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Mapping QTLs for drought tolerance in an $F_{2:3}$ population from an inter-specific cross between *Gossypium tomentosum* and *Gossypium hirsutum*

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ABSTRACT. Cotton is one of the most important natural fiber crops in the world. Its growth and yield is greatly limited by drought. A quantitative trait locus (QTL) analysis was therefore conducted to investigate the genetic basis of drought tolerance in cotton (*Gossypium* spp) using 188 $F_{2:3}$ lines developed from an inter-specific cross between a wild cotton species, *G. tomentosum*, and an upland cotton, *G.*

hirsutum (CRI-12). A genetic map was constructed using 1295 simple sequence repeat markers, which amplified 1342 loci, distributed on 26 chromosomes, covering 3328.24 cM. A field experiment was conducted in two consecutive years (2014 and 2015) and 11 morphological and physiological traits were recorded under water-limited (W1)/well-watered (W2) regimes at three growth stages (bud, flowering, and full boll). The traits measured included chlorophyll content, plant height, leaf area, leaf number, leaf fresh weight, leaf dry weight, boll weight, number of bolls per plant, and the number of fruiting branches. Sixty-seven and 35 QTLs were found under the W1 and W2 conditions, respectively. Of these, the majority exhibited partial dominance or over-dominance genetic effects for increasing the trait values. Four consistent QTLs were found under the W1 treatment on chromosomes 5, 8, 9, and 16, whereas no consistent QTL was found in W2. Thirteen QTL clusters were also identified on nine chromosomes (2, 3, 5, 6, 9, 14, 15, 16, and 21). These results will help to elucidate the genetic basis of drought tolerance in cotton.

Key words: *Gossypium*; Genetic map; Drought tolerance; Simple sequence repeat markers; Quantitative trait loci

INTRODUCTION

Cotton (*Gossypium* spp) is an important crop with great economic importance as a leading raw material for the world's textile industry. However, cotton production is hindered by abiotic stresses such as drought and salt. Stress due to drought is a complex phenomenon that affects cotton physiology (Grimes and El-Zik, 1990), growth and productivity (Chu et al., 1995). Inadequate water supplies in many cotton growing regions result in lower yield of the leading natural fiber crop (Jia et al. 2014). Plants inhabiting drought prone areas have developed various strategies to cope with this stress, including developing larger and deeper root systems to increase water absorption from the deep soil; regulating stomata closure to reduce water loss; accumulation of compatible solutes and protective proteins; and increasing the level of antioxidants (Chaves et al., 2003). It is desirable to develop drought-tolerant cotton cultivars with some of these traits that can sustain high production, even in drought-affected regions.

Cotton breeders have made significant contributions towards developing drought tolerant cotton cultivars through conventional breeding (Iqbal et al., 2013). However, drought tolerance is a quantitative trait with a complex phenotype and genetic control (McWilliam, 1989), making conventional breeding both tedious and likely to produce little progress even after several years of selection. Molecular breeding techniques are therefore necessary to supplement the efforts made by conventional breeders. Drought tolerance is regulated by numerous loci, each with little effect, and hundreds of genes that control various morphological and physiological responses to drought (Hu and Xiong, 2014). These loci can be located by conducting quantitative trait locus (QTL) mapping studies, which can then be used for marker-assisted breeding. Hundreds of QTLs for drought resistance traits in various plants, including rice and barley (Iqbal et al., 2013), have been mapped. However, only a small portion of these have been repeatedly detected in different environments and populations and few of these

QTLs have been verified or cloned (Hu and Xiong, 2014) because they are not "stable" in different environments (Fleury et al., 2010). In cotton, researchers have mapped QTLs for physiological traits including yield, earliness, and fiber traits under water-limited environments (Saleem et al., 2015). These previous QTL studies have shown that the productivity of cotton under drought and well-watered conditions is controlled by different sets of genetic markers (Saranga et al., 2004).

Breeding for drought tolerance and water usage efficiency in cotton has been limited by the narrow genetic variability within the species, which has been unintentionally caused by the intensive selection to produce large quantities of lint (Rosenow et al., 1983). Genomic exploration of wild tetraploid cotton (*G. tomentosum*, *G. darwinii*, and *G. mustelinum*) may yield additional valuable alleles (Saranga et al., 2004). Due to their potential as a genetic resource, the wild species are being used in various breeding programs aimed at improving cultivated cotton (Mehetre et al., 2004). However, the use of inter-specific crosses in cotton breeding have been limited due to numerous barriers to gene flow (Jiang et al., 2000). The use of marker-assisted selection (MAS) mitigates many of the problems associated with inter-specific crosses (Jiang et al., 2000). Previous reports indicate that wild cotton species harbor a large number of unique genes, which may provide novel diversity for genetic improvement upon introgression (Hulse-Kemp et al., 2014). Mapping QTLs for both physiological and morphological parameters related to drought tolerance using an inter-specific cross will, therefore, further help to elucidate the molecular mechanisms controlling drought tolerance under drought stress. Furthermore, it will facilitate the development of new varieties with improved drought tolerance.

