

1 Genome-wide quantitative trait loci mapping on *Verticillium*
2 wilt resistance in 300 chromosome segment substitution lines
3 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 resistant VW. However, multiple attempts to improve the VW resistance of the most widely cultivated Upland cotton have brought in little significant progress, and it seems necessary and urgent to develop Chromosome segment substitution lines (CSSLs) for merging the superior genes related with high yield and wide adaptation from *G. hirsutum* and VW resistance and excellent fiber quality from *G. barbadense*. In this study, 300 CSSLs were chosen from the developed BC₅F_{3:5} CSSLs constructed by *G. hirsutum* CCRI36 and *G. barbadense* Hai1 to conduct quantitative trait locus (QTL) mapping on VW resistance, and a total of 53 QTLs relevant to VW disease index (DI) were identified together with the phenotypic data of 2 years investigations in two fields with two replications per year. All the QTLs were distributed on 20 chromosomes with phenotypic variation of 3.74-11.89%, of which 29 stable ones were consistent in at least two environments. Based on Meta-analysis on the 53 QTLs, 43 novel ones were identified, while 10 ones consistent to previously identified QTLs. Meanwhile, 32 QTL hotspot regions were detected, including 15 ones were novel. This study concentrates on QTL identification and screening hotspot region related with VW in the 300 CSSLs, which lay a solid platform 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 program for resistant cotton varieties.

Keywords: CSSLs, *Verticillium* wilt, Disease Index, Quantitative Trait Loci,
Meta-analysis

1. Introduction

Cotton (*Gossypium* spp. L.) is not only the most significant cash crop producing the main source of natural fiber for the textile industry, but also the second important oilseed crop [1]. The cultivation history of cotton could retrospect to 7000 years ago[2], which is widely grown in approximately 100 countries principally located in tropical and sub-tropical arena [3]. The genus *Gossypium* consists of 53 species all over the world, including 46 diploid ones ($2n = 2 \times = 26$) and 7 allotetraploid ones ($2n = 2 \times = 52$) [4], of which the emergence of the latter dated from a polyploidization event between A and D genome 1-2 million years ago [3]. Only 4 cultivated species (2 diploids and 2 tetraploids) are extant and widely planted all over the world, while the rest of the 53 species are wild but important reservoir of beneficial agronomic traits for improvement of the cultivated ones [5, 6]. Nowadays, *G. hirsutum* and *G. barbadense* are the most widely cultivated species, and could contribute for 97% and 3% of world cotton production, respectively, which attributes to the facts that the former harbors high yield and wide adaptability, while the latter possesses superior fiber quality and high VW resistance [7].

Plenty of restraining factors during organism growth are generally divided into abiotic and biotic stresses [8], while plant diseases might be the dominating threat in cotton production [9], of which *Verticillium* wilt (VW) infected by soil-borne fungus

Verticillium dahliae Kleb has been the most significant disease in cotton production due to causing substantial yield loss and serious fiber quality reduction [10 - 12]. As a result of cotton VW infestation, fiber loss is estimated to approximately stand at 80% [13]. What is worse, this disease can attack more than 400 plant species and exist in soil for a long period in dormant form in the vascular system of perennial plants. Thus, it is completely impossible to control VW disease through conventional method [14]. 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 [15, 16].

Despite multiple methods put forward to control VW, it remains one of the most efficient and economical measures to develop elite cotton cultivars harboring genetic factors tolerant or completely resistant against pathogen in cotton breeding [17-19]. There are only four subsistent cultivars of *Gossypium* species, while the tetraploid cultivars cover more than 95% of planting areas around the world, namely as *G. barbadense* (Sea Island cotton) and *G. hirsutum* (Upland cotton), which present resistant and susceptible to VW disease, respectively [20, 21]. Hybrid breeding via conventional techniques has been utilized earlier to improve VW resistance in upland cottons, while some hindrances like infertility and hybrid break down/low parent heterosis hindered the way of conducting resistant gene introgression from *G. barbadense* into *G. hirsutum* [21]. Therefore, it has become a challenging task for cotton breeders to achieve synchronous improvement in cultivating novel varieties simultaneously harboring high yield, superior fiber quality, and high disease resistance. QTL Mapping approaches make it possible for the discovery of

quantitative genetic factors responsible for disease resistance as well as high fiber quality and yield with the utilization of marker-assisted selection (MAS). Thus, we can take full advantage of genetic markers presenting linkage disequilibrium with disease resistance to confirm the contribution of key candidate genes in cotton research, which will be transferred from Sea Island cotton into Upland cotton to improve the VW resistance [22].

Chromosome Segment Substitution Lines (CSSLs) have perpetual effects as accompanied with similar genetic base to their recurrent parent thereby acting as favorable implement in mining of elite QTLs and alleles; ultimately carrying out advanced functional genomic techniques devoid of any non-additive genetic effects [23-28]. Optimal utilization of upland cotton as well as island cottons can be brought about via MAS and conventional breeding techniques of inbreeding, outcrossing and backcrossing with the provision of CSSLs. Therefore, CSSLs are extensively exploited especially in QTL mapping approaches for discovering genetic factors responsible for economic traits such as fiber quality, yield, biotic and abiotic stress tolerance or resistance [29-37].

Nowadays, cotton genomics research like other crop species, has been successively performed by QTL mapping on the significant traits based upon comprehensive deployment of molecular markers, of which simple sequence repeats (SSRs) are the most extensively utilized genetic markers in cotton [38]. To date, approximately 19010 SSRs have been accounted for cotton genomics research in Cotton Data Base (<http://cottondb.org/>), and almost 100,290 microsatellites have been

newly extracted from genome while about 77,996 ones have been established successfully.

In the recent days, there is a newly emerging technique of mapping renowned as Meta-analysis of QTLs in tetraploid cotton research, which has been intensively activated for the identification of hotspot regions and known to harbor a massive amount of QTLs [32, 33]. Consensus map positions for QTLs and merging of datasets are the fundamental properties for meta-analysis approach, making this technique unique and widely adoptable. Not only previously declared QTLs positions can be reassured with identification of hotspot regions, but also the pleotropic effects of QTLs for different traits can be identified with Meta QTL analysis [32]. Moreover, this beneficial aspect of meta-analysis can be exploited to create hotspot region refuting stable QTLs for any disease by reassembling the previously identified QTLs for the relevant disease. Facilitation of breeders and geneticists can be brought about by employing this technique as they would only need to identify that specific chromosome region enriched with genetic factors controlling disease resistance for MAS or advanced mapping techniques [7, 39].

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 program, and to confirm consistent and stable QTLs through meta-analysis for MAS application in cotton breeding for VW prevention and control. The results in this study are of importance for VW resistance as well as breeding improvements in cotton.

2. Results

2.1 Phenotypic disease index (DI) of parents and controls

At Anyang in July 2015, the highest DI value of VW was obtained in the susceptible Jimian11 (41.95%), followed by CCRI36 (31.03%), while the lowest one was observed in the parental line Hai1 (6.21%) (Table 2), indicating a significant difference of DI values between Hai1 and Jimian11. At Anyang in August 2015, the highest DI was found in Jimian11 (48.30%), followed by CCRI36 (47.70%) and by Hai1 (19.50%). The difference of DI values between the parental lines was significant while that of DI values between CCRI36 and Jimian11 was insignificant (Figure 1. A). In both case of Xinjiang in July and August 2015, highly significant differences were observed between parental lines (Figure 1. B).

At 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 2), identifying no significant difference of DI values between CCRI36 and Jimian11. At 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 1. C). The difference of DI values between CCRI36 and Jimian11 was also insignificant. In both case of Xinjiang in July and August 2016, we observed highly significant difference of resistance against the VW disease between the parents, while no significant difference between CCRI36 and Jimian11 was observed (Figure 1. D).

2.2 Evaluation of CSSLs for VW resistance

The ANOVA results displayed the P-value was 0.002, suggesting significant differences of resistance against VW in CSSLs (Table 1). Results of the descriptive statistical analysis of CSSLs and parental lines across 8 environments were illustrated in Table 2. Less than one absolute value of skewness of the mean values of VW in CSSLs across 8 environments indicated a normal distribution. The DI of CSSLs presented a perpetual and 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 2). The average DI value in XJJuly15 was 6.52%, showing not significant to either of parents. On the other hand, 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%, of which the highest heritability was observed in AYJuly15 while the lowest in XJAug15 (Table 2). For all the environments of two years and developmental stages, wide variations of heritability were found in CSSLs to VW disease onset with some lines showing introgressive segregation over their parents.

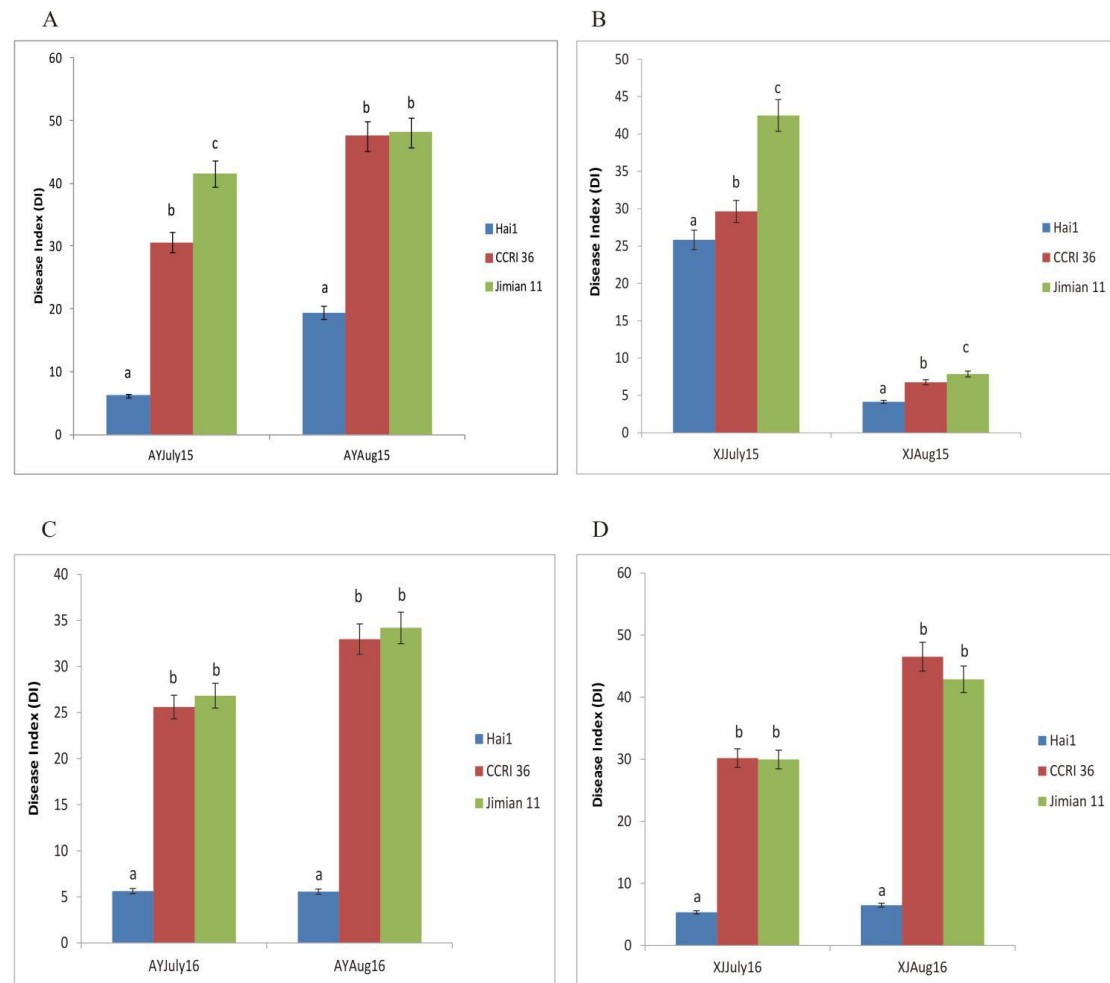


Figure 1. *Verticillium* wilt disease index of parent CCRI36, resistant control Hai1 and susceptible control Jimian11;

(A) Anyang 2015; (B) Xinjiang 2015; (C) Anyang 2016; (D) Xinjiang 2016. The error bar shows the standard deviation. a, b, c indicate the significance at 5%.

2.3 Correlation coefficient among DI in different stages growth and environments

Highly significant positive correlations were visible among the disease index of *Verticillium* wilt in the fields except between XJJul15 and AYJul16 (Table 3).

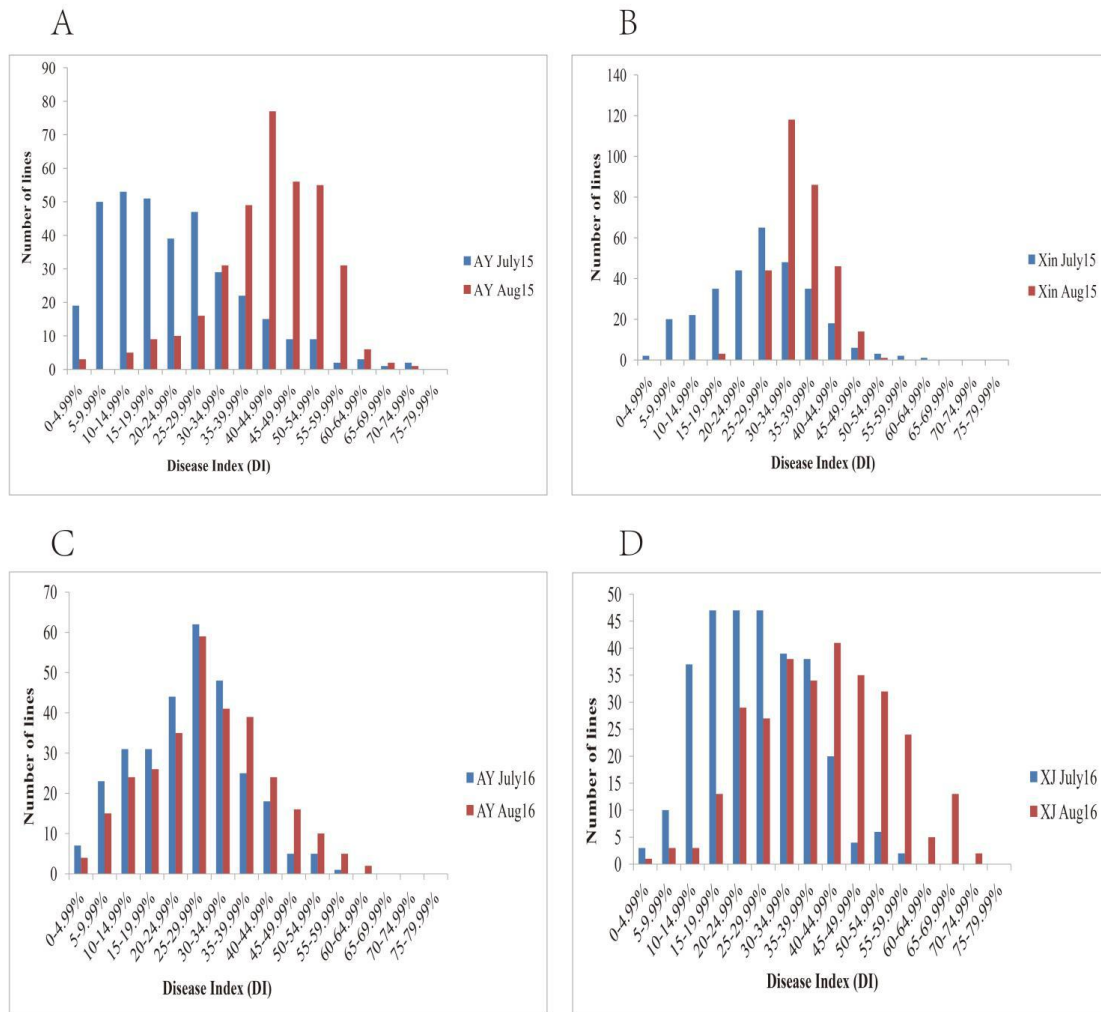


Figure 2. Normal distribution of DI phenotype in CSSLs; (A) Anyang 2015; (B) Xinjiang 2015; (C) Anyang 2016; D Xinjiang 2016.

