bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

Md Harun or Rashid[1,2†], Peng-tao Li[3†], Tingting Chen[1†], Koffi Kibalou Palanga[1†], Wan-kui Gong[1], Qun Ge[1], Ju-wu Gong[1], Ai-ying Liu[1], Quan-wei Lu[3], Latyr Diouf[1], Zareen Sarfraz[1], Muhammad Jamshed[1], Yu-zhen Shi[1]* and You-lu Yuan[1]*

1. State Key Laboratory of Cotton Biology, Key Laboratory of Biological and Genetic Breeding of Cotton, The Ministry of Agriculture, Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang 455000, Henan, China

2. Senior Scientific Officer, Breeding Division, Bangladesh Jute Research Institute, Dhaka-1207, Bangladesh

3. School of Biotechnology and Food Engineering, Anyang Institute of Technology, Anyang 455000, Henan, China

Md Harun or Rashid: harunbjri@yahoo.com

Peng-tao Li: lipengtao1056@126.com

Tingting Chen: chentingting7039@126.com

Koffi Kibalou Palanga: palangaeddieh@yahoo.fr

Wan-kui Gong: wkgong@aliyun.com

Qun Ge: gequn@caas.cn

Ju-wu Gong: gongjuwu@caas.cn

Ai-ying Liu: liuaiying@caas.cn

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Quan-wei Lu: 13707667581@163.com

Latyr Diouf: latyr@cricaas.com.cn

Zareen Sarfraz: zareensarfraz88@ciit.net.pk

Muhammad Jamshed: jamshed_muhammad@yahoo.com

Yu-zhen Shi: shiyuzhen@caas.cn

You-lu Yuan: yuanyoulu@caas.cn

†: These authors made equal contributions

*: Corresponding authors:

Youlu Yuan (yuanyoulu@caas.cn);

Yuzhen Shi (shiyuzhen@caas.cn);

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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 $BC_5F_{3:5}$ 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.

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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× = 26) and 7 allotetraploid ones (2n = 2× = 52) [4], of which the emergence of the latte dated from a polyploidization event between A and D genome1-2 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 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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

93 *Verticillium dahliae* Kleb has been the most significant disease in cotton production
94 due to causing substantial yield loss and serious fiber quality reduction [10 - 12]. As a
95 result of cotton VW infestation, fiber loss is estimated to approximately stand at 80%
96 [13]. What is worse, this disease can attack more than 400 plant species and exist in
97 soil for a long period in dormant form in the vascular system of perennial plants. Thus,
98 it is completely impossible to control VW disease through conventional method [14].
99 The general symptoms of the disease are vascular browning, stunting, leaf epinasty
100 and chlorosis, curling or necrosis, wilt and finally death of the entire plant [15, 16].
101 Despite multiple methods put forward to control VW, it remains one of the most
102 efficient and economical measures to develop elite cotton cultivars harboring genetic
103 factors tolerant or completely resistant against pathogen in cotton breeding [17-19].
104 There are only four subsistent cultivars of *Gossypium* species, while the tetraploid
105 cultivars cover more than 95% of planting areas around the world, namely as *G.*
106 *barbadense* (Sea Island cotton) and *G. hirsutum* (Upland cotton), which present
107 resistant and susceptible to VW disease, respectively [20, 21]. Hybrid breeding via
108 conventional techniques has been utilized earlier to improve VW resistance in upland
109 cottons, while some hindrances like infertility and hybrid break down/low parent
110 heterosis hindered the way of conducting resistant gene introgression from *G.*
111 *barbadense* into *G. hirsutum* [21]. Therefore, it has become a challenging task for
112 cotton breeders to achieve synchronous improvement in cultivating novel varieties
113 simultaneously harboring high yield, superior fiber quality, and high disease
114 resistance. QTL Mapping approaches make it possible for the discovery of

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

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

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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).

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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.

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

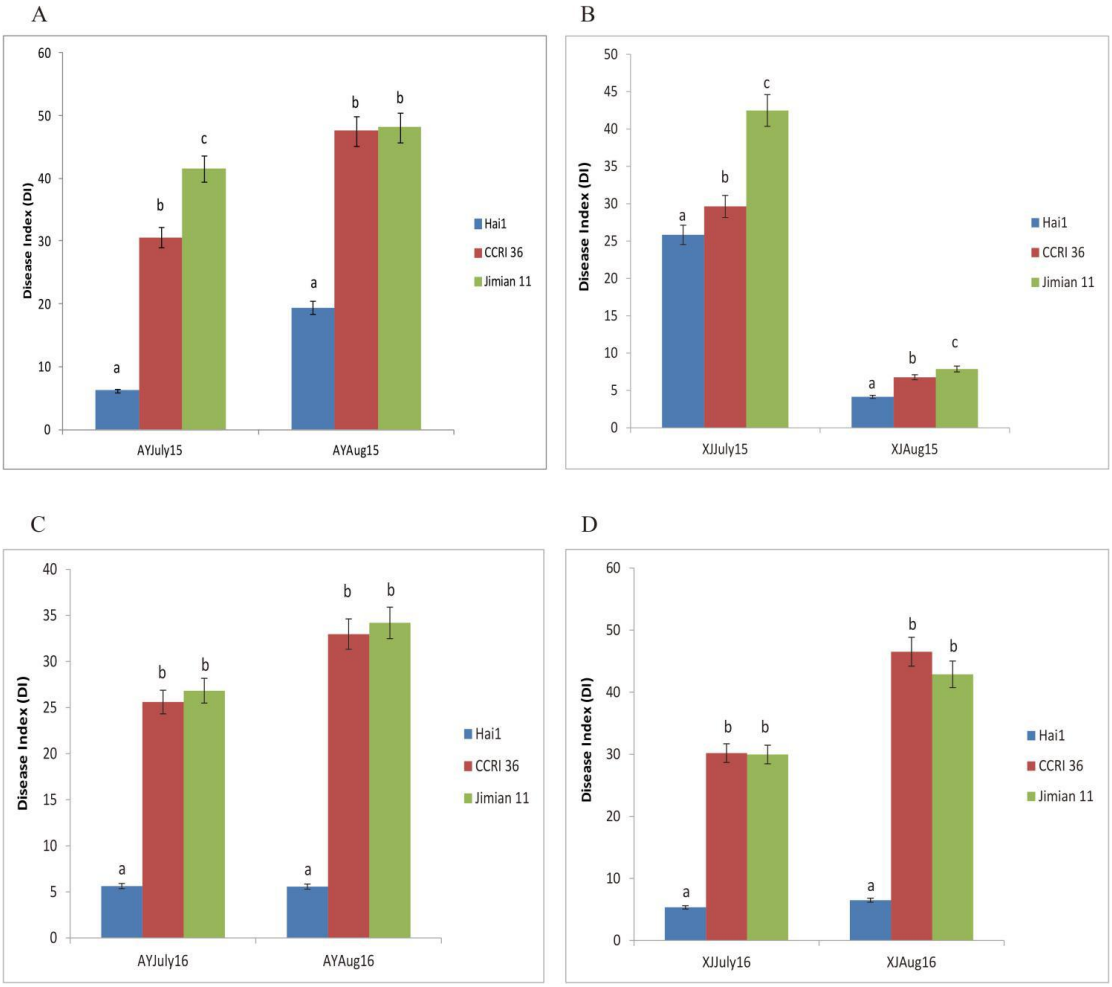


Figure 1.*Verticillium* wilt disease index of parent CCRI36, resistant control Hai1 and susceptible control Jimian11;

(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).

