and their recipient parents

Source of variation	df ^a	F ratio				
		Lint	OP	$\delta^{13}\text{C}$	Chl	SLW
<i>G. barbadense</i> genotypes						
Genotype (G)	12 (12)	29.30***	3.23***	9.62***	5.77***	4.27***
Irrigation (I)	1 (1)	3.47	24.61**	0.84	113.36***	129.92***
G × I	12 (12)	3.48***	2.67**	1.51	1.71	1.88*
Block (B)	5 (3)	0.35	0.40	0.64	1.08	0.77
Error a (B × I)	5 (3)	2.61*	6.36***	6.75***	2.80*	2.18
Error b (residual)	119 (70)					
<i>G. hirsutum</i> genotypes						
Genotype (G)	6 (6)	29.00***	1.70	1.24	12.91***	6.10***
Irrigation (I)	1 (1)	7.82*	35.90*	4.90	156.59***	60.91***
G × I	6 (6)	2.96**	1.21	9.10***	2.91*	2.53*
Block (B)	5 (3)	4.83*	1.89	1.43	1.02	0.38
Error a (B × I)	5 (3)	0.82	1.48	1.94	1.76	2.69*
Error b (residual)	60 (33)					

Traits are abbreviated as: Lint, lint yield; OP, osmotic potential; $\delta^{13}\text{C}$, carbon isotope ratio; Chl, leaf chlorophyll content; SLW, specific leaf weight

^a df number for $\delta^{13}\text{C}$ are indicated in parenthesis

*, **, *** Indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively

tested environments (Table 4). The NILs introgressed with target regions #2 and #3 usually did not differ from their recipient parent in trial 1 and exhibited significantly lower yields in trial 2. NILs introgressed with target #5 (QTL associated with higher productivity) as well as with targets #8 and 9 (no productivity QTL) exhibited in most cases lower yield as compared with their recipient parents.

Osmotic potential (OP) under the well-watered treatment of trial 1 ranged from -0.9 to -1.2 MPa, and under water-limited treatment from -1.3 to -1.9 MPa (Table 5). Higher OP values were obtained in trial 2, ranging from -0.8 to -1.0 MPa under control conditions and from -1.0 to -1.5 MPa under water limitation. Nevertheless, OA values were similar in both trials ranging between 0.1 and 0.7 MPa.

Three sets of NILs (#s 1, 2 and 3) were introgressed with target regions conferring lower OP (Table 1). All NILs carrying target region #1 exhibited in both trials under the water-limited treatment significantly lower OP (nearly significant in NIL 1-2) as compared with their recipient parent (Table 5). The lower OP was associated with greater OA, which was found significant in several cases. NILs carrying

target region #2 did not differ significantly from the recipient parent in OP, yet in several cases they exhibited significantly greater OA values. Among the NILs introgressed with target region #3, one line (3-2) exhibited in trial 1 lower OP and greater OA as compared to the recipient parent, however, opposite trends were found in trial 2. A similar phenomenon was found for all three NILs introgressed with target region #8, in which OP QTLs were not detected previously. NILs introgressed with targets #5 and 9 (not containing OP QTLs) did not show an advantage in OP in any trial or environment.

Carbon isotope ratio ($\delta^{13}\text{C}$) ranged from -25.7 to -27.9% in trial 1, whereas lower values were obtained in trial 2, ranging from -26.3 to -29.4% (Table 6). Two sets of NILs were introgressed with target regions conferring higher $\delta^{13}\text{C}$: #9 with the *GB* recipient parent and #8 with the *GH* recipient parent (Table 1). All NILs carrying target region #9 showed significantly greater $\delta^{13}\text{C}$ in all tested environments (except 9-2 in the water-limited treatment of trial 2). Among NILs carrying target region #8, only one NIL (8-1) exhibited significantly higher $\delta^{13}\text{C}$ under well-watered conditions in both trials (Table 6).

Table 4 Lint yield of cotton NILs and their recipient parents F-177 (*G. barbadense*) and Siv'on (*G. hirsutum*) under four tested environments

Genotype	Lint yield (g m ⁻²)			
	Trial 1		Trial 2	
	Well-watered	Water-limited	Well-watered	Water-limited
<i>G. barbadense</i> genotypes				
1-1	147	82 (0.06)	190	176
1-2	174*	103	209	162*
1-4	171 (0.06)	100	143***	206
2-2	158	97	47***	98***
2-4	121	112	117***	156**
2-5	148	80*	82***	95***
5-2	77***	55***	99***	131***
5-3	115 (0.09)	74**	179	167*
5-4	91***	73**	67***	101***
9-1	135	70**	113***	142***
9-2	132	69**	159*	151**
9-3	130*	47**	92***	82***
F-177	140	108	198	199
<i>G. hirsutum</i> genotypes				
3-1	214	174	220*	189 (0.09)
3-2	223	141**	194*	171**
3-3	209	168	177***	211
8-1	87***	103***	129***	112***
8-3	202	146**	150***	151***
8-4	112***	95***	153***	123***
Siv'on	216	181	245	216
Mean yield				
<i>GB</i> genotypes	134	82	130	144
<i>GH</i> genotypes	181	144	181	167

In bold are NILs introgressed with QTLs for the targeted trait
 *, **, *** Indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively, between each NIL to the recipient parent. Probability levels between $0.05 \leq P \leq 0.1$ are indicated in parenthesis

Among the NILs that were not introgressed with $\delta^{13}\text{C}$ QTLs, higher $\delta^{13}\text{C}$ were manifested in NILs carrying target regions #5 and #2 (both treatments), whereas target region #3 was associated usually with reduced $\delta^{13}\text{C}$, except for the trial 2 control treatment showing the opposite trend.

Leaf chlorophyll contents (Chl) exhibited similar values in all NILs and recipient parents across years (Table 7). Three NILs were introgressed with target region #1, which was associated with high Chl, two of which (1-2, 1-4) exhibited higher Chl compared with the recipient parent (significant in one or two environments). Target regions #8 and #9, which were selected primarily for their high $\delta^{13}\text{C}$ in the mapping study, were also associated with low Chl. However, low Chl was found only in one case (line 9-1, well-watered), while high Chl was found in a few other cases (8-1, both

treatments; 8-3, well-watered) and no effect in the remaining cases. Among the NILs that were not introgressed with Chl QTLs, lower Chl was associated with target region #5 (both treatments) and target region #3 (water-limited), whereas target region #2 was associated with mixed trends.

Specific leaf weight (SLW) was not mapped in our previous study and hence it was not targeted in the current introgressions. Nevertheless, several target regions were associated with significant modifications in SLW as compared with their recipient parents (Table 8). NILs introgressed with target regions #5 and 3 exhibited lower SLW in most lines and environments, whereas NILs carrying target region #8 showed higher SLW in trial 1.

Stomatal conductance, which was not mapped in our previous study, was assessed in trial 1. Three sets

Table 5 Osmotic potential (OP) and osmotic adjustment (OA) of cotton NILs and their recipient parents F-177 (*G. barbadense*) and Siv'on (*G. hirsutum*) under four tested environments

Genotype	Osmotic potential and osmotic adjustment (Mpa)					
	Trial 1			Trial 2		
	OP, well-watered	OP, water-limited	OA	OP, well-watered	OP, water-limited	OA
<i>G. barbadense</i> genotypes						
1-1	-1.19	-1.86***	0.67**	-0.95	-1.39*	0.44
1-2	-1.16	-1.64 (0.06)	0.48	-0.99	-1.38 (0.07)	0.39
1-4	-1.16	-1.77*	0.61*	-0.92	-1.50**	0.58*
2-2	-0.97	-1.58	0.61*	-0.83	-1.37 (0.09)	0.54*
2-4	-1.07	-1.52	0.45	-0.98	-1.18	0.20
2-5	-1.02	-1.43	0.41	-0.82	-1.30	0.48*
5-2	-0.99	-1.33*	0.34	-0.86	-1.03 (0.09)	0.17
5-3	-1.11	-1.38	0.27	-0.90	-1.03 (0.08)	0.13 (0.09)
5-4	-1.06	-1.46	0.40	-0.91	-1.03 (0.07)	0.12 (0.09)
9-1	-0.98	-1.27**	0.29	-0.84	-1.11	0.27
9-2	-1.04	-1.39	0.35	-0.95	-1.19	0.17
9-3	-1.03	-1.47	0.44	-0.82	-1.24	0.42
F-177	-1.20	-1.49	0.39	-0.89	-1.20	0.31
<i>G. hirsutum</i> genotypes						
3-1	-0.91	-1.31	0.40	-0.87	-1.05*	0.18*
3-2	-0.97	-1.48*	0.51**	-0.88	-0.99**	0.11**
3-3	-0.96	-1.28	0.32	-0.88	-1.08*	0.20*
8-1	-1.07	-1.53**	0.46*	-0.87	-1.09 (0.07)	0.22 (0.08)
8-3	-1.10	-1.67***	0.57**	-0.87	-1.09*	0.22*
8-4	-1.02	-1.48*	0.46*	-0.91	-1.24	0.33
Siv'on	-1.00	-1.33	0.33	-0.88	-1.29	0.41

