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Genetic dissection of tetraploid cotton resistant to *Verticillium* wilt using interspecific chromosome segment introgression lines

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ABSTRACT

Verticillium wilt (caused by the pathogen *Verticillium dahliae*) is of high concern for cotton producers and consumers. The major strategy for controlling this disease is the development of resistant cotton (*Gossypium* spp.) cultivars. We used interspecific chromosome segment introgression lines (CSILs) to identify quantitative trait loci (QTL) associated with resistance to *Verticillium* wilt in cotton grown in greenhouse and inoculated with three defoliating *V. dahliae* isolates. A total of 42 QTL, including 23 with resistance-increasing and 19 with resistance-decreasing, influenced host resistance against the three isolates. These QTL were identified and mapped on 18 chromosomes (chromosomes A1, A3, A4, A5, A7, A8, A9, A12, A13, D1, D2, D3, D4, D5, D7, D8, D11, and D12), with LOD values ranging from 3.00 to 9.29. Among the positive QTL with resistance-increasing effect, 21 conferred resistance to only one *V. dahliae* isolate, suggesting that resistance to *V. dahliae* conferred by most QTL is pathogen isolate-specific. The At subgenome of cotton had greater effect on resistance to *Verticillium* wilt than the Dt subgenome. We conclude that pyramiding different resistant QTL could be used to breed cotton cultivars with broad-spectrum resistance to *Verticillium* wilt.

1. Introduction

Cotton (*Gossypium* spp.) is one of the most important fiber crops in the world and serves as a source of oil and biofuel [1]. *Verticillium* wilt has worldwide distribution and causes serious economic losses [2]. The disease is caused by the soilborne fungus *Verticillium dahliae* Kleb. The fungus infects the roots of the cotton plant in the soil by entering through cortical cells.

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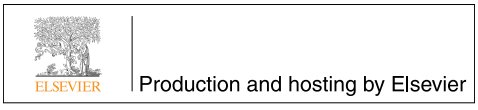

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Once inside, the spores and mycelia of the pathogen block the vessels of the plant. *V. dahliae* toxins and acidic glycoproteins are also important pathogenicity factors that can rapidly induce wilting [3]. Strains of the pathogen in China can be divided into two types according to their virulence: defoliating and non-defoliating [4]. An early symptom seen in the host plant after infection by a defoliating pathogen is downward curling and epinasty of the terminal leaf, followed by epinasty of most of the other leaves. These epinastic leaves then exhibit general chlorosis, which eventually leads to defoliation. If cotton plants are infected with a non-defoliating pathogen, the lower leaves exhibit interