G3, 2021, 11(5), jkab027
DOI: 10.1093/g3journal/jkab027
Advance Access Publication Date: 12 April 2021
Investigation

Genome-wide quantitative trait loci mapping on *Verticillium* wilt resistance in 300 chromosome segment substitution lines from *Gossypium hirsutum* × *Gossypium barbadense*

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Abstract

Cotton *Verticillium* wilt (VW) is a devastating disease seriously affecting fiber yield and quality, and the most effective and economical prevention measure at present is selection and extension of *Gossypium* varieties harboring high resistance to VW. However, multiple attempts to improve the VW resistance of the most widely cultivated upland cottons have made little significant progress. The introduction of chromosome segment substitution lines (CSSLs) provide the practical solutions for merging the superior genes related with high yield and wide adaptation from *Gossypium hirsutum* and VW resistance and the excellent fiber quality from *Gossypium barbadense*. In this study, 300 CSSLs were chosen from the developed $BC_5F_{3:5}$ CSSLs constructed from CCRI36 (*G. hirsutum*) and Hai1 (*G. barbadense*) to conduct quantitative trait locus (QTL) mapping of VW resistance, and a total of 40 QTL relevant to VW disease index (DI) were identified. Phenotypic data were obtained from a 2-year investigation in two fields with two replications per year. All the QTL were distributed on 21 chromosomes, with phenotypic variation of 1.05%–10.52%, and 21 stable QTL were consistent in at least two environments. Based on a meta-analysis, 34 novel QTL were identified, while 6 loci were consistent with previously identified QTL. Meanwhile, 70 QTL hotspot regions were detected, including 44 novel regions. This study concentrates on QTL identification and screening for hotspot regions related with VW in the 300 CSSLs, and the results lay a solid foundation not only for revealing the genetic and molecular mechanisms of VW resistance but also for further fine mapping, gene cloning and molecular designing in breeding programs for resistant cotton varieties.

Keywords: CSSLs; *Verticillium* wilt; disease index; quantitative trait loci; meta-analysis

Introduction

Cotton (*Gossypium* spp. L.) produces the major natural fiber for textile industries, and is also the important resource of edible oil and plant protein, which is of significance for human economic and social development (Xu *et al.* 2008). The cultivation history of cotton dates to 7,000 years ago (Lee *et al.* 2015), and cotton is widely grown in approximately 100 countries generally located in tropical and sub-tropical areas (Alkuddsi *et al.* 2013). The genus *Gossypium* consists of 53 species worldwide, with 46 diploid species ($2n = 2 \times = 26$) and 7 allotetraploids ($2n = 2 \times = 52$) (Wendel and Grover 2015); the emergence of the latter dated from a polyploidization event between the A and D genomes 1–2 million years ago (Alkuddsi *et al.* 2013). Only four cultivated species (two diploids and two tetraploids) are extant and widely planted, while the rest of the 53 species are wild but important reservoirs of beneficial agronomic traits for improvement of the cultivated species (Mehetre *et al.* 2004; Grover *et al.* 2015). Nowadays, *Gossypium hirsutum* and *Gossypium barbadense* are the most widely cultivated species, accounting for more than 95% and 3% of world cotton production, respectively. This dominance is attributes to the fact that the former presents high yield and wide adaptability, while the latter possesses superior fiber quality and high VW resistance (Zhang *et al.* 2015).

Most limiting factors during organism growth are generally divided into abiotic and biotic stresses (DeVay *et al.* 1997), while plant diseases might be the dominating threat in cotton production (Blasingame and Patel 2013). *Verticillium* wilt (VW) infection by the soil-borne fungus Verticillium *dahliae* Kleb has been the

Received: September 20, 2020. **Accepted:** February 02, 2021

most significant disease in cotton production due to its causing substantial yield loss and serious fiber quality reduction, which is the main reason being called "cotton cancer"(Cai *et al.* 2009; Xu *et al.* 2010; Yu *et al.* 2011). As a result of cotton VW infestation, fiber loss is estimated to be approximately 80% (Wei *et al.* 2015). Even worse, this disease can attack more than 400 plant species and can exist in the soil for long periods in a dormant form within the vascular system of perennial plants. Thus, it is impossible to control VW disease through conventional methods (Zhang *et al.* 2016). The general symptoms of the disease are vascular browning, stunting, leaf epinasty and chlorosis, curling or necrosis, wilt and finally death of the entire plant (Bell and Hillocks 1992; Li *et al.* 2016).

Despite multiple methods proposed to control VW, the most efficient and economical measure involve developing elite cotton cultivars harboring genetic factors tolerant or completely resistant against the pathogen (Zhang *et al.* 2000; Mert *et al.* 2005; Wang *et al.* 2014). There are only four subsistent cultivars of *Gossypium* species; the tetraploid cultivars *G. barbadense* and *G. hirsutum* comprise more than 95% of the planted cotton area worldwide, with the former being resistant and the latter susceptible to VW disease (Wilhelm *et al.* 1974; Fang *et al.* 2013). Hybrid breeding via conventional techniques has been utilized to improve VW resistance in upland cottons, while some factors such as infertility and hybrid breakdown/low parent heterosis have hindered using resistant gene introgression from *G. barbadense* into *G. hirsutum* (Fang *et al.* 2013). Therefore, it has become a challenging task for cotton breeders to achieve synchronous improvement in cultivating novel varieties simultaneously displaying high yield, superior fiber quality, and high disease resistance. Quantitative trait locus (QTL) mapping approaches make it possible to discover quantitative genetic factors responsible for disease resistance as well as for high fiber quality and yield. Thus, we can take full advantage of genetic markers presenting linkage disequilibrium with disease resistance to confirm the contribution of key candidate genes that can be transferred from *G. barbadense* into *G. hirsutum* to improve VW resistance (Shi *et al.* 2016).

Chromosome segment substitution lines (CSSLs) have constant effects accompanied by similar genetic bases to their recurrent parent, thereby acting as effective agents in the mining of elite QTL and alleles. The use of CSSLs facilitates advanced functional genomic techniques devoid of nonadditive genetic effects (Takershi *et al.* 2005; Chen *et al.* 2009; Zhao *et al.* 2009; Zhu *et al.* 2009; Ali *et al.* 2010; Ye *et al.* 2010). Optimal utilization of *G. hirsutum* as well as *G. barbadense* can be brought about via marker-assisted selection (MAS) and conventional techniques of inbreeding, outcrossing and backcrossing with the provision of CSSLs. Therefore, CSSLs are extensively exploited in QTL mapping approaches for discovering genetic factors responsible for economic traits such as fiber quality, yield, biotic and abiotic stress tolerance or resistance (Wang *et al.* 2008; Lacape *et al.* 2010; Said *et al.* 2014a, 2015b; Yu *et al.* 2014; Shi *et al.* 2015; Wu *et al.* 2016; Zhai *et al.* 2016; Zheng *et al.* 2016).

Modern cotton genomics research, as for other crop species, has successively incorporated QTL mapping of significant traits based upon comprehensive deployment of molecular markers, of which simple sequence repeats (SSRs) are the most extensively utilized genetic markers in cotton (Wang *et al.* 2015). Recently, there has been a newly emerging technique of mapping known as meta-analysis of QTL in tetraploid cotton research that has been intensively employed for the identification of hotspot regions known to harbor a large number of QTL (Said *et al.* 2014a, 2015b). Consensus map positions for QTL and merging of datasets are the fundamental bases of the meta-analysis approach, making this technique unique and widely adoptable. Meta QTL analysis of previously declared QTL positions can be confirmed via the identification of hotspot regions, and the pleotropic effects of QTL for different traits can be identified (Said *et al.* 2014a). Moreover, this beneficial aspect of meta-analysis can be exploited to create hotspot regions harboring stable QTL for any disease by reassembling the previously identified QTL for the relevant disease. Breeders and geneticists can employ this technique, as they only need to identify the specific chromosome regions enriched with genetic factors controlling disease resistance for MAS or advanced mapping techniques (Said *et al.* 2013c; Zhang *et al.* 2015).

The goals of this study therefore are to identify favorable QTL alleles linked with VW resistance, to screen SSR markers that can be implemented in marker-assisted breeding programs, and to confirm consistent and stable QTL through meta-analysis for MAS application in cotton breeding for VW prevention and control. The results of this study are of importance for VW resistance as well as for breeding improvements in cotton.