Table 1. 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			

Table 2. Descriptive statistics of VW resistance with broad sense Heritability (H^2) measured in

BC₅F_{3:5} population

Traits	Env	CSSL population							Parents		Mid parent	Jimian11 (Control)	H^2 (%)
		Mean	Max	Mini	SD	Skew	Kurt	Var	CCRI36	Hail			
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

Table 3. Correlation coefficient among the DI in the different stages of growth of BC₅F_{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**

2.4 QTL mapping

In total, 53 QTLs for VW were detected during different stages of growth and environments at Anyang and Xinjiang fields in the year of 2015 and 2016, which explained from 3.74 to 11.89% of the total phenotypic variation (PV) with LOD scores ranging 2.50 to 6.96. They were located on 20 chromosomes except Chr04, Chr08, Chr13, Chr16, Chr18 and Chr25. Among them, 35 QTLs (66%) had negative

additive effects, indicating that their favorable alleles come from *G. barbadense*, which enhanced VW resistance and decremented DI by 2.64 to 13.23. On the other hand, 18 QTLs (34%) had positive additive effects, indicating that the *G. barbadense* alleles decremented VW resistance and enhanced phenotypic DI values by 2.27 to 19.47. Thirty-one QTLs were identified in 2015 and 86 QTLs in 2016, of which eleven ones were found in the both years. The highest number of QTLs (11) was detected on Chromosome 5 (Figure 3, Table S1).

2.4.1 QTLs for VW resistance in Anyang in 2015

In July 2015, there were ten QTLs identified in Anyang and mapped on 5 chromosomes, explaining 4.39–11.89% of overall PV with LOD scores ranging 2.89–4.87, of which five ones were found on Chr05 while two ones on Chr19. All QTLs except *qVW-Chr05-8* and *qVW-Chr19-5* had negative additive effects, indicating that their favorable alleles derived from donor parent Hail incremented VW resistance and decremented phenotypic DI by 4.75-7.98 (Table S1).

In August 2015, thirteen QTLs were identified at Anyang and mapped on 8 chromosomes, explaining 3.79–7.67% of the overall phenotypic variation with LOD scores ranging 2.51–5.22. Five QTLs were found on Chr05 and two QTLs on Chr19, which was consistent with the results in July 2015. Except for *qVW-Chr01-1*, *qVW-Chr12-1* and *qVW-Chr26-1*, the whole QTLs had negative additive effects, which suggested that donor parent *G. barbadense* alleles incremented VW resistance and decremented DI by 2.64-13.23 (Table S1).

2.4.2 QTLs for VW resistance at Xinjiang in 2015

In July 2015, there were six QTLs detected at Xinjiang, which were mapped on 6 Chromosomes with 3.78–9.33% of the total PV explained. All the QTLs showed positive additives, which suggested the Hai1 alleles decremented resistance against VW and incremented phenotypic DI by 2.27-13.25 (Table S1).

In August 2015, two QTLs were found at Xinjiang, namely as *qVW-Chr05-10* and *qVW-Chr06-1* which were mapped on Chr5 and Chr6 with 5.00 and 5.59% of PV and LOD scores of 3.35 and 3.94, respectively. These QTLs also presented positive additives, suggesting their alleles derived from *G. barbadense* decreased resistance of the disease and increased DI by 2.81 and 9.40 (Table S1).

2.4.3 QTLs for VW resistance in Anyang in 2016

In July 2016, there were fourteen QTLs detected at Anyang and mapped on 9 chromosomes, explaining 4.27–7.71% of the total PV. Four QTLs were located on Chr05, while each two QTLs were identified on Chr06 and Chr19, respectively. All the QTLs had negative additives, which suggested their parent Hai alleles incremented VW resistance and decremented DI by 4.43-9.57 (Table S1).

In August 2016, ten QTLs were recorded at Anyang and mapped on 8 chromosomes, explaining 3.76–6.15% of the overall PV, of which three ones were identified on Chr05. All the QTLs except *qVW-Chr02-3* had negative additives, suggesting their alleles derived from parent Hai1 incremented resistance and decremented DI by 4.19-10.47 (Table S1).

2.4.4 QTLs for VW resistance in Xinjiang in 2016

In July 2016, there were twenty-eight QTLs detected at Xinjiang and mapped on 14 chromosomes with 3.74–11.14% of total PV explained, of which LOD score ranging was 2.55–6.96. In addition, nine QTLs were found on Chr05, and three QTLs were located on Chr19. All the QTLs except *qVW-Chr09-1*, *qVW-Chr10-2*, *qVW-Chr15-1*, and *qVW-Chr22-2* had negative additives, suggesting their parent Hail alleles enhanced resistance against VW and decreased DI by 2.96-7.65 (Table S1).

In August 2016, thirty-four QTLs were found at Xinjiang and mapped on 15 chromosomes, explaining 3.79–10.22% of total PV. Nine QTLs were identified on Chr05, while five and three QTLs were separately located on Chr19 and Chr10. Except for *qVW-Chr10-2*, *qVW-Chr10-3*, *qVW-Chr15-1*, and *qVW-Chr22-2*, all the QTLs had negative additives, which suggested their alleles derived from parent *G. barbadense* incremented resistance against VW and decremented phenotypic value of DI by 3.94-10.48 (Table S1).

2.5 Identification of stable QTLs over environments and developmental periods

In total, 53 QTLs of VW disease index were detected in CSSLs during different stages of growth and environments, which were separately located on 20 different chromosomes. There were 11 and 7 QTLs identified on Chr05 and Chr19, respectively, and each 3 QTLs were separately located on Chr01, Chr06, Chr10, and Chr22. Each 2 QTLs were found on Chr02, Chr03, Chr09, Chr11, Chr14, Chr15, Chr17, Chr21, and Chr23, respectively, while Chr07, Chr12, Chr20, Chr24, and Chr26 separately contained only 1 QTL (Table S1).

Among 53 QTLs, 29 stable QTLs were identified in at least two environment, explaining 3.74-11.89% of the overall PV (Table 4). There were 25 stable QTLs (86%) showing negative additive effects, which suggested thier Hai1 alleles enhanced resistance against VW and decreased phenotypic DI. Among 29 stable QTLs, Chr05 harbored 09 stable QTLs, and Chr19 contained 3 stable QTLs. Each 2 stable QTLs were separately located on Chr06, Chr10, Chr17, and Chr22, while Chr01, Chr03, Chr07, Chr11, Chr14, Chr15, Chr20, Chr23, and Chr26 contained 1 stable QTL, respectively.

Four stable QTLs, namely as *qVW-Chr05-2*, *qVW-Chr05-3*, *qVW-Chr05-6*, and *qVW-Chr20-1*, were detected in six environments explaining 4.56-11.89%, 4.56-10.03%, 4.15-10.17% and 4.53-11.14% of PV, respectively. Only one stable QTL (*qVW-Chr19-2*) was identified in five environments with 3.82-9.40% of the observed PV, while three stable QTLs (*qVW-Chr05-4*, *qVW-Chr10-1*, and *qVW-Chr19-1*) were investigated in four environments separately explaining the observed PV of 4.47-7.62%, 4.09-5.17%, and 4.66-7.96%. Moreover, there were nine stable QTLs detected in three environments, namely as *qVW-Chr05-1*, *qVW-Chr05-11*, *qVW-Chr06-2*, *qVW-Chr06-3*, *qVW-Chr07-1*, *qVW-Chr11-2*, *qVW-Chr19-6*, *qVW-Chr22-1*, and *qVW-Chr23-2*, which presented 7.67-9.13%, 4.69-5.98%, 3.98-5.52%, 5.28-6.67%, 4.05-5.97%, 6.06-9.04%, 3.79-5.20%, 4.39-7.06%, and 5.32-7.59% of the observed PV, respectively. Twelve stable QTLs were detected in two environments with overall 3.74-10.22% of PV. The stable QTLs, including *qVW-Chr05-2*, *qVW-Chr05-3*, *qVW-Chr05-6*, *qVW-Chr05-7*, and

317 *qVW-Chr20-1*, had major effects and explained 11.89%, 10.03%, 10.17%, 10.22%
 318 and 11.14% of the observed PV, respectively (Table 4).

319 **Table 4.** Identification of QTLs for VW disease index during different development and
 320 environments in BC₅F_{3:5} populations

SL. No.	QTLs	Growth stage	Env	Chr	Location (cM)	Nearest marker	LOD	Add	PV (%)
1	qVW-Chr01-3	July	XJJul16	Chr01	122.7	TMB152	3.15	-3.37	5.60
		August	XJAug16	Chr01	122.7	TMB152	4.27	-5.05	7.59
2	qVW-Chr03-2	July	XJJul16	Chr03	114.4	HAU0195	3.95	-5.99	6.38
		August	XJAug16	Chr03	114.4	HAU0195	4.88	-8.50	7.71
3	qVW-Chr05-1	August	AYAUG15	Chr05	30.5	CIR224b	5.22	-3.81	7.67
		July	XJJul16	Chr05	30.5	CIR224b	6.87	-4.25	9.13
		August	XJAug16	Chr05	30.5	CIR224b	5.95	-5.09	7.88
4	qVW-Chr05-2	July	AYJul15	Chr05	32.3	CIR102	3.84	-5.44	11.89
		August	AYAUG15	Chr05	32.3	CIR102	3.48	-4.07	5.31
		July	AYJul16	Chr05	32.3	CIR102	3.47	-4.43	5.42
		August	AYAUG16	Chr05	32.3	CIR102	2.88	-4.46	4.56
		July	XJJul16	Chr05	32.3	CIR102	6.66	-5.65	9.77
		August	XJAug16	Chr05	32.3	CIR102	4.59	-6.16	6.97
		July	AYJul15	Chr05	35.4	DPL0063	3.32	-5.08	4.89
5	qVW-Chr05-3	August	AYAUG15	Chr05	35.4	DPL0063	3.57	-4.23	5.46
		July	AYJul16	Chr05	35.4	DPL0063	5.07	-5.42	7.71
		August	AYAUG16	Chr05	35.4	DPL0063	2.98	-4.57	4.56
		July	XJJul16	Chr05	35.4	DPL0063	6.96	-5.87	10.03
		August	XJAug16	Chr05	35.4	DPL0063	4.45	-6.15	6.61
		July	AYJul15	Chr05	38.2	HAU0746	3.19	-5.69	4.76
		July	AYJul16	Chr05	38.2	HAU0746	2.99	-4.68	4.47
6	qVW-Chr05-4	July	XJJul16	Chr05	38.2	HAU0746	5.18	-5.80	7.62
		August	XJAug16	Chr05	38.2	HAU0746	3.26	-5.98	4.86
		July	XJJul16	Chr05	40.2	CGR5025	3.92	-4.74	5.84
		August	XJAug16	Chr05	40.2	CGR5025	6.30	-7.69	9.22
7	qVW-Chr05-5	July	XJJul16	Chr05	40.2	CGR5025	6.30	-7.69	9.22
		August	XJAug16	Chr05	40.2	CGR5025	6.30	-7.69	9.22
8	qVW-Chr05-6	July	AYJul15	Chr05	43.1	HAU1712	3.48	-5.39	5.23
		August	AYAUG15	Chr05	43.1	HAU1712	2.83	-3.78	4.15
		July	AYJul16	Chr05	43.1	HAU1712	3.47	-4.56	5.18
		August	AYAUG16	Chr05	43.1	HAU1712	2.82	-4.53	4.25
		July	XJJul16	Chr05	43.1	HAU1712	6.96	-6.06	10.17
		August	XJAug16	Chr05	43.1	HAU1712	4.34	-6.23	6.44
		July	XJJul16	Chr05	45.0	DPL0138	2.82	-3.90	6.84
9	qVW-Chr05-7	August	XJAug16	Chr05	45.0	DPL0138	3.59	-4.48	10.22
		July	XJJul16	Chr05	89.9	MUSS317	3.23	-6.92	4.85
10	qVW-Chr05-9	August	XJAug16	Chr05	89.9	MUSS317	3.84	-9.71	5.74
		July	XJJul16	Chr05	197.4	HAU1050	3.11	-2.64	4.69
11	qVW-Chr05-11	August	AYAUG15	Chr05	197.4	HAU1050	3.80	-2.96	5.62
		July	XJJul16	Chr05	197.4	HAU1050	4.03	-3.94	5.98
		August	XJAug16	Chr05	197.4	HAU1050	4.03	-3.94	5.98
12	qVW-Chr06-2	July	AYJul16	Chr06	44.5	CER0086b	5.11	-9.57	7.50
		August	AYAUG16	Chr06	44.5	CER0086b	2.78	-7.83	4.17