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

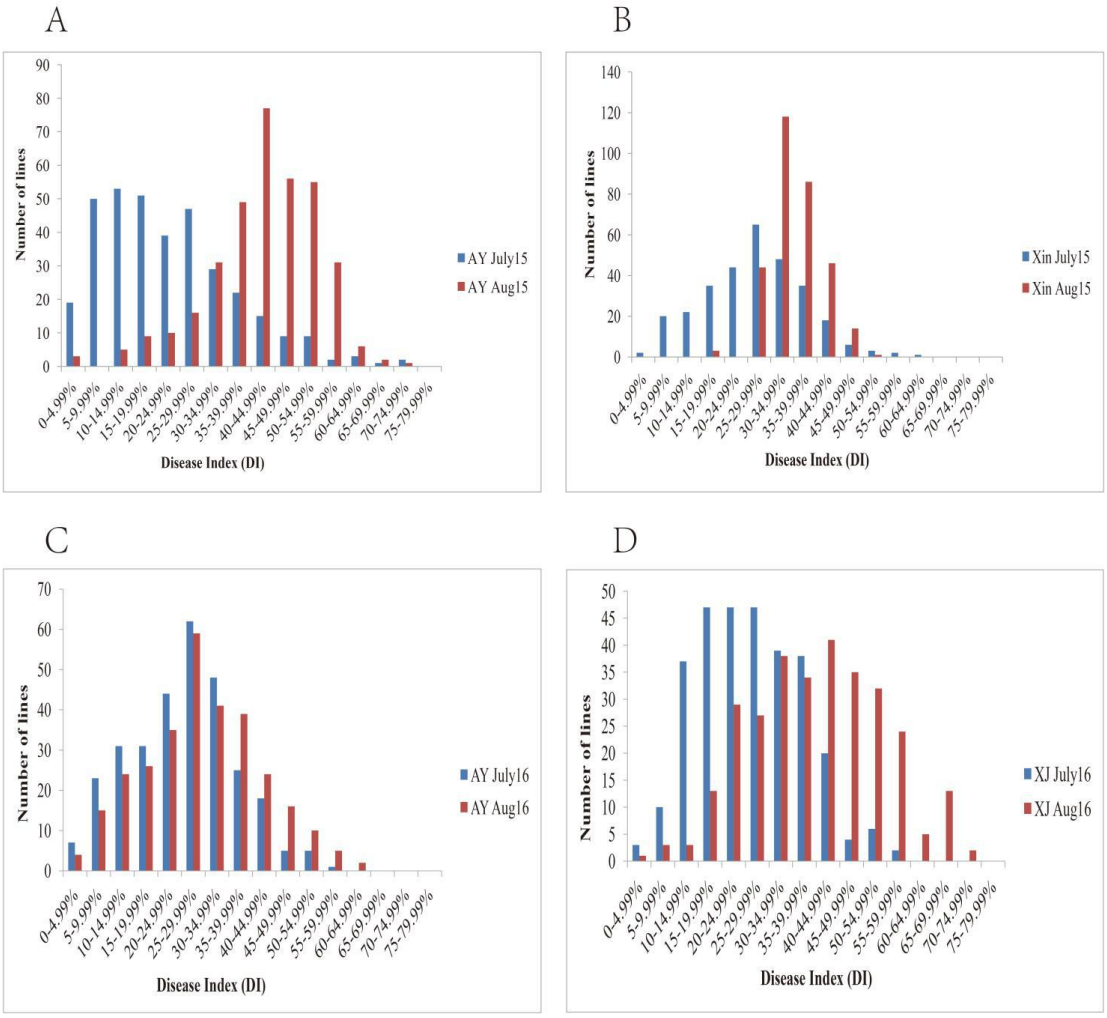


Figure 2. Normal distribution of DI phenotype in CSSLs; (A) Anyang 2015; (B) Xinjiang 2015; (C) Anyang 2016; D Xinjiang 2016.

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			

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Table 2. Descriptive statistics of VW resistance with broad sense Heritability (H^2) measured in $BC_5F_{3:5}$ population

Traits	Env	CSSL population							Parents		Mid parent	Jimian11 (Control)	H^2 (%)
		Mean	Max	Mini	SD	Skew	Kurt	Var	CCRI36	Hai1			
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 $BC_5F_{3:5}$ 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**

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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 Hai1 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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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 Hai1 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).

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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 $BC_5F_{3:5}$ populations

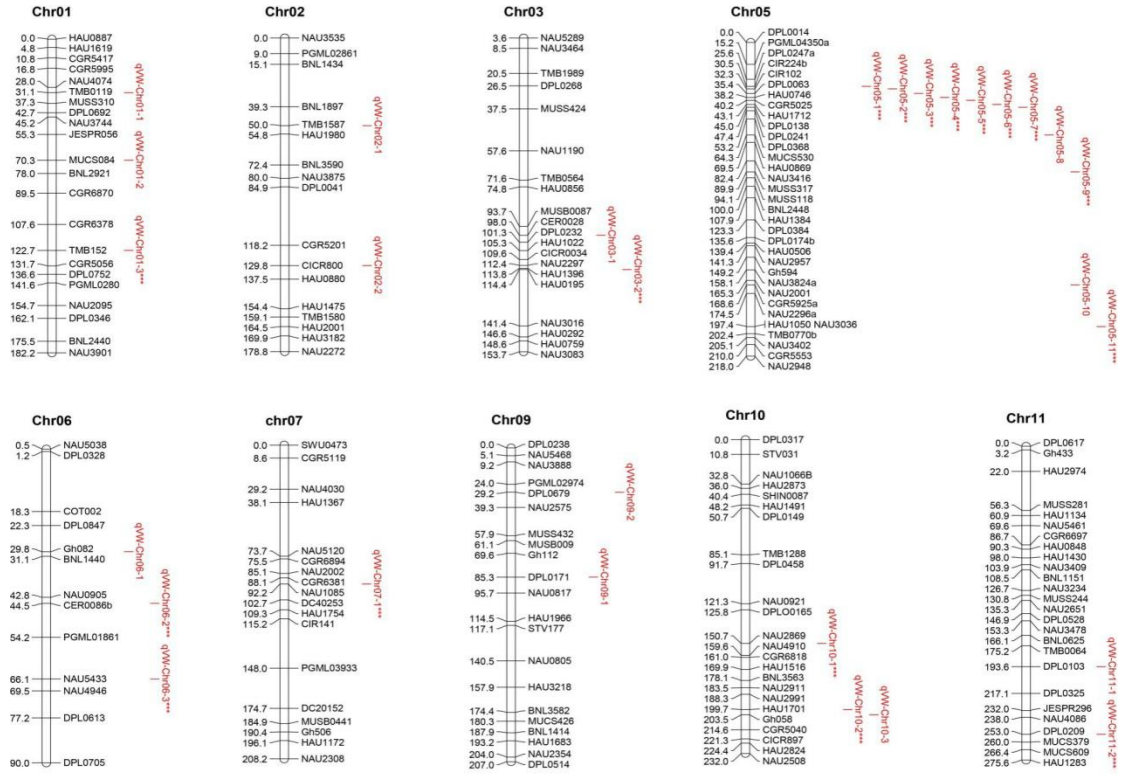
SL. No.	QTLs	Growth stage	Env	Chr	Location (cM)	Nearest marker	LOD	Add	PV (%)
1	qVW-Chr01-3	July	XJJul16	Chr01	122.7	TMB152	3.15	-3.37	5.60
		August	XJAug16	Chr01	122.7	TMB152	4.27	-5.05	7.59
2	qVW-Chr03-2	July	XJJul16	Chr03	114.4	HAU0195	3.95	-5.99	6.38
		August	XJAug16	Chr03	114.4	HAU0195	4.88	-8.50	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
5	qVW-Chr05-3	July	AYJul15	Chr05	35.4	DPL0063	3.32	-5.08	4.89
		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
6	qVW-Chr05-4	July	AYJul15	Chr05	38.2	HAU0746	3.19	-5.69	4.76
		July	AYJul16	Chr05	38.2	HAU0746	2.99	-4.68	4.47
		July	XJJul16	Chr05	38.2	HAU0746	5.18	-5.80	7.62
		August	XJAug16	Chr05	38.2	HAU0746	3.26	-5.98	4.86
7	qVW-Chr05-5	July	XJJul16	Chr05	40.2	CGR5025	3.92	-4.74	5.84
		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
9	qVW-Chr05-7	July	XJJul16	Chr05	45.0	DPL0138	2.82	-3.90	6.84
		August	XJAug16	Chr05	45.0	DPL0138	3.59	-4.48	10.22
10	qVW-Chr05-9	July	XJJul16	Chr05	89.9	MUSS317	3.23	-6.92	4.85
		August	XJAug16	Chr05	89.9	MUSS317	3.84	-9.71	5.74
11	qVW-Chr05-11	August	AYAug15	Chr05	197.4	HAU1050	3.11	-2.64	4.69
		July	XJJul16	Chr05	197.4	HAU1050	3.80	-2.96	5.62
		August	XJAug16	Chr05	197.4	HAU1050	4.03	-3.94	5.98
12	qVW-Chr06-2	July	AYJul16	Chr06	44.5	CER0086 b	5.11	-9.57	7.50
		August	AYAug16	Chr06	44.5	CER0086 b	2.78	-7.83	4.17

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

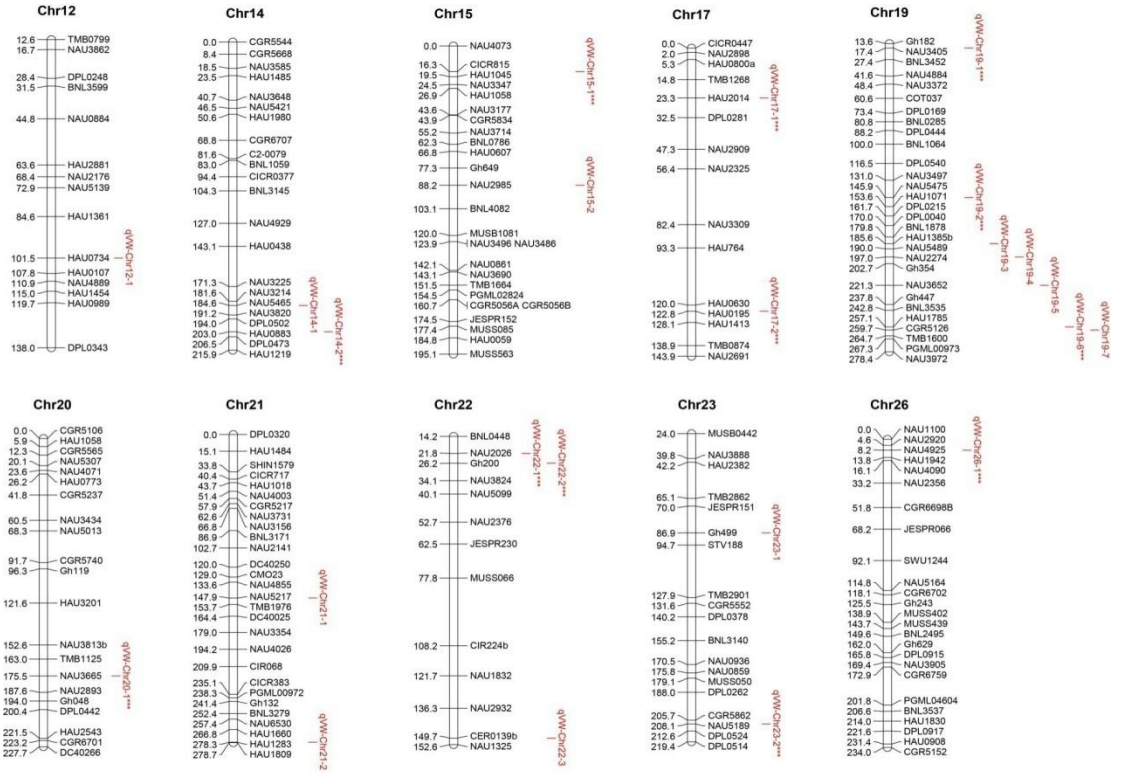
		July	XJJul16	Chr06	44.5	CER0086b	2.67	-6.62	3.98
		August	XJAug16	Chr06	44.5	CER0086b	3.74	-10.06	5.52
13	qVW-Chr06-3	July	AYJul16	Chr06	66.1	NAU5433	3.55	-6.89	5.28
		July	XJJul16	Chr06	66.1	NAU5433	3.69	-6.68	5.50
		August	XJAug16	Chr06	66.1	NAU5433	4.53	-9.50	6.67
14	qVW-Chr07-1	August	AYAug16	Chr07	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	-10.48	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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