In bold are NILs introgressed with QTLs for the targeted trait

*, **, *** Indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively, between each NIL to the recipient parent. Probability levels between $0.05 \leq P \leq 0.1$ are indicated in parenthesis

of NILs carrying target regions #2, 5 and 9 on a *GB* background showed significantly lower stomatal conductance as compared with their recipient parent under well-watered conditions (Table 9). NILs introgressed with target region #1, also on *GB* background, exhibited similar stomatal conductance as compared with the recipient parent. Two sets of NILs carrying target regions #3 and 8 on a *GH* background did not show a consistent trend with respect to stomatal conductance.

Leaf morphology in two sets of NILs was considerably different from that of the recipient parent. NILs introgressed with target region #1 showed highly significant reduction in leaf size as compared with the recipient parent (Fig. 1a). NILs carrying

target region #3 exhibited significantly higher stomatal density (Fig. 1b) and smaller epidermal cells (Fig. 2) as compared with the recipient parent.

Association between lint yield and physiological traits

Correlation analyses were employed to study the association between lint yield and physiological traits. Analyses were conducted separately for each species under each environment. In trial 1, correlations between lint yield and physiological traits were significant or nearly significant in most cases (Table 10). Similar trends were obtained in trial 2, though usually not significant.

Table 6 Carbon isotope ratio of cotton NILs and their recipient parents F-177 (*G. barbadense*) and Siv'on (*G. hirsutum*) under four tested environments

Genotype	Carbon isotope ratio (‰)			
	Trial 1		Trial 2	
	Well-watered	Water- limited	Well-watered	Water- limited
<i>G. barbadense</i> genotypes				
1-1	-27.0**	-27.5	-29.0	-29.1 (0.09)
1-2	-27.8	-27.6	-29.4	-28.8
1-4	-27.9	-27.7	-29.4	-28.9
2-2	-27.5	-26.5**	-27.9**	-28.0
2-4	-27.2*	-27.0 (0.09)	-28.0*	-28.4
2-5	-27.8	-26.7**	-28.4	-27.4 (0.06)
5-2	-27.0**	-26.1***	-27.7**	-27.7
5-3	-26.7***	-26.6**	-27.6**	-27.9
5-4	-26.5***	-25.9***	-28.1*	-26.8**
9-1	-27.4 (0.09)	-26.8*	-27.3***	-26.9**
9-2	-26.7***	-25.7***	-27.3***	-27.6
9-3	-26.8***	-26.2***	-27.9*	-27.1**
F-177	-27.8	-27.4	-29.0	-28.3
<i>G. hirsutum</i> genotypes				
3-1	-27.6	-27.2 (0.07)	-27.3**	-28.1
3-2	-27.9*	-27.1	-27.5**	-28.0
3-3	-27.6 (0.07)	-26.9	-27.9	-27.5
8-1	-26.6*	-26.3 (0.08)	-27.5**	-27.5
8-3	-27.2	-26.7	-28.0	-27.3
8-4	-26.9	-26.5	-28.6	-26.3***
Siv'on	-27.2	-26.7	-28.4	-27.7

In bold are NILs introgressed with QTLs for the targeted trait

*, **, *** Indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively, between each NIL to the recipient parent. Probability levels between $0.05 \leq P \leq 0.1$ are indicated in parenthesis

A comparison between coefficients obtained with *GB* versus *GH* showed considerable differences. Chl and SLW, which were correlated with each other ($P < 0.05$, data not shown), exhibited positive correlation with yield in *GB* genotypes but negative correlations in *GH* genotypes. OP exhibited negative correlation with lint yield in *GB*, while positive or no association between these variables was found in *GH*. In agreement with this, the correlations between OA and yield in trail 1 exhibited positive trend in *GB* and negative trend in *GH*, thought in both cases it did not reach statistical significance. Stomatal conductance was positively correlated with lint yield in *GB*, but showed no association in *GH*. Finally, $\delta^{13}\text{C}$ was negatively correlated with lint yield in *GB* and *GH*, thus being the only variable showing similar association in both species. It worth noting that the physiological variables used represent leaf-level spot measurements, whereas yield integrates the whole plant and leaf performances over the entire season.