		July	XJJul16	Chr06	44.5	CER0086 b	2.67	-6.62	3.98
		August	XJAug16	Chr06	44.5	CER0086 b	3.74	-10.0 6	5.52
13	qVW-Chr06-3	July	AYJul16	Chr06	66.1	NAU5433	3.55	-6.89	5.28
		July	XJJul16	Chr06	66.1	NAU5433	3.69	-6.68	5.50
		August	XJAug16	Chr06	66.1	NAU5433	4.53	-9.50	6.67
14	qVW-Chr07-1	August	AYAug16	Chr07	92.2	NAU1085	2.62	-4.19	4.05
		July	XJJul16	Chr07	92.2	NAU1085	3.91	-4.41	5.97
		August	XJAug16	Chr07	92.2	NAU1085	3.37	-5.29	5.18
15	qVW-Chr10-1	July	AYJul16	Chr10	150.7	NAU2869	3.37	-6.75	5.12
		August	AYAug16	Chr10	150.7	NAU2869	3.28	-7.31	5.00
		July	XJJul16	Chr10	150.7	NAU2869	2.69	-5.73	4.09
		August	XJAug16	Chr10	150.7	NAU2869	3.41	-8.31	5.17
16	qVW-Chr10-2	July	XJJul16	Chr10	199.7	HAU1701	2.90	6.64	4.36
		August	XJAug16	Chr10	199.7	HAU1701	2.61	7.80	3.94
17	qVW-Chr11-2	July	AYJul16	Chr11	253.0	DPL0209	3.97	-7.00	6.06
		July	XJJul16	Chr11	253.0	DPL0209	5.40	-7.65	8.01
		August	XJAug16	Chr11	253.0	DPL0209	6.06	-10.4 8	9.04
18	qVW-Chr14-2	July	AYJul16	Chr14	203.0	HAU0883	3.32	-5.89	5.42
		August	XJAug16	Chr14	203.0	HAU0883	4.27	-8.15	6.91
19	qVW-Chr15-1	July	XJJul16	Chr15	16.3	CICR815	2.64	6.63	3.96
		August	XJAug16	Chr15	16.3	CICR815	2.56	8.43	3.85
20	qVW-Chr17-1	July	XJJul16	Chr17	23.3	HAU2014	4.58	-5.46	6.78
		August	XJAug16	Chr17	23.3	HAU2014	5.25	-7.49	7.66
21	qVW-Chr17-2	July	XJJul16	Chr17	122.8	HAU0195	2.55	-3.87	3.87
		August	XJAug16	Chr17	122.8	HAU0195	2.50	-4.94	3.79
22	qVW-Chr19-1	July	AYJul16	Chr19	17.4	NAU3405	3.12	-5.01	4.66
		August	AYAug16	Chr19	17.4	NAU3405	4.05	-6.23	6.00
		August	XJAug16	Chr19	17.4	NAU3405	5.04	-7.75	7.43
		July	XJJul16	Chr19	17.4	NAU3405	5.42	-6.22	7.96
23	qVW-Chr19-2	July	AYJul15	Chr19	145.9	NAU5475	4.03	-6.38	5.98
		August	AYAug15	Chr19	145.9	NAU5475	2.54	-4.01	3.82
		July	AYJul16	Chr19	145.9	NAU5475	2.85	-4.57	4.27
		July	XJJul16	Chr19	145.9	NAU5475	6.45	-6.45	9.40
		August	XJAug16	Chr19	145.9	NAU5475	5.58	-7.76	8.18
24	qVW-Chr19-6	August	XJAug16	Chr19	257.1	HAU1785	3.47	-8.39	5.20
		August	AYAug15	Chr19	257.1	HAU1785	2.51	-5.42	3.79
		July	XJJul16	Chr19	257.1	HAU1785	3.01	-6.07	4.53
25	qVW-Chr20-1	July	AYJul15	Chr20	175.5	NAU3665	3.64	-6.31	6.74
		August	AYAug15	Chr20	175.5	NAU3665	2.73	-4.07	4.53
		July	AYJul16	Chr20	175.5	NAU3665	2.80	-4.56	4.89
		August	AYAug16	Chr20	175.5	NAU3665	3.41	-5.61	6.15
		July	XJJul16	Chr20	175.5	NAU3665	6.52	-6.54	11.14
		August	XJAug16	Chr20	175.5	NAU3665	5.73	-7.87	9.69
26	qVW-Chr22-1	July	AYJul15	Chr22	21.8	NAU2026	2.89	-4.75	4.39
		August	XJAug16	Chr22	21.8	NAU2026	5.51	-6.28	7.06
		July	XJJul16	Chr22	21.8	NAU2026	4.84	-4.58	6.26
27	qVW-Chr22-2	July	XJJul16	Chr22	26.2	Gh200	2.90	12.75	3.74
		August	XJAug16	Chr22	26.2	Gh200	2.97	16.66	3.83
28	qVW-Chr23-2	July	AYJul15	Chr23	208.1	NAU5189	3.32	-7.98	5.32

Figure 3. Identification of QTLs for VW disease index and linkage map in BC₅F_{3:5} populations.

Note: stars indicate stable QTLs

2.6 QTL hotspots and meta-analysis

Based on Meta-analysis, 32 QTL hotspot regions were totally detected on 18 chromosomes, including Chr01, Chr03, Chr05, Chr06, Chr07, Chr09, Chr11, Chr12, Chr14, Chr15, Chr17, Chr19, Chr20, Chr21, Chr22, Chr23, Chr24 and Chr26 (Figure S1, Table 5). Among them, 17 QTL hotspot regions were consistent with those detected earlier by [7, 22, 33] (Table 5), and the other 15 were identified as novel ones. Each 3 QTL hotspot regions were separately located on Chr05, Chr19, and Chr26, while each 2 QTL hotspot regions were detected on Chr01, Chr03, Chr07, Chr09, Chr20, Chr21, Chr22, and Chr23, respectively. In addition, Chr06, Chr11, Chr12, Chr14, Chr15, Chr17, and Chr24 separately contained 1 QTL hotspot region (Table 5).

Among 32 QTL hotspot regions, 9 hotspot regions located on seven different chromosomes had more QTLs (Figure S1, Table 5), which could be very important for further studies and utilized for molecular breeding via MAS. As for chr05, 40 QTLs were selected to project on consensus chromosome 05 (Cons.Chr05), resulting in 3 identified QTL hotspot regions. There were 18, 5, and 17 QTLs on Chr05-DI-Hotspot-1, Chr05-DI-Hotspot-2, and Chr05-DI-Hotspot-3, respectively (Figure 4, Table 5). Eleven QTLs were selected to project on chromosome 09 (Cons.Chr09), and 2 QTL hotspot regions were identified, of which Chr09-DI-Hotspot-1 had 9 QTLs, while Chr09-DI-Hotspot-2 had 2 QTLs. Sixteen

QTLs were identified and projected on consensus Chr19 to perform meta-analysis, identifying 3 QTL hotspot regions. Chr19-DI-Hotspot-1, Chr19-DI-Hotspot-2 and Chr19-DI-Hotspot-3 contained 5, 4 and 7 QTLs, respectively. Twelve QTLs were selected to project on chromosome 22 (Cons.Chr22), and 2 QTL hotspot regions were identified, of which Chr22-DI-Hotspot-1 had 5 QTLs, while Chr22-DI-Hotspot-2 had 7 QTLs (Figure 4). Fifty six QTLs were selected to project on Cons.Chr23, identifying 2 QTL hotspot regions. Chr23-DI-Hotspot-1 and Chr23-DI-Hotspot-2 contained 30 and 26 QTLs, respectively. Sixteen QTLs were selected to project on Cons.Chr26, and 3 QTL hotspot regions were identified. Chr26-DI-Hotspot-1, Chr26-DI-Hotspot-2 and Chr26-DI-Hotspot-3 contained 6, 5 and 5 QTLs, respectively. The details of all QTLs are described in Table 5.

As for the hotspots on Cons.Chr05, Chr05-DI-Hotspot-1 from the 25–36 cM region was located between markers Gh243 and HAU3395, and Chr05-DI-Hotspot-2 from 31 to 42 cM region and Chr05-DI-Hotspot-3 from 39 to 54 cM region were separately located between markers NAU3204 and CIR301 and between markers TMB0131b and NAU2948. There were two hotspots on Cons.Chr09, and Chr09-DI-Hotspot-1 from the 34–60 cM region and Chr09-DI-Hotspot-2 from 87 to 93 cM region were located between markers CGR6170 and CGR6719 and between markers BNL0597 and BNL4053, respectively. With regard to the hotspots on Cons.Chr19, Chr19-DI-Hotspot-1 from the 2–27 cM region was located between markers CIR415 and BNL3452, and Chr19-DI-Hotspot-2 from 32–55 cM region and Chr19-DI-Hotspot-3 from 123 to 148 cM region were separately located between

386

387 **Table 5.** QTL hotspots detected for VW resistance on the consensus map through meta-analysis

Hotspot name	Chr	Location (cM)	No. of QTLs	No. of QTLs in this paper	Reported earlier
Chr01-DI-Hotspot-1	Chr01	14-33 cM	6	3	
Chr01-DI-Hotspot-2	Chr01	34-55 cM	5	0	
Chr03-DI-Hotspot-1	Chr03	20-34 cM	5	1	Shi et al., 2016
Chr03-DI-Hotspot-2	Chr03	34-44 cM	5	1	
Chr05-DI-Hotspot-1	Chr05	25-36 cM	18	8	Shi et al., 2016
Chr05-DI-Hotspot-2	Chr05	31-42 cM	5	2	Shi et al., 2016; Said et al., 2015
Chr05-DI-Hotspot-3	Chr05	39-54 cM	17	1	Shi et al., 2016; Zhang et al., 2015
Chr06-DI-Hotspot-1	Chr06	35-51 cM	6	2	
Chr07-DI-Hotspot-1	Chr07	51-76 cM	6	0	Zhang et al., 2015
Chr07-DI-Hotspot-2	Chr07	178-193 cM	8	1	
Chr09-DI-Hotspot-1	Chr09	34-60 cM	9	2	Zhang et al., 2015
Chr09-DI-Hotspot-2	Chr09	87-93 cM	2	0	
Chr11-DI-Hotspot-1	Chr11	72-99 cM	6	2	
Chr12-DI-Hotspot-1	Chr12	13-28 cM	6	1	Shi et al., 2016
Chr14-DI-Hotspot-1	Chr14	19-34 cM	7	2	Shi et al., 2016
Chr15-DI-Hotspot-1	Chr15	41-68 cM	10	1	
Chr17-DI-Hotspot-1	Chr17	6-23 cM	4	2	
Chr19-DI-Hotspot-1	Chr19	2-27 cM	5	1	Shi et al., 2016; Zhang et al., 2015
Chr19-DI-Hotspot-2	Chr19	32-55 cM	4	0	Zhang et al., 2015
Chr19-DI-Hotspot-3	Chr19	123-148 cM	7	4	Shi et al., 2016
Chr20-DI-Hotspot-1	Chr20	12-24 cM	4	0	Shi et al., 2016; Zhang et al., 2015
Chr20-DI-Hotspot-2	Chr20	26-45 cM	5	1	
Chr21-DI-Hotspot-1	Chr21	3-29 cM	6	0	Zhang et al., 2015
Chr21-DI-Hotspot-2	Chr21	35-60 cM	7	2	
Chr22-DI-Hotspot-1	Chr22	0-25 cM	5	1	Zhang et al., 2015
Chr22-DI-Hotspot-2	Chr22	30-54 cM	7	2	
Chr23-DI-Hotspot-1	Chr23	40- 65 cM	30	1	Zhang et al., 2015
Chr23-DI-Hotspot-2	Chr23	67-92 cM	26	1	
Chr24-DI-Hotspot-1	Chr24	0-25 cM	5	1	Zhang et al., 2015
Chr26-DI-Hotspot-1	Chr26	4-29 cM	6	1	Zhang et al., 2015
Chr26-DI-Hotspot-2	Chr26	33-54 cM	5	0	
Chr26-DI-Hotspot-3	Chr26	85-102 cM	5	0	

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389

390 **3. Discussion**

391 **3.1 Field status and phenotypic assessment**

392 Without the inoculation provision and just under natural environmental conditions, a
 393 population of CSSLs developed from interspecific cross between Upland cotton
 394 CCRI36 and Sea Island cotton Hai1, which has been investigated for resistance
 395 against VW together with parents and controls. The VW resistance was assessed
 396 based on the leaf tissue damage in the mature stages, of which the results indicated the
 397 parent Hai1 appeared to be more resistant to the disease compared to CCRI36, while
 398 the control Jimian11 displayed slightly higher susceptibility over CCRI36. Most of
 399 the CSSLs exhibited higher DI values than mid parents (Table 2), and this unclear
 400 phenomenon might be due to DI values fluctuation across the environments. The
 401 same remark was made in a study using an interspecific chromosome segment line
 402 with different VW strains and according to the authors, that fact can be explained the
 403 resistance to different VW isolates is controlled by distinct single genes and that in the
 404 presence of a mixture of isolates, interactions occurred [[19](#)].

405 Over different years of study and across variable environments, the investigated
 406 population of CSSLs has displayed a broad range of sensitivity ranging between
 407 highly susceptible to highly resistant. Having taken the previous studies [[40](#)] into
 408 consideration, the hypothesis came into being regarding inheritance of VW in
 409 recessive fashion, which is that both the paternal and maternal contributors should
 410 harbor genetic factors for resistance. For the verification of the generated hypothesis,

the CSSL population has been investigated on phenological basis over different environments at various growth stages. In this study, we observed that DI values susceptible to VW infection were higher in August than those in July, to be specific to presenting that the susceptible control (Jimian11) showed above 35% DI values except in XJJul15 and AYJul16, while the DI values of CCRI36 were lower than 35% except in AYAUG15 and XJAUG16 (Table 2). This lesser DI percentage is the evidence for the occurrence of high pressure projected by variable VW strains under natural environmental conditions. Few more reasons behind this phenological variation include intensity and virulence of strains, fungal amount in soil and developmental stages as well as environmental influences [41]. The similar findings have been reported earlier in which the host plant proved to be resistant against inoculum of VW while remained susceptible under natural environmental conditions [21]. We also have synchrony with previous findings with a display of lesser disease index (DI<40%) by CCRI36 progenitor whereas some of the offspring depicted a prominent resistance level comparable to susceptible control Jimian11. Besides this, a noteworthy level of transgressive segregation has been witnessed under field conditions, which are in accordance with previous reports [42, 43]. Across different environment during whole investigation period, few CSSLs remained consistent in resistance display to pressurizing mixture of strains present in the vicinity as compared to most of the lines which displayed a high level of susceptibility (Figure 2). This fact can be justified by the presence of wider range of environmental variation occurrence during two experimental years of study, where the VW strains keep on

changing their genetic make up for being more resistant. Previous reports [19] justified our such findings for the confirmation of reality that there must exists an antagonistic interaction between resistance QTLs/genes and different strains of fungi plus large number of genes are responsible for controlling the resistance mechanism against *V. dahliae* isolates.

The phenological parameters measured in two 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 weak correlation coefficient values (Table 3). It realizes the fact regarding alteration of genes on exposure to VW strains at varying growth stages. In a study on backcross inbreed lines regarding VW resistance, there observed a weak but positive correlation among disease index under field conditions [44].

Due to varying environmental stresses in both years at two locations, erroneous frequency was very high and because of this heritability values ranged between weak to moderate only. This happening suggests a wider range of phenology regarding DI has been caused by varying environmental influences. However, this is not a surprising truth as cotton resistance levels to *V. dahliae* are greatly inclined to environmental influences, resistance genes, inoculum concentrations and their interactions [45].

3.2 Genetic Map used for QTLs identification

Through utilization of hybridization technique including interspecific [7, 18, 21, 42, 43, 45-48] and intraspecific [21, 46, 47, 49, 50] crossing wide range of genetic maps have been constructed. However, lesser genome coverage i.e. < 50% has been achieved by using interspecific crossing, which appeared as bottleneck in the detection of QTLs from whole genome with ultra-resolution. The fact has been proved by the discovery of about 57.90% of tetraploid cotton genome from Zhang et al. [7] study, 27% i.e. 1143.1cM and 35% with 279 markers of genome coverage in Fang et al. [21] and [47] reports. To date, one exclusive report has found that covered more than 50% of genome i.e. 55.7% accounting for 882 genetic markers in total, including 414 SNPs, 36 RGA-RFLPs (resistance gene analog-amplified fragment length polymorphism) and 432 SSRs. Therefore, the whole genome coverage of allotetraploid cotton with resistant QTLs for VW is not yet to be achieved. This study paced to cover approximately 100% of cotton genome enclosing about 5115.6cM [37], which is really a comprehensive distance accomplished so far. It's neonatal to take in account all the 26 genetic threads of allotetraploid cotton with use of CSSLs in quest of QTLs for VW resistance. An announce-worthy amount of QTLs (53) were identified to be related to VW resistance from 20 chromosomes, which exposed the reality that these QTLs are extensively distributed in whole genome chromosomes. These results would be not easy to achieve if *G.barbadense* genome will be used as template with restricted amount of markers and lesser polymorphism.

3.3 Distribution of QTLs of Verticillium wilt through the whole genome

There were fewer chromosomes yet to have been explored regarding VW resistance QTLs in the previous studies, specifically including Chromosome 6, Chromosome 10, Chromosome 12, and Chromosome 18 together with almost 100 plus related QTLs [45, 51], which left these gaps from completing the whole tetraploid genome. Our findings have contributed plenty of valuable information to filling up there gaps to greater extent, leaving just Chromosome 18 to be explored. There were three QTLs detected on Chromosome 6 and 10, while only one DI QTL was identified on Chromosome 12. Like previous findings such as Zhang et al. [7] from meta-analysis done by different researchers, we also remained unable to discover any hotspot region on Chromosomes 10 and 18. However, few chromosomes were found to be heavily loaded with DI QTLs like Chromosome 5 with 11 DI QTLs, and Chromosome 19 with 7 DI QTLs. Each 3 QTLs were separately located on Chromosome 1 and 22 like Chromosome 6 and 10 as mentioned earlier. Also in our findings we remained, successful in identifying some stable QTLs across six different environments, which was not the case in any of the previous reports.

As mentioned earlier, 20 chromosomes were explored in our study with 53 QTLs using BC₅F_{3:5} populations, of which 30 QTLs were located on A sub-genome chromosomes covering Chr01, Chr02, Chr03, Chr05, Chr06, Chr07, Chr09, Chr10, Chr11 and Chr12 accounting 56.66%, while 23 QTLs were explored on D sub-genome covering Chr14, Chr15, Chr17, Chr19, Chr20, Chr21, Chr22, Chr23, Chr24 and Chr26 estimating about 43.44%. There results provided an evidence of the fact that A sub-genome enclosed more resistant QTLs for VW resistance as compared

to D sub-genome. Consistent discoveries have been made by Yang et al. [46], Ning et al. [47] and Bolek et al. [42].

3.4 Stability with earlier studies VW resistance QTLs

In this study, 53 QTLs related to VW resistance were totally identified in 300 CSSLs. Among all the QTLs, 35 ones (66%) had negative additive effects, which indicated that the *G. barbadense* alleles increased *Verticillium* wilt resistance and decreased disease index values by about 2.64 to 13.23. On the other hand, 18 QTLs (34%) had positive additives effects, which indicated that the *G. hirsutum* alleles enhanced VW wilt resistance and decremented phenotypic disease index values by about 2.27 to 19.47. As for different years, 31 QTLs were identified in the year of 2015, while 86 QTLs in the year of 2016, of which 11 QTLs were found in the both years. The maximum number of QTLs (11) was detected on Chr05 (Figure 3, Table S2).

Among 53 QTLs, 29 QTLs were detected consistently in at least two environments, which were deemed as stable QTLs. Out of 29 stable QTLs, 25 QTLs (86%) had negative additive effects, which indicated that the *G. barbadense* alleles incremented VW resistance and decreased DI. Based on Meta-analysis of the identified 53 QTLs, 10 QTLs were consistent to previously identified QTLs, and they had common SSR markers [19, 45-47, 52]. One QTL, *qVW-Chr01-3* positioned on Chr01 for VW resistance was the similar as Ning's *qVW-A1-1* [47], which were identified with common markers of Gh215. Another QTL, *qVW-Chr03-2* was the similar as *qVW-C3-2* in the results of Shi et al. [22], and they were associated with the

shared marker CER0028. In addition, *qVW-Chr05-1* on Chr05 was similar as Shi et al's *qVW-C5-1* [22] based on common marker CIR224b. The *qVW-Chr05-11* mapped on Chr05 was similar as the *qVLBP2-A5-1RIL* in the results of Yang et al. [46], which were associated with shared markers NAU5210. The QTL *qVW-Chr05-4* was similar as the *qVW-C5-3* in the results of Shi et al [22] with the association of shared marker HAU0746 [22]. The *qVW-Chr07-1* was similar as *qVW-A7-1* in the results of Ning et al., [47] based on shared marker Gh527. *qVW-Chr09-1* mapped on Chr09 was the similar as Shi's *qVW-C9-1* [22], with the association of common markers of DPL0783. The QTL *qVW-Chr12-1* was the similar as *qVWR-06-C12* in the results of Zhang et al. [7], which were associated with the common marker CIR272. Besides, *qVW-Chr23-2* was similar as Fang's *qDR52T2-C23-2* [48] associated with the shared marker DPL1938. Lastly, the QTL *qVW-Chr05-1* was similar as the *qVW-C5-2* in the results of Shi et al [22] with the association of shared marker CIR102 [22]. The remaining 43 QTLs for VW resistance could be allowed as novel ones in this study.

Based on meta-analysis, 32 QTLs hotspot regions were detected, of which 17 ones were consistent with the earlier studies [7, 22, 33], while another 15 ones were novel and unreported hotspot regions (Figure 4, Table 5). These hotspot regions and QTLs could be very important information for further comparative studies and utilized for marker assisted selection.

3.5 Further utilization of QTLs for VW resistance

According to previous reports on the CSSLs in cotton, the prominent characteristics of high fiber quality and high yielding traits have deliberately been explained [53-58]. Nowadays in this whole experimental study, a total of 300 CSSLs from Upland cotton CCRI36 and Sea Island cotton Hail have been keenly investigated regarding their resistance to VW. The segments of chromosome introgressed from *G. barbadense* into *G. hirsutum* made these lines little bit different from their recurrent parent by reducing the influences of genetic background of recipient, which makes the CSSLs as efficient breeding materials to conduct quantitative genetics researchs. Thus the experimented work proves to be beneficial in paving the way towards whole genome study of cotton by laying a solid platform stuffed with molecular findings related to fine mapping, functional genomics, gene pyramiding and ultimately marker assisted breeding.

4. Materials and Methods

4.1 Plant materials and development of cotton CSSLs

Mapping population based on 300 CSSLs along with their parents, specifically as CCRI36 (*G. hirsutum*) as recurrent while Hail (*G. barbadense*) as donor parent, was sown at the farm area of ICR, CAAS (Anyang, Henan) and Shihezi, Xinjiang Province, respectively. The reason behind selection of Hail as donor parent is its characteristic features of producing high quality fiber, resistant genes residence for VW in its genome and also the presence of glandless producing factors which act in dominant fashion [59]. However, CCRI36 developed by ICR, CAAS (State Approval Certificate of Cotton 990007) [36] is a commercially grown renowned variety of

upland cotton has the obvious property of high yielding as well as early maturing in growth patterns but susceptible to *Verticillium* wilt. The two cultivars Hail 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 2660 plants of BC₅F₃ was obtained by using CCRI36 as recurrent parent. In 2010 and 2011, BC₅F_{3:4} population was planted via plant-to row method at Anyang and Xinjiang, respectively. In 2014, at Xinjiang province, BC₅F_{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. These selected lines were then grown at Anyang and Xinjiang in 2015 and 2016, respectively. The details of development of CSSLs was brought about by following the same procedure as described earlier [60]. Stable performance regarding resistance to VW was displayed by some lines in multiple environments over different years of study.

4.2 Field investigations and experimental design

Two field stations of ICR, CAAS in Anyang, Henan and Shihezi, Xinjiang were used to grow the experimental material for two years. In 2015 and 2016, phenotypic data were collected in months of July and August from Anyang and Xinjiang, respectively. Under natural environmental conditions, there occurred intensive attack of *V. dahliae* strains. Randomized complete block design (RCBD) under two replications was established for 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 upto 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. The detail of field layout is mentioned in Table 6. Wide/narrow row to row distance pattern was followed and plastic membranes were utilized for covering of seedlings. Standard agronomic performs were established during whole experiment at all locations.

Table 6. 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+0.1) m
	Xinjiang August	XJAUG15	2	3× (0.66+0.1) 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+0.1) m
	Xinjiang August	XJAUG16	2	3× (0.66+0.1) m

4.3 Verticillium wilt phenotypic evaluation

For scoring of diseased portion of plant, a percentage based scale was used for evaluation ranging between 0-4 [61]. The scale used is a standard one being used deliberately in China especially for *Verticillium* disease rating indices by classifying the damaged portion of matured stage leaves into five groups [46, 51, 62]. The scoring pattern is considered in ascending order regarding resistance level accounting 0-2 as resistant and 3-4 as susceptible. The disease rating scale of VW is comprehensively discussed in Table 7.

Table 7. Scoring of symptoms of *Verticillium* wilt

Rate	Degree of susceptibility	Symptoms
0	Immune	Without symptom (healthy plants)
1	Extremely resistance	<25 % diseased leaves
2	Resistance	25–50 % diseased leaves
3	Susceptible	50–75 % diseased leaves
4	Extremely susceptible	>75 % diseased leaves or plant death

The disease Index (DI) was estimated following the formulae below [Z, 61].

$$DI(\%) = \frac{\sum(d_c \times n_c)}{n_t \times 4} \times 100$$

Where, d_c is disease rate

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

4.4 Analysis of phenotypic trait

The software SPSS 20.0 was used for analyzing the observed phenotypic data and the Pearson's rank correlation coefficient was used for evaluating the correlation among the disease index. The statistical package SAS version 9.1 was employed for Analysis of variance (ANOVA) of disease index and Tukey's test was used to compare treatment means. The broad-sense heritability (H^2) was calculated following the formulae described by [63].

$$\text{Broad sense Heritability (\%)} = \left(\frac{\text{Var(G)}}{\text{Var(P)}} \right) \times 100$$

Where, Var(G) =

Genotypic variance

Var(P)

= Phenotypic variance

4.5 Genetic analysis

Genomic DNA of CSSLs from BC₅F_{3.5} population and its parents was extracted by following a modified procedure of CTAB method [64] by using young leaves which were sampled from each line and kept at -80°C. The working concentration of DNA was adjusted at 30ng/μL; quantified on NanoDrop2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE USA). Further the integrity of DNA was patterned on agarose gel (1%) using Lambda DNA/HindIII Markers[65] as ladder. Scoring pattern followed for SSRs fragments include ‘—’ for missing, ‘1’ for presence and ‘0’ for absence of bands.

4.6 SSR markers and SSR molecular detection

Based on the genetic map [37], in total, 597 pairs markers were screened out by using 2292 pairs of markers to be used to screen 300 CSSLs DNA. The sequences of these SSR primers were downloaded from the CMD database (<http://www.cottongen.org/>). First of all, we diluted these primer pairs. For dilution, we centrifuge primer pairs at 12000rpm at 4°C for 10 minutes to settle down the contents at the bottom. We diluted these primer pairs 100X and shake it vigorously for 2 minutes. Centrifuge it again and store at -20°C. The details of these SSR primers are mentioned in Table S2.

4.7 QTL mapping

QTL IciMapping V4.0 software developed by Wang et al. [66] was used to map QTLs of CSSLs. A LOD (likelihood of odds) of threshold 2.5 was used to declare significant additive QTLs. The percentage of phenotypic variance (PV%) explained

individual QTL and additive effects at the LOD peaks were determined through stepwise regression (RSTEP-LRT). The graphical presentation of QTLs was done by using the MapChart2.2 software [67].

Positive additive effects showed that CCRI36 alleles decremented the phenotypic disease index values and enhanced resistance against VW. On the other hand, negative scores indicated that Hail alleles decremented the phenotypic disease index values and incremented the values of VW resistance. The QTL nomenclature was designed as follows: the QTL designations begin with “q” come after the trait abbreviation, the chromosome name, and the number of QTL on that chromosome [68, 69]. Stable QTL was declared when it is found in at least two environments.

4.8 Meta-analysis of QTLs

Biomercator 4.2 [70] software was considered suitable for our data in order to perform Meta-analysis[32]. Already performed QTL meta-analysis has established a database[33]of QTLs including approximately 2,274 QTLs regarding 66 traits; accounting 201 QTLs regarding resistance for VW [13, 21, 43, 46-48, 50, 61, 71]. In our study, we kept the standard reference of Said et al [33]for information of mapped QTLs controlling VW resistance. Remaining previous studies, including 113 QTLs responsible for VW resistance have also been mentioned later[7, 19, 22, 45, 72, 73]. In aggregate 367 QTLs related to VW resistance have been utilized to build a platform for meta-analysis in which 53 QTLs were from our discovery in current study. On manual basis, new QTL hotspots have been identified by considering a

consistent QTL region as if four or more QTLs were occurring in an interval of 25cM. However, the same consistent QTL region was possessing QTLs for only one trait then it was taken as a ‘QTL Hotspot’ [7].

Meta-analysis was performed by taking two files as input i.e. QTL file and map file. Map file was based on the information regarding names of parents, cross type and markers position on chromosomes. The QTL file was loaded with QTL in given environment as row information and QTL name, trait name, trait ontology, location, year, chromosome number, linkage group, LOD score, observed phenotypic variation (R^2), most likely position of QTL, CI start position and CI end position. Initially, the two files were uploaded successfully and map connectivity was investigated for construction of consensus map. After that QTLs projection on consensus map was done, followed by meta-analysis regarding trait. Ultimately four model were obtained with different AIC (Akaike information criterion) value. The lowest AIC value holding model was considered suitable for the identification of mQTL position or QTL hotspot. The criteria described by Said et al.[32]of occurrence of mQTLs in 20 cM interval was kept standard for the identification of hotspot.

5. Conclusions

In this study, 300 CSSLs developed from *Gossypium hirsutum* CCRI36 $\times$ *Gossypium 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 population (CSSL), population size and the presence of control (Jimian11)

in our study showed us to lower the experimental error and to check the accurateness of data.

In total, 53 QTLs for VW resistance were identified in CSSLs populations, of which 29 ones were found as stable QTLs. Ten QTLs were similar to previously reported QTLs, while 43 ones were novel QTLs. Based on meta-analysis, 32 QTLs hotspot regions were detected, including 15 novel ones. These consistent QTLs and hotspot regions form critical steps, which will contribute to molecular breeders in developing and improving the VW resistance in upland cotton. The outcomes of this study also provide most important message for further studies of the molecular basis of VW resistance in cotton.

Supplementary Materials

Supplementary materials can be found at www.mdpi.com/link.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

Y.Y.L., T.T.C., Y.Z.S conceived and designed the experiments; M.H.R., L.P.T., K.K.P., Q.G., A.Y.L., J.W.G., Q.W.L., L.D., R.O.M., M.S.I., M.J. and W.K.G. performed the experiments; M.H.R. and L.P.T. analyzed the data; M.H.R. contributed reagents/materials/analysis tools: Y.Y.L., Y.Z.S., M.H.R., and L.P.T. drafted the manuscript.