		July	XJJul16	Chr23	208.1	NAU5189	4.35	-7.26	6.77
		August	XJAug16	Chr23	208.1	NAU5189	4.98	-9.92	7.59
29	qVW-Chr26-1	August	AYAug15	Chr26	8.2	NAU4925	4.03	7.97	6.08
		July	XJJul15	Chr26	8.2	NAU4925	3.09	2.27	4.61



321



322

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Figure 3. Identification of QTLs for VW disease index and linkage map in $BC_5F_{3:5}$ 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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

346 QTLs were identified and projected on consensus Chr19 to perform meta-analysis,
347 identifying 3 QTL hotspot regions. Chr19-DI-Hotspot-1, Chr19-DI-Hotspot-2 and
348 Chr19-DI-Hotspot-3 contained 5, 4 and 7 QTLs, respectively. Twelve QTLs were
349 selected to project on chromosome 22 (Cons.Chr22), and 2 QTL hotspot regions were
350 identified, of which Chr22-DI-Hotspot-1 had 5 QTLs, while Chr22-DI-Hotspot-2 had
351 7 QTLs (Figure 4). Fifty six QTLs were selected to project on Cons.Chr23,
352 identifying 2 QTL hotspot regions. Chr23-DI-Hotspot-1 and Chr23-DI-Hotspot-2
353 contained 30 and 26 QTLs, respectively. Sixteen QTLs were selected to project on
354 Cons.Chr26, and 3 QTL hotspot regions were identified. Chr26-DI-Hotspot-1,
355 Chr26-DI-Hotspot-2 and Chr26-DI-Hotspot-3 contained 6, 5 and 5 QTLs,
356 respectively. The details of all QTLs are described in Table 5.

357 As for the hotspots on Cons.Chr05, Chr05-DI-Hotspot-1 from the 25–36 cM
358 region was located between markers Gh243 and HAU3395, and Chr05-DI-Hotspot-2
359 from 31 to 42 cM region and Chr05-DI-Hotspot-3 from 39 to 54 cM region were
360 separately located between markers NAU3204 and CIR301 and between markers
361 TMB0131b and NAU2948. There were two hotspots on Cons.Chr09, and
362 Chr09-DI-Hotspot-1from the 34–60 cM region and Chr09-DI-Hotspot-2 from 87 to
363 93 cM region were located between markers CGR6170 and CGR6719 and between
364 markers BNL0597 and BNL4053, respectively. With regard to the hotspots on
365 Cons.Chr19, Chr19-DI-Hotspot-1 from the 2–27 cM region was located between
366 markers CIR415 and BNL3452, and Chr19-DI-Hotspot-2 from 32–55 cM region and
367 Chr19-DI-Hotspot-3 from 123 to 148 cM region were separately located between

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

markers NAU2894 and COT037 and between markers DPL0216 and Gh354. Moreover, three hotspots were identified on Cons.Chr26, of which Chr26-DI-Hotspot-1 from 4 to 29 cM region was located between markers HAU1845 and DPL0888, while Chr26-DI-Hotspot-2 from 33 to 54 cM region and Chr26-DI-Hotspot-3 from 85 to 102 cM region were lacated between markers NAU2356 and CIR167 and between markers C2-0528 and DPL1283, respectively.

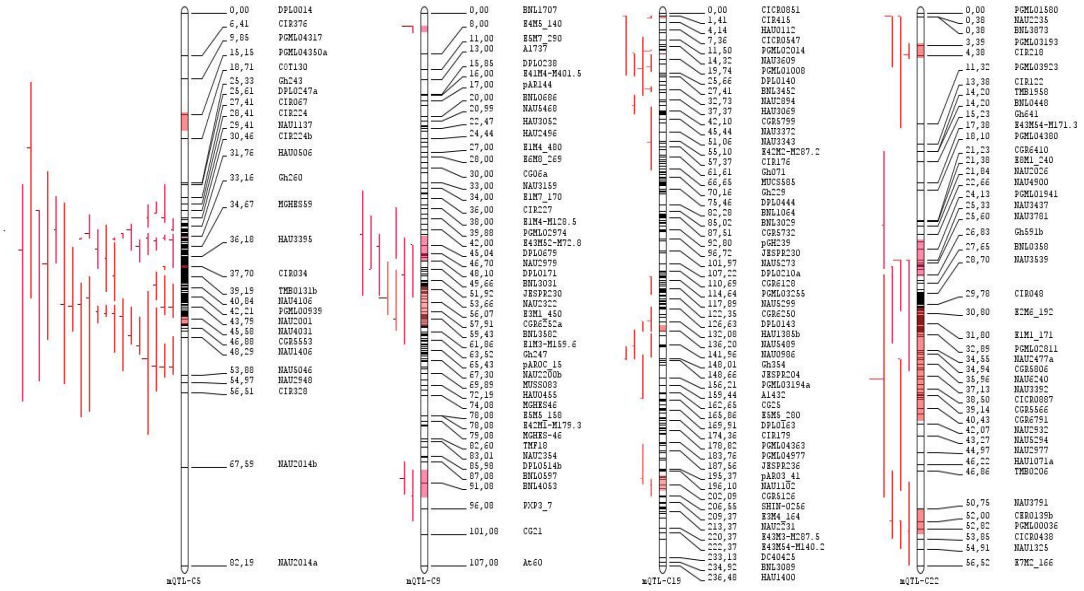


Figure 4. QTL hotspots and QTLs for VW resistance on the consensus map by a meta-analysis. Consensus Chromosome 05 (Cons.Chr05) has three hotspots, Cons.Chr09 has 2, Cons.Chr19 has three and Cons.Chr22 has 2 hotspots.

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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	

388

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

3. Discussion

3.1 Field status and phenotypic assessment

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

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

411 the CSSL population has been investigated on phenological basis over different
412 environments at various growth stages. In this study, we observed that DI values
413 susecptible to VW infection were higher in August than those in July, to be specific to
414 presenting that the susceptible control (Jimian11) showed above 35% DI values
415 except in XJJul15 and AYJul16, while the DI values of CCRI36 were lower than 35%
416 except in AYAug15 and XJAug16 (Table 2). This lesser DI percentage is the
417 evidence for the occurrence of high pressure projected by variable VW strains under
418 natural environmental conditions. Few more reasons behind this phenological
419 variation include intensity and virulence of strains, fungal amount in soil and
420 developmental stages as well as environmental influences [41]. The similar findings
421 have been reported earlier in which the host plant proved to be resistant against
422 inoculum of VW while remained susceptible under natural environmental conditions
423 [21]. We also have synchrony with previous findings with a display of lesser disease
424 index (DI<40%) by CCRI36 progenitor whereas some of the offspring depicted a
425 prominent resistance level comparable to susceptible control Jimian11. Besides this, a
426 noteworthy level of transgressive segregation has been witnessed under field
427 conditions, which are in accordance with previous reports [42, 43]. Across different
428 environment during whole investigation period, few CSSLs remained consistent in
429 resistance display to pressurizing mixture of strains present in the vicinity as
430 compared to most of the lines which displayed a high level of susceptibility (Figure 2).
431 This fact can be justified by the presence of wider range of environmental variation
432 occurrence during two experimental years of study, where the VW strains keep on

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

438 The phenological parameters measured in two years of study at both locations
439 depicted rare weak correlations. Expression of different genetic factors in variable
440 environments at different growth stages confirmed the reason behind weak correlation
441 coefficient values (Table 3). It realizes the fact regarding alteration of genes on
442 exposure to VW strains at varying growth stages. In a study on backcross inbreed
443 lines regarding VW resistance, there observed a weak but positive correlation among
444 disease index under field conditions [44].

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

452

453 **3.2 Genetic Map used for QTLs identification**

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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 $BC_5F_{3:5}$ 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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

498 to D sub-genome. Consistent discoveries have been made by Yang et al. [46], Ning et
499 al. [47] and Bolek et al. [42].
500
501 **3.4 Stability with earlier studies VW resistance QTLs**
502 In this study, 53 QTLs related to VW resistance were totally identified in 300 CSSLs.
503 Among all the QTLs, 35 ones (66%) had negative additive effects, which indicated
504 that the *G. barbadense* alleles increased *Verticillium* wilt resistance and decreased
505 disease index values by about 2.64 to 13.23. On the other hand, 18 QTLs (34%) had
506 positive additives effects, which indicated that the *G. hirsutum* alleles enhanced VW
507 wilt resistance and decremented phenotypic disease index values by about 2.27 to
508 19.47. As for different years, 31 QTLs were identified in the year of 2015, while 86
509 QTLs in the year of 2016, of which 11 QTLs were found in the both years. The
510 maximum number of QTLs (11) was detected on Chr05 (Figure 3, Table S2).
511 Among 53 QTLs, 29 QTLs were detected consistently in at least two
512 environments, which were deemed as stable QTLs. Out of 29 stable QTLs, 25 QTLs
513 (86%) had negative additive effects, which indicated that the *G. barbadense* alleles
514 incremented VW resistance and decreased DI. Based on Meta-analysis of the
515 identified 53 QTLs, 10 QTLs were consistent to previously identified QTLs, and they
516 had common SSR markers [19, 45-47, 52]. One QTL, *qVW-Chr01-3* positioned on
517 Chr01 for VW resistance was the similar as Ning's *qVW-A1-1* [47], which were
518 identified with common markers of Gh215. Another QTL, *qVW-Chr03-2* was the
519 similar as *qVW-C3-2* in the results of Shi et al. [22], and they were associated with the

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

541 According to previous reports on the CSSLs in cotton, the prominent characteristics
542 of high fiber quality and high yielding traits have deliberately been explained [53-58].
543 Nowadays in this whole experimental study, a total of 300 CSSLs from Upland cotton
544 CCRI36 and Sea Island cotton Hai1 have been keenly investigated regarding their
545 resistance to VW. The segments of chromosome introgressed from *G. barbadense*
546 into *G. hirsutum* made these lines little bit different from their recurrent parent by
547 reducing the influences of genetic background of recipient, which makes the CSSLs
548 as efficient breeding materials to conduct quantitative genetics researchs. Thus the
549 experimented work proves to be beneficial in paving the way towards whole genome
550 study of cotton by laying a solid platform stuffed with molecular findings related to
551 fine mapping, functional genomics, gene pyramiding and ultimately marker assisted
552 breeding.