Materials and methods

Plant materials and development of cotton CSSLs

A mapping population based on 300 CSSLs along with their parents, specifically as CCRI36 (*G. hirsutum*) as recurrent and Hail (*G. barbadense*) as the donor parent, was sown at the farm areas of ICR, CAAS (Anyang, Henan of East longitude 114.355 and North latitude 36.108) and Shihezi, Xinjiang Province of East longitude 86.079 and North latitude 44.307, respectively. The reason behind selection of Hail as the donor parent is its characteristic features of producing high quality fiber, resistance genes for VW and the presence of glandless producing factors that act in a dominant fashion (Sun *et al.* 2010). However, CCRI36 developed by ICR, CAAS (State Approval Certificate of Cotton 990007) (Zhai *et al.* 2016) is a commercially grown variety of upland cotton that has the desirable properties of high yield as well as early maturing in growth but is susceptible to VW. The two cultivars Hai1 and CCRI36 used as paternal and maternal parents were hybridized followed by backcross in 2003 at Anyang to construct CSSLs. In 2009, a mapping population comprising 2,660 plants of BC_5F_3 was obtained by using CCRI36 as the recurrent parent. In 2010 and 2011, a $BC_5F_{3:4}$ population was planted via the plant-to-row method at Anyang and Xinjiang, respectively. In 2014, at Xinjiang province, the $BC_5F_{3:5}$ population was grown again. From these populations, a random selection process was conducted, and 300 CSSLs were obtained for the evaluation of VW disease index (DI). These selected lines were then grown at Anyang and Xinjiang in 2015 and 2016, respectively. The details of development of CSSLs followed the same procedure as described earlier (Li *et al.* 2017). Stable performance regarding resistance to VW was displayed by some lines in multiple environments over different years of study.

Field investigations and experimental design

Two field stations of ICR, CAAS in Anyang (Henan Province) and Shihezi (Xinjiang Province) were used to grow the experimental material for 2 years. In 2015 and 2016, phenotypic data were collected in July and August from Anyang and Xinjiang, respectively. Under natural environmental conditions, there occurred intensive attacks by *V. dahliae* strains. A randomized complete block design with two replications was established for the study. By following the specifications prescribed for crop management

according to the locality, seeds were sown in single row plots. At research farm areas of Anyang, planting rows were kept 5 m long with an interval of 0.8 m, whereas thinning of seedlings was done up to 20 plants in a row. However, in Xinjiang row length was kept at 3 m, with plant-to-plant distance of 0.1 m following two narrow by row plots methodology. Row spacing alternation was 0.1 m by 0.66 m (Table 1). Wide/narrow row-to-row distance patterns were followed, and plastic membranes were utilized for covering of seedlings. Standard agronomic performs were established during each experiment at all locations.

VW phenotypic evaluation

For scoring of diseased portions of plants, a percentage-based scale ranging between 0 and 4 was used for evaluation (Zhao *et al.* 2014). The scale used is a standard one being used in China, especially for VW rating indices, by classifying the damaged portion of mature leaves into five groups (Wu *et al.* 1999; Yang *et al.* 2008; Zhang *et al.* 2014). The scoring pattern is considered in ascending order regarding resistance level, counting 0–2 as resistant and 3–4 as susceptible (Table 2).

The DI was estimated following the formulae below (Zhao *et al.* 2014; Zhang *et al.* 2015).

$$DI\ (\%) = [\Sigma (d_c \times d_t)/(n_t \times 4)] \times 100$$

where d_c is disease rating between 0 and 4;

n_c is number of plants with interrelated disease rate;

n_t is total number of plants tested for each CSSL.

Analysis of phenotypic traits

The software SPSS 20.0 was used for analyzing the observed phenotypic data, and Pearson's rank correlation coefficients were used for evaluating the correlations among the disease indices. The one-way analysis of variance (ANOVA) of environments and genotypes was performed by the statistical package SAS version 9.1, and Tukey's test was used to compare treatment means. The broad-sense heritability (H2 b) was calculated by the formulae H2 b = Var (G)/Var (P), where Var (G) represents genotypic variance and Var (P) represents phenotypic variance (PV) (Khan *et al.* 2010).

Genetic analysis

Genomic DNA of CSSLs from the $BC_5F_{3:5}$ population and its parents was extracted by following a modified procedure of the CTAB method (Paterson *et al.* 1993) using young leaves sampled from each line and kept at −80°C. The working concentration of DNA was adjusted to 30 ng/μL and was quantified using a NanoDrop2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The integrity of the DNA was verified on agarose gels (1%) using Lambda DNA/HindIII Markers (Niu *et al.* 2008) as a ladder. The scoring pattern followed for SSR fragments included "–" for missing, "1" for presence and "0" for absence of bands.

Table 1 Details of 8 environments of fields used to evaluate CSSL population

Year	Environments	Abbreviation used	Replication	Layout
2015	Anyang July	AYJul15	2	5 × 0.8 m
	Anyang August	AYAug15	2	5 × 0.8 m
	Xinjiang July	XJJul15	2	3× (0.66 + 0.10) m
	Xinjiang August	XJAug15	2	3× (0.66 + 0.10) m
2016	Anyang July	AYJul16	2	5 × 0.8 m
	Anyang August	AYAug16	2	5 × 0.8 m
	Xinjiang July	XJJul16	2	3× (0.66 + 0.10) m
	Xinjiang August	XJAug16	2	3× (0.66 + 0.10) m

SSR markers and SSR molecular detection

Based on the genetic map (Shi *et al.* 2015), a total of 597 pairs of markers were screened using 2,292 pairs of primers to screen 300 CSSLs DNA. The sequences of these SSR primers were downloaded from the CMD database (http://www.cottongen.org/). First, we diluted these primer pairs. For dilution, we centrifuged primer pairs at 12,000 rpm at 4°C for 10 minutes to settle the contents at the bottom. We diluted these primer pairs 100x with vigorous shaking for 2 minutes. The supernatant was centrifuged again and stored at −20°C.

QTL mapping

Inclusive Composite Inter Mapping (ICIM) method and QTL IciMapping V4.0 software were utilized in this study to conduct QTL mapping on VW resistance in CSSLs, and the corresponding CSL (QTL mapping in CSSLs) functionality and LOD (likelihood of odds) thresholds calculated with 1,000 permutation tests ($P < 0.05$) were adopted to declare significant additive epistasis loci related with significant phenotypic traits (Wang *et al.* 2006). The percentage of PV (PV%) explained by individual QTL and additive effects at the LOD peaks were determined through stepwise regression (RSTEP-LRT). The PV% explained by QTL was calculated as followed: $PVE_Q = (4pq\hat{a}^2)/V_P$, of which p and q separately represent the frequencies of background fragments and donor fragments in mapping population, while â and V_P represent the additive effect of QTL and the PV, respectively. The graphical presentation of QTL was done by using the MapChart2.2 software (Voorrips 2002).

Positive additive effects showed that CCRI36 alleles decremented the phenotypic DI values and enhanced resistance against VW. In contrast, negative scores indicated that Hai1 alleles decremented the phenotypic DI values and incremented the values of VW resistance. The QTL nomenclature was designed as follows: the QTL designations that begin with "q" come after the trait abbreviation; the chromosome name and the number of QTL on that chromosome follow (Sun *et al.* 2012; Jamshed *et al.* 2016). A stable QTL was declared when it was found in at least two environments.

Meta-analysis of QTL

Biomercator 4.2 (Arcade *et al.* 2004) software was considered suitable for our data in order to perform meta-analysis (Said *et al.* 2014a). The previous QTL meta-analysis has established a database (Said *et al.* 2015b) of QTL including 2,274 QTL for 66 traits; this includes 201 QTL regarding resistance for VW (Wang *et al.* 2007; Yang *et al.* 2008; Jiang *et al.* 2009; Zhang *et al.* 2012; Fang *et al.* 2013, 2014; Ning *et al.* 2013; Zhao *et al.* 2014; Wei *et al.* 2015). In our study, we kept the standard reference for information concerning mapped QTL controlling VW resistance (Said *et al.* 2015b). Other previous studies have identified 113 QTL responsible for VW resistance (Cai *et al.* 2009; Wang *et al.* 2014; Zhang *et al.* 2014, 2015; Zhou *et al.* 2014; Shi *et al.* 2016). In aggregate, 367 QTL related to VW resistance have been utilized to build a platform for meta-analysis in which 40 QTL were from our current study. The QTL hotspots have been identified by considering a consistent QTL region if four or more QTL occurred in an interval of 25 cM. However, if the same consistent QTL region possessed QTL for only one trait then it was considered as a QTL Hotspot (Zhang *et al.* 2015).

Meta-analysis was performed by taking two files as input, *i.e.*, a QTL file and a map file. The map file was based on the information regarding the names of parents, cross type, and marker

Table 2 Scoring of symptoms of *Verticillium* wilt

Score	Degree of susceptibility	Symptoms
0	Immune	No symptom (healthy plants)
1	Highly resistance	<25% chlorotic/necrotic leaves
2	Resistance	25%–50% chlorotic/necrotic leaves
3	Susceptible	50%–75% chlorotic/necrotic leaves
4	Highly susceptible	>75% chlorotic/necrotic leaves or plant death

positions on chromosomes. The QTL file was loaded with QTL in a given environment as row information and QTL name, trait name, trait ontology, location, year, chromosome number, linkage group, LOD score, observed phenotypic variation (PV) (R^2), most likely position of QTL, CI start position, and CI end position. Initially, the two files were uploaded and map connectivity was investigated for construction of a consensus map. Afterward, QTL projection on the consensus map was done, followed by meta-analysis regarding the trait. Ultimately, four models were obtained with different Akaike information criterion (AIC) values, which were calculated as follow: ACI = 2*k* - 2ln (*L*), of which *k* and *L* separately represent the number of parameters in the model and the maximized likelihood for the model, respectively. The lowest AIC value model was considered suitable for the identification of mQTL positions or QTL hotspots. The criteria described of occurrence of mQTL in 20 cM intervals was kept standard for the identification of hotspots (Said *et al.* 2014a).

Results

Phenotypic disease index of parents and controls

In Anyang in July 2015, the highest DI value of VW was obtained in the susceptible Jimian11 strain (41.95%) followed by CCRI36 (31.03%), while the lowest was observed in the parental line Hai1 (6.21%) (Table 3), indicating a significant difference in DI values between Hai1 and Jimian11. In Anyang during August 2015, the highest DI was found in Jimian11 (48.30%) followed by CCRI36 (47.70%) and by Hai1 (19.50%). The difference in DI values between the parental lines was significant, while that of DI values between CCRI36 and Jimian11 was insignificant (Figure 1A). In Xinjiang in July and August 2015, highly significant differences were observed between parental lines (Figure 1B).

In Anyang in July 2016, the DI value of Jimian11 (26.83%) was the highest, followed by CCRI36 (25.57%), while the DI value of Hai1 (5.59%) was the lowest (Table 4), with no significant difference in DI values between CCRI36 and Jimian11. In Anyang in August 2016, the highest DI was recorded in Jimian11 (35.19%) followed by CCRI36 (32.89%), while the DI value of Hai1 (5.60%) was the lowest (Figure 1C). The difference in DI values between CCRI36 and Jimian11 was also insignificant. In Xinjiang during both July and August 2016, we observed highly significant differences in resistance against the VW disease between the parents, while no significant difference between CCRI36 and Jimian11 was observed (Figure 1D).

Evaluation of CSSLs for VW resistance

The ANOVA results ($P = 0.002$) suggested significant differences in resistance against VW among the CSSLs (Table 4). Less than one absolute value of skewness of the mean values of VW in CSSLs across eight environments indicated a normal distribution. The DI of CSSLs presented a constant normal distribution, which was in consistent with multi-gene inheritance patterns for VW resistance (Figure 2).

The average DI values of CSSLs varied from 0.30% to 18.50% in XJJuly15 and from 16.67% to 53.29% in XJAug15 (Table 3). The average DI value in XJJuly15 was 6.52 and was not significantly different from either parent. In contrast, the average DI values of CSSLs varied from 0% to 59.72% in AYJuly16. The average DI value in AYJuly16 was 25.02%, which was close to the recurrent parent CCRI36 (25.57%). The broad-sense heritability varied from 67.90% to 97.07%; the highest heritability was observed in AYJuly15, while the lowest was in XJAug15 (Table 3). For each environment over 2 years and various developmental stages, wide variation in heritability was found in CSSLs to VW disease onset, with some lines showing introgressive segregation over their parents.

Correlations among DIs at different stages of growth and in different environments

DI correlations were investigated among the different environments by pairwise comparisons, and the results showed that highly significant positive correlations ($P < 0.05$) were visible among the DIs of VW in the field, except between XJJul15 and AYJul16 (Table 5).

QTL mapping

In total, 40 QTLs for VW were detected during different stages of growth and in the various environments at the fields in Anyang

Table 3 Descriptive statistics of resistance to *Verticillium* wilt with broad sense heritability (H2 b) measured in the $BC_5F_{3:5}$ population

		CSSL population							Parents				H2 b(%)
Traits	Env	Mean	Max	Mini	SD	Skew	Kurt	Var	CCRI36	Hai1	Mid parent	Jimian11 (Control)	
DI (%)	AYJul15	21.90	73.20	0.00	13.10	0.94	1.33	171.55	31.03	6.21	18.62	41.95	97.07
	AYAug15	43.33	73.50	14.30	9.54	−0.18	0.09	91.06	47.70	19.50	33.60	48.30	94.87
	XJJul15	6.52	18.50	0.30	3.44	0.56	0.00	11.81	6.76	4.14	5.45	7.87	72.03
	XJAug15	35.10	53.29	16.67	5.45	0.23	0.35	29.69	29.63	25.83	27.83	42.48	67.90
	AYJul16	25.02	59.72	0.00	11.32	0.06	−0.20	128.11	25.57	5.59	15.58	26.83	96.60
	AYAug16	28.96	63.24	0.00	12.41	0.16	−0.35	153.91	32.89	5.60	19.25	35.19	96.56
	XJJul16	26.21	56.61	2.81	10.75	0.29	−0.46	115.56	33.18	5.43	19.31	35.20	82.79
	XJAug16	39.94	72.64	3.37	13.87	0.03	−0.47	192.27	46.52	6.41	26.47	42.89	85.33

DI: Disease Index; Env: Environment; Max: Maximum; Mini: Minimum; SD: Standard deviation; Skew: Skewness; Kurt: Kurtosis; Var: Variance

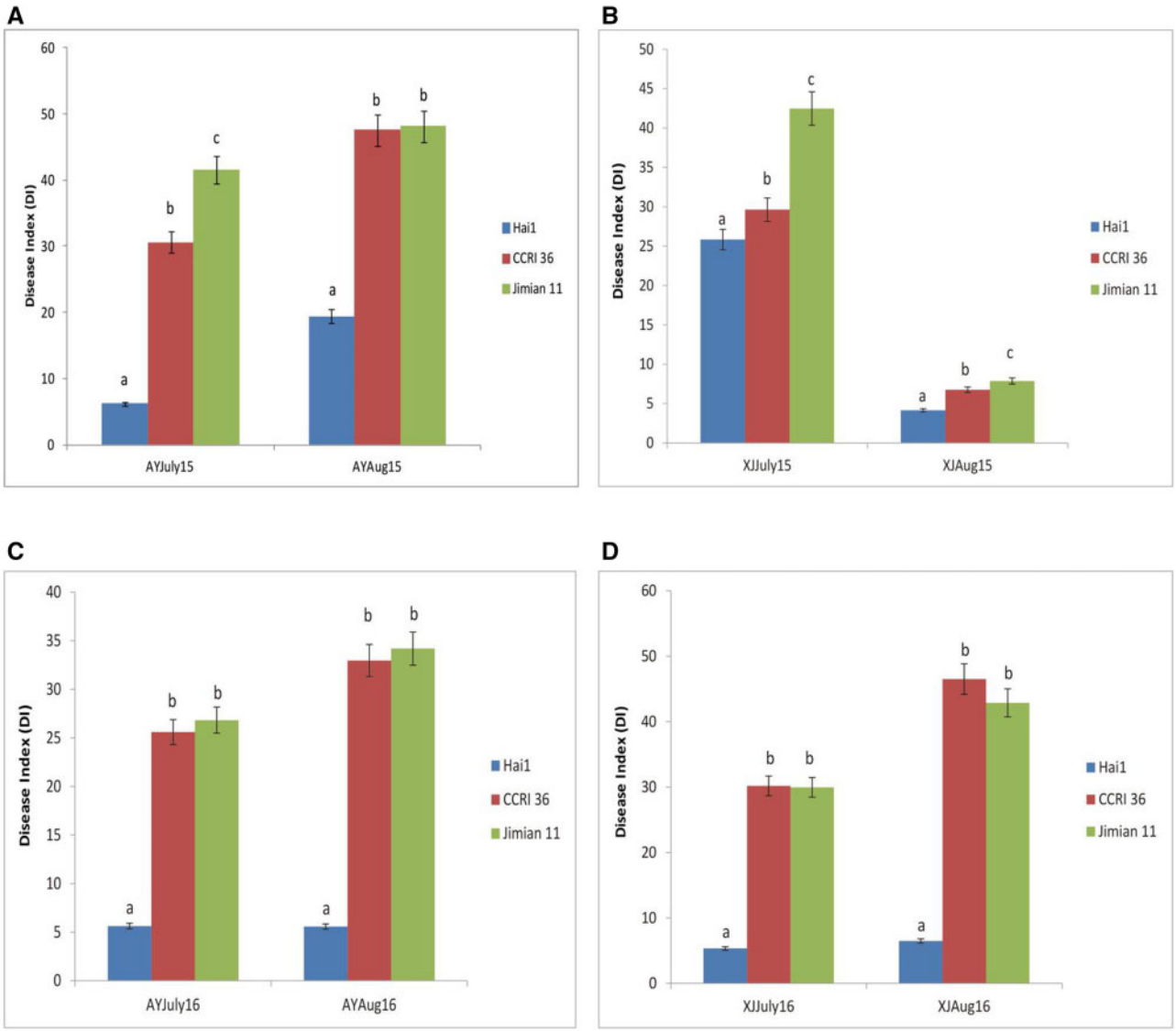

Figure 1 *Verticillium* wilt disease index of parent CCRI36, resistant control Hai1 and susceptible control Jimian11: (A) Anyang 2015; (B) Xinjiang 2015; (C) Anyang 2016 and (D) Xinjiang 2016. The error bar shows the standard deviation. a–c indicate significance at 5%.