Discussion

Effects of irrigation treatments on plant performances

Two trials were conducted in the current study in different years and locations. Similar amounts of water were applied in both years in the well-watered and water-limited treatments, nevertheless plant responses to these water regimes differed considerably between years. In trial 1, the water-limited treatment exhibited 40% reduced lint yield as compared with the well-watered conditions. In trial 2, *GH* genotypes showed a significant (Table 3) but small reduction (9%) in yield under the water-limited treatment, whereas, yield of the *GB* genotypes was not significantly affected by irrigation regimes. Apparently, the well-watered treatment in trial 2 may have been over-irrigated, and hence the

Table 7 Leaf chlorophyll content of cotton NILs and their recipient parents F-177 (*G. barbadense*) and Siv'on (*G. hirsutum*) under four tested environments

Genotype	Chlorophyll content (SPAD values)			
	Trial 1		Trial 2	
	Well-watered	Water- limited	Well-watered	Water- limited
<i>G. barbadense</i> genotypes				
1-1	38.5	46.1	38.5	47.5
1-2	43.4*	45.6	37.5	49.1*
1-4	41.1	47.4	37.3	48.4*
2-2	37.2*	45.3	35.6*	43.7
2-4	41.8	47.9 (0.06)	37.8	51.9**
2-5	40.2	46.4	37.9	47.3
5-2	33.7***	40.7***	31.9***	41.6 (0.06)
5-3	36.4**	43.5	36.1 (0.08)	43.3
5-4	35.9**	44.0	36.6	42.0
9-1	37.9 (0.1)	46.1	36.7	45.1
9-2	38.5	44.5	37.6	44.5
9-3	40.5	47.0	38.7	47.4
F-177	40.0	45.4	39.3	44.9
<i>G. hirsutum</i> genotypes				
3-1	40.5	47*	37.8	46.5*
3-2	40.4 (0.09)	46.9*	37.9	47.0 (0.07)
3-3	40.9	47.9 (0.07)	36.2	43.2***
8-1	43.8	56.1***	43.2***	54.9**
8-3	44.0	50.5	40.7**	48.2
8-4	41.3	50.7	36.9	48.7
Siv'on	42.8	50.4	35.4	50.0

In bold are NILs introgressed with QTLs for the targeted trait

*, **, *** Indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively, between each NIL to the recipient parent. Probability levels between $0.05 \leq P \leq 0.1$ are indicated in parenthesis

respective water-limited treatment experienced a minor stress. This is supported by the higher OP values in trial 2 as compared with trial 1 (Table 5). Nevertheless, in both trials OP was significantly affected by the irrigation treatments. Despite the differences between the two trials with respect to lint yield, a high degree of similarity was observed between them in the physiological responses to water limitation.

Efficiency of marker-assisted selection for yield and physiological traits

The effect of QTLs on yield production was examined in 12 NILs (set #s 1, 2, 3, 5) of which only two lines (1-2 and 1-4) exhibited superior yield over the recipient parent under the trial 1 well-watered treatment (Table 4). Among nine NILs introgressed with OP QTL, four NILs (Table 5, targets #1, 2) exhibited significantly reduced OP under water-limited conditions or improved OA capacity in both trials, and one

NIL (3-2) exhibited such an effect only in trial 1. Among the six NILs introgressed with $\delta^{13}\text{C}$ QTLs, four NILs exhibited higher $\delta^{13}\text{C}$ under most tested environments. Out of the three NILs introgressed with QTL for high Chl, two exhibited higher Chl under specific environments compared with the recipient parent. In summary, while introgression of QTLs for higher yield had poor success, a considerable number of NILs introgressed with QTLs for physiological traits exhibited the expected phenotype.

The application of MAS for improvement of simply inherited traits, such as disease resistances and crop quality, is gaining considerable successes and increasing importance (Francia et al. 2005 and references therein). However, the complexity of polygenic traits, such as yield, makes MAS extremely difficult. MAS studies for yield improvement in crop plants resulted in opposite effects of those expected (Bouchez et al. 2002), non-significant effect (Kandemir et al. 2000; Reyna and Sneller 2001), or non-

Table 8 Specific leaf weight of cotton NILs and their recipient parents F-177 (*G. barbadense*) and Siv'on (*G. hirsutum*) under four tested environments