Abbreviations

English Abbr.	English Full Name
VW	<i>Verticillium</i> Wilt
DI	Disease Index
SSR	Simple Sequence Repeats
CSSL	Chromosome Segment Substitution Lines
AIC	Akaike Information Criterion
CI	Confidence Interval

Chr	Chromosome
cM	Centi- Morgan
CMD	Cotton Marker Database
H ² _B	Broad sense Heritability
LOD	Logarithm of Odds
MAS	Marker Assisted Selection
QTL	Quantitative Trait Loci
PV	Phenotypic Variation
CTAB	Cetyl-Trimethyl Ammonium Bromide
mya	Million Years Ago

References

1. Xu, Z.Y.; Kohel, R.J.; Song, G.L.; Cho, J.M.; Alabady, M.; Yu, J. et al. Gene-rich islands for fiber development in the cotton genome. *Genomics***2008**, *92*, 173-183.
2. Lee, J.A. and Fang, D.D. Cotton as a world crop: origin, history, and current status. *In: Fang DD, Percy RG (eds) Cotton, 2nd edn. ASA-CSSA-SSSA, Madison, WI, USA***2015**, pp. 1-23.
3. Alkuddsi, Y.A.; Rao, M.G.; Patil, S.S.; Joshi, M. and Gowda, T.H. Heterosis Studies and Per se Performance of Intra Hirsutum Hybrids (*G.hirsutum* x *G.hirsutum*) for Kapas Yield and Its Components in Cotton. *Cotton Genomics and Genetics***2013**, *4*, 73-92, 10.5376/cgg.2013.04.0006.
4. Wendel, J.F. and Grover, C.E. Taxonomy and evolution of the cotton genus, *Gossypium*. *In: Fang DD, Percy RG (eds) Cotton, 2nd edn. ASA-CSSA-SSSA, Madison, WI, USA***2015**, pp. 1-23.
5. Mehetre, S.S.; Patil, S.C.; Pawar, S.V.; Pardedhi, S.U.; Shinde, G.C. and Aher, A.R. Ovulo embryo cultured hybrid between amphidiploids (*Gossypium arboreum*&*Gossypium anomalum*) and *Gossypium hirsutum*. *Curr Sci* **2004**, *87*, 286-289.
6. Grover, C.E.; Zhu, X. and Grupp, K.K. Molecular confirmation of species status for the allopolyploid cotton species, *Gossypium ekmanianum* Wittmack. *Genet Resour Crop Evol* **2015**, *62*, 103-114.
7. Zhang, J.F.; Yu, J.W.; Pei, W.F.; Li, X.L.; Said, J.; Song, M.Z. et al. Genetic analysis of *Verticillium* wilt resistance in a backcross inbred line population and a meta-analysis of quantitative trait loci for disease resistance in cotton. *BMC Genomics***2015**, *16*, 577. doi: 10.1186/s12864-015-1682-2.
8. DeVay, J.E.; Weir, B.L.; Wakeman, R.J. and Stapleton, J.J. Effects of *Verticillium dahliae* infection of cotton plants (*Gossypium hirsutum*) on potassium levels in leaf petioles. *Plant Dis***1997**, *81*, 1089-1092.
9. Blasingame, D. and Patel, M.V. Cotton disease loss estimate committee report. *San Antonio, TX: Proc, Beltwide Cotton Conf***2013**, 1242-5.

- 768 10. Cai, Y.; Xiaohong, H.; Mo, J.; Sun, Q.; Yang, J. and Liu, J. Molecular research and
769 genetic engineering of resistance to *Verticillium wilt* in cotton: A review. *Afr. J.*
770 *Biotechnol***2009**, 8, 7363-7372.
- 771 11. Yu, Y.; Daojun, Y.; Shaoguang, L.; Ximei, L.; Xiaqing, W.; Zhongxu, L. et al. Genome
772 structure of cotton revealed by a genome-wide SSR genetic map constructed from a BC₁
773 population between *Gossypium hirsutum* and *G. barbadense*. *BMC Genomics* **2011**, 12.
- 774 12. Xu, J.J.; Zhao, Q.; Du, P.N.; Xu, C.W.; Wang, B.H. and Feng, Q. Developing high
775 throughput genotyped chromosome segment substitution lines based on population
776 whole-genome re-sequencing in rice (*Oryza sativa* L.). *BMC Genomics***2010**, 24, 656. doi:
777 10.1186/1471-2164-11-656.
- 778 13. Wei, F.; Fan, R.; Dong, H.-T.; Shang, W.-J.; Xu, X.-M.; Zhu, H.-Q. et al. Threshold
779 microsclerotial inoculum for cotton *Verticillium* wilt determined through wet-sieving and
780 real-time quantitative PCR. *Phytopathology* **2015**, 105, 220-229.
- 781 14. Zhang, T.; Yun, J.Y.; Zhao, J.H.; Gao, F.; Zhou, B.J.; Fang, Y.Y. et al. Host-Induced
782 Gene Silencing of the Target Gene in Fungal Cells Confers Effective Resistance to the
783 Cotton Wilt Disease Pathogen *Verticillium dahliae*. *Molecular Plant***2016**, 9, 939–942,
784 doi: org/10.1016/j.molp.2016.02.008.
- 785 15. Bell, A.A. and Hillocks, R.J. *Verticillium wilt*. Wallingford: *CAB International***1992**,
786 87-126.
- 787 16. Li, F.; Shen, H.; Wang, M.; Fan, K.; Bibi, N.; Ni, M. et al. A synthetic antimicrobial
788 peptide BTDS expressed in *Arabidopsis thaliana* confers enhanced resistance to
789 *Verticillium dahliae*. *Mol Genet Genomics***2016**, 291, 1647-1661, doi:
790 10.1007/s00438-016-1209-9.
- 791 17. Zhang, B.H.; Liu, F.; Yao, C.B. and Wang, K.B. Recent progress in cotton biotechnology
792 and genetic engineering in China. *Curr. Sci.***2000**, 79, 37-44.
- 793 18. Mert, M.; Kurt, S.; Gencer, O.; Akiscan, Y.; Boyaci, K. and Tok, F.M. Inheritance of
794 resistance to *Verticillium wilt* (*Verticillium dahliae*) in cotton (*Gossypium hirsutum* L.).
795 *Plant Breed***2005**, 124, 102–104, doi: 10.1111/j.1439-0523.2004.01040.x.
- 796 19. Wang, P.; Ning, Z.; Lin, L.; Chen, H.; Mei, H. and Zhao, J., et al. Genetic dissection of
797 tetraploid cotton resistant to *Verticillium* wilt using interspecific chromosome segment
798 introgression lines. *Crop J.***2014**, 2, 278 – 288, doi:10.1016/j.cj.2014.06.007.
- 799 20. Wilhelm, S.; Sagen, J.E. and Tietz, H. Resistance to *Verticillium* wilt in cotton: sources,
800 techniques of identification, inheritance trends, and resistance potential of multiline
801 cultivars. *Phytopathology***1974**, 64, 924–31.
- 802 21. Fang, H.; Zhou, H.P.; Sanogo, S.; Flynn, R.; Percy, R.G.; Hughs, S.E. et al. Quantitative
803 trait locus mapping for *Verticillium* wilt resistance in a backcross inbred line population
804 of cotton (*Gossypium hirsutum* X *Gossypium barbadense*) based on RGA-AFLP analysis.
805 *Euphytica***2013**, 194, 79-91, doi: 10.1007/s10681-013-0965-4.
- 806 22. Shi, Y.; Zhang, B.; Liu, A.; Li, W.; Li, J. and Lu, Q., et al. Quantitative trait loci analysis
807 of *Verticillium* wilt resistance in interspecific backcross populations of *Gossypium*
808 *hirsutum* × *Gossypium barbadense*. *BMC Genomics***2016**, 17, 877, doi:
809 10.1186/s12864-016-3128-x.
- 810 23. Zhu, W.Y.; Lin, J.; Yang, D.W.; Zhao, L.; Zhang, Y.D.; Zhu, Z. et al. Development of
811 chromosome segment substitution lines derived from backcross between two sequenced

- 812 rice cultivars, Indica recipient 93–11 and *Japonica* donor Nipponbare. *Plant Mol Biol*
- 813 *Rep***2009**, 27, 126–131, doi:10.1007/s11105-008-0054-3.
- 814 24. Zhao, L.N.; Zhou, H.J.; Lu, L.X.; Liu, L.; Li, X.H.; Lin, Y.J. et al. Identification of
- 815 quantitative trait loci controlling rice mature seed cultivability chromosomal segment
- 816 substitution lines. *Plant Cell Rep***2009**, 28, 247–256. doi:10.1007/s00299-008-0641-7.
- 817 25. Ali, M.L.; Paul, L.; Yu, S.B.; Lorieux, M. and Eizenga, G.C. Chromosome Segment
- 818 Substitution Lines: A Powerful Tool for the Introgression of Valuable Genes from *Oryza*
- 819 Wild Species into Cultivated Rice (*O. sativa*). *Rice***2010**, 3, 218–234, doi:
- 820 10.1007/s12284-010-9058-3.
- 821 26. Takershi, E.; Yoshinobu, T.; Yasunori, N.; Toshio Y.; Kasumi, T. and Masahiro, Y.
- 822 Construction and evaluation of chromosome segment substitution lines carrying
- 823 overlapping chromosome segments of indica rice cultivar ‘Kasalath’ in a genetic
- 824 background of japonica elite cultivar ‘Kooshihikari’. *Breed Sci***2005**, 55, 65–73.
- 825 27. Chen, H.; Qian, N.; Guo, W.; Song, Q.; Li, B.; Deng, F. et al. Using three overlapped
- 826 RILs to dissect genetically clustered QTL for Wber strength on Chro.D8 in Upland cotton.
- 827 *Theor Appl Genet***2009**, 119, 605–612, doi: 10.1007/s00122-009-1070-x.
- 828 28. Ye, G.Y.; Liang, S.S. and Wan, J.M. QTL mapping of protein content in rice using single
- 829 chromosome segment substitution lines. *Theor Appl Genet***2010**, 121, 741–750, doi:
- 830 10.1007/s00122-010-1345.
- 831 29. Wang, P.; Ding, Y.Z.; Lu, Q.X.; Guo, W.Z. and Zhang, T.Z. Development of *Gossypium*
- 832 *barbadense* chromosome segment substitution lines in the genetic standard line TM-1 of
- 833 *Gossypium hirsutum*. *Chinese Sci. Bullet***2008**, 53, 1512–1517, doi:
- 834 10.1007/s11434-008-0220-x.
- 835 30. Lacape, J.M.; Llewellyn, D.; Jacobs, J.; Arioli, T.; Becker, D. and Calhoun, S.
- 836 Meta-analysis of cotton fiber quality QTLs across diverse environments in a *Gossypium*
- 837 *hirsutum* × *G. barbadense* RIL population. *BMC Plant Biol***2010**, 10, 132.
- 838 31. Yu, J.Z.; Ulloa, M.; Hoffman, S.M.; Kohel, R.J.; Pepper, A.E.; Fang, D.D. et al. Mapping
- 839 genomic loci for cotton plant architecture, yield components, and fiber properties in an
- 840 interspecific (*Gossypium hirsutum* L. × *G. barbadense* L.) RIL population. *Mol Genet*
- 841 *Genomics***2014**, 289, 1347–1367, doi: 10.1007/s00438-014-0930-5.
- 842 32. Said, J.I.; Song, M.; Wang, H.; Lin, Z.; Zhang, X. and Fang, D.D. A comparative
- 843 metaanalysis of QTL between intraspecific *Gossypium hirsutum* and interspecific *G.*
- 844 *hirsutum* × *G. barbadense* populations. *Mol Gen Genomics***2014**, 290, 1003–25, doi:
- 845 10.1007/s00438-014-0963-9.
- 846 33. Said, J.I.; Knapka, J.A.; Song, M. and Zhang, J. Cotton QTLdb: a cotton QTL database
- 847 for QTL analysis, visualization, and comparison between *Gossypium hirsutum* and *G.*
- 848 *hirsutum* × *G. barbadense* populations. *Mol Gen Genomics***2015**, 290, 1615–25, doi:
- 849 10.1007/s00438-015-1021-y.
- 850 34. Wu, M.; Zhang, L.; Li, X.; Xie, X.; Pei, W.; Yu, J. et al. A comparative transcriptome
- 851 analysis of two sets of backcross inbred lines differing in lint yield derived from a
- 852 *Gossypium hirsutum* × *Gossypium barbadense* population. *Mol Genet Genomics***2016**,
- 853 291, 1749–1767, doi: 10.1007/s00438-016-1216-x.
- 854 35. Zheng, J.Y.; Oluoch, G.; Riaz Khan, M.K.; Wang, X.X.; Cai, X.Y. and Zhou, Z.L., et al.
- 855 Mapping QTLs for drought tolerance in an F2:3 population from an inter-specific cross

- 856 between *Gossypium tomentosum* and *Gossypium hirsutum*. *Genet. Mol. Res.***2016**, *15*,
857 gmr.15038477.
- 858 36. Zhai, H.; Gong, W.; Tan, Y.; Liu, A.; Song, W. and Li, J., et al. Identification of
859 Chromosome Segment Substitution Lines of *Gossypium barbadense* Introgressed in *G.*
860 *hirsutum* and Quantitative Trait Locus Mapping for Fiber Quality and Yield Traits. *PLoS*
861 *One***2016**, *11*, e0159101. doi:10.1371/journal.pone.0159101.
- 862 37. Shi, Y.; Li, W.; Li, A.; Ge, R.; Zhang, B. and Li, J., et al. Constructing a high-density
863 linkage map for *Gossypium hirsutum* X *Gossypium barbadense* and identifying QTLs for
864 lint percentage. *J Integr Plant Biol***2015**, *57*, 450-67, doi: 10.1111/jipb.12288.
- 865 38. Wang, Q.; Fang, L.; Chen, J.; Hu, Y.; Si, Z.; Wang, S. et al. Genome-Wide Mining,
866 Characterization, and Development of Microsatellite Markers in *Gossypium* Species.
867 *Scientific Reports***2015**, *5*, 10638, doi: 10.1038/srep10638.
- 868 39. Said, J.I.; Zhongxu Lin, Z., X., ; Zhang, X.L.; Song, M.Z. and Zhang, J.F. A
869 comprehensive meta QTL analysis for fiber quality, yield, yield related and
870 morphological traits, drought tolerance, and disease resistance in tetraploid cotton. *BMC*
871 *Genomics* **2013**, *14*, 776, doi:10.1186/1471-2164-14-776.
- 872 40. Devey, M.E. and Roose, M.L. Genetic analysis of *Verticillium* wilt tolerance in cotton
873 using pedigree data from three crosses. *Theor Appl Genet***1987**, *74*, 162-167.
- 874 41. Bejarano, A.Z.; Blanco-Lo' Pez, M.A.; Melero-Vara, J.M. and Jime' Nez-Di'Az, R.M.
875 The influence of *verticillium* wilt epidemics on cotton yield in southern Spain. *Plant*
876 *Pathol***1997**, *46*, 168–178, doi: 10.1046/j.1365-3059.1997.d01-221.x.
- 877 42. Bolek, Y.; El-Zik, K.M.; Pepper, A.E.; Bell, A.A.; Magill, C.W.; Thaxton, P.M. et al.
878 Mapping of *verticillium* wilt resistance genes in cotton. *Plant Sci***2005**, *168* 1581–1590,
879 doi: 10.1016/j.plantsci.2005.02.008.
- 880 43. Wang, H.M.; Lin, Z.X.; Zhang, X.L.; Chen, W.; Guo, X.P.; Nie, Y.C. et al. Mapping and
881 Quantitative Trait Loci Analysis of *Verticillium* Wilt Resistance Genes in Cotton. *J.*
882 *Integr. Plant Biol.***2008**, *50*, 174–182, doi: 10.1111/j.1744-7909.2007.00612.x.
- 883 44. Zhang, J.F.; Sanogo, S.; Flynn, R.; Baral, J.B.; Bajaj, S.; Hughs, S.E. et al. Germplasm
884 evaluation and transfer of *Verticillium* wilt resistance from Pima (*Gossypium barbadense*)
885 to upland cotton (*G. hirsutum*). *Euphytica***2012**, *187*, 147–60,
886 doi:10.1007/s10681-011-0549-0.
- 887 45. Zhang, X.; Yuan, Y.; Wei, Z.; Guo, X. and Guo, Y. Molecular Mapping and Validation of
888 a Major QTL Conferring Resistance to a Defoliating Isolate of *Verticillium* Wilt in Cotton
889 (*Gossypium hirsutum* L.). *PLoS ONE***2014**, *9*, e96226, doi:
890 10.1371/journal.pone.0096226.
- 891 46. Yang, C.; Guo, W.Z.; Li, G.Y.; Gao, F.; Lin, S.S. and Zhang, T.Z. QTLs mapping for
892 *Verticillium* wilt resistance at seedling and maturity stages in *Gossypium barbadense* L.
893 *Plant Science* **2008**, *174* 290–298.
- 894 47. Ning, Z.Y.; Zhao, R.; Chen, H.; Ai, N.J.; Zhang, X.; Zhao, J. et al. Molecular Tagging of
895 a Major Quantitative Trait Locus for Broad-Spectrum Resistance to *Verticillium* Wilt in
896 Upland Cotton Cultivar Prema. *Crop Science***2013**, *53*, 2304-2312,
897 10.2135/cropsci2012.12.0694.
- 898 48. Fang, H.; Zhou, H.P.; Sanog, S.; Lipka, A.E.; Fang, D.D.; Percy, R.G. et al. Quantitative
899 trait locus analysis of *Verticillium* wilt resistance in an introgressed recombinant inbred