In the present study, we constructed a dense genetic map using 1295 simple sequence repeats (SSR) from an inter-specific cross between a wild cotton, *G. tomentosum* and upland cotton CRI-12, *G. hirsutum*. The map was used to locate QTLs for traits related to drought tolerance at the bud, flowering, and full boll stages. This is the first effort in which an inter-specific cross between wild cotton and an upland cotton population is used for QTL mapping. The aims of this study were to discover molecular markers linked with QTLs and to identify common genetic regions controlling various traits under drought stress. The identified QTLs can facilitate future molecular breeding programs and will be helpful in the evolution of drought tolerant cotton varieties.

MATERIAL AND METHODS

Population used for QTL analysis

One hundred and eighty-eight $F_{2:3}$ lines were developed from an inter-specific cross between *G. hirsutum*, CRI-12 (G09091801-2) and wild cotton *G. tomentosum*, AD_3-00 (P0601211). CRI-12 is a stable cultivar maintained by strict self-pollination. CRI-12 was in its 11th generation when used as female parent. *G. tomentosum* is native to Hawaii, and is found in rocky, arid, or clay coastal plains (DeJoode and Wendel, 1992). It is considered a dry-land plant (Stephens, 1964).

Experimental design

The field experiments were performed at the National Wild Cotton Nursery, Sanya, Hainan, China, from October 2013 to February 2015. The laboratory work was carried out at the Institute of Cotton Research, Chinese Academy of Agricultural Sciences (ICR-CAAS),

Anyang, Henan, China. The $F_{2:3}$ population and their parents were evaluated for drought tolerance, using W1/W2 regimes. Healthy seeds of the cotton genotypes were delinted, using concentrated sulfuric acid. Excess acid was removed by rinsing the seeds in running tap water. Due to their hard epidermis, the seeds were slitted slightly to allow for easier germination. A completely randomized block design was used for the phenotypic evaluation with the irrigation regimes (W1 and W2) in the main blocks and $F_{2:3}$ lines in the sub-blocks with three replications. All other standard agronomic practices for normal crop growth were applied equally to both treatments. This included addition of fertilizer (urea) at a rate of 150 kg/ha and a compound fertilizer at the rate of 255 kg/ha in both W1 and W2 regimes. All plant lines were kept under rainproof installations, and the experimental irrigation was therefore the sole source of water.

The W1 regime consisted of one sowing irrigation and two supplement sprinkling irrigations at day 35 and 70 after sowing. When the soil moisture content reached 3%, the drought stress was allowed to persist for half a month. Sprinkling irrigation was done at a rate of 1.58×10^3 m^3/ha. The well-watered (W2) control regime consisted of one sowing irrigation and nine subsequent sprinkling irrigations as required for normal crop growth and development. The rate of sprinkling irrigation was 5.25×10^3 m^3/ha.

Trait measurements and analysis

The morphological and physiological data were measured at three different cotton growth stages (bud, flowering, and full boll). Chlorophyll content at the bud (BCC) and flowering (FCC) stages was measured using a leaf chlorophyll meter (SPAD-502 meter, Minolta, Osaka, Japan). Mean leaf chlorophyll content for each genotype was derived from three readings taken at the base, middle, and tip of the youngest fully expanded leaf. Plant height at the bud (BPH), flowering (FPH), and full boll (FBPH) stages was measured from the cotyledonary node to the apical bud, using a measuring tape. The leaf area at bud stage (BLA) of the biggest leaf was determined using a LAI 3000 portable leaf area meter (LI-COR ®Biosciences, LI-COR Inc., Lincoln, NE, USA), whereas the main stem leaf number at the bud stage (BLN) was counted from the first true leaf to the apical meristem. The leaf fresh weight (LFW) at the flowering stage was measured by weighing the 2nd leaf from the top using an electronic balance. Leaf dry weight (LDW) was determined after drying the fresh leaves in an oven at 100°C until they reached a constant weight. Boll weight at full boll stage (FBBW) was determined by dividing the total weight of the collected cotton seeds by the total number of collected bolls. The number of fruiting branches at the full boll stage was recorded by counting from the first branch to the growing point. All the measurements were taken from ten plants per line in each treatment and the average trait values were used for QTL analysis. The percentage reduction for all measured parental traits was calculated as described by Tiwari et al. (2013). SAS 9.1 (SAS Institute Inc., USA) was used to calculate broad-sense heritability (H^2). The VARCOMP procedure was followed to estimate genotypic variance, environmental variance, genotype-environmental interaction variance, and error variance. The H^2 of each measured trait was calculated as previously described by Hallauer and Miranda (1988).