553 **4. Materials and Methods**

554 **4.1 Plant materials and development of cotton CSSLs**

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

563 upland cotton has the obvious property of high yielding as well as early maturing in
564 growth patterns but susceptible to *Verticillium* wilt. The two cultivars Hail and
565 CCRI36 used as paternal and maternal parents were hybridized followed by backcross
566 in 2003 at Anyang to construct CSSLs. In 2009, a mapping population comprising
567 2660 plants of BC_5F_3 was obtained by using CCRI36 as recurrent parent. In 2010 and
568 2011, $BC_5F_{3:4}$ population was planted via plant-to row method at Anyang and
569 Xinjiang, respectively. In 2014, at Xinjiang province, $BC_5F_{3:5}$ population was grown
570 again. From these populations, a random selection process was conducted and 300
571 CSSLs were obtained for the evaluation of VW disease index. These selected lines
572 were then grown at Anyang and Xinjiang in 2015 and 2016, respectively. The details
573 of development of CSSLs was brought about by following the same procedure as
574 described earlier [60]. Stable performance regarding resistance to VW was displayed
575 by some lines in multiple environments over different years of study.
576

577 **4.2 Field investigations and experimental design**

578 Two field stations of ICR, CAAS in Anyang, Henan and Shihezi, Xinjiang were used
579 to grow the experimental material for two years. In 2015 and 2016, phenotypic data
580 were collected in months of July and August from Anyang and Xinjiang, respectively.
581 Under natural environmental conditions, there occurred intensive attack of *V. dahliae*
582 strains. Randomized complete block design (RCBD) under two replications was
583 established for study. By following the specifications prescribed for crop management
584 according to the locality, seeds were sown in single row plots. At research farm areas

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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.

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

603 **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

604

605 The disease Index (DI) was estimated following the formulae below [7, 61].

606

607 $$DI\,(\%) = \frac{\Sigma(d_c \times n_c)}{n_t \times 4} \times 100$$

608

609 Where, d_c is disease rate
610 between 0 and 4;
611 n_c is number of plants with interrelated disease rate;
612 n_t is total number of plants tested for each CSSL
613

614 **4.4 Analysis of phenotypic trait**

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

621

622 $$\text{Broad sense Heritability (\%)} = \left(\frac{\text{Var(G)}}{\text{Var(P)}}\right) \text{X } 100$$

623

624 Where, Var(G) =
625 Genotypic variance
626 Var(P)
627 = Phenotypic variance
628

629 **4.5 Genetic analysis**

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

630 Genomic DNA of CSSLs from $BC_5F_{3:5}$ population and its parents was extracted by
631 following a modified procedure of CTAB method [64] by using young leaves which
632 were sampled from each line and kept at -80°C. The working concentration of DNA
633 was adjusted at 30ng/µL; quantified on NanoDrop2000 spectrophotometer (NanoDrop
634 Technologies, Wilmington, DE USA). Further the integrity of DNA was patterned on
635 agarose gel (1%) using Lambda DNA/HindIII Markers[65] as ladder. Scoring pattern
636 followed for SSRs fragments include '–' for missing, '1' for presence and '0' for
637 absence of bands.
638
639 **4.6 SSR markers and SSR molecular detection**
640 Based on the genetic map [37], in total, 597 pairs markers were screened out by using
641 2292 pairs of markers to be used to screen 300 CSSLs DNA. The sequences of these
642 SSR primers were downloaded from the CMD database (http://www.cottongen.org/).
643 First of all, we diluted these primer pairs. For dilution, we centrifuge primer pairs at
644 12000rpm at 4^0C for 10 minutes to settle down the contents at the bottom. We diluted
645 these primer pairs 100X and shake it vigorously for 2 minutes. Centrifuge it again and
646 store at -20^0C. The details of these SSR primers are mentioned in Table S2.
647
648 **4.7 QTL mapping**
649 QTL IciMapping V4.0 software developed by Wang et al. [66] was used to map QTLs
650 of CSSLs. A LOD (likelihood of odds) of threshold 2.5 was used to declare
651 significant additive QTLs. The percentage of phenotypic variance (PV%) explained

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

655 Positive additive effects showed that CCRI36 alleles decremented the phenotypic
656 disease index values and enhanced resistance against VW. On the other hand,
657 negative scores indicated that Hai1alleles decremented the phenotypic disease index
658 values and incremented the values of VW resistance. The QTL nomenclature was
659 designed as follows: the QTL designations begin with "q" come after the trait
660 abbreviation, the chromosome name, and the number of QTL on that chromosome [68,
661 69]. Stable QTL was declared when it is found in at least two environments.

662

663 **4.8 Meta-analysis of QTLs**

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

677 Meta-analysis was performed by taking two files as input i.e. QTL file and map
678 file. Map file was based on the information regarding names of parents, cross type and
679 markers position on chromosomes. The QTL file was loaded with QTL in given
680 environment as row information and QTL name, trait name, trait ontology, location,
681 year, chromosome number, linkage group, LOD score, observed phenotypic variation
682 (R^2), most likely position of QTL, CI start position and CI end position. Initially, the
683 two files were uploaded successfully and map connectivity was investigated for
684 construction of consensus map. After that QTLs projection on consensus map was
685 done, followed by meta-analysis regarding trait. Ultimately four model were obtained
686 with different AIC (Akaike information criterion) value. The lowest AIC value
687 holding model was considered suitable for the identification of mQTL positon or QTL
688 hotspot. The criteria described by Said et al.[32]of occurrence of mQTLs in 20 cM
689 interval was kept standard for the identification of hotspot.
690

691 **5. Conclusions**

692 In this study, 300 CSSLs developed from *Gossypium hirsutum* CCRI36 × *Gossypium*
693 *barbadense* Hai1 were used to detect QTL for VW resistance in various environments
694 (Anyang and Xinjiang) and different developmental stages (July and August). The
695 nature of population (CSSL), population size and the presence of control (Jimian11)

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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

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

706

707

708 **Supplementary Materials**

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

710

711 **Ethics approval and consent to participate**

712 Not applicable

713

714 **Consent for publication**

715 Not applicable

716

717 **Competing interests**

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

718 The authors declare that they have no competing interests.

719

720 **Funding**

721 This study was supported by the National Natural Science Foundation of China
722 (31621005 and 31801404) and Joint Funds of the National Natural Science
723 Foundation (U1804103), the National Agricultural Science and Technology
724 Innovation project for CAAS (CAAS-ASTIP-2016-ICR), and the Central Level of the
725 Scientific Research Institutes for Basic R & D Special Fund Business (Y2017PT51).

726

727 **Author Contributions**

728 Y.Y.L., T.T.C., Y.Z.S conceived and designed the experiments; M.H.R., L.P.T.,
729 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.
730 performed the experiments; M.H.R. and L.P.T. analyzed the data; M.H.R. contributed
731 reagents/materials/analysis tools: Y.Y.L., Y.Z.S., M.H.R., and L.P.T. drafted the
732 manuscript.

733

734 **Abbreviations**

735

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

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

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 arboretum*&*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.

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

10. Cai, Y.; Xiaohong, H.; Mo, J.; Sun, Q.; Yang, J. and Liu, J. Molecular research and genetic engineering of resistance to *Verticillium wilt* in cotton: A review. *Afr. J. Biotechnol***2009**, *8*, 7363-7372.

11. Yu, Y.; Daojun, Y.; Shaoguang, L.; Ximei, L.; Xiaqing, W.; Zhongxu, L. et al. Genome structure of cotton revealed by a genome-wide SSR genetic map constructed from a BC_1 population between *gossypium hirsutum* and *G. barbadense*. *BMC Genomics* **2011**, *12*.

12. Xu, J.J.; Zhao, Q.; Du, P.N.; Xu, C.W.; Wang, B.H. and Feng, Q. Developing high throughput genotyped chromosome segment substitution lines based on population whole-genome re-sequencing in rice (*Oryza sativa* L.). *BMC Genomics***2010**, *24*, 656. doi: 10.1186/1471-2164-11-656.

13. Wei, F.; Fan, R.; Dong, H.-T.; Shang, W.-J.; Xu, X.-M.; Zhu, H.-Q. et al. Threshold microsclerotial inoculum for cotton *Verticillium* wilt determined through wet-sieving and real-time quantitative PCR. *Phytopathology* **2015**, *105*, 220-229.

14. Zhang, T.; Yun, J.Y.; Zhao, J.H.; Gao, F.; Zhou, B.J.; Fang, Y.Y. et al. Host-Induced Gene Silencing of the Target Gene in FungalCells ConfersEffectiveResistance to the Cotton Wilt Disease Pathogen *Verticillium dahliae*. *Molecular Plant***2016**, *9*, 939–942, doi: org/10.1016/j.molp.2016.02.008.