- 900 population of Upland cotton. *Mol Breeding***2014**, 33, 709–720, doi:
901 10.1007/s11032-013-9987-9.
- 902 49. Wang, Y.; Ning, Z.; Hu, Y.; Chen, J.; Zhao, R. and Chen, H., et al. Molecular Mapping of
903 Restriction-Site Associated DNA Markers in Allotetraploid Upland Cotton. *PLoS*
904 *ONE***2015**, 10(4): e0124781.
- 905 50. Jiang, F.; Zhao, J.; Zhou, L.; Guo, W.Z. and Zhang, T.Z. Molecular mapping of
906 *Verticillium* wilt resistance QTL clustered on chromosomes D7 and D9 in upland cotton.
907 *Science China-Life Sciences***2009**, 52, 872-884, doi: 10.1007/s11427-009-0110-8.
- 908 51. Zhang, J.F.; Fang, H.; Zhou, H.; Sanogo, S. and Ma, Z. Genetics, Breeding, and
909 Marker-Assisted Selection for Verticillium Wilt Resistance in Cotton. *Crop Sci.***2014**, 54,
910 1–15, doi: 10.2135/cropsci2013.08.0550.
- 911 52. Wu, C.C.; Jian, G.L.; Wang, A.; Liu, F.; Zhang, X.L.; Song, G.L. et al. Primary detection
912 of QTL for *Verticillium* wilt resistance in cotton *Mole Plant Breed***2010**, 18, 680–686, doi:
913 10.3969/mpb.008.000680.
- 914 53. He, R.; Shi, Y.Z.; Zhang, J.F.; Liang, Y.; Zhang, B.C.; Li, J.W. et al. QTL mapping for
915 plant height using chromosome segment substitution lines in upland cotton. *Acta Agron*
916 *Sin***2014**, 40, 457-65.
- 917 54. Liang, Y.; Jia, Y.J.; Li, A.G.; Zhang, B.C.; Liu, G.P.; Li, J.Z. et al. Phenotyping traits
918 related to yield and quality of BC₅F₂ substitution lines in cotton (*Gossypium*) and their
919 QTL mapping. *Mole Plant Breed***2010**, 8, 221–30, doi: 10.3969/mpb.008.000221.
- 920 55. Zhang, J.F.; Shi, Y.Z.; Liang, Y.; Jia, Y.J.; Zhang, B.C.; Li, J.W. et al. Evaluation of
921 Yield and Fiber Quality Traits of Chromosome Segment Substitution Lines Population
922 (BC₅F₃ and BC₅F_{3:4}) in Cotton. *J Plant Genetic Res.***2012**, 13, 773-81, doi:
923 10.3724/SP.J.1006.2014.00457.
- 924 56. Yang, Z.M.; Li, J.Z.; Li, A.G.; Zhang, B.C.; Liu, G.P.; Li, J.W. et al. Developing
925 chromosome segment substitution lines (CSSLs) in Cotton (*Gossypium*) using advanced
926 backcross and MAS. *Mol Plant Breed***2009**, 7, 233-41.
- 927 57. Lan, M.J.; Yang, Z.M.; Shi, Y.Z.; Ge, R.H.; Li, A.G.; Zhang, B.C. et al. Assessment of
928 substitution lines and identification of QTL related to fiber yield and quality traits in
929 BC₄F₂ and BC₄F₃ populations from *Gossypium hirsutum* × *Gossypium barbadense*. *Sci*
930 *Agric Sin***2011**, 44, 3086–97.
- 931 58. Ma, L.J.; Shi, Y., Z.; Lan, M., J.; Yang, Z., M.; Zhang, J., F.; Zhang, B., C. et al.
932 Evaluation of chromosome segment substitution lines related to fiber yield and quality
933 traits from *Gossypium hirsutum* × *Gossypium barbadense*. *Cotton Sci***2013**, 25, 486-95.
- 934 59. Sun, Q.; Cai, Y.F.; Xie, Y.F.; Mo, J.C.; Youlu, Y. and Shi, Y.Z., et al. Gene expression
935 profiling during gland morphogenesis of a mutant and a glandless upland cotton.
936 *Molecular Biology Reports***2010**, 37, 3319–3325.
937 doi:10.1007/s11033-009-9918-3 PMID:19888674.
- 938 60. Li, P.T.; Wang, M.; Lu, Q.W.; Ge, Q.; Rashid, M.H.; Liu, A.Y. et al. Comparative
939 transcriptome analysis of cotton fiber development of Upland cotton (*Gossypium*
940 *hirsutum*) and Chromosome Segment Substitution Lines from *G. hirsutum* × *G.*
941 *barbadense*. *BMC Genomics* **2017**, 18, DOI 10.1186/s12864-017-4077-8.

- 942 61. Zhao, Y.; Wang, H.; Chen, W. and Li, Y. Genetic Structure, Linkage Disequilibrium and
943 Association Mapping of Verticillium Wilt Resistance in Elite Cotton (*Gossypium*
944 *hirsutum* L.) Germplasm Population. *PLoS ONE***2014**, *9*, e86308.
- 945 62. Wu, Z.B.; Li, J.; Feng, C.D. and Zhang, J.F. Studies on the identification techniques of
946 cotton resistance to Verticillium wilt. *Hubei Agric Sci.***1999**, *5*, 16-19.
- 947 63. Khan, N.U.; Marwat, K.B.; Farhatullah, G.H.; Batool, S.; Makhdoom, K.; Ahmad, W. et
948 al. Genetic variation and heritability for cotton seed, fiber and oil traits in *Gossypium*
949 *hirsutum* L. *Pak. J. Bot.***2010**, *42*, 615-625.
- 950 64. Paterson, A.H.; Brubaker, C.L. and Wendel, F.J. A Rapid Method for Extraction of
951 Cotton (*Gossypium* spp.) Genomic DNA Suitable for RFLP or PCR Analysis. *Plant Mol.*
952 *Biol. Rep.***1993**, *11*, 122-127.
- 953 65. Niu, C.; Lister, H.E.; Nguyen, B.; Wheeler, T.A. and Wright, R.J. Resistance to
954 *Thielaviopsis basicola* in the cultivated A genome cotton. *Theor Appl Genet***2008**, *117*,
955 1313–1323, doi: 10.1007/s00122-008-0865-5.
- 956 66. Wang, J.; Wan, X.; Crossa, J.; Crouch, J.; Weng, J. and Zhai, H., et al. QTL mapping of
957 grain length in rice (*Oryza sativa* L.) using chromosome segment substitution lines.
958 *Genetical Research***2006**, *88*, 93-104, PMID:1712558.
- 959 67. Voorrips, R.E. Map Chart: Software for the graphical presentation of linkage maps and
960 QTLs. *Journal of Heredity***2002**, *93*, 77–78. PMID:12011185.
- 961 68. Sun, F.D.; Zhang, J.H.; Wang, S.F.; Gong, W.K.; Shi, Y.Z. and Liu, A.Y., et al. QTL
962 mapping for fiber quality traits across multiple generations and environments in upland
963 cotton. *Mol. Breed.***2012**, *30*, 569-582, doi: 10.1007/s11032-011-9645-z .
- 964 69. Jamshed, M.; Jia, F.; Gong, J.; Palanga, K.K.; Shi, Y.; Li, J. et al. Identification of stable
965 quantitative trait loci (QTLs) for fiber quality traits across multiple environments in
966 *Gossypium hirsutum* recombinant inbred line population. *BMC Genomics***2016**, *17*, 197,
967 doi: 10.1186/s12864-016-2560-2.
- 968 70. Arcade, A.; Labourdette, A.; Falque, M.; Mangin, B.; Chardon, F.; Charcosset, A. et al.
969 BioMercator: integrating genetic maps and QTL towards discovery of candidate genes.
970 *Bioinformatics***2004**, *20*, 2324-6.
- 971 71. Wang, F.R.; Liu, R.Z.; Wang, L.M.; Zhang, C.Y.; Liu, G.D.; Liu, Q.H. et al. Molecular
972 markers of *Verticillium* wilt resistance in Upland cotton (*Gossypium hirsutum* L.) cultivar
973 and their effect on Assisted Phenotypic Selection. *Cotton Sci.***2007**, *19*, 424-430.
- 974 72. Cai, Y.F.; Xiaohong, H.; Mo, J.C.; Sun, Q.; Yang, J.P. and Liu, J.G. Molecular research
975 and genetic engineering of resistance to Verticillium wilt in cotton: A review. *Afr. J.*
976 *Biotechnol.***2009**, *8*, 7363-7372.
- 977 73. Zhou, H.P.; Fang, H.; Sanogo, S.; Hughs, S.E.; Jones, D.C. and Zhang, J. Evaluation of
978 *Verticillium* wilt resistance in commercial cultivars and advanced breeding lines of cotton.
979 *Euphytica***2014** *196*, 437-448, doi: 10.1007/s10681-013-1045-5.

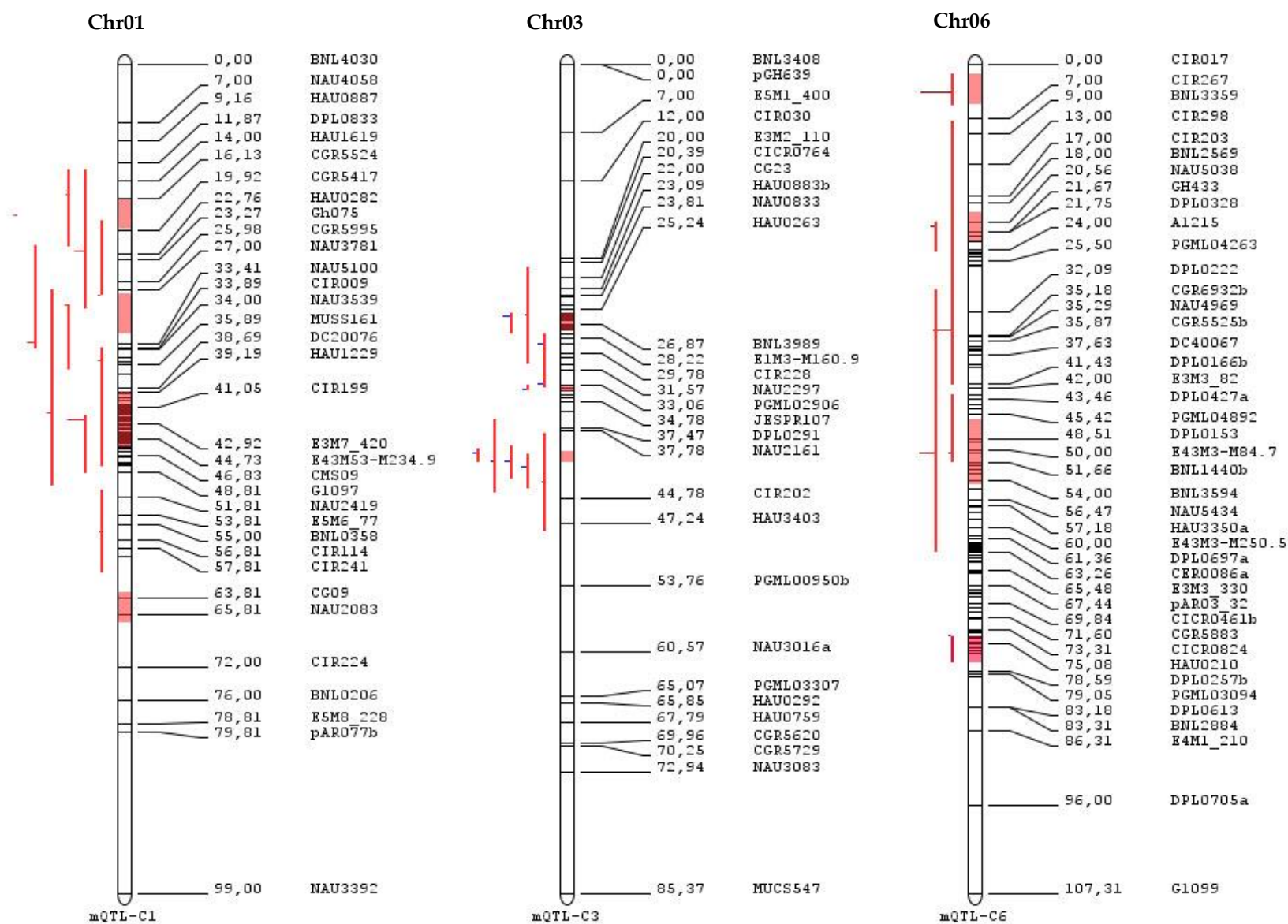


Figure S2. QTLs and QTL hotspot for *Verticillium* wilt resistance on the consensus map by a meta-analysis