Genotype	Specific leaf weight (mg cm ⁻²)			
	Trial 1		Trial 2	
	Well-watered	Water- limited	Well-watered	Water- limited
<i>G. barbadense</i> genotypes				
1-1	6.4	7.1*	6.0	7.9
1-2	6.1	6.6	5.0**	7.5
1-4	6.1	7.0 (0.09)	6.0	8.3
2-2	5.7**	6.2	6.1	8.3
2-4	6.2	6.9	6.1	8.4
2-5	6.6	6.7	5.7	8.5
5-2	5.1***	5.5***	5.0**	6.5***
5-3	6.1	6.7	6.3	7.3
5-4	5.6**	6.1 (0.08)	5.8	6.9*
9-1	6.1	6.6	5.7	8.0
9-2	6.9 (0.08)	6.6	6.2	7.6
9-3	6.5	7.0 (0.07)	6.0	8.5
F-177	6.4	6.5	6.1	7.9
<i>G. hirsutum</i> genotypes				
3-1	5.5**	6.2***	6.9	9.1**
3-2	5.6**	6.6**	7.1	8.4***
3-3	5.9	6.6**	6.6	8.8**
8-1	8.2***	8.5**	8.2 (0.07)	10.3
8-3	7.4**	7.7	7.7	10.0
8-4	7.6***	7.9	7.0	10.8
Siv'on	6.4	7.6	7.3	10.3

*, **, *** Indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively, between each NIL to the recipient parent. Probability levels between $0.05 \leq P \leq 0.1$ are indicated in parenthesis

consistent effects across environments (Schmierer et al. 2004). There are only a few success examples in which introgression of QTLs for yield improvement resulted in consistent effects across environments, with one QTL (Tuinstra et al. 1998), or with a few out of several QTLs (Stuber 1995; Romagosa et al. 1999; Concibido et al. 2003). Compared to the poor efficiency MAS for yield, better success was reported for the less complex physiological traits such as root morphology (Shen et al. 2001; Steele et al. 2006), stay-green (Tuinstra et al. 1998; Harris et al. 2007), anthesis-silking interval (in maize) (Ribaut and Ragot 2007) and earliness (Bouchez et al. 2002).

The discrepancy between the predictions of mapping studies and the observed values in introgression lines could be derived from several sources (reviewed by Francia et al. 2005; Cattivelli et al. 2008): (i) change in the testing environments affecting $G \times E$ interactions; (ii) change in genetic background of the introgressed QTL from the donor to the final recipient

line, which can alter epistatic interactions between QTLs and other genetic factors; and (iii) small effects of each of the individual genes involved in the plant phenotype. In the current study, each of these factors could affect the performance of the NILs. In most tested variables, significant $G \times E$ interactions were found in both years (Tables 2, 3). This is in accordance with our mapping study in which a considerable number of QTLs were expressed under specific irrigation regimes (Saranga et al. 2004). Under each of the respective genetic backgrounds, OP and Chl exhibited contrasting association with yield (Table 10). The QTLs introgressed into our NILs explained usually <10% of the variability in the segregating mapping populations (Saranga et al. 2004). Weak effects are more difficult to assess particularly for traits with a strong $G \times E$ interaction. This implies that the effect of individual genetic regions is not readily identifiable and that several regions (i.e. QTLs) must be manipulated simultaneously in order to obtain a significant impact

Table 9 Stomatal conductance of cotton NILs and their recipient parents F-177 (*G. barbadense*) and Siv'on (*G. hirsutum*) under the two tested environments of trial 1

Genotype	Stomatal conductance (mol m ⁻² s ⁻¹)	
	Well-watered	Water-limited
<i>G. barbadense</i> genotypes		
1-1	1.8	0.9
1-2		
1-4	1.7	0.7
2-2	1.0***	0.7
2-4	1.3**	0.8
2-5	1.3**	0.7
5-2	0.9***	0.5
5-3	0.9***	0.9
5-4	0.6***	0.5
9-1	0.7***	0.4
9-2	0.7***	0.3
9-3	0.7***	0.5
F-177	1.9	0.6
<i>G. hirsutum</i> genotypes		
3-1	1.48	0.69
3-2	1.27	0.79
3-3	0.86**	0.72
8-1	0.94***	0.70
8-3	2.15 (0.08)	0.89
8-4	1.64	0.84
Siv'on	1.67	0.90

*, **, *** Indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively, between each NIL to the recipient parent. Probability levels between $0.05 \leq P \leq 0.1$ are indicated in parenthesis

(Cattivelli et al. 2008). Finally, we cannot preclude the possibility that non-targeted introgressions were also involved in determining the phenotype of the studied NILs.