Linkage map

A new genetic map was constructed from the genotype data obtained from ICR-CAAS. The data were first generated by Kashif et al. (2015), who developed a dense genetic

map from the same population consisting of 2823 SSR primers mainly composed of EST-SSR primers available at http://www.cottonmarker.org. However, some of the primers were highly distorted and hence could not be used for QTL mapping. A new linkage map was therefore constructed, using JoinMap 4.0 (Van Ooijen 2006). Segregation ratios of the two genotype classes at each locus were tested using a chi-square test. Skewed segregation were detected at P = 0.05 significance level. The segregation ratio at a locus deviating from the expected ratio of 3:1 indicates segregation distortion. Markers for which this was found to be true were excluded from the QTL analysis. The loci that remained after removing those distorted were assigned to linkage groups based on the log of odds (LOD) score (≥ 4) and a maximum recombination fraction of 0.4. Map distances in cM were calculated using Kosambi's (1944) mapping function. The final linkage map consisted of 1295 markers that amplified 1342 loci (**Tables S1** and **S2**). A complete cotton chromosome assignment for this map was done based on the previously chromosome-anchored map (Riaz et al., 2013).

QTL analysis

Composite interval mapping (CIM) was conducted using WinQTL Cartographer 2.5 (Wang et al., 2010). QTLs were declared based on an LOD threshold of ≥3.0. QTLs with an LOD of ≥ 2.5 in both environments were considered common QTLs, based on the explanation by Lander and Kruglyak (1995). In addition, a test with 1000 permutations was performed for each trait to identify the minimum significant LOD threshold score. The CIM was performed using a stepwise forward-backward regression procedure with a probability (into and out) of 0.01 and model six with a walking speed of 1 cM. LOD peaks were automatically localized using WinQTL Cartographer. Consistent QTLs were declared when two LOD intervals for two QTLs overlapped. Graphical representations were then generated using MapChart 2.0 (Voorrips 2002). Gene actions were estimated as described by Stuber et al. (1987).

RESULTS

Phenotypic variation between parents

The percentage reduction parameter was used to assess the effect of drought stress on parental growth at the bud, flowering, and full-boll stages. There was a significant difference for most of the plant traits measured under both the W1 and W2 growth conditions (Figure 1). In both parents, all traits were reduced when exposed to drought. However, the level of reduction differed significantly between parents for most of the traits. At the bud stage, *G. tomentosum* had a higher percentage reduction in BLA, whereas CRI-12 had a higher percentage reduction in FPH and FCC at the flowering stage. At the full boll stage, CRI-12 had higher percentage reduction in FBPH and FBBW, whereas no significant differences were found in LFW or LDW.

Descriptive statistics and heritability of $F_{2:3}$ population

The frequency distribution of all traits in the $F_{2:3}$ generation showed typical quantitative variation and all variables fitted a normal distribution (**Figure S1**). The phenotypic variation in all measured traits for the drought and control treatments are presented in Tables 1 and 2,

respectively. BLN had the lowest H^2 (44.6%), whereas BPH had the highest (66.6%). The average H^2 for all the traits was 57.88% (Table 3).

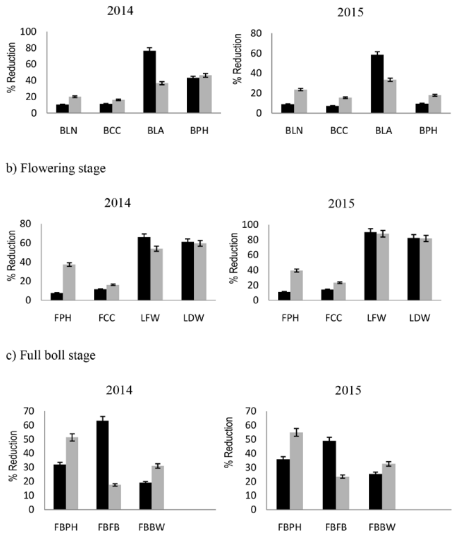

Figure 1. Phenotypic variation in parents based on percentage reduction at the bud (**a**), flowering (**b**), and full boll (**c**) stages. Black and grey charts represent *Gossypium tomentosum* and CRI-12, respectively. BCC = bud chlorophyll content; FCC = flowering chlorophyll content; BPH = bud plant height; FPH = flowering plant height; FBPH = full boll plant height; BLA = bud leaf area; BLN = bud leaf number; LFW = leaf fresh weight; LDW = leaf dry weight; FBBW = full boll, boll weight; FBFB = full boll, fruiting branch.