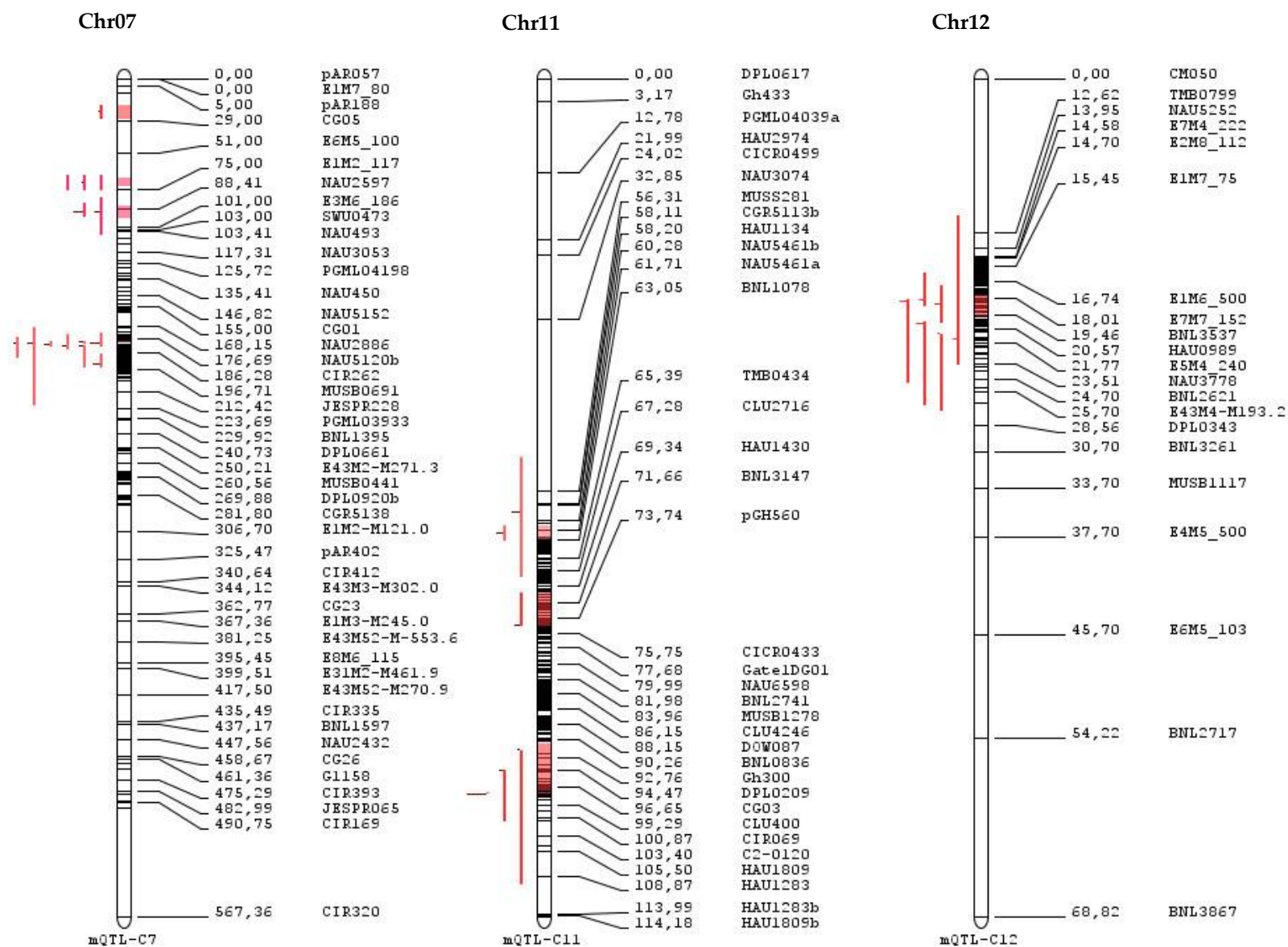


Figure S2. Continued

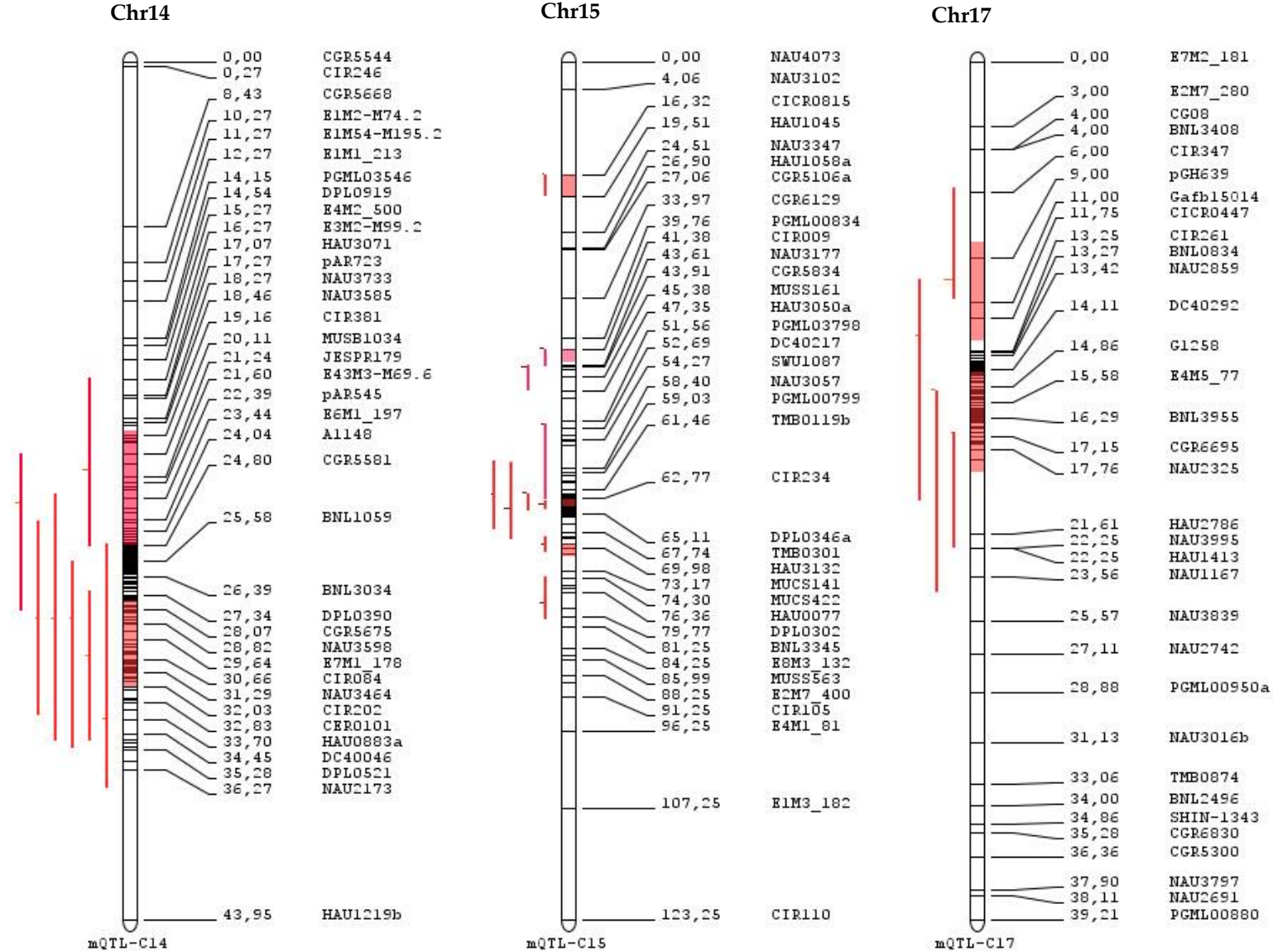
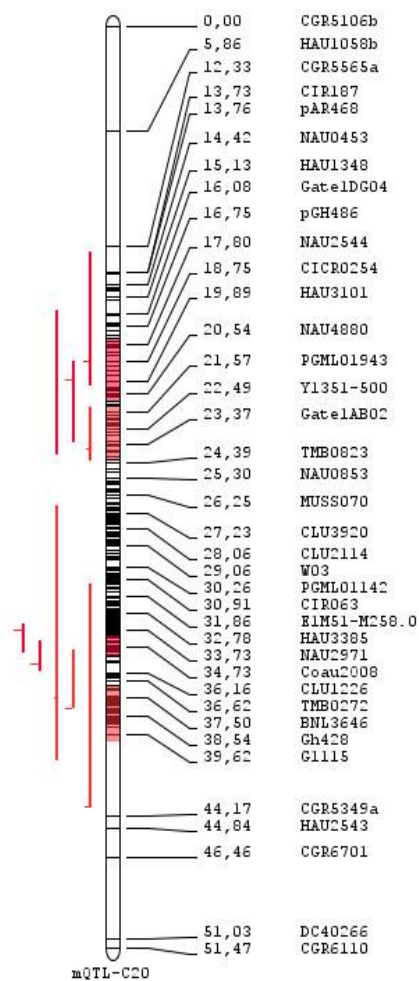
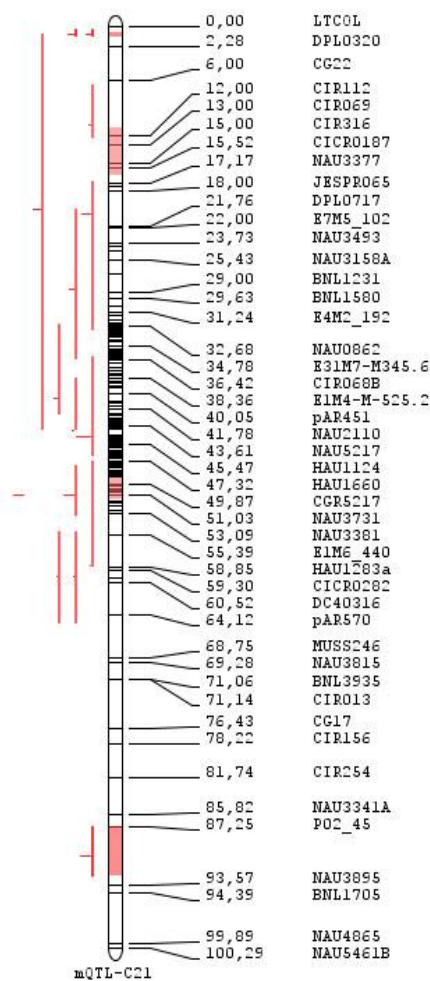


Figure S2. Continued

Chr20



Chr21



Chr23

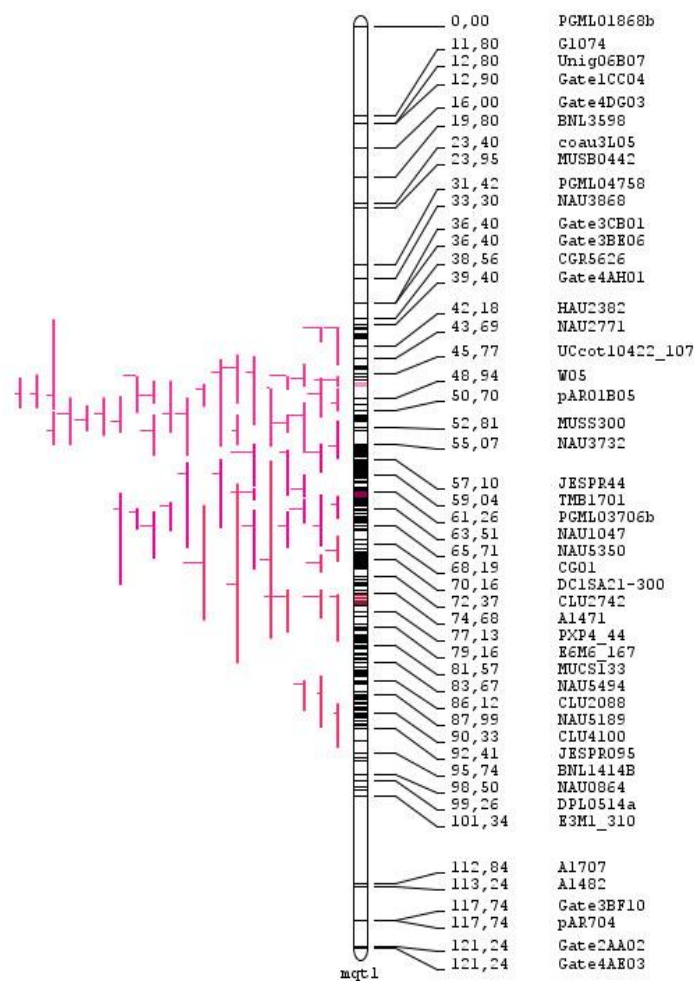


Figure S2. Continued

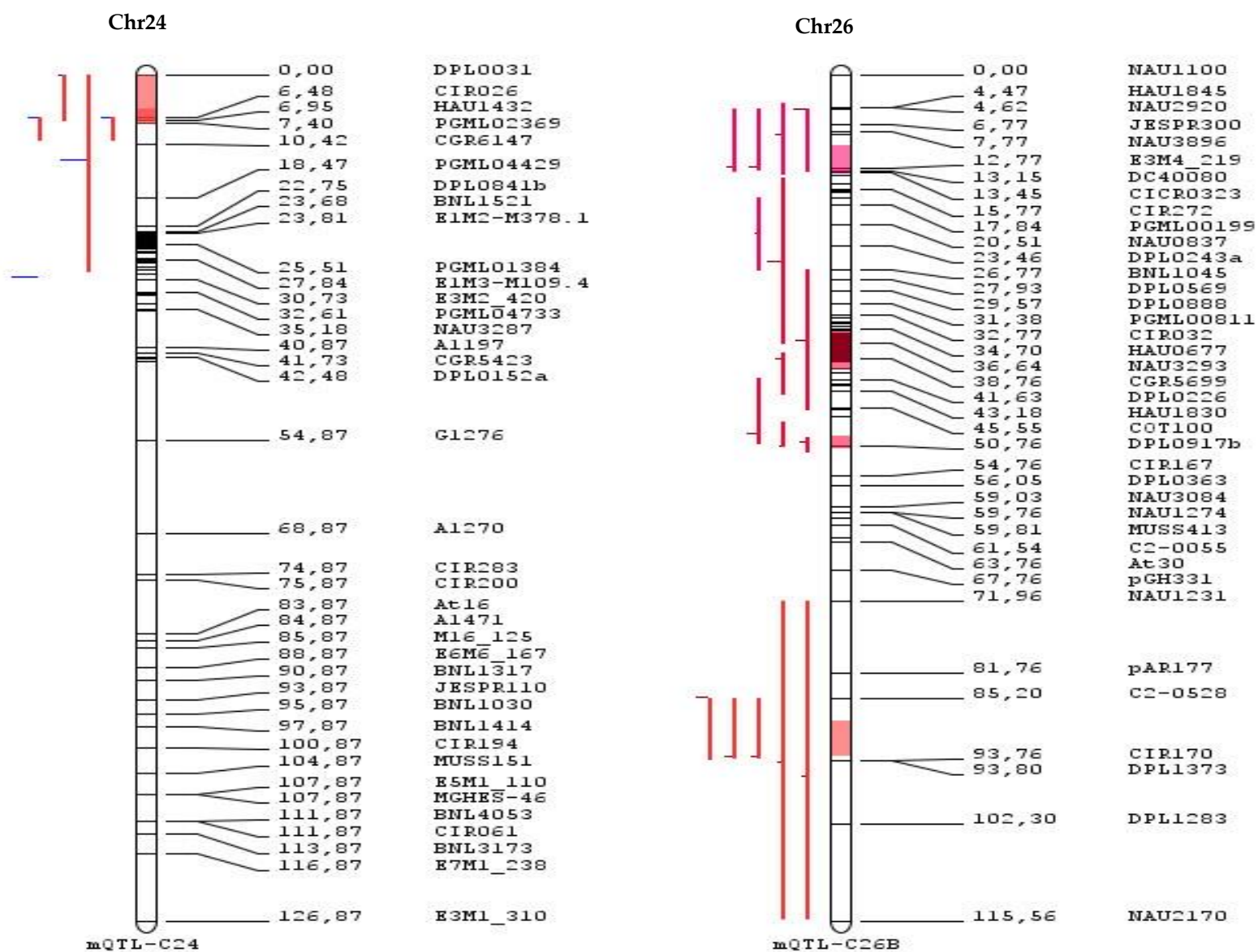


Figure S2. Continued

Table S1. Details QTLs for Verticillium wilt resistance detected during different stages of growth and environments in BC5F3:5