Table 4 Analysis of variance of VW resistance ratings showed by DI across 8 environments

Source of variation	DF	Sum of square	Mean square	*F*	*P*-value
Environments	7	281489	40212.71	508.556	0**
Genotypes	299	101795.6	340.4534	4.306	<0.001**
Error	2093	165498.4	79.07235	—	—
Total	2399	548783	—	—	—

and Xinjiang for the years 2015 and 2016, and the QTL explained from 1.05% to 10.52% of the total PV, with LOD scores ranging from 1.83 to 7.30. The QTL was located on 22 chromosomes except Chr04, Chr08, Chr13, and Chr24. Among these, 15 QTLs (37.5%) had negative additive effects, indicating that their favorable alleles originated from *G. barbadense* as they enhanced VW resistance and decremented DI by 2.47 to 14.07. The remaining 25 QTLs (62.5%) had positive additive effects, indicating that the *G. barbadense* alleles decremented VW resistance; these QTLs enhanced phenotypic DI values by 0.92 to 10.05. Twenty-three QTL were identified in 2015 and 30 QTLs in 2016, of which 14 were found in the both years. The highest number of QTL (4) was detected on Chr19 and Chr23, respectively (Figure 3).

QTL for VW resistance in Anyang in 2015: In July 2015, there were 7 QTLs identified in Anyang mapped onto seven chromosomes, explaining 2.95%–5.96% of overall PV with LOD scores ranging from 1.83 to 5.96. All QTLs had positive additive effects, indicating that their favorable alleles derived from donor parent Hai1, incrementing phenotypic DI and decrementing VW resistance by 3.76–5.88.

In August 2015, 10 QTLs were identified at Anyang and mapped onto nine chromosomes, explaining 2.91%–7.26% of the overall PV. The LOD scores ranged from 1.91 to 7.26, and two QTLs were separately located on Chr05 and Chr20, consistent with the results from July 2015. Except for *qVW-Chr12-2* and *qVW-Chr26-1*, all other QTLs had positive additive effects, suggesting that donor parent *G. barbadense* alleles decremented VW resistance and incremented DI by 2.45–8.99.

QTL for VW resistance at Xinjiang in 2015: In July 2015, there were 6 QTLs detected at Xinjiang that were mapped onto six chromosomes, with 1.05%–4.58% of the total PV explained. All

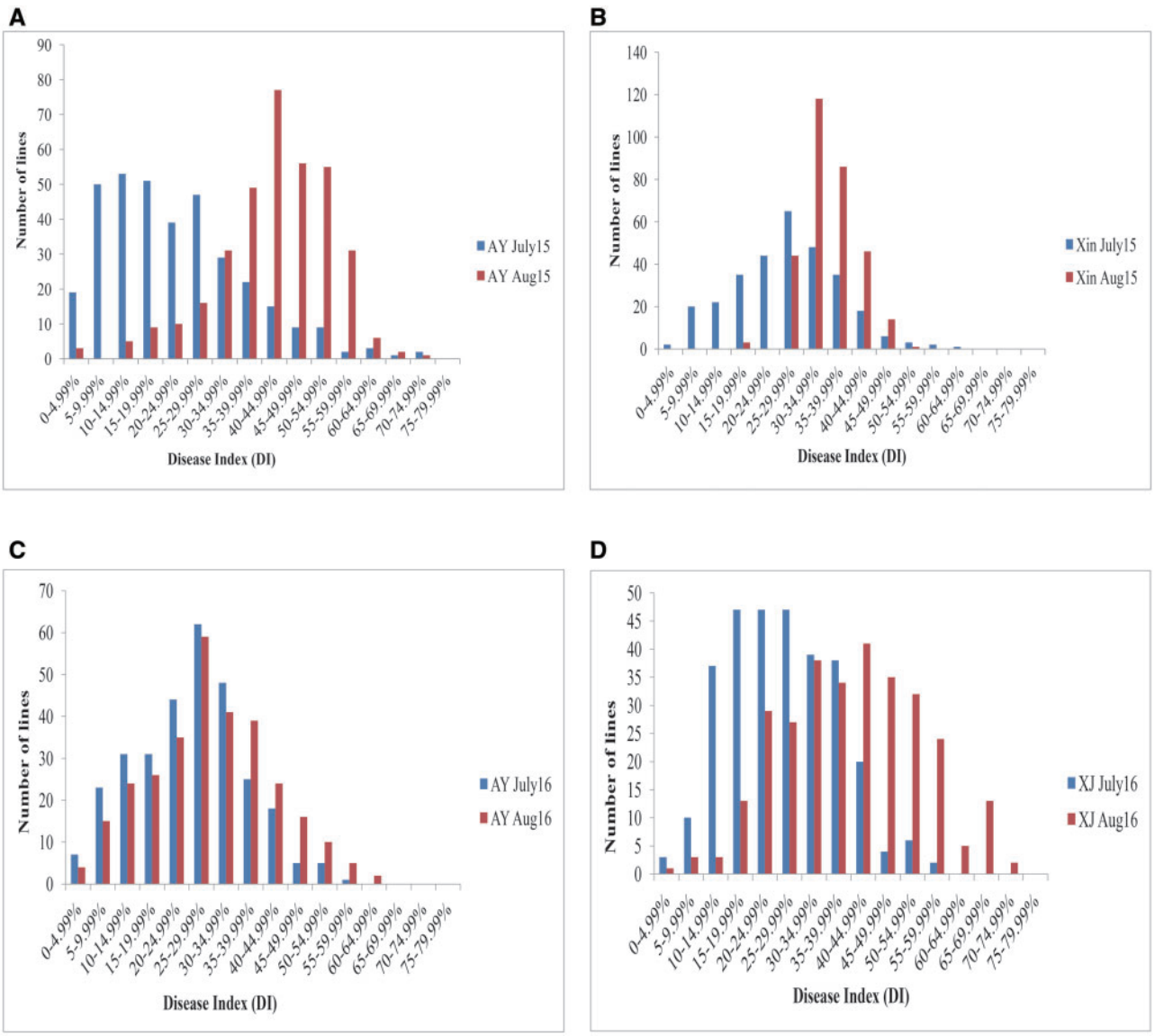

Figure 2 Normal distribution of DI phenotype in CSSLs: (A) Anyang 2015; (B) Xinjiang 2015; (C) Anyang 2016, and (D) Xinjiang 2016.

Table 5 Correlations among the disease indexes at different stages of growth in the $BC_5F_{3:5}$ population

Traits	AYJul15	AYAug15	XJJul15	XJAug15	AYJul16	AYAug16	XJJul16
AYAug15	0.407**	—	—	—	—	—	—
XJJul15	0.202**	0.164**	—	—	—	—	—
XJAug15	0.187**	0.136*	0.314**	—	—	—	—
AYJul16	0.119*	0.164**	0.04	0.123*	—	—	—
AYAug16	0.315**	0.326**	0.169**	0.188**	0.401**	—	—
XJJul16	0.445**	0.485**	0.210**	0.157**	0.248**	0.376**	—
XJAug16	0.437**	0.481**	0.163**	0.164**	0.240**	0.379**	0.919**

* present significant differences.
** extremely significant differences.

QTLs except for *qVW-Chr20-1* showed negative additive effects, suggesting that the Hai1 alleles incremented resistance against VW and decremented phenotypic DI by 1.46–6.01.

In August 2015, 1 QTL were found at Xinjiang, namely *qVW-Chr15-3*. The QTL was mapped onto Chr15 with 3.16% of PV and LOD scores of 2.09. The QTL had also negative additive effects, suggesting that their alleles derived from *G. barbadense*, as they increased resistance to the disease and decreased DI by 2.47.

QTL for VW resistance in Anyang in 2016: In July 2016, there were 8 QTLs detected at Anyang, which were mapped onto eight chromosomes, explaining 3.41%–5.79% of the total PV. All the QTL had positive additive effects, which suggested their parent Hai1 alleles decremented VW resistance and incremented DI by 2.75–9.57.

In August 2016, 10 QTLs were recorded at Anyang and mapped onto 10 chromosomes, explaining 3.22%–5.99% of the overall PV with LOD scores ranging from 2.08 to 4.03. All the QTLs had positive additive effects, suggesting that their alleles derived from parent Hai1 as they decremented resistance and incremented DI by 2.80–7.82.

QTL for VW resistance in Xinjiang in 2016: In July 2016, there were 19 QTLs detected at Xinjiang mapped onto 15 chromosomes and explaining 2.09%–10.13% of total PV with LOD scores ranging from 1.83–7.19. All QTLs except *qVW-Chr15-1*, *qVW-Chr18-2*, *qVW-Chr18-3*, and *qVW-Chr22-2* had positive additive effects, suggesting that their parent Hai1 alleles decremented resistance against VW and incremented DI by 2.96–7.65.