Table 1. Phenotypic variation of the measured traits in the $F_{2:3}$ generation under the W1 (drought) treatment.

Stage	Trait	Year	Mean	SD	Kurtosis	Skewness	Min	Max
Bud	BCC	2014	36.29	3.69	0.08	0.09	27.40	46.40
		2015	47.59	2.86	0.17	0.38	40.94	56.36
	BLA	2014	25.02	10.41	0.38	0.76	7.25	59.54
		2015	64.22	17.21	0.54	0.29	16.77	111.97
	BLN	2014	7.88	1.66	0.51	0.39	4.40	13.85
		2015	13.21	1.50	0.28	0.08	9.31	17.70
	BPH	2014	15.76	3.78	-0.27	0.37	6.88	26.20
		2015	37.39	7.57	0.42	0.46	18.87	62.30
Flowering	FCC	2014	35.58	3.12	0.67	-0.13	25.20	44.50
		2015	54.75	2.94	-0.10	0.37	47.85	62.64
	FPH	2014	19.33	5.94	0.10	0.65	7.38	34.80
		2015	48.05	8.66	0.38	0.52	27.93	75.00
	LDW	2014	0.16	0.08	0.65	1.01	0.03	0.43
		2015	0.12	0.03	0.09	0.31	0.04	0.21
	LFW	2014	0.64	0.36	0.80	1.07	0.10	1.83
		2015	0.76	0.20	0.43	0.55	0.25	1.35
Full boll	FBBW	2014	1.09	0.29	0.57	0.56	0.43	2.11
		2015	0.92	0.29	-0.08	0.58	0.29	1.70
	FBFB	2014	7.17	4.42	-0.37	0.23	0.00	19.60
		2015	8.89	2.86	-0.06	0.38	2.61	17.10
	FBPH	2014	44.89	17.56	0.14	0.27	9.00	102.20
		2015	68.30	11.65	-0.49	0.31	44.88	96.61

BCC = bud chlorophyll content; BLA = bud leaf area; BLN = bud leaf number; BPH = bud plant height; FCC = flowering chlorophyll content; FPH = flowering plant height; LDW = leaf dry weight; LFW = leaf fresh weight; FBBW = full boll boll weight; FBFB = full boll first branch; FBPH = full boll plant height; Min = minimum; Max = maximum; SD = standard deviation.

Table 2. Phenotypic variation of the measured traits in $F_{2:3}$ generation under the W2 (control) treatment.

Stage	Trait	Year	Mean	SD	Kurtosis	Skewness	Min	Max
Bud	BCC	2014	40.33	3.14	-0.66	0.33	33.80	46.90
		2015	45.13	3.47	1.23	0.02	36.10	57.30
	BLA	2014	69.68	32.45	0.80	0.66	21.57	226.49
		2015	100.10	26.70	0.87	0.53	44.67	193.29
	BLN	2014	11.24	2.16	-0.95	0.18	7.00	15.50
		2015	15.75	2.54	0.38	0.22	9.50	24.00
	BPH	2014	23.37	5.26	-0.46	0.22	12.50	35.75
		2015	45.91	14.93	0.02	0.31	18.25	86.50
Flowering	FCC	2014	39.87	3.96	1.53	-0.86	26.40	46.70
		2015	47.04	3.88	0.94	-0.09	33.10	56.80
	FPH	2014	44.12	12.35	-0.82	0.20	21.57	72.50
		2015	65.19	18.28	-0.02	0.06	17.00	115.39
	LDW	2014	0.64	0.26	1.10	0.92	0.23	1.57
		2015	0.42	0.13	-0.74	0.07	0.12	0.72
	LFW	2014	2.59	1.08	1.01	0.89	0.84	6.13
		2015	8.81	2.66	-0.66	0.13	2.70	14.45
Full boll	FBBW	2014	1.08	0.39	0.75	0.77	0.35	2.31
		2015	0.74	0.26	0.21	0.56	0.26	1.54
	FBFB	2014	13.83	3.48	0.19	0.71	7.00	23.20
		2015	16.88	2.91	0.25	0.09	10.29	25.50
	FBPH	2014	108.15	19.51	0.61	-0.71	50.00	148.40
		2015	104.40	24.16	0.78	-0.45	26.00	158.50

For abbreviations, see legend to Table 1.

Table 3. Broad sense heritability estimates for the morphological and physiological parameters of the $F_{2:3}$ lines grown under the W1 condition.