Modifications of non-targeted traits

The modified expression of non-targeted traits in NILs carrying target regions #1 and 3 warrants special attention. NILs introgressed with target region #1, associated with Chl, OP and yield (Saranga et al. 2004), exhibited considerably lower leaf size as compared with the recipient parent (Fig. 1a) and high stomatal conductance, though not significantly different, from the recipient parent (Table 9). Lu et al. (1994) reported that selection for high yield in

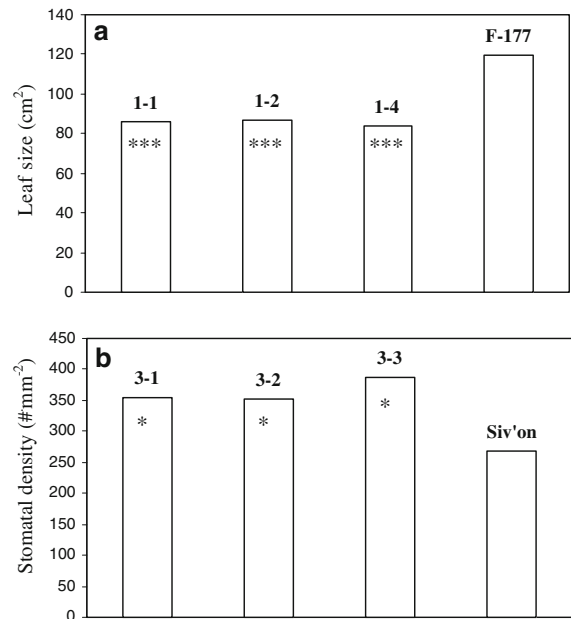


Fig. 1 (a) Leaf size of NILs introgressed with target region #1 and their recipient parent F-177 (*G. barbadense*). (b) Stomatal density of NILs introgressed with target region #3 and their recipient parent Siv'on (*G. hirsutum*). * and *** indicate significant difference between each NIL to its recipient parent at $P \leq 0.05$ and $P \leq 0.001$, respectively

advanced lines of *GB* cotton was associated with reduced leaf size and higher stomatal conductance. Crop cultivars selected for yield under water-limited environments often have constitutively reduced leaf area associated with smaller leaves (Blum 2005). Reduced leaf size caused by moisture stress was speculated to have led to greater concentration of 'photosynthetic machinery' (i.e. higher Chl values) per unit leaf area, which enables greater photosynthetic rate during the morning (Pettigrew and Gerek 2007). The concurrent effect of target region #1 on leaf size, Chl, OP and to some extent also lint yield, may indicate that these traits are under common genetic control. This suggests that genetic manipulation of NILs carrying this target region, aiming to eliminate negative epistatic effects, may result in improved lint yield.

NILs introgressed with target region #3, associated with OP and yield (Saranga et al. 2004), exhibited considerably lower SLW (leaf thickness; Table 8), higher stomatal density (Fig. 1b), smaller epidermal cells (Fig. 2) and, in some cases, also lower $\delta^{13}\text{C}$ (Table 6) as compared with the recipient parent.

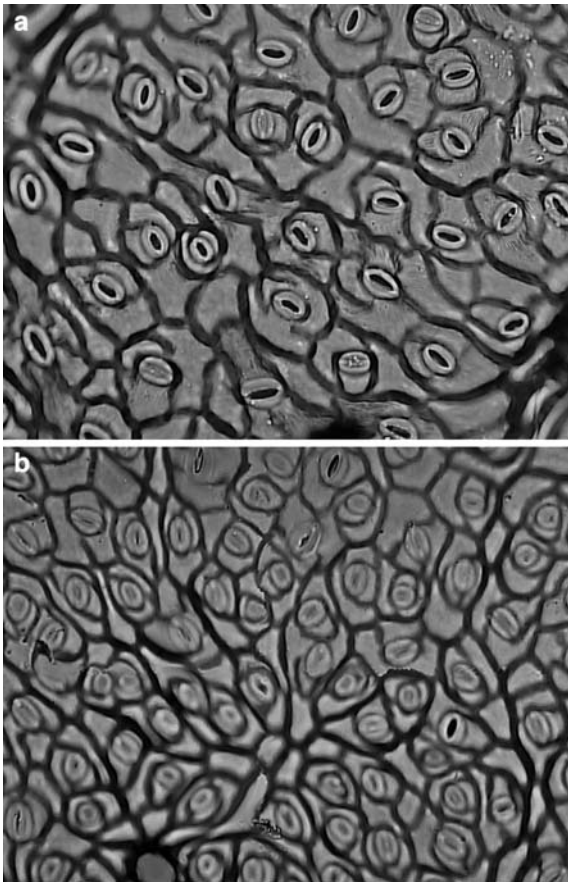


Fig. 2 Abaxial leaf surface of NILs introgressed with target region #3 (**b**) and its recipient parent *G. hirsutum* cv. Siv'on (**a**) showing differences in stomatal density and epidermis cell size