SL. No.	QTLs	Growth stage	Env	Chr	Position (cM)	Nearest marker	LOD	Add
1	qVW-Chr01-1	August	AYAUG15	Chr01	31.1	TMB0119	2.63	7.73
2	qVW-Chr01-2	July	AYJul16	Chr01	70.3	MUCS084	2.97	-8.77
3	qVW-Chr01-3	July	XJJul16	Chr01	122.7	TMB152	3.15	-3.37
		August	XJAug16	Chr01	122.7	TMB152	4.27	-5.05
4	qVW-Chr02-1	July	XJJul15	Chr02	50.0	TMB1587	6.48	13.25
5	qVW-Chr02-2	August	AYAUG16	Chr02	129.8	CICR800	2.59	9.95
6	qVW-Chr03-1	August	XJAug16	Chr03	98.0	CER0028	2.85	-6.02
7	qVW-Chr03-2	July	XJJul16	Chr03	114.4	HAU0195	3.95	-5.99
		August	XJAug16	Chr03	114.4	HAU0195	4.88	-8.50
8	qVW-Chr05-1	August	AYAUG15	Chr05	30.5	CIR224b	5.22	-3.81
		July	XJJul16	Chr05	30.5	CIR224b	6.87	-4.25
		August	XJAug16	Chr05	30.5	CIR224b	5.95	-5.09
9	qVW-Chr05-2	July	AYJul15	Chr05	32.3	CIR102	3.84	-5.44
		August	AYAUG15	Chr05	32.3	CIR102	3.48	-4.07
		July	AYJul16	Chr05	32.3	CIR102	3.47	-4.43
		August	AYAUG16	Chr05	32.3	CIR102	2.88	-4.46
		July	XJJul16	Chr05	32.3	CIR102	6.66	-5.65
		August	XJAug16	Chr05	32.3	CIR102	4.59	-6.16
		July	AYJul15	Chr05	35.4	DPL0063	3.32	-5.08
10	qVW-Chr05-3	August	AYAUG15	Chr05	35.4	DPL0063	3.57	-4.23
		July	AYJul16	Chr05	35.4	DPL0063	5.07	-5.42
		August	AYAUG16	Chr05	35.4	DPL0063	2.98	-4.57
		July	XJJul16	Chr05	35.4	DPL0063	6.96	-5.87
		August	XJAug16	Chr05	35.4	DPL0063	4.45	-6.15
11	qVW-Chr05-4	July	AYJul15	Chr05	38.2	HAU0746	3.19	-5.69
		July	AYJul16	Chr05	38.2	HAU0746	2.99	-4.68
		July	XJJul16	Chr05	38.2	HAU0746	5.18	-5.80
		August	XJAug16	Chr05	38.2	HAU0746	3.26	-5.98
12	qVW-Chr05-5	July	XJJul16	Chr05	40.2	CGR5025	3.92	-4.74
		August	XJAug16	Chr05	40.2	CGR5025	6.30	-7.69
13	qVW-Chr05-6	July	AYJul15	Chr05	43.1	HAU1712	3.48	-5.39
		August	AYAUG15	Chr05	43.1	HAU1712	2.83	-3.78
		July	AYJul16	Chr05	43.1	HAU1712	3.47	-4.56
		August	AYAUG16	Chr05	43.1	HAU1712	2.82	-4.53
		July	XJJul16	Chr05	43.1	HAU1712	6.96	-6.06
		August	XJAug16	Chr05	43.1	HAU1712	4.34	-6.23
14	qVW-Chr05-7	July	XJJul16	Chr05	45.0	DPL0138	2.82	-3.90
		August	XJAug16	Chr05	45.0	DPL0138	3.59	-4.48
15	qVW-Chr05-8	July	AYJul15	Chr05	64.3	MUCS530	4.87	12.73
16	qVW-Chr05-9	July	XJJul16	Chr05	89.9	MUSS317	3.23	-6.92
		August	XJAug16	Chr05	89.9	MUSS317	3.84	-9.71
17	qVW-Chr05-10	August	XJAug15	Chr05	168.6	CGR5925a	3.35	2.81
18	qVW-Chr05-11	August	AYAUG15	Chr05	197.4	HAU1050	3.11	-2.64
		July	XJJul16	Chr05	197.4	HAU1050	3.80	-2.96
		August	XJAug16	Chr05	197.4	HAU1050	4.03	-3.94

19	qVW-Chr06-1	August	XJAug15	Chr06	29.8	Gh082	3.94	9.40
20	qVW-Chr06-2	July	AYJul16	Chr06	44.5	CER0086b	5.11	-9.57
		August	AYAug16	Chr06	44.5	CER0086b	2.78	-7.83
		July	XJJul16	Chr06	44.5	CER0086b	2.67	-6.62
		August	XJAug16	Chr06	44.5	CER0086b	3.74	-10.06
21	qVW-Chr06-3	July	AYJul16	Chr06	66.1	NAU5433	3.55	-6.89
		July	XJJul16	Chr06	66.1	NAU5433	3.69	-6.68
		August	XJAug16	Chr06	66.1	NAU5433	4.53	-9.50
22	qVW-Chr07-1	August	AYAug16	Chr07	92.2	NAU1085	2.62	-4.19
		July	XJJul16	Chr07	92.2	NAU1085	3.91	-4.41
		August	XJAug16	Chr07	92.2	NAU1085	3.37	-5.29
23	qVW-Chr09-1	July	XJJul16	Chr09	85.3	DPL0679	2.64	10.77
24	qVW-Chr09-2	August	XJAug16	Chr09	29.2	DPL0171	3.55	-5.09
25	qVW-Chr10-1	July	AYJul16	Chr10	150.7	NAU2869	3.37	-6.75
		August	AYAug16	Chr10	150.7	NAU2869	3.28	-7.31
		July	XJJul16	Chr10	150.7	NAU2869	2.69	-5.73
		August	XJAug16	Chr10	150.7	NAU2869	3.41	-8.31
26	qVW-Chr10-2	July	XJJul16	Chr10	199.7	HAU1701	2.90	6.64
		August	XJAug16	Chr10	199.7	HAU1701	2.61	7.80
27	qVW-Chr10-3	August	XJAug16	Chr10	203.5	Gh058	2.53	8.37
28	qVW-Chr11-1	July	XJJul15	Chr11	193.6	DPL0103	2.72	6.01
29	qVW-Chr11-2	July	AYJul16	Chr11	253.0	DPL0209	3.97	-7.00
		July	XJJul16	Chr11	253.0	DPL0209	5.40	-7.65
		August	XJAug16	Chr11	253.0	DPL0209	6.06	-10.48
30	qVW-Chr12-1	August	AYAug15	Chr12	101.5	HAU0734	2.80	8.38
31	qVW-Chr14-1	August	AYAug16	Chr14	184.6	NAU5465	2.50	-10.47
32	qVW-Chr14-2	July	AYJul16	Chr14	203.0	HAU0883	3.32	-5.89
		August	XJAug16	Chr14	203.0	HAU0883	4.27	-8.15
33	qVW-Chr15-1	July	XJJul16	Chr15	16.3	CICR815	2.64	6.63
		August	XJAug16	Chr15	16.3	CICR815	2.56	8.43
34	qVW-Chr15-2	August	XJAug16	Chr15	88.2	NAU2985	2.77	-5.79
35	qVW-Chr17-1	July	XJJul16	Chr17	23.3	HAU2014	4.58	-5.46
		August	XJAug16	Chr17	23.3	HAU2014	5.25	-7.49
36	qVW-Chr17-2	July	XJJul16	Chr17	122.8	HAU0195	2.55	-3.87
		August	XJAug16	Chr17	122.8	HAU0195	2.50	-4.94
37	qVW-Chr19-1	July	AYJul16	Chr19	17.4	NAU3405	3.12	-5.01
		August	AYAug16	Chr19	17.4	NAU3405	4.05	-6.23
		August	XJAug16	Chr19	17.4	NAU3405	5.04	-7.75
		July	XJJul16	Chr19	17.4	NAU3405	5.42	-6.22
38	qVW-Chr19-2	July	AYJul15	Chr19	145.9	NAU5475	4.03	-6.38
		August	AYAug15	Chr19	145.9	NAU5475	2.54	-4.01
		July	AYJul16	Chr19	145.9	NAU5475	2.85	-4.57
		July	XJJul16	Chr19	145.9	NAU5475	6.45	-6.45
		August	XJAug16	Chr19	145.9	NAU5475	5.58	-7.76
39	qVW-Chr19-3	August	XJAug16	Chr19	185.6	HAU1385b	2.61	-5.77
40	qVW-Chr19-4	July	XJJul15	Chr19	197.0	NAU2274	2.72	6.01
41	qVW-Chr19-5	July	AYJul15	Chr19	221.3	NAU3652	3.83	19.47

42	qVW-Chr19-6	August	XJAug16	Chr19	257.1	HAU1785	3.47	-8.39
		August	AYAug15	Chr19	257.1	HAU1785	2.51	-5.42
		July	XJJul16	Chr19	257.1	HAU1785	3.01	-6.07
43	qVW-Chr19-7	August	XJAug16	Chr19	259.7	CGR5126	2.56	-7.22
44	qVW-Chr20-1	July	AYJul15	Chr20	175.5	NAU3665	3.64	-6.31
		August	AYAug15	Chr20	175.5	NAU3665	2.73	-4.07
		July	AYJul16	Chr20	175.5	NAU3665	2.80	-4.56
		August	AYAug16	Chr20	175.5	NAU3665	3.41	-5.61
		July	XJJul16	Chr20	175.5	NAU3665	6.52	-6.54
		August	XJAug16	Chr20	175.5	NAU3665	5.73	-7.87
45	qVW-Chr21-1	July	XJJul15	Chr21	147.9	NAU5217	2.72	6.01
46	qVW-Chr21-2	August	AYAug15	Chr21	278.3	HAU1283	2.54	-10.28
47	qVW-Chr22-1	July	AYJul15	Chr22	21.8	NAU2026	2.89	-4.75
		August	XJAug16	Chr22	21.8	NAU2026	5.51	-6.28
		July	XJJul16	Chr22	21.8	NAU2026	4.84	-4.58
48	qVW-Chr22-2	July	XJJul16	Chr22	26.2	Gh200	2.90	12.75
		August	XJAug16	Chr22	26.2	Gh200	2.97	16.66
49	qVW-Chr22-3	July	XJJul15	Chr22	149.7	CER0139b	2.52	3.91
50	qVW-Chr23-1	July	AYJul16	Chr23	86.9	Gh499	2.92	-8.40
51	qVW-Chr23-2	July	AYJul15	Chr23	208.1	NAU5189	3.32	-7.98
		July	XJJul16	Chr23	208.1	NAU5189	4.35	-7.26
		August	XJAug16	Chr23	208.1	NAU5189	4.98	-9.92
52	qVW-Chr24-1	August	AYAug15	Chr24	0.0	DPL0031	2.90	-13.23
53	qVW-Chr26-1	August	AYAug15	Chr26	8.2	NAU4925	4.03	7.97
		July	XJJul15	Chr26	8.2	NAU4925	3.09	2.27

5 populations. Stable QTL are in bold

PV (%)	Status
3.80	New
4.48	New
5.60	Confirmed
7.59	Confirmed
9.33	New
3.89	New
4.26	Confirmed
6.38	Confirmed
7.71	Confirmed
7.67	Confirmed
9.13	Confirmed
7.88	Confirmed
11.89	Confirmed
5.31	Confirmed
5.42	Confirmed
4.56	Confirmed
9.77	Confirmed
6.97	Confirmed
4.89	New
5.46	New
7.71	New
4.56	New
10.03	New
6.61	New
4.76	Confirmed
4.47	Confirmed
7.62	Confirmed
4.86	Confirmed
5.84	New
9.22	New
5.23	New
4.15	New
5.18	New
4.25	New
10.17	New
6.44	New
6.84	Confirmed
10.22	Confirmed
7.18	New
4.85	New
5.74	New
5.00	Confirmed
4.69	New
5.62	New
5.98	New

5.59	New
7.50	New
4.17	New
3.98	New
5.52	New
5.28	New
5.50	New
6.67	New
4.05	Confirmed
5.97	Confirmed
5.18	Confirmed
4.09	Confirmed
5.28	New
5.12	New
5.00	New
4.09	New
5.17	New
4.36	New
3.94	New
3.79	New
4.08	New
6.06	New
8.01	New
9.04	New
4.19	Confirmed
3.76	New
5.42	New
6.91	New
3.96	New
3.85	New
4.15	New
6.78	New
7.66	New
3.87	New
3.79	New
4.66	New
6.00	New
7.43	New
7.96	New
5.98	New
3.82	New
4.27	New
9.40	New
8.18	New
3.91	New
4.08	New
6.80	New

5.20	New
3.79	New
4.53	New
3.84	New
6.74	New
4.53	New
4.89	New
6.15	New
11.14	New
9.69	New
4.08	New
3.81	New
4.39	New
7.06	New
6.26	New
3.74	New
3.83	New
3.78	New
4.42	New
5.32	Confirmed
6.77	Confirmed
7.59	Confirmed
4.31	New
6.08	New
4.61	New

Table 2. Details of primers used in this study

SL. No.	Primer Name	No. of polym.	Discovered by/Source
1	BNL	43	Brookhaven National Laboratory, NY
2	C2	1	Monsanto Company, USA
3	CER	5	Monsanto Company, USA
4	CGR	48	Monsanto Company, USA
5	CICR	13	ICR, CAAS, Anyang, China
6	CIR	9	CIRAD, France
7	CM	1	Texas A & M University, USA
8	COT	4	Texas A & M University, USA
9	DC	10	Monsanto Company, USA
10	DPL	77	Delta and Pine Land, USA
11	Gh	25	Texas A & M University, USA
12	HAU	100	Huazhong Agricultural University, CHN
13	JESPR	7	Texas A & M University, USA
14	MGHES	2	USDA-ARS, Texas
15	MUCS	7	University of California Davis, USA
16	MUSB	7	University of California Davis, USA
17	MUSS	13	University of California Davis, USA
18	NAU	173	Nanjing Agricultural University, CHN
19	PGML	15	Plant Genome Mapping Lab
20	SHIN	5	Monsanto Company, USA
21	STV	4	Stoneville, USA
22	TMB	23	USDA-ARS, Texas
23	SWU	5	South West University, CHN
Total		597	

Manufacturer
Invitrogen Co. Ltd. Shanghai
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Beijing Genomics Inst.