15. Bell, A.A. and Hillocks, R.J. Verticillium wilt. *Wallingford: CAB International***1992**, 87-126.

16. Li, F.; Shen, H.; Wang, M.; Fan, K.; Bibi, N.; Ni, M. et al. A synthetic antimicrobial peptide BTDS expressed in *Arabidopsis thaliana* confers enhanced resistance to *Verticillium dahliae*. *Mol Genet Genomics***2016**, *291*, 1647-1661, doi: 10.1007/s00438-016-1209-9.

17. Zhang, B.H.; Liu, F.; Yao, C.B. and Wang, K.B. Recent progress in cotton biotechnology and genetic engineering in China. *Curr. Sci.***2000**, *79*, 37-44.

18. Mert, M.; Kurt, S.; Gencer, O.; Akiscan, Y.; Boyaci, K. and Tok, F.M. Inheritance of resistance to Verticillium wilt (*Verticillium dahliae)* in cotton (*Gossypium hirsutum* L.). *Plant Breed***2005**, *124*, 102–104, doi: 10.1111/j.1439-0523.2004.01040.x.

19. Wang, P.; Ning, Z.; Lin, L.; Chen, H.; Mei, H. and Zhao, J., et al. Genetic dissection of tetraploid cotton resistant to *Verticillium* wilt using interspecific chromosome segment introgression lines. *Crop J.***2014**, *2*, 278 – 288, doi:10.1016/j.cj.2014.06.007.

20. Wilhelm, S.; Sagen, J.E. and Tietz, H. Resistance to*Verticillium*wilt in cotton: sources, techniques of identification, inheritance trends, and resistance potential of multiline cultivars. *Phytopathology***1974**, *64*, 924–31.

21. Fang, H.; Zhou, H.P.; Sanogo, S.; Flynn, R.; Percy, R.G.; Hughs, S.E. et al. Quantitative trait locus mapping for Verticillium wilt resistance in a backcross inbred line population of cotton (*Gossypium hirsutum X Gossypium barbadense)* based on RGA-AFLP analysis. *Euphytica***2013**, *194*, 79-91, doi: 10.1007/s10681-013-0965-4.

22. Shi, Y.; Zhang, B.; Liu, A.; Li, W.; Li, J. and Lu, Q., et al. Quantitative trait loci analysis of Verticillium wilt resistance in interspecific backcross populations of *Gossypium hirsutum* × *Gossypium barbadense*. *BMC Genomics***2016**, *17*, 877, doi: 10.1186/s12864-016-3128-x.

23. Zhu, W.Y.; Lin, J.; Yang, D.W.; Zhao, L.; Zhang, Y.D.; Zhu, Z. et al. Development of chromosome segment substitution lines derived from backcross between two sequenced

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

rice cultivars, Indica recipient 93–11 and *Japonica* donor Nipponbare. *Pant Mol Biol Rep***2009**, *27*, 126-131, doi:10.1007/s11105-008-0054-3.

24. Zhao, L.N.; Zhou, H.J.; Lu, L.X.; Liu, L.; Li, X.H.; Lin, Y.J. et al. Identification of quantitative trait loci controlling rice mature seed cultivability chromosomal segment substitution lines. *Plant Cell Rep***2009**, *28*, 247–256. doi:10.1007/s00299-008-0641-7.

25. Ali, M.L.; Paul, L.; Yu, S.B.; Lorieux, M. and Eizenga, G.C. Chromosome Segment Substitution Lines: A Powerful Tool for the Introgression of Valuable Genes from Oryza Wild Species into Cultivated Rice (*O. sativa)*. *Rice***2010**, *3*, 218–234, doi: 10.1007/s12284-010-9058-3.

26. Takershi, E.; Yoshinobu, T.; Yasunori, N.; Toshio Y.; Kasumi, T. and Masahiro, Y. Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of indica rice cultivar 'Kasalath' in a genetic background of japonica elite cultivar 'Kooshihikari'. *Breed Sci***2005**, *55*, 65–73.

27. Chen, H.; Qian, N.; Guo, W.; Song, Q.; Li, B.; Deng, F. et al. Using three overlapped RILs to dissect genetically clustered QTL for Wber strength on Chro.D8 in Upland cotton. *Theor Appl Genet***2009**, *119*, 605–612, doi: 10.1007/s00122-009-1070-x.

28. Ye, G.Y.; Liang, S.S. and Wan, J.M. QTL mapping of protein content in rice using single chromosome segment substitution lines. *Theor Appl Genet***2010**, *121*, 741–750, doi: 10.1007/s00122-010-1345.

29. Wang, P.; Ding, Y.Z.; Lu, Q.X.; Guo, W.Z. and Zhang, T.Z. Development of *Gossypium barbadense* chromosome segment substitution lines in the genetic standard line TM-1 of *Gossypium hirsutum*. *Chinese Sci. Bullet.***2008**, *53*, 1512-1517, doi: 10.1007/s11434-008-0220-x.

30. Lacape, J.M.; Llewellyn, D.; Jacobs, J.; Arioli, T.; Becker, D. and Calhoun, S. Meta-analysis of cotton fiber quality QTLs across diverse environments in a *Gossypium hirsutum x G. barbadense* RIL population. *BMC Plant Biol***2010**, *10*, 132.

31. Yu, J.Z.; Ulloa, M.; Hoffman, S.M.; Kohel, R.J.; Pepper, A.E.; Fang, D.D. et al. Mapping genomic loci for cotton plant architecture, yield components, and fiber properties in an interspecific (*Gossypium hirsutum* L. × *G. barbadense* L.) RIL population. *Mol Genet Genomics***2014**, *289*, 1347–1367, doi: 10.1007/s00438-014-0930-5.

32. Said, J.I.; Song, M.; Wang, H.; Lin, Z.; Zhang, X. and Fang, D.D. A comparative metaanalysis of QTL between intraspecific *Gossypium hirsutum* and interspecific *G. hirsutum* × *G. barbadense* populations. *Mol Gen Genomics***2014**, *290*, 1003–25, doi: 10.1007/s00438-014-0963-9.

33. Said, J.I.; Knapka, J.A.; Song, M. and Zhang, J. Cotton QTLdb: a cotton QTL database for QTL analysis, visualization, and comparison between *Gossypium hirsutum* and *G. hirsutum*×*G. barbadense* populations. *Mol Gen Genomics***2015**, *290*, 1615-25, doi: 10.1007/s00438-015-1021-y.

34. Wu, M.; Zhang, L.; Li, X.; Xie, X.; Pei, W.; Yu, J. et al. A comparative transcriptome analysis of two sets of backcross inbred lines differing in lint yield derived from a *Gossypium hirsutum* × *Gossypium barbadense* population. *Mol Genet Genomics***2016**, *291*, 1749–1767, doi: 10.1007/s00438-016-1216-x.

35. Zheng, J.Y.; Oluoch, G.; Riaz Khan, M.K.; Wang, X.X.; Cai, X.Y. and Zhou, Z.L., et al. Mapping QTLs for drought tolerance in an F2:3 population from an inter-specific cross

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

between *Gossypium tomentosum* and *Gossypium hirsutum*. *Genet. Mol. Res.***2016**, *15*, gmr.15038477.

36. Zhai, H.; Gong, W.; Tan, Y.; Liu, A.; Song, W. and Li, J., et al. Identification of Chromosome Segment Substitution Lines of *Gossypium barbadense* Introgressed in *G. hirsutum* and Quantitative Trait Locus Mapping for Fiber Quality and Yield Traits. *PLoS One***2016**, *11*, e0159101. doi:10.1371/journal. pone.0159101.
37. Shi, Y.; Li, W.; Li, A.; Ge, R.; Zhang, B. and Li, J., et al. Constructing a high-density linkage map for *Gossypium hirsutum* X*Gossypium barbadense* and identifying QTLs for lint percentage. *J Integr Plant Biol***2015**, *57*, 450-67, doi: 10.1111/jipb.12288.
38. Wang, Q.; Fang, L.; Chen, J.; Hu, Y.; Si, Z.; Wang, S. et al. Genome-Wide Mining, Characterization, and Development of Microsatellite Markers in *Gossypium* Species. *Scientific Reports***2015**, *5*, 10638, doi: 10.1038/srep10638.
39. Said, J.I.; Zhongxu Lin, Z., X., ; Zhang, X.L.; Song, M.Z. and Zhang, J.F. A comprehensive meta QTL analysis for fiber quality, yield, yield related and morphological traits, drought tolerance, and disease resistance in tetraploid cotton. *BMC Genomics* **2013**, *14*, 776, doi:10.1186/1471-2164-14-776.
40. Devey, M.E. and Roose, M.L. Genetic analysis of *Verticillium* wilt tolerance in cotton using pedigree data from three crosses. *Theor Appl Genet***1987**, *74*, 162-167.
41. Bejarano, A.Z.; Blanco-Lo´ Pez, M.A.; Melero-Vara, J.M. and Jime´ Nez-Di´Az, R.M. The influence of *verticillium* wilt epidemics on cotton yield in southern Spain. *Plant Pathol***1997**, *46*, 168–178, doi: 10.1046/j.1365-3059.1997.d01-221.x.
42. Bolek, Y.; El-Zik, K.M.; Pepper, A.E.; Bell, A.A.; Magill, C.W.; Thaxton, P.M. et al. Mapping of *verticillium wilt* resistance genes in cotton. *Plant Sci***2005**, *168* 1581–1590, doi: 10.1016/j.plantsci.2005.02.008.
43. Wang, H.M.; Lin, Z.X.; Zhang, X.L.; Chen, W.; Guo, X.P.; Nie, Y.C. et al. Mapping and Quantitative Trait Loci Analysis of *Verticillium* Wilt Resistance Genes in Cotton. *J. Integr. Plant Biol.***2008**, *50*, 174–182, doi: 10.1111/j.1744-7909.2007.00612.x.
44. Zhang, J.F.; Sanogo, S.; Flynn, R.; Baral, J.B.; Bajaj, S.; Hughs, S.E. et al. Germplasm evaluation and transfer of *Verticillium*wilt resistance from Pima (*Gossypium barbadense*) to upland cotton (*G. hirsutum*). *Euphytica***2012**, *187*, 147–60, doi:10.1007/s10681-011-0549-0.
45. Zhang, X.; Yuan, Y.; Wei, Z.; Guo, X. and Guo, Y. Molecular Mapping and Validation of a Major QTL Conferring Resistance to a Defoliating Isolate of *Verticillium* Wilt in Cotton (*Gossypium hirsutum* L.). *PLoS ONE***2014**, *9*, e96226, doi: 10.1371/journal.pone.0096226.
46. Yang, C.; Guo, W.Z.; Li, G.Y.; Gao, F.; Lin, S.S. and Zhang, T.Z. QTLs mapping for *Verticillium* wilt resistance at seedling and maturity stages in *Gossypium barbadense* L. *Plant Science* **2008**, *174* 290–298.
47. Ning, Z.Y.; Zhao, R.; Chen, H.; Ai, N.J.; Zhang, X.; Zhao, J. et al. Molecular Tagging of a Major Quantitative Trait Locus for Broad-Spectrum Resistance to Verticillium Wilt in Upland Cotton Cultivar Prema. *Crop Science***2013**, *53*, 2304-2312, 10.2135/cropsci2012.12.0694.
48. Fang, H.; Zhou, H.P.; Sanog, S.; Lipka, A.E.; Fang, D.D.; Percy, R.G. et al. Quantitative trait locus analysis of *Verticillium* wilt resistance in an introgressed recombinant inbred

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

population of Upland cotton. *Mol Breeding***2014**, *33*, 709–720, doi: 10.1007/s11032-013-9987-9.

49. Wang, Y.; Ning, Z.; Hu, Y.; Chen, J.; Zhao, R. and Chen, H., et al. Molecular Mapping of Restriction-Site Associated DNA Markers in Allotetraploid Upland Cotton. *PLoS ONE***2015**, *10(4): e0124781*.

50. Jiang, F.; Zhao, J.; Zhou, L.; Guo, W.Z. and Zhang, T.Z. Molecular mapping of *Verticillium* wilt resistance QTL clustered on chromosomes D7 and D9 in upland cotton. *Science China-Life Sciences***2009**, *52*, 872-884, doi: 10.1007/s11427-009-0110-8.

51. Zhang, J.F.; Fang, H.; Zhou, H.; Sanogo, S. and Ma, Z. Genetics, Breeding, and Marker-Assisted Selection for Verticillium Wilt Resistance in Cotton. *Crop Sci.***2014**, *54*, 1–15, doi: 10.2135/cropsci2013.08.0550.

52. Wu, C.C.; Jian, G.L.; Wang, A.; Liu, F.; Zhang, X.L.; Song, G.L. et al. Primary detection of QTL forVerticillium wilt resistance in cotton *Mole Plant Breed***2010**, *18*, 680–686, doi: 10.3969/mpb.008.000680.

53. He, R.; Shi, Y.Z.; Zhang, J.F.; Liang, Y.; Zhang, B.C.; Li, J.W. et al. QTL mapping for plant height using chromosome segment substitution lines in upland cotton. *Acta Agron Sin***2014**, *40*, 457-65.

54. Liang, Y.; Jia, Y.J.; Li, A.G.; Zhang, B.C.; Liu, G.P.; Li, J.Z. et al. Phenotyping traits related to yield and quality of BC_5F_2 substitution lines in cotton (*Gossypium*) and their QTL mapping. *Mole Plant Breed.***2010**, *8*, 221–30, doi: 10.3969/mpb.008.000221.

55. Zhang, J.F.; Shi, Y.Z.; Liang, Y.; Jia, Y.J.; Zhang, B.C.; Li, J.W. et al. Evaluation of Yield and Fiber Quality Traits of Chromosome Segment Substitution Lines Population ($BC_5F_3$and $BC_5F_{3:4}$) in Cotton. *J Plant Genetic Res.***2012**, *13*, 773-81, doi: 10.3724/SP.J.1006.2014.00457.

56. Yang, Z.M.; Li, J.Z.; Li, A.G.; Zhang, B.C.; Liu, G.P.; Li, J.W. et al. Developing chromosome segment substitution lines (CSSLs) in Cotton (*Gossypium*) using advanced backcross and MAS. *Mol Plant Breed***2009**, *7*, 233-41.

57. Lan, M.J.; Yang, Z.M.; Shi, Y.Z.; Ge, R.H.; Li, A.G.; Zhang, B.C. et al. Assessment of substitution lines and identification of QTL related to fiber yield and quality traits in BC_4F_2 and BC_4F_3 populations from *Gossypium hirsutum* × *Gossypium barbadense*. *Sci Agric Sin***2011**, *44*, 3086–97.

58. Ma, L.J.; Shi, Y., Z, ; Lan, M., J, ; Yang, Z., M, ; Zhang, J., F, ; Zhang, B., C, et al. Evaluation of chromosome segment substitution lines related to fiber yield and quality traits from *Gossypium hirsutum* × *Gossypium barbadense*. *Cotton Sci***2013**, *25*, 486-95.

59. Sun, Q.; Cai, Y.F.; Xie, Y.F.; Mo, J.C.; Youlu, Y. and Shi, Y.Z., et al. Gene expression profiling during gland morphogenesis of a mutant and a glandless upland cotton. *Molecular Biology Reports***2010**, *37*, 3319–3325. doi:10.1007/s11033-009-9918-3PMID:19888674.

60. Li, P.T.; Wang, M.; Lu, Q.W.; Ge, Q.; Rashid, M.H.; Liu, A.Y. et al. Comparative transcriptome analysis of cotton fiber development of Upland cotton (*Gossypium hirsutum)* and Chromosome Segment Substitution Lines from *G. hirsutum* × *G. barbadense*. *BMC Genomics* **2017**, *18*, DOI 10.1186/s12864-017-4077-8.

bioRxiv preprint doi: https://doi.org/10.1101/788901; this version posted October 1, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

61. Zhao, Y.; Wang, H.; Chen, W. and Li, Y. Genetic Structure, Linkage Disequilibrium and Association Mapping of Verticillium Wilt Resistance in Elite Cotton (*Gossypium hirsutum* L.) Germplasm Population. *PLoS ONE***2014**, *9*, e86308.

62. Wu, Z.B.; Li, J.; Feng, C.D. and Zhang, J.F. Studies on the identification techniques of cotton resistance toVerticillium wilt. *Hubei Agric Sci.***1999**, *5*, 16-19.

63. Khan, N.U.; Marwat, K.B.; Farhatullah, G.H.; Batool, S.; Makhdoom, K.; Ahmad, W. et al. Genetic variation and heritability for cotton seed, fiber and oil traits in *gossypium hirsutum* L. *Pak. J. Bot.***2010**, *42*, 615-625.

64. Paterson, A.H.; Brubaker, C.L. and Wendel, F.J. A Rapid Method for Extraction of Cotton (*Gossypium* spp.) Genomic DNA Suitable for RFLP or PCR Analysis. *Plant Mol. Biol. Rep.***1993**, *11*, 122-127.

65. Niu, C.; Lister, H.E.; Nguyen, B.; Wheeler, T.A. and Wright, R.J. Resistance to *Thielaviopsis basicola* in the cultivated A genome cotton. *Theor Appl Genet***2008**, *117*, 1313–1323, doi: 10.1007/s00122-008-0865-5.

66. Wang, J.; Wan, X.; Crossa, J.; Crouch, J.; Weng, J. and Zhai, H., et al. QTL mapping of grain length in rice *(Oryza sativa* L.) using chromosome segment substitution lines. *Genetical Research***2006**, *88*, 93-104, PMID:1712558.

67. Voorrips. RE. Map Chart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity***2002**, *93*, 77–78. PMID:12011185.

68. Sun, F.D.; Zhang, J.H.; Wang, S.F.; Gong, W.K.; Shi, Y.Z. and Liu, A.Y., et al. QTL mapping for fiber quality traits across multiple generations and environments in upland cotton. *Mol. Breed.***2012**, *30*, 569-582, doi: 10.1007/s11032-011-9645-z. .

69. Jamshed, M.; Jia, F.; Gong, J.; Palanga, K.K.; Shi, Y.; Li, J. et al. Identification of stable quantitative trait loci (QTLs) for fiber quality traits across multiple environments in *Gossypium hirsutum* recombinant inbred line population. *BMC Genomics***2016**, *17*, 197, doi: 10.1186/s12864-016-2560-2.

70. Arcade, A.; Labourdette, A.; Falque, M.; Mangin, B.; Chardon, F.; Charcosset, A. et al. BioMercator: integrating genetic maps and QTL towards discovery of candidate genes. *Bioinformatics***2004**, *20*, 2324-6.

71. Wang, F.R.; Liu, R.Z.; Wang, L.M.; Zhang, C.Y.; Liu, G.D.; Liu, Q.H. et al. Molecular markers of *Verticillium* wilt resitance in Upland cotton (*Gossypium hirsutum* L.) cultivar and their effect on Assisted Phenotypic Selection. *Cotton Sci.***2007**, *19*, 424-430.

72. Cai, Y.F.; Xiaohong, H.; Mo, J.C.; Sun, Q.; Yang, J.P. and Liu, J.G. Molecular research and genetic engineering of resistance to Verticillium wilt in cotton: A review. *Afr. J. Biotechnol.***2009**, *8*, 7363-7372.

73. Zhou, H.P.; Fang, H.; Sanogo, S.; Hughs, S.E.; Jones, D.C. and Zhang, J. Evaluation of *Verticillium* wilt resistance in commercial cultivars and advanced breeding lines of cotton. *Euphytica***2014** *196*, 437-448, doi: 10.1007/s10681-013-1045-5.

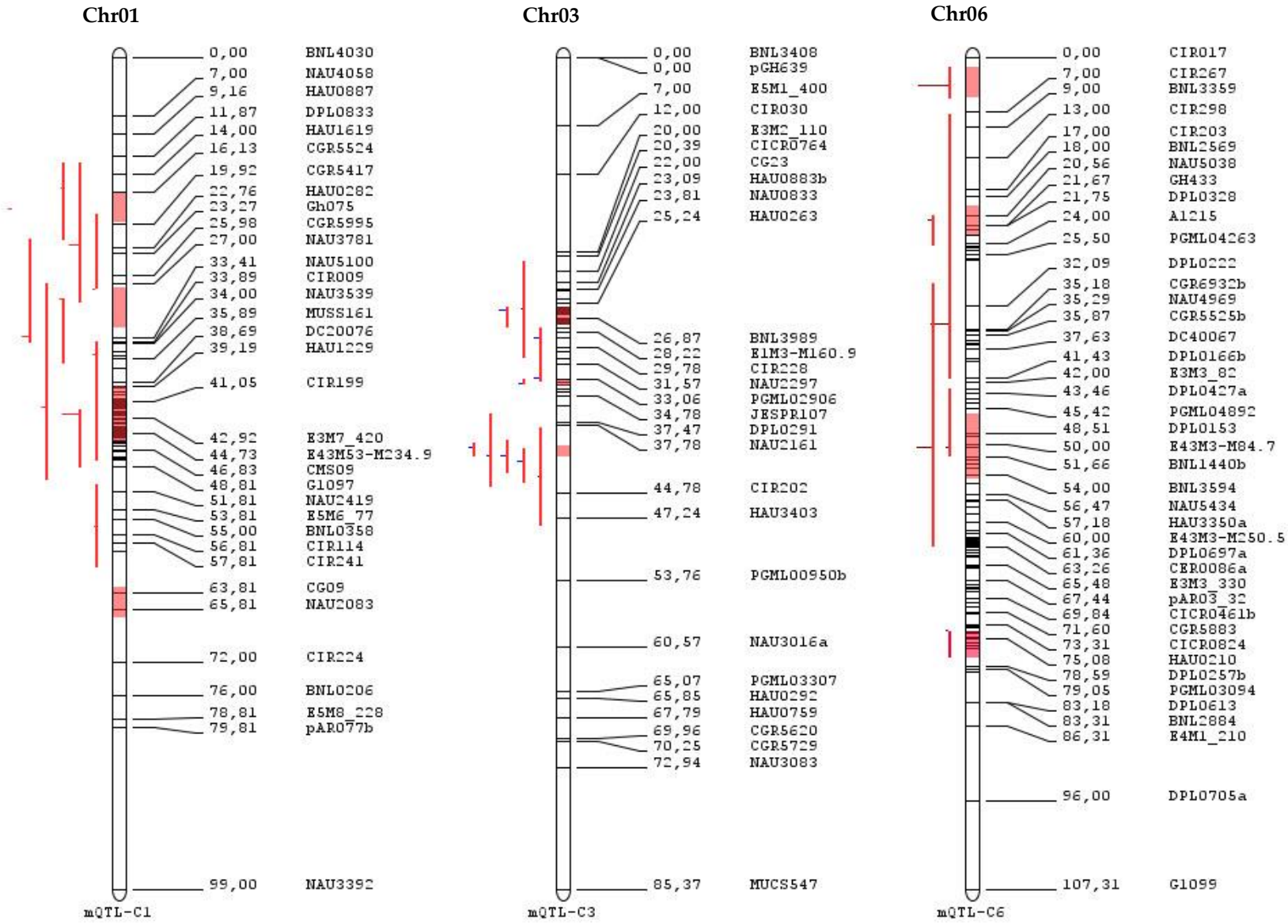


Figure S2. QTLs and QTL hotspot for *Verticillium* wilt resistance on the consensus map by a meta-analysis

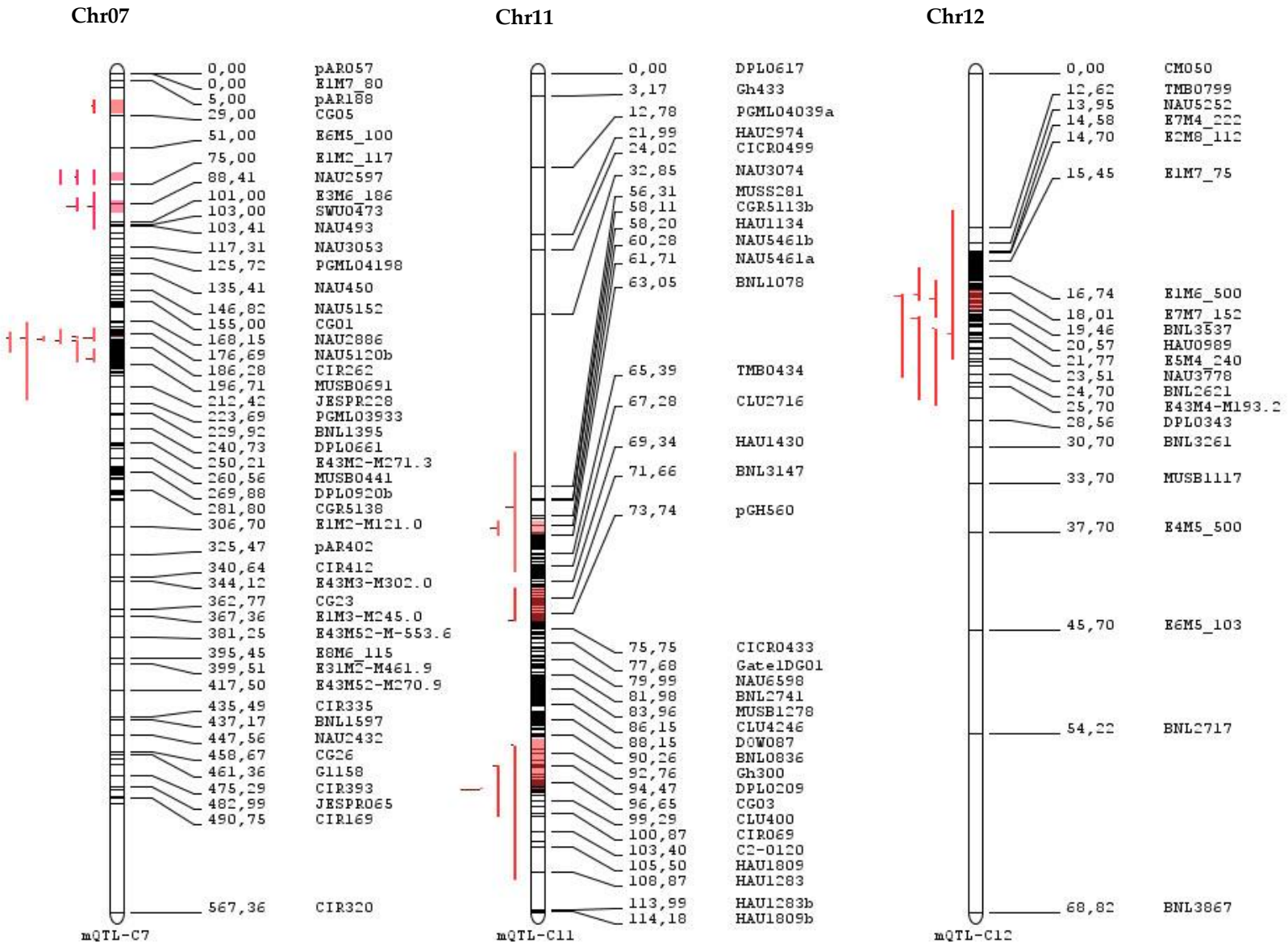


Figure S2. Continued

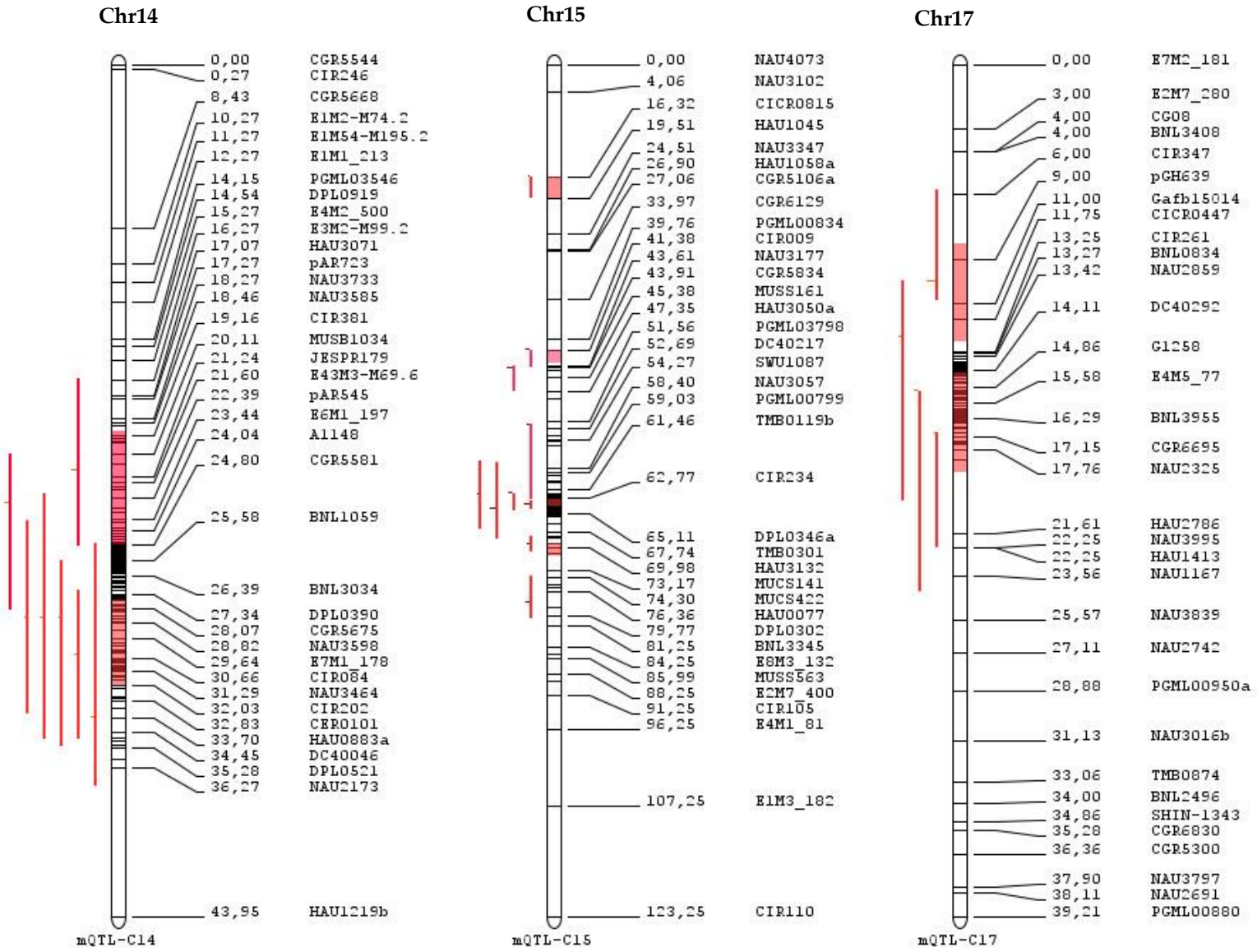


Figure S2. Continued

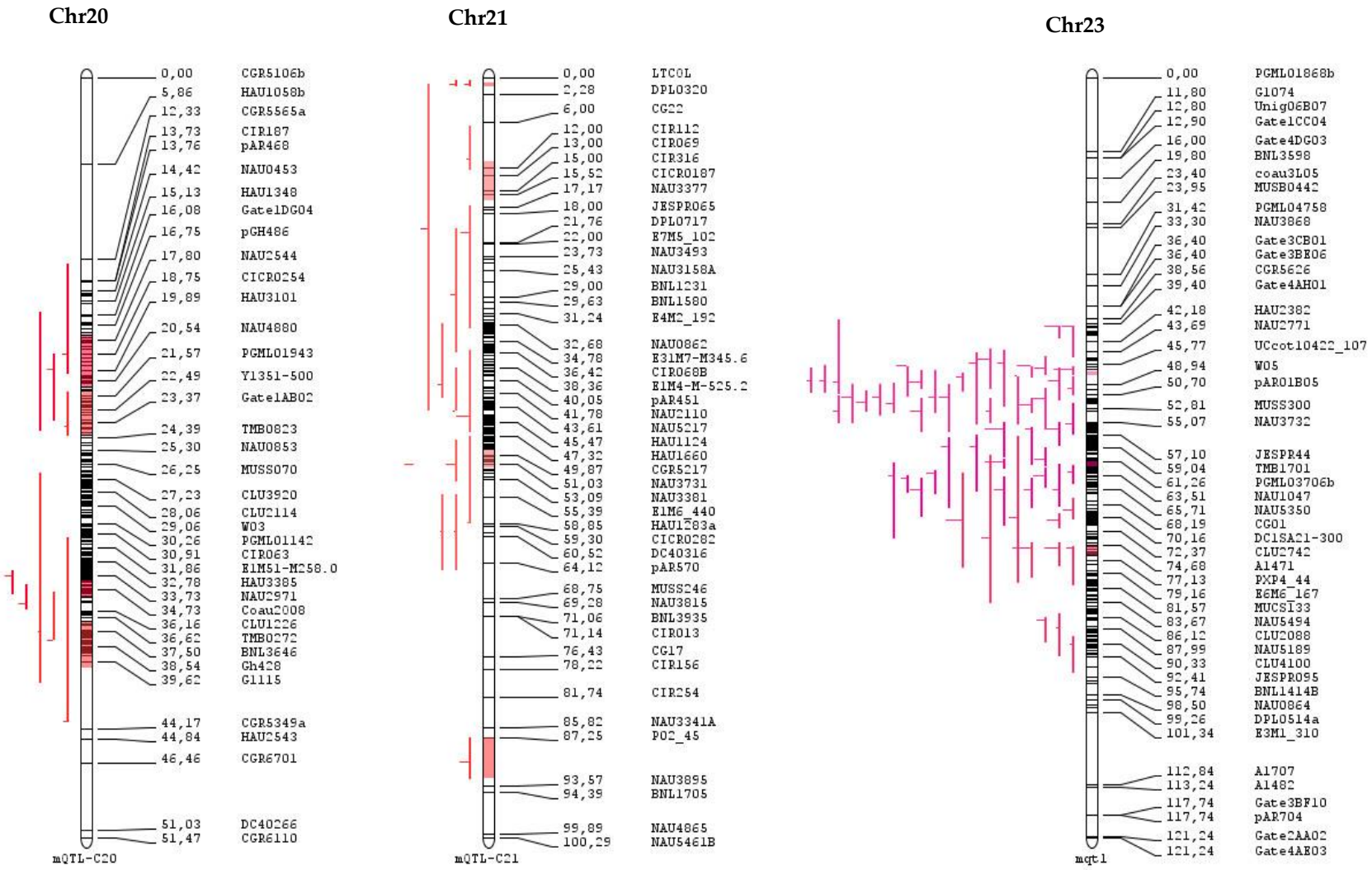


Figure S2. Continued

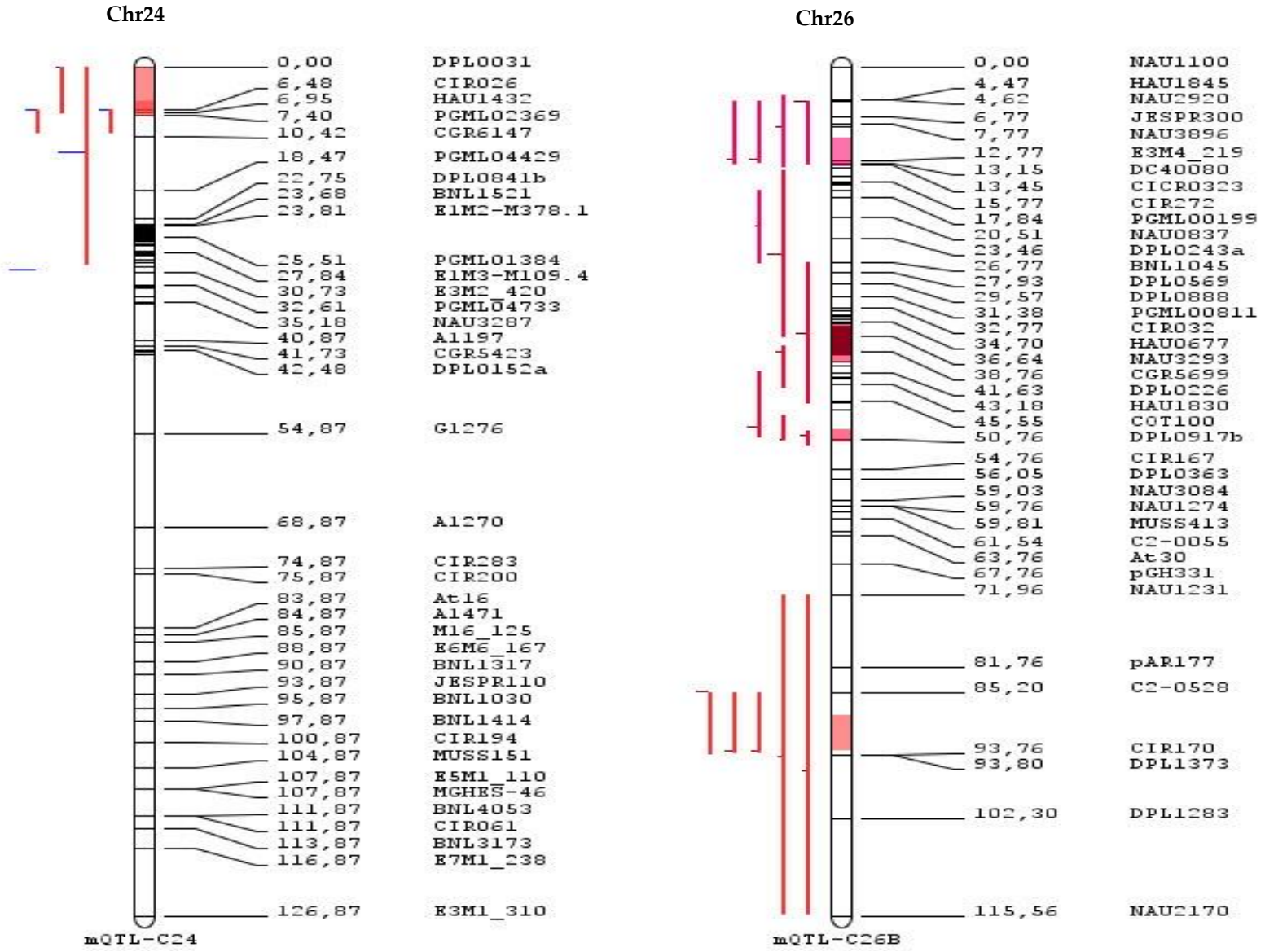


Figure S2. Continued

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
10	**qVW-Chr05-3**	July	AYJul15	Chr05	35.4	DPL0063	3.32	-5.08
		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
do
do
do
do
do
do
do
do
do
do
do
do
do
do
do
do
do
do
do
Beijing Genomics Inst.