Arabidopsis mutant lines carrying null or non-functional copy of the *ERECTA* gene, which regulates transpiration efficiency, exhibited modified mesophyll anatomy, smaller epidermal cells, greatly increased stomatal density, and lower $\delta^{13}\text{C}$ (Mastle et al. 2005). These mutant lines did not exhibit systematic differences in leaf blade thickness, however, association between mesophyll anatomy differences and SLW or leaf thickness were previously reported in other studies (Syvertsen et al. 1995; Hanba et al. 1999) including *GH* cotton (Volkanburgh and Davies 1977). *ERECTA* homologs were identified in diverse species (Mastle et al. 2005). Using Tblastn program (Altschul et al. 1990), we found several cotton mRNAs showing a high sequence similarity (>80%) to the *ERECTA* protein (CottonDB URL; <http://cottondb.org/cdbhome.html>), indicating a highly conserved function of this protein in cotton. It is therefore possible that an *ERECTA*

homolog is involved in target region #3, thus influencing SLW (leaf thickness), stomatal density and $\delta^{13}\text{C}$.

Associations between lint yield and physiological variables in *GB* versus *GH* genotypes

In the original mapping population, QTL likelihood intervals for low OP corresponded with QTLs for high yield in three genomic regions, suggesting a partly common genetic basis of these traits (Saranga et al. 2004). In agreement with this, under the severe stress imposed in trial 1, low OP and high OA were associated with higher lint yield in *GB* genotypes, whereas in the *GH* the opposite trends were found (Table 10). Both turgid OP measured under water-limited conditions and OA may represent the active solute accumulation in response to water deficiency, as reflected by the highly significant correlations between these two variables ($P < 0.0001$, in all trials and genotype groups, data not shown). However, OA is calculated from two independent OP measurements, and therefore it is subjected to a greater (~double) experimental error. This reduces the association between OA and yield, compared with OP versus yield, as found in the current study. Previous findings on the relation between OA and yield were inconsistent, showing either positive (Reviewed by Blum 2005), negative (Singh et al. 1990), or no association between these traits (Leport et al. 1999; Turner et al. 2007), as reported in *GH* cotton as well (Leidi et al. 1999). Serraj and Sinclair (2002) claimed that crop yields benefit from OA particularly under extremely low-yielding environments, however, some benefit may be observed in cases where OA increases the extraction of water from deep soil layers.

Previous studies showed that advances in yield of *GB* cotton during the second half of the Twentieth century were associated with increased photosynthetic rate and heat avoidance via increased stomatal conductance (Cornish et al. 1991; Lu et al. 1994; Radin et al. 1994) which is detrimental to WUE (Lu et al. 1996). In agreement with this, in our *GB* genotypes higher lint yield was associated with higher stomatal conductance and lower $\delta^{13}\text{C}$ (Table 10), with the two latter variables being negatively correlated with one another ($P < 0.05$, data not shown). Our results suggest that the unfavorable association between yield and stomatal conductance in *GB* cotton

Table 10 Coefficients of correlation (r) between lint yield and drought-related physiological traits in cotton NILs and their recipient parents F-177 (*G. barbadense*) and Siv' on (*G. hirsutum*) under four tested environments

Physiology traits	Trial 1		Trial 2	
	Control $n = 17$	Water-limited $n = 17$	Control $n = 13$	Water-limited $n = 13$
<i>GB</i> genotypes				
Osmotic potential	-0.46 (0.06)	-0.44 (0.07)	-0.67*	-0.25
Osmotic adjustment		0.33		-0.05
Carbon isotope ratio	-0.64**	-0.71**	-0.47	-0.73**
Chlorophyll content	0.68**	0.53*	0.38	0.26
Specific leaf weight	0.63**	0.26	-0.008	-0.04
Stomatal conductance ^a	0.47 (0.08)	0.50 (0.06)		
	$n = 13$	$n = 13$	$n = 7$	$n = 7$
<i>GH</i> genotypes				
Osmotic potential	0.19	0.63* ^b	0.14	-0.06
Osmotic adjustment		-0.23 ^b		0.03
Carbon isotope ratio	-0.54*	-0.53 (0.06)	0.03	-0.56
Chlorophyll content	-0.16	-0.51 (0.07)	-0.72 (0.07)	-0.64
Specific leaf weight	-0.76**	-0.66*	-0.51	-0.51
Stomatal conductance	0.05	-0.24		

*, **, *** Indicate significance at $P \leq 0.05$, 0.01 and 0.001, respectively. Probability levels between $0.05 \leq P \leq 0.1$ are indicated in parenthesis

^a $n = 15$

^b One outlier data point omitted, $n = 12$

was not 'broken' by introgression of the targeted *GH* genomic regions. Low OP is known to maintain leaf turgor pressure and stomatal conductance under water deficit (Serraj and Sinclair 2002). As opposed to *GB*, yield in *GH* was not related to stomatal conductance (or OP), whereas in both species yield was negatively correlated with $\delta^{13}\text{C}$.

Chl per unit of leaf area was highly correlated with SLW in both species. However, while in *GB* Chl and SLW were positively correlated with yield, an opposite trend was evident in *GH* (Table 10). SLW was positively associated with Chl also in wheat and barley (Araus et al. 1997a, b). In *GH* cotton differences in SLW reflected differences in leaf thickness (Volkanburgh and Davies 1977). Therefore, it is likely that changes in Chl in the NILs were derived from changes in SLW (leaf thickness). In *GH* cotton, Leidi et al. (1999) reported significant negative correlation between SLW and seed cotton yield whereas Lopez et al. (1995) reported a positive association. Thicker leaves, containing more photosynthetic tissue per unit leaf area, can result in higher

photosynthetic capacity thus enabling greater yield (see Araus et al. 1997a, b and references therein; Terashima et al. 2001). However, greater leaf thickness can be associated with increased mesophyll resistance (Syvertsen et al. 1995; Terashima et al. 2001), which limits CO_2 influx to carboxylation sites and reduces photosynthetic rate (Evans 1999) and productivity. A simulation study of cotton suggested that thicker leaves cause an increase in the sink strength of leaves leading to carbohydrate investment in leaves rather than in fruits (Landivar et al. 1983). It is therefore possible that while in *GB* genotypes high Chl and SLW enhanced photosynthetic capacity, in *GH* the opposite occurred due to increased mesophyll resistance or a different balance between vegetative and reproductive sinks.

Conclusions

The importance of this study arises from the concurrent introgression of QTLs for yield and major

drought related traits (OP, $\delta^{13}\text{C}$ and Chl) in the same NILs, which permitted direct testing of MAS efficiency for yield versus underlying physiological variables. We are aware of only two prior MAS studies in which QTLs for yield and a single physiological trait were targeted, ‘stay green’ in sorghum (Tuinstra et al. 1998) and earliness in maize (Bouchez et al. 2002). Another unique aspect of the current study is the mutual introgression between two cotton species, which enabled us to characterize the studied traits under two distinct genetic backgrounds and reveal the considerable differences between the respective species in physiological mechanisms underlying plant productivity.

Yield, the ultimate objective of most breeding programs, is known as a low-heritability complex trait, influenced by multiple gene networks and epistatic interactions among genetic elements, as well as between genetic and environmental variables. Breeding for yield under stress conditions is even more complex due to the difficulty to define and apply a precise set of environmental condition relevant to the range of naturally occurring stress scenarios. This emphasizes the power of genetic mapping, allowing the dissection of complex traits and distinguishing common heredity from casual associations (Paterson et al. 1988), which cannot be achieved by conventional approaches. Targeting specific genomic regions and characterizing their effects can enable the reconstruction of favorable loci into elite cultivars.

Some advantages and difficulties involved in the application of MAS for practical breeding are highlighted in the current study. Cotton NILs exhibited an improvement in drought related traits such as greater OA, higher $\delta^{13}\text{C}$, and reduced leaf size, compared with the recipient parents. From a purely mechanistic point of view such changes can be defined as drought tolerance (Specht and Williams 1984). However, from an empirical point of view, or yield-based definition of drought tolerance, NILs containing putatively favorable alleles did not exhibit a clear advantage. Elite cotton cultivars, such as the recipient parents used in our study, are the outcome of intense selection over many generations among huge number of individual genotypes. The unique combination of gene networks resulting from such processes is likely to be interrupted due to introgression of either targeted or non-targeted genomic regions. This possibly is a major reason for the poor success of MAS for improved yield in

previous studies (Cattivelli et al. 2008) as well as in the current study. Theoretical and analytical investigations have shown that maximal selection efficiency for quantitative traits may be obtained by using a combination of molecular and phenotypic information (Lande and Thompson 1990; Wittaker et al. 1995). The relatively successful introgression of QTLs for drought related traits in this study may serve as a basis for future breeding. It is suggested that elite genetic materials introgressed with a QTL(s) for drought adaptive traits (e.g. OA) via MAS should be subjected to conventional breeding to identify lines taking advantage of the newly introduced QTL(s) while retaining high yield potential.

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