In August 2016, 24 QTLs were found at Xinjiang and mapped onto 17 chromosomes, explaining 1.98%–10.52% of total PV. Except *qVW-Chr10-2*, *qVW-Chr15-1*, *qVW-Chr18-1*, *qVW-Chr18-3*, and *qVW-Chr22-2*, all the QTLs had positive additive effects, suggesting that their alleles

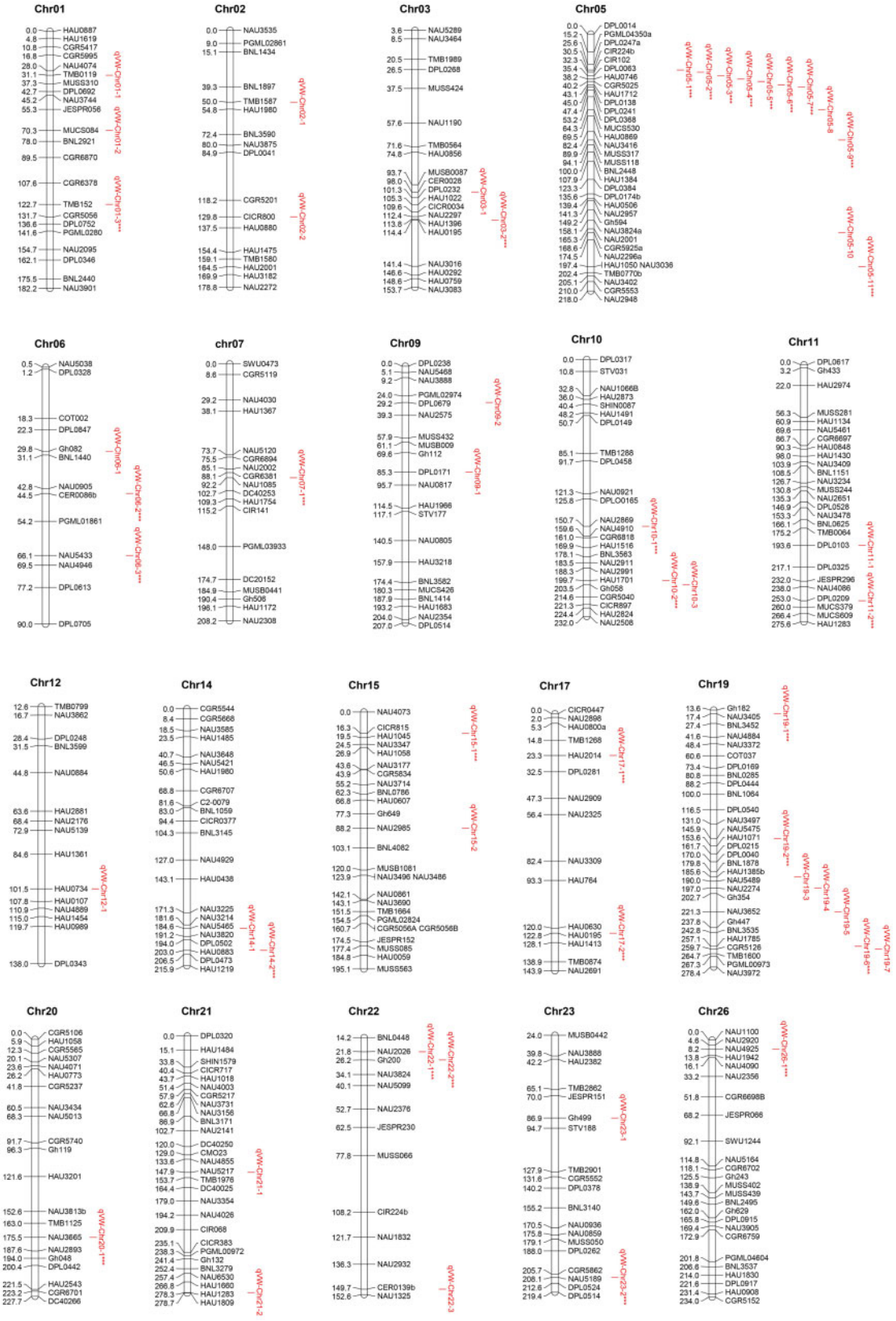

Figure 3 Identification of QTL for VW and linkage map in $BC_5F_{3:5}$ population.

derived from parent *G. barbadense* decremented resistance against VW and incremented phenotypic value of DI by 4.02–9.49.

Identification of stable QTL over environments and developmental periods

In total, 40 QTLs for VW resistance were detected in CSSLs during different stages of growth and in various environments. These QTLs were located on 21 different chromosomes. There were 4 QTLs each identified on Chr19 and Chr23, respectively, and 3 QTLs each were located on Chr12, Chr15, Chr18, and Chr23. Two QTLs were found on Chr01, Chr03, Chr05, Chr10, Chr17, and Chr22, while Chr02, Chr06, Chr07, Chr09, Chr14, Chr20, Chr21, Chr25, and Chr26 each contained only one QTL.

Among the 40 QTLs, 21 stable loci were identified in at least two environments, explaining 0.92%–10.05% of the overall PV (Table 6). There were 18 stable QTLs (85.7%) had negative additive effects, which suggested that their Hai1 alleles decremented resistance against VW and incremented phenotypic DI. Among the 21 stable QTLs, Chr19 harbored three stable QTLs, and two stable QTLs apiece were located on Chr05, Chr17, and Chr22, while Chr01, Chr03, Chr06, Chr07, Chr10, Chr11, Chr14, Chr15, Chr18, Chr20, Chr23, and Chr25 contained one stable QTL.

Two stable QTLs, namely as *qVW-Chr05-1* and *qVW-Chr20-1* were detected in six environments, explaining 4.43%–10.13% and 3.21%–10.52% of PV, respectively. There were six stable QTLs (*qVW-Chr06-1*, *qVW-Chr07-1*, *qVW-Chr14-1*, *qVW-Chr22-1*, *qVW-Chr23-3*, and *qVW-Chr25-1*) were detected in four environments, separately explaining respective observed PV of 2.95%–7.26%, 2.93%–5.49%, 3.06%–7.50%, 4.28%–6.18%, 4.22%–10.06%, and 4.96%–8.68%. Moreover, there were eight stable QTLs; (*qVW-Chr01-2*, *qVW-Chr03-2*, *qVW-Chr05-2*, *qVW-Chr10-1*, *qVW-Chr17-1*, *qVW-Chr17-2*, *qVW-Chr19-2*, and *qVW-Chr19-4*) detected in three environments that presented 7.26%–9.35%, 5.27%–7.51%, 4.06%–6.27%, 3.69%–5.10%, 3.03%–7.37%, 3.06%–5.58%, 5.96%–9.16%, and 3.83%–4.51% of the observed PV, respectively. Twelve stable QTLs were detected in two environments, with overall 3.74%–10.22% of PV. The stable QTL *qVW-Chr05-1*, *qVW-Chr20-1*, and *qVW-Chr23-3* had major effects and explained 10.13%, 10.52%, and 10.06% of the observed PV, respectively (Table 6).

QTL hotspots and meta-analysis

Based on the meta-analysis, 70 QTLs hotspot regions were detected on 14 chromosomes: Chr01, Chr03, Chr05, Chr07, Chr09, Chr11, Chr12, Chr14, Chr15, Chr19, Chr20, Chr22, Chr23, and Chr26 (Supplementary Figure S1, Table 7). Among these, 17 QTLs hotspot regions were consistent with those detected earlier (Said *et al.* 2015b; Zhang *et al.* 2015; Shi *et al.* 2016; Table 5), and the other 15 were identified as novel regions. Three QTLs hotspot regions each were located on Chr05, Chr19, and Chr26, while two QTLs hotspot regions were detected on Chr01, Chr03, Chr07, Chr09, Chr20, Chr21, Chr22, and Chr23. In addition, Chr06, Chr11, Chr12, Chr14, Chr15, Chr17, and Chr24 each contained 1 QTL hotspot region (Table 7).

Among 70 QTLs hotspot regions, six located on three different chromosomes had more than 10 QTLs (Supplementary Figure S1, Table 7), which might be very important for further studies and could be utilized for molecular breeding via MAS. As for chr05, 40 QTLs were selected to project on consensus chromosome 05 (Cons.Chr05), resulting in seven identified QTLs hotspot regions. There were 6, 9, 2, 4, 13, 14, and 7 QTLs on Chr05-DI-Hotspot-1, Chr05-DI-Hotspot-2, Chr05-DI-Hotspot-3, Chr05-DI-Hotspot-4, Chr05-DI-Hotspot-5, Chr05-DI-Hotspot-6, and Chr05-DI-Hotspot-7, respectively (Table 7). Eleven QTLs were selected to project on chromosome 09 (Cons.Chr09), and five QTLs hotspot regions were identified, where Chr09-DI-Hotspot-1, Chr09-DI-Hotspot-2, and Chr09-DI-Hotspot-3 separately had 2, 7, and 6 QTLs, while 3 QTL each were observed in Chr09-DI-Hotspot-4 and Chr09-DI-Hotspot-5. Sixteen QTLs were identified and projected on consensus Chr19, and the meta-analysis identified five QTLs hotspot regions. Chr19-DI-Hotspot-1, Chr19-DI-Hotspot-2, Chr19-DI-Hotspot-3, Chr19-DI-Hotspot-4, and Chr19-DI-Hotspot-5 contained 7, 3, 4, 8, and 6 QTLs, respectively. Twelve QTLs were selected to project on chromosome 22 (Cons.Chr22), and five QTLs hotspot regions were identified, where Chr22-DI-Hotspot-1 and Chr22-DI-Hotspot-1 had 4 QTLs, while Chr22-DI-Hotspot-2 and Chr22-DI-Hotspot-4 had 2 QTLs (Figure 4). Fifty-six QTLs were selected to project on Cons.Chr23, identifying eight QTLs hotspot regions. The largest numbers of QTL were observed in Chr23-DI-Hotspot-1, Chr23-DI-Hotspot-2, and Chr23-DI-Hotspot-3, and the specific numbers were 13, 22, and 20, respectively. Sixteen QTLs were selected to project on Cons.Chr26, and six QTLs hotspot regions were identified. There were 2, 4, 6, 2, 5, and 4 QTLs separately identified in Chr26-DI-Hotspot-1, Chr26-DI-Hotspot-2, Chr26-DI-Hotspot-3, Chr26-DI-Hotspot-4, Chr26-DI-Hotspot-5, and Chr26-DI-Hotspot-6.