Stage	Trait	σ^2_G	σ^2_E	$\sigma^2_{G\times E}$	σ^2_e	H^2 (%)
Bud	BCC	3.740	1.280	2.460	4.800	64.800
	BLA	109.120	435.750	144.870	456.230	59.500
	BLN	3.067	0.617	11.566	11.020	44.600
	BPH	25.476	113.010	30.560	61.810	66.600
Flowering	FCC	4.303	2.306	3.010	4.919	64.900
	FPH	49.870	252.470	84.890	105.450	62.400
	LDW	0.002	0.028	0.002	0.012	40.000
	LFW	0.078	0.735	0.094	0.180	50.500
Full boll	FBPH	129.560	629.880	187.200	269.120	65.200
	FBFB	3.881	11.392	3.684	4.914	59.300
	FBBW	0.019	0.003	-0.002	0.080	58.900

σ^2_G = genotypic variance; σ^2_E = environmental variance; σ^2_{GxE} = genotype by environment interaction variance; σ^2_e = error variance; H^2 = broad-sense heritability.

Phenotypic correlations

Pearson's correlation coefficient analysis was carried out to establish associations among the measured traits at the different growth stages during 2014 and 2015. All traits measured correlated positively with each other, with the exception of BPH and FPH, which correlated negatively (Table 4).

QTL mapping

A total of 71 QTLs under W1 and 35 under W2 were identified for the 11 traits measured (**Table S3**). At all growth stages, the number of QTLs detected under W1 was

Table 4. Phenotypic correlations among the traits in the well-watered (W1) treatment based on mean values for chlorophyll content (BCC and FCC) and various morphological traits in 2014 and 2015.

	BCC	BPH	FCC	FPH	LDW	LFW	FBPH
BCC	1	-0.076	0.744**	-0.042	-0.046	-0.003	-0.127
BPH	-0.056	1	-0.024	0.600**	0.227*	0.279**	0.284**
FCC	0.366**	0.135	1	-0.068	-0.056	-0.049	0.052
FPH	-0.058	0.879**	0.132	1	0.399**	0.487**	0.696**
LDW	0.090	0.498**	0.206*	0.647**	1	0.942**	0.194*
LFW	0.036	0.478**	0.179*	0.637**	0.971**	1	0.264**
FBPH	0.055	0.537**	0.205*	0.640**	0.669**	0.643**	1

BCC = bud chlorophyll content; BPH = bud plant height; FCC = flowering chlorophyll content; FPH = flowering plant height; LDW = leaf dry weight; LFW = leaf fresh weight; FBPH = full boll plant height; **Correlation significant at $P \leq 0.01$ (2-tailed); *Correlation significant at $P \leq 0.05$ (2-tailed). Below diagonal for 2014 and the above diagonal for 2015.

higher than under W2 conditions. Twenty-eight (39.4%) of the QTLs obtained under W1 and 12 (34%) of the QTLs identified under W2 were due to partial dominance (PD) gene effects. Over-dominance (OD) gene effects accounted for 33.8 and 34% of the QTLs under the W1 and W2 conditions, respectively. Dominance effects accounted for 8.4 and 8.5% of the QTLs under W1 and W2, respectively, whereas additive effects were identified in 18 and 22% of the QTLs under W1 and W2 treatments, respectively. Four of the QTLs for BLA, BCC, FCC, and FBBW were consistently found within overlapping intervals in 2014 and 2015 under the W1 condition on chromosomes 5, 8, 9, and 16 (Table 5 and Figure 2). No consistent QTL was found under the control (W2) treatment. Two of the consistent QTLs, *qBcc-Chr9-1* and *qFcc-Chr8-1*, were related to chlorophyll contents at the bud and flowering stages, respectively, and their alleles were derived from *G. tomentosum*. A major QTL for leaf area, *qBla-Chr5-1*, was found on chromosome 5 with high additive and PD genetic effects.

Table 5. QTLs detected in 2014 and 2015 under W1.

QTL	Year	Marker interval	LOD	A^a	D^b	d/a	PVE
qBla-Chr5-1	2014	HAU0878(b)-NAU5181	3.0*	4.283	-2.400	PD	13.3
	2015	NAU1221(b)-TMB1750	3.1*	6.740	-1.396	PD	9.0
qFcc-Chr8-1	2014	DOW098-HAU0333	2.9*	0.053	-2.022	OD	4.1
	2015	NBRI-0149-MON_DPL0551	3.2*	0.952	-1.487	OD	16.0
qBcc-Chr9-1	2014	BNL4099-MUSS022	2.8	0.929	-1.381	OD	9.6
	2015	Gh423-NAU5494	2.8	0.792	-1.563	OD	12.6
qFbbw-Chr16-1	2014	MON_DC30209-BNL580	4.5*	-0.149	-0.063	PD	7.0
	2015	HAU2481-NAU2999	3.1	-0.144	-0.040	PD	6.4

LOD = log of odds score (asterisk denotes LOD scores higher than the permutation test LOD); A^a = additive effect (positive values indicate that *Gossypium tomentosum* alleles increase the trait value); D^b = dominant effect; d/a: estimation of gene action (PD = partial dominance, 0.21-0.80; OD = over dominance, >1.20); PVE = phenotypic variation explained by a single QTL.

QTL clusters

QTLs for various traits were found co-located within an overlapping interval in 2014 and 2015. Under the W1 condition, 13 QTL clusters were found on nine chromosomes (2, 3, 5, 6, 9, 14, 15, 16, and 21) (**Table S4**), with chromosome 16 having the largest number of clusters

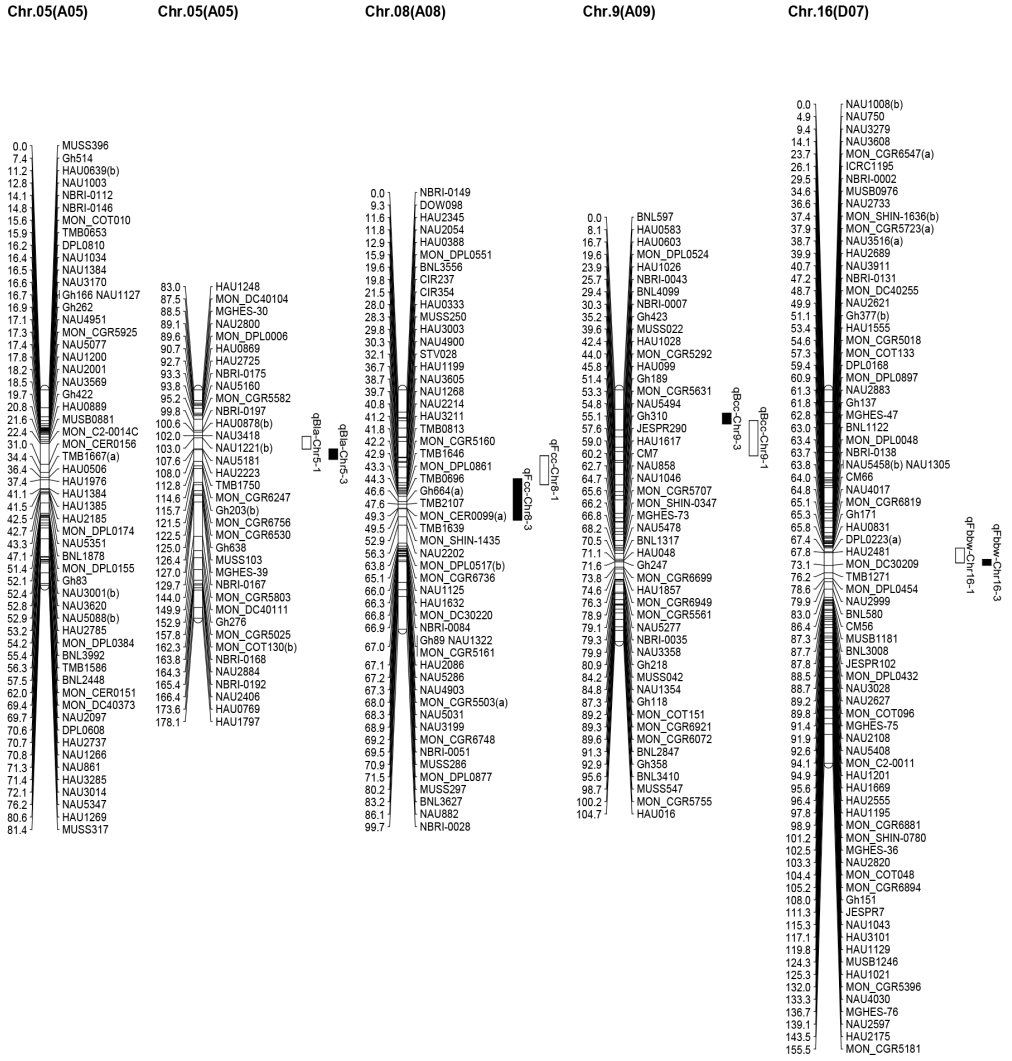

Figure 2. QTL locations for traits related to drought tolerance in the $F_{2:3}$ population subjected to the water-limited treatment. The QTLs are indicated on the right side of each chromosome and marker positions are shown on the left. QTLs detected in 2014 are represented by the empty box while those found in 2015 are represented by filled boxes.