Discussion

Field status and phenotypic assessment

The CSSLs utilized in this study were firstly subjected to VW resistance investigation together with their parents and controls, which was performed without the inoculation provision and under natural environmental conditions. The VW resistance was assessed based on the amount of leaf tissue damage in the mature stages. The results indicated that the parent Hai1 appeared to be more resistant to the disease compared to CCRI36, while the control Jimian11 displayed slightly higher susceptibility compared to CCRI36. Most of the CSSLs exhibited higher DI values than mid parents (Table 3), and this phenomenon might be due to DI value fluctuation across the environments. The same remark was made in a study using an interspecific chromosome segment line with different VW strains; according to the authors, this result may missing be explained by the resistance to different VW isolates being controlled by distinct single genes, and that in the presence of a mixture of isolates, interactions occurred (Wang *et al.* 2014).

Over different years of study and across variable environments, the investigated population of CSSLs has displayed a broad range of sensitivity, from highly susceptible to highly resistant. For the verification of this hypothesis, the CSSL population has been investigated on a phenological basis over different environments at various growth stages. In this study, we observed that DI values of susceptible to VW infection were higher in August than in July, where the susceptible control (Jimian11) showed greater than 35% DI values except in XJJul15 and AYJul16, while the DI values of CCRI36 were less than 35% except in AYAug15 and XJAug16 (Table 3). This lesser DI percentage is evidence for the selective pressure exerted by variable VW strains under natural environmental conditions. Other reasons behind this phenological variation include intensity and virulence of strains, fungal amount in the soil, and developmental stages as well as environmental influences (Bejarano *et al.* 1997). Similar findings have been reported earlier in which the host plant was shown to be resistant against inoculum of VW while remaining susceptible under natural environmental conditions (Fang *et al.* 2013). We also have compared the results with previous findings

Table 6 Identification of QTLs for VW disease index during different development and environments in $BC_5F_{3:5}$ populations

SL. No.	QTLs	Growth stage	Env	Chr	Nearest marker	LOD	Add	PV (%)
1	qVW-Chr01-1	July	AYJul16	1	MUCS084	2.8787	5.5549	7.7591
2	qVW-Chr01-2	August	AYAug15	1	TMB1152	3.1935	7.2645	2.9729
		July	XJJul16	1	TMB1152	4.9111	8.0573	3.8104
		August	XJAug16	1	TMB1152	5.6252	9.3546	5.291
3	qVW-Chr02-1	July	XJJul15	2	TMB1578	2.6201	3.9423	−3.4229
4	qVW-Chr03-1	August	XJAug16	3	CER0028	2.0002	2.9425	4.8718
5	qVW-Chr03-2	August	AYAug16	3	HAU01953	3.5605	5.266	4.4108
		July	XJJul16	3	HAU01953	3.8574	6.1216	3.9558
		August	XJAug16	3	HAU01953	4.8213	7.5116	5.6721
6	qVW-Chr05-1	July	AYJuly15	5	DPL0063	3.6862	5.4796	5.6668
		August	AYAug15	5	DPL0063	3.2146	4.4283	3.8677
		July	AYJul16	5	DPL0063	3.569	5.2103	4.7711
		August	AYAug16	5	DPL0063	3.41	4.6642	5.0786
		July	XJJul16	5	DPL0063	7.1896	10.1321	6.0874
		August	XJAug16	5	DPL0063	5.8282	8.1755	7.0881
7	qVW-Chr05-2	August	AYAug15	5	HAU1050	2.9203	4.0577	2.4472
		July	XJJul16	5	HAU1050	4.7384	6.2683	3.165
		August	XJAug16	5	HAU1050	4.4496	6.0124	4.018
8	qVW-Chr06-1	July	AYJul15	6	NAU5433	1.826	2.9497	5.8819
		July	AYJul16	6	NAU5433	3.5397	5.7939	6.8909
		July	XJJul16	6	NAU5433	3.6745	5.8739	6.6781
		August	XJAug16	6	NAU5433	4.5085	7.2619	9.4894
9	qVW-Chr07-1	August	AYAug15	7	NAU1085	1.9384	2.932	2.8305
		August	AYAug16	7	NAU1085	2.4494	3.6901	3.7526
		July	XJJul16	7	NAU1085	3.6792	5.491	3.9666
		August	XJAug16	7	NAU1085	3.0662	4.5979	4.6818
10	qVW-Chr09-1	August	XJAug16	9	DPL0171	3.5231	5.2645	5.0756
11	qVW-Chr10-1	July	AYJul16	10	NAU2869	3.4096	5.0996	5.6771
		August	AYAug16	10	NAU2869	2.9018	4.3569	5.7526
		August	XJAug16	10	NAU2869	2.4481	3.6874	5.8022
12	qVW-Chr10-2	August	XJAug16	10	Gh058	2.4296	3.6591	−8.0612
13	qVW-Chr11-1	July	XJJul15	11	DPL0103	2.7115	4.0768	−6.0087
14	qVW-Chr11-2	July	XJJul16	11	DPL0209	5.8426	7.7311	6.7518
		August	XJAug16	11	DPL0209	5.979	8.3431	8.8115
15	qVW-Chr12-1	July	AYJul16	12	NAU3862	2.4149	3.8166	6.6937
16	qVW-Chr12-2	August	AYAug15	12	HAU0734	2.7961	4.2012	−8.3897
17	qVW-Chr12-3	July	XJJul15	12	HAU0107	1.9456	1.0464	−1.462
18	qVW-Chr14-1	August	AYAug15	14	HAU0883	2.1496	3.0607	2.654
		July	AYJul16	14	HAU0883	2.0086	3.4097	2.7486
		July	XJJul16	14	HAU0883	2.4247	3.9706	2.8612
		August	XJAug16	14	HAU0883	4.8176	7.5025	5.1472
19	qVW-Chr15-1	July	XJJul16	15	CICR815	2.6404	3.9722	−6.6383
		August	XJAug16	15	CICR815	2.4482	3.688	−8.0786
20	qVW-Chr15-2	August	XJAug16	15	NAU2985	2.6351	3.9759	5.5483
21	qVW-Chr15-3	August	XJAug15	16	JESPR297	2.0905	3.1578	−2.4666
22	qVW-Chr17-1	July	AYJul15	17	HAU2014	1.8459	3.0291	4.3604
		July	XJJul16	17	HAU2014	4.5595	7.3702	5.4556
		August	XJAug16	17	HAU2014	3.4264	6.7461	5.9106
23	qVW-Chr17-2	August	AYAug15	17	HAU0195	1.9109	3.0598	2.7468
		August	AYAug16	17	HAU0195	3.5605	5.266	4.4108
		August	XJAug16	17	HAU0195	2.8499	5.5841	4.223
24	qVW-Chr18-1	August	XJAug16	18	DPL0795	2.0555	1.9806	−12.26
25	qVW-Chr18-2	July	XJJul16	18	DPL0348	1.9904	2.0916	−7.2709
26	qVW-Chr18-3	July	XJJul16	18	NAU4860	2.0829	2.1872	−8.299
		August	XJAug16	18	NAU4860	2.3375	2.2476	−11.3297
27	qVW-Chr19-1	July	AYJul16	19	NAU3405	3.1073	4.6577	5.0053
		August	AYAug16	19	NAU3405	4.0269	5.9947	6.2251
28	qVW-Chr19-2	July	AYJul15	19	NAU5475	4.0064	5.9648	6.361
		July	XJJul16	19	NAU5475	6.0373	9.1624	6.1133
		August	XJAug16	19	NAU5475	5.1611	7.7824	7.2948
29	qVW-Chr19-3	July	XJJul15	19	NAU2274	2.7115	4.0768	−6.0087
30	qVW-Chr19-4	August	AYAug15	19	HAU1785	2.5011	3.9863	5.4126
		July	XJJul16	19	HAU1785	2.5732	3.8268	5.355
		August	XJAug16	19	HAU1785	3.0245	4.5145	7.5305
31	qVW-Chr20-1	July	AYJul15	20	NAU3665	3.3275	5.121	3.7565
		August	AYAug15	20	NAU3665	3.1754	5.0552	2.8752
		July	XJJul15	20	NAU3665	3.0179	4.5781	0.9208
		August	AYAug16	20	NAU3665	2.1177	3.2158	2.7996
		July	XJJul16	20	NAU3665	6.5696	9.4454	4.1522
		August	XJAug16	20	NAU3665	7.304	10.5203	5.644
32	qVW-Chr21-1	July	XJJul15	21	NAU5217	2.7115	4.0768	−6.0087

(continued)

Table 6. (continued)