(five). Three QTL clusters for BCC and FCC were found on each of chromosomes 14, 16, and 21. The one located on chromosome 16 had obtained its alleles from *G. tomentosum*, whereas the other two originated from CRI-12. A further three sets of QTL clusters; one on chromosome 3 and two on chromosome 16, were detected for FPH and FBPH. An important cluster of major QTLs for BLA (detected in both 2014 and 2015) and BLN (detected in 2014) was found on chromosome 5 with the alleles controlling QTL expression derived from *G. tomentosum*.

DISCUSSION

Molecular markers are powerful tools that have been used to locate QTLs utilized for both MAS and as landmarks for map-based cloning of genes responsible for many important traits in cotton. The inter-specific $F_{2:3}$ population used in this study will serve as an indispensable genomic resource for QTL mapping. Previous reports have shown that intra-specific crosses of upland cotton have a low polymorphism detection (Mei et al., 2004), which indicates a lack of diversity in cultivated cotton. This confirms the need to use inter-specific crosses. *G. tomentosum* is a wild species of cotton that has a high tolerance to drought with certain xerophytic features such as woody perennial growth form, slow growth, and deep root penetration (MacCaughey, 1917).

In this study, eleven different traits were measured at three different growth stages (bud, flowering, and full boll) to detect the various polygenes expressed at each stage. This was done because gene expression can be stage-specific; for example, genes responding to stress at the seedling stage need not be the same as those responding at the flowering stage (Blum, 1988). Cotton drought tolerance begins to decline from the early germination stage, it reaches its lowest level at the bud stage followed by a gradual increase until it reaches a stable level at the real leaf stage (Zhang et al., 2007). The sensitivity of cotton plants to drought stress at the flowering and boll development stages have previously been reported (Turner et al., 1986). Therefore, identification of QTLs at the bud, flowering, and full boll stages will contribute greatly to marker assisted breeding of drought tolerant cotton cultivars.

Percentage reduction was used to compare the among parent phenotypic variation, because it is an accurate method to determine response of various genotypes under stress (Tiwari et al., 2013). *G. tomentosum* had a high percentage reduction in BLA at the bud stage as compared to CRI-12. The ability of *G. tomentosum* to reduce its leaf area under drought stress is a xerophytic feature that enables a reduction in the surface area exposed for transpiration and, thus, reduced water loss. The results found here in relation to leaf area, indicate the potential of *G. tomentosum* to withstand drought stress. A major QTL, *qBla-Chr5-1*, was found on chromosome 5 explaining 13.3 and 9.0% of the phenotypic variation found in 2014 and 2015, respectively. The alleles from *G. tomentosum* caused a reduction in leaf area in both years.

Chlorophyll is one of the basic pigments in plants and a reduction in the chlorophyll concentration causes chlorosis as well as a reduction in both growth and yield (Khosh and Ando, 1995). For example, corn stress-resistant genotypes that had higher yield potential and chlorophyll content were found to be more drought tolerant (Homayoun et al., 2011). Hence, loss of chlorophyll content is a negative consequence of water stress. In this study, the percentage reduction of chlorophyll content in *G. tomentosum* was lower than that in CRI-12 at both the bud and flowering stages in both years. A higher chlorophyll content has been reported in resistant genotypes compared to in sensitive genotypes in both wheat and corn under stress (Pastori and Trippi, 1992). Two consistent QTLs, *qBcc-Chr9-1* and *qFcc-Chr8-1*, at the bud and flowering stages, respectively, were found with the alleles responsible for their expression derived from *G. tomentosum*. Both QTLs showed OD gene action and can, therefore, be used to facilitate conventional breeding of hybrid cotton capable of delaying wilting under drought stress.

The use of $F_{2:3}$ lines in mapping QTLs enables an estimation of genetic effects of the detected QTLs. Gene actions were determined on the basis of the dominant and additive parameters obtained from QTL cartographer as described by Stuber et al. (1987). PD gene action accounted for 39.4 and 34% of all the QTLs obtained under the W1 and W2 conditions,

respectively. OD accounted for 33.8 and 34% of the QTLs obtained under the W1 and W2 treatments, respectively. These results indicate that the PD and OD types of gene actions governed the inheritance of most of the traits measured, indicating that QTLs with these effects are a common phenomenon in the cotton genome. Previous studies have implicated an OD genetic effect in playing an important role in the genetic control of heterosis in rice and QTLs with OD genetic effects have been reported to be common in the rice genome (Zhuang et al., 2001). Analyses of QTLs for yield and yield components in cotton indicated that the effects of PD and OD simultaneously contributed to heterosis in upland cotton (Liang et al., 2015). Hence, QTLs with PD and OD effects may play an important role in the development of drought tolerant hybrid cotton varieties that are tolerant to drought stress through conventional and male sterility methods. The low number of QTLs identified to have additive effects may be due in part to the low heritability for the traits measured in this study.