SL. No.	QTLs	Growth stage	Env	Chr	Nearest marker	LOD	Add	PV (%)
33	qVW-Chr22-1	July	AYJuly15	22	NAU2026	2.8677	5.0498	4.7381
		August	AYAug16	22	NAU2026	2.0829	4.2836	3.7289
		July	XJJul16	22	NAU2026	2.9621	5.3729	3.8318
		August	XJAug16	22	NAU2026	3.4532	6.1844	5.2768
34	qVW-Chr22-2	July	XJJul16	22	Gh200	1.8326	3.2056	−10.6602
		August	XJAug16	22	Gh200	1.9384	3.3896	−14.0703
35	qVW-Chr23-1	July	AYJul16	23	Gh499	2.1085	4.7953	6.214
36	qVW-Chr23-2	August	AYAug15	23	NAU0859	4.0104	5.9711	7.1328
37	qVW-Chr23-3	July	AYJul15	23	NAU5189	2.5209	4.2164	4.8607
		August	AYAug16	23	NAU5189	3.6538	5.9953	5.4211
		July	XJJul16	23	NAU5189	5.2507	8.1358	5.6123
		August	XJAug16	23	NAU5189	6.6099	10.0631	8.0675
38	qVW-Chr23-4	August	AYAug15	23	DPL0524	2.0507	2.9142	8.9899
39	qVW-Chr25-1	July	AYJul16	25	CER0086b	5.0908	8.6807	9.5675
		August	AYAug16	25	CER0086b	2.7679	4.957	7.8214
		July	XJJul16	25	CER0086b	2.6554	5.0476	6.6149
		August	XJAug16	25	CER0086b	3.7238	6.8359	10.0519
40	qVW-Chr26-1	August	AYAug15	26	NAU4925	2.2945	3.3359	−5.1952

of lesser (DI < 40%) by CCRI36's progenitor, whereas some of the offspring displayed a significant resistance level comparable to the susceptible control Jimian11. Besides this, a noteworthy level of transgressive segregation has been observed under field conditions, in accordance with previous reports (Bolek *et al.* 2005; Wang *et al.* 2008). Across different environments during the investigation period, several CSSLs remained consistent in their resistance to the mixture of strains present in the area, as compared to most of the lines that displayed a high level of susceptibility (Figure 2). This fact can be explained by the presence of a wider range of environmental variation occurring during two years of study, where the VW strains constantly change their genetic makeup to be more resistant. Previous reports (Wang *et al.* 2014) justified our findings for the confirmation of the idea that there must exist an antagonistic interaction between resistance QTL/genes and different strains of fungi and that a large number of genes are responsible for controlling the resistance mechanism against *V. dahliae* isolates.

The phenological parameters measured in 2 years of study at both locations depicted rare weak correlations. Expression of different genetic factors in variable environments at different growth stages confirmed the reason behind the weak correlation coefficient values (Table 3). This follows from the alteration of genes on exposure to VW strains at varying growth stages. In a study of backcross inbred lines regarding VW resistance, there was a weak but positive correlation among DIs under field conditions (Zhang *et al.* 2012).

Due to varying environmental stresses in both years at two locations, error variances were very high; because of this, heritability values ranged from weak to moderate. This suggests that the wide range of phenology regarding DI has been caused by varying environmental influences. However, this is not surprising, as cotton resistance levels to *V. dahliae* are greatly influenced by environmental factors, resistance genes, inoculum concentrations and their interactions (Zhang *et al.* 2014).

Genetic map used for QTL identification

Through utilization of hybridization techniques including interspecific (Bolek *et al.* 2005; Mert *et al.* 2005; Wang *et al.* 2008; Yang *et al.* 2008; Fang *et al.* 2013; Ning *et al.* 2013; Fang *et al.* 2014; Zhang *et al.* 2014, 2015) and intraspecific (Yang *et al.* 2008; Fang *et al.* 2013; Ning *et al.* 2013; Wang *et al.* 2015) crossing, a wide range of genetic maps were constructed. However, lesser genome coverage, *i.e.*, < 50%, has been achieved by using interspecific crossing, and this has led to a bottleneck in the detection of QTL from the whole genome with ultra-resolution. This has been confirmed by the sequencing of about 57.90% of the tetraploid cotton genome (Zhang *et al.* 2015), where 27%, *i.e.*, 1143.1 cM, and 35% with 279 markers of genome coverage (Fang *et al.* 2013; Ning *et al.* 2013). To date, one exclusive study has been reported that covered more than 50% of the genome, *i.e.*, 55.7% accounting for 882 genetic markers in total, including 414 SNPs, 36 RGA-RFLPs (resistance gene analog-amplified fragment length polymorphisms) and 432 SSRs. Therefore, the whole-genome coverage of allotetraploid cotton with resistant QTL for VW has not yet been achieved. This study aimed to cover 100% of the cotton genome, comprising about 5115.6 cM (Shi *et al.* 2015). It would be premature to consider all 26 genetic threads of allotetraploid cotton found via the use of CSSLs in the quest for QTL involved in VW resistance. A noteworthy number of QTL (53) from 20 chromosomes were identified as being related to VW resistance, meaning that these QTLs are extensively distributed throughout the whole genome. These results would be not easy to achieve if the *G. barbadense* genome was used as a template with a restricted number of markers and lesser polymorphism.

Distribution of QTL of VW through the whole genome

There were several chromosomes as yet unexplored regarding VW resistance QTL in previous studies, specifically Chr06, Chr10, Chr12, and Chr18 together with almost 100+ related QTL (Zhang *et al.* 2014). This omission has left gaps in our knowledge of the tetraploid genome. Our findings have contributed a significant amount of valuable information regarding these gaps. There were three QTLs each detected on Chr12 and Chr18, while only one and two DI QTL were identified on Chr06 and Chr12, respectively. As in previous findings from meta-analyses by different researchers (Zhang *et al.* 2015), we were unable to discover any hotspot regions on Chr10 and Chr18. However, several chromosomes were found to be heavily loaded with DI QTL as on Chr19 and Chr23 with each 4 DI QTLs. Three QTLs were located on Chr15, as on Chr12 and Chr18 as mentioned earlier. Also, we successfully identified stable QTL across six different environments, which was not the case in any of the previous reports.

As mentioned earlier, 21 chromosomes were explored in our study with 40 QTLs using $BC_5F_{3:5}$ populations, of which 17 QTLs

Table 7 QTL hotspots detected for VW resistance on the consensus map through meta-analysis

SL	Hotspot name	Chr	Location (cM)	No. of QTLs	No. of QTLs in this paper	Reported earlier
1	Chr01-MetaQTL-1	Chr01	70–78cM	5	0	Said *et al.* 2015b; Shi *et al.* 2016
2	Chr01-MetaQTL-2	Chr01	101–117cM	5	1	—
3	Chr01-MetaQTL-3	Chr01	166–170cM	3	0	Said *et al.* 2015b; Shi *et al.* 2016
4	Chr01-MetaQTL-4	Chr01	181–187cM	2	1	—
5	Chr01-MetaQTL-5	Chr01	202–212cM	3	0	—
6	Chr03-MetaQTL-1	Chr03	95–101cM	5	1	—
7	Chr03-MetaQTL-2	Chr03	103–112cM	2	0	—
8	Chr03-MetaQTL-3	Chr03	113–121cM	8	1	Shi *et al.* 2016
9	Chr03-MetaQTL-4	Chr03	124–132cM	4	0	Shi *et al.* 2016
10	Chr05-MetaQTL-1	Chr05	30–36cM	6	1	—
11	Chr05-MetaQTL-2	Chr05	35–43cM	9	0	—
12	Chr05-MetaQTL-3	Chr05	59–68cM	2	0	—
13	Chr05-MetaQTL-4	Chr05	100–106cM	4	0	—
14	Chr05-MetaQTL-5	Chr05	126–136cM	13	0	Said *et al.* 2015b
15	Chr05-MetaQTL-6	Chr05	161–173cM	14	0	Shi *et al.* 2016
16	Chr05-MetaQTL-7	Chr05	191–204cM	7	1	Shi *et al.* 2016
17	Chr07-MetaQTL-1	Chr07	74–77cM	2	0	—
18	Chr07-MetaQTL-2	Chr07	87–98cM	11	1	Shi *et al.* 2016
19	Chr07-MetaQTL-3	Chr07	130–139cM	2	0	Shi *et al.* 2016
20	Chr07-MetaQTL-4	Chr07	154–164cM	3	0	—
21	Chr07-MetaQTL-5	Chr07	195–204cM	7	0	—
22	Chr09-MetaQTL-1	Chr09	54–59cM	2	0	—
23	Chr09-MetaQTL-2	Chr09	75–84cM	7	1	Said *et al.* 2015b; Shi *et al.* 2016
24	Chr09-MetaQTL-3	Chr09	112–123cM	6	0	Zhang *et al.* 2015; Said *et al.* 2015b; Shi *et al.* 2016
25	Chr09-MetaQTL-4	Chr09	159–168cM	3	0	—
26	Chr09-MetaQTL-5	Chr09	194–197cM	3	0	Shi *et al.* 2016
27	Chr11-MetaQTL-1	Chr11	67–80cM	3	0	—
28	Chr11-MetaQTL-2	Chr11	104–124cM	2	0	Shi *et al.* 2016
29	Chr11-MetaQTL-3	Chr11	185–202cM	2	1	—
30	Chr11-MetaQTL-4	Chr11	252–253cM	2	1	—
31	Chr12-MetaQTL-1	Chr12	104–111cM	6	2	—
32	Chr12-MetaQTL-2	Chr12	120–123cM	2	0	Said *et al.* 2015b; Shi *et al.* 2016
33	Chr12-MetaQTL-3	Chr12	128–129cM	2	0	Shi *et al.* 2016
34	Chr14-MetaQTL-1	Chr14	58–65cM	3	0	Shi *et al.* 2016
35	Chr14-MetaQTL-2	Chr14	72–99cM	2	0	—
36	Chr14-MetaQTL-3	Chr14	170–179cM	4	0	—
37	Chr14-MetaQTL-4	Chr14	202–203cM	2	1	—
38	Chr15-MetaQTL-1	Chr15	46–53cM	2	1	Said *et al.* 2015b
39	Chr15-MetaQTL-2	Chr15	61–69cM	3	0	Shi *et al.* 2016
40	Chr15-MetaQTL-3	Chr15	79–98cM	2	1	—
41	Chr15-MetaQTL-4	Chr15	135–145cM	4	0	—
42	Chr15-MetaQTL-5	Chr15	153–168cM	4	0	—
43	Chr15-MetaQTL-6	Chr15	179–190cM	2	0	Shi *et al.* 2016
44	Chr19-MetaQTL-1	Chr19	33–44cM	7	0	Shi *et al.* 2016
45	Chr19-MetaQTL-2	Chr19	84–97cM	3	0	—
46	Chr19-MetaQTL-3	Chr19	140–152cM	4	1	—
47	Chr19-MetaQTL-4	Chr19	188–198cM	8	1	—
48	Chr19-MetaQTL-5	Chr19	218–256cM	6	0	Zhang *et al.* 2015
49	Chr20-MetaQTL-1	Chr20	44–48cM	5	0	Said *et al.* 2015b
50	Chr20-MetaQTL-2	Chr20	51–59cM	5	0	—
51	Chr20-MetaQTL-3	Chr20	154–195cM	3	1	—
52	Chr22-MetaQTL-1	Chr22	21–30cM	4	2	—
53	Chr22-MetaQTL-2	Chr22	70–75cM	2	0	Said *et al.* 2015b
54	Chr22-MetaQTL-3	Chr22	100–110cM	8	0	Zhang *et al.* 2015
55	Chr22-MetaQTL-4	Chr22	136–140cM	2	0	—
56	Chr22-MetaQTL-5	Chr22	146–151cM	4	0	Said *et al.* 2015b
57	Chr23-MetaQTL-1	Chr23	49–57cM	13	0	—
58	Chr23-MetaQTL-2	Chr23	65–70cM	22	0	—
59	Chr23-MetaQTL-3	Chr23	73–79cM	20	0	—
60	Chr23-MetaQTL-4	Chr23	84–90cM	9	1	—
61	Chr23-MetaQTL-5	Chr23	95–102cM	5	0	—
62	Chr23-MetaQTL-6	Chr23	111–115cM	4	0	—
63	Chr23-MetaQTL-7	Chr23	121–141cM	9	0	Zhang *et al.* 2015
64	Chr23-MetaQTL-8	Chr23	184–225cM	6	2	—
65	Chr26-MetaQTL-1	Chr26	5–12cM	2	1	—
66	Chr26-MetaQTL-2	Chr26	10–37cM	4	0	—
67	Chr26-MetaQTL-3	Chr26	90–95cM	6	0	—
68	Chr26-MetaQTL-4	Chr26	105–110cM	2	0	
69	Chr26-MetaQTL-5	Chr26	129–146cM	5	0	—
70	Chr26-MetaQTL-6	Chr26	216–232cM	4	0	—

were located on the A sub-genome chromosomes covering Chr01, Chr02, Chr03, Chr05, Chr06, Chr07, Chr09, Chr10, Chr11, and Chr12 accounting for 42.5%, while 23 QTLs were explored on the D sub-genome covering Chr14, Chr15, Chr17, Chr18, Chr19, Chr20, Chr21, Chr22, Chr23, Chr25, and Chr26, estimated as about 57.5%. The results provide evidence for the conclusion that the D sub-genome encloses more QTLs for VW resistance as compared to the A sub-genome which was not consistent results obtained by previous studies (Bolek *et al.* 2005; Yang *et al.* 2008; Ning *et al.* 2013).

Stability with earlier studies VW resistance QTL

In this study, 40 QTLs related to VW resistance were identified in 300 CSSLs. Among the QTL, 14 (35%) had negative additive effects, which indicated that the *G. barbadense* alleles increased VW resistance and decreased DI values, by about 1.46 to 14.07. In contrast, 26 QTLs (65%) had positive additives effects, indicating that the *G. hirsutum* alleles enhanced VW wilt resistance and decremented phenotypic DI values, by about 0.92 to 10.05. As for different years, 23 QTLs were identified in 2015, while 30 QTLs were found in 2016, and 14 QTLs were found in both years. The maximum number of QTL (4) was detected on Chr19 and Chr22 (Figure 3, Table 6).

Among the 40 QTLs, 21 were detected consistently in at least two environments, and these were deemed as stable QTL. Out of 21 stable QTLs, 18 (85.71%) had positive additive effects, indicating that the *G. barbadense* alleles decremented VW resistance and increased DI. Based on meta-analysis of the identified 40 QTLs, 6 ones were consistent with previously identified QTL, and they had common SSR markers (Yang *et al.* 2008; Wu *et al.* 2010; Ning *et al.* 2013; Wang *et al.* 2014; Zhang *et al.* 2014). One QTL, *qVW-Chr01-1* positioned on Chr01, was similar to Ning's *qVW-A1-1* (Ning *et al.* 2013) that was identified with common markers of Gh215. Another QTL, *qVW-Chr03-2*, was similar to *qVW-C3-2* in the results of Shi's article (Shi *et al.* 2015), and both were associated with the shared marker CER0028. The QTL *qVW-Chr12-2* was similar to *qVWR-06-C12* in the results of Zhang's article (Zhang *et al.* 2015) associated with the common marker CIR272. In addition, *qVW-Chr23-2* was similar to Fang's *qDR52T2-C23-3* (Fang *et al.* 2014) associated with the shared marker DPL1938. The remaining 34 QTLs for VW resistance could be considered as novel loci in this study.

Based on the meta-analysis, 70 QTLs hotspot regions were detected, of which 26 were consistent with earlier studies (Said *et al.* 2015b; Zhang *et al.* 2015; Shi *et al.* 2016), while another 44 were novel and unreported hotspot regions (Figure 4, Table 6). These hotspot regions and QTL could be very important for further comparative studies, and they can be utilized for marker assisted selection.

Further utilization of QTL for VW resistance

According to previous reports on the CSSLs in cotton, the prominent characteristics of high-fiber quality and high yield have deliberately been exploited (Yang *et al.* 2009; Liang *et al.* 2010; Lan *et al.* 2011; Zhang *et al.* 2012; Ma *et al.* 2013; He *et al.* 2014). At present, a total of 300 CSSLs from *G. hirsutum* CCRI36 and *G. barbadense* Hai1 have been investigated regarding their resistance to VW. The segments of chromosomes introgressed from *G. barbadense* into *G. hirsutum* made these lines slightly different from their recurrent parent lines by reducing the influence of genetic background of the recipient, making the CSSLs efficient breeding materials for conducting quantitative genetics research. Thus, the experimental work proves to be beneficial in paving the way towards whole genome study of cotton by laying a solid platform of molecular findings related to fine mapping, functional genomics, gene pyramiding and ultimately marker-assisted breeding.

Conclusions

In this study, 300 CSSLs developed from *G. hirsutum* CCRI36 × *G. barbadense* Hai1 were used to detect QTL for VW resistance in various environments (Anyang and Xinjiang) and different developmental stages (July and August). The nature of the population (CSSLs), population size, and the presence of controls (Jimian11) in our study allowed us to lower the experimental error and to check the accuracy of the data.

In total, 40 QTLs for VW resistance were identified in CSSLs populations, of which 21 were found as stable QTLs. Six QTLs were similar to previously reported QTL, while 34 were novel QTLs. Based on a meta-analysis, 70 QTL hotspot regions were detected, including 44 novel regions. These consistent QTL and hotspot regions form critical steps will contribute to molecular breeding in developing and improving the VW resistance in upland cotton. The outcomes of this study also provide an important foundation for further studies of the molecular basis of VW resistance in cotton.

Data Availability

All the sequences of SSR markers in this study are available at CottonGen (https://www.cottongen.org/). All the supplementary material files are available at https://doi.org/10.6084/m9.figshare.13619309, including Supplementary Figure S1 and phenotype and genotype data used to map QTL in this study.

Funding

This study was supported by the National Natural Science Foundation of China (31801404 and 32070560) and Joint Funds of the National Natural Science Foundation (U1804103), and Science and Technology development Project of Henan Province (192102110127).

Conflicts of interest: The authors declared that they have no conflicts of interest to this work.

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Communicating editor: A. E. Lipka