The extent of transmission of traits from the parents to the offspring is estimated by heritability. Traits with high heritability are easier to manipulate than those with low heritability (Saba et al., 2001). High values of heritability indicate that the trait can be improved by selection breeding, while selection on the basis of low heritability may be misleading due to the influence of the environment on the genetic make-up (Nadarajan and Gunasekaran, 2005). In the present study, the H^2 estimates ranged from low to moderate (44.6-66.6%) for BLN and BPH, respectively, with an average of 57.88%. The lack of a high heritability for these traits may be due to the presence of many QTLs with dominant and OD genetic effects in the cotton genome. These results indicate that these traits could be used as a selection criterion to improve drought tolerance in cotton.

Boll weight, boll number, and lint percentage are three yield components that significantly determine cotton lint yield (Wu et al., 2004). Studies have shown that drought stress decreases yield, boll number, and boll weight (Alishah and Ahmadikhah, 2009). Hence, one way to reduce the detrimental effects of drought is by developing drought tolerant cotton cultivars through identification of QTLs for boll weight. The boll weight of CRI-12 was much higher than that of *G. tomentosum* in both treatments. This is probably due to the former being an established upland cotton variety that has already undergone several selection cycles, whereas the latter is a wild type. Both parents, however, recorded a reduction in boll weight under drought stress with *G. tomentosum* having a lower percentage reduction than CRI-12.A consistent QTL (*qFbbw-Chr16-1*) for boll weight was detected on chromosome 16 under W1 treatment explaining a PVE of 6.9 and 6.4 in 2014 and 2015, respectively, with the alleles derived from CRI-12. This QTL will facilitate breeding of cotton varieties with higher boll weight under drought.

Some of the QTLs were not evenly distributed across the cotton genome but were concentrated to specific regions on some chromosomes. It has previously been proposed that there might be QTL-rich regions along a chromosome congruent between mapping populations, generations, and locations (Lacape et al., 2005). In this study, 11 clusters on six chromosomes (2, 3, 5, 6, 16, and 21) were identified with each cluster having two or more QTLs for different traits. BCC and FCC QTLs were found co-localized on chromosomes 16 and 21, suggesting that the same set of genes could be influencing chlorophyll content at both the bud and flowering stages. This co-localization is supported by the highly significant positive correlation between BCC and FCC. However, these QTLs were not detected to have significant LOD thresholds in 2014, which may be due to environmental influences. Five QTL clusters for plant height were detected. Of these, three were for FPH and FBPH on chromosomes 3 and 16, respectively, and two for BPH and FPH on chromosomes 6 and 21, respectively. The clustering can be explained

by the significant correlations between plant height at the bud, flowering, and full boll stages. This could also be an indication that plant height is controlled by the same genes at the different growth stages. Among the clustered QTLs, alleles for each cluster were contributed by the same parent, indicating a strong genetic linkage of the QTLs.

QTL mapping for traits associated with drought tolerance has previously been reported in many crops such as rice, maize, barley, soybean, and wheat (Saleem et al., 2015). In cotton, a few studies have mapped genomic regions associated with drought tolerance. These studies include one involving an inter-specific cross between *G. hirsutum* cv. Siv'on and *G. barbadense* (Saranga et al., 2001; Saranga et al., 2004) and an intra-specific cross of *G. hirsutum* L. (Saeed et al., 2011). However, the present study is the first QTL mapping study involving a wild cotton species (*G. tomentosum*) and therefore the results obtained are unique compared to previous reports. As reported earlier by MacCaughey (1917), *G. tomentosum* has xerophytic features such as deep root penetration and it is endemic to the dry coastal regions of Hawaii. The importance of root length for drought tolerance in cotton has previously been established (Riaz et al., 2013). Therefore, further research should focus on examining the root growth parameters of cotton under drought conditions and compare them with those of upland cultivated cotton. This important drought stress trait (root length) could potentially be transferred to upland cotton through marker-assisted breeding, in order to improve drought tolerance. This study examined only two water regimes and future studied should involve a broader range of drought/watering conditions that may provide more detailed information about cotton drought tolerance. The QTLs identified here may be used to identify key genes involved in various tolerance mechanisms in cotton and the markers flanking the QTLs may be used for marker-assisted breeding in cotton. However, this study should be replicated to confirm the identified QTLs.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

Figure S1. Frequency distribution of traits at bud, flowering and full boll stages under W1 (drought) and W2 (control) in 2014 and 2015.

Table S1. Distribution of loci in a genetic map constructed using SSR markers.

Table S2. Characteristics of the genetic map.

Table S3. All the QTLs detected under W1 and W2.

Table S4. QTL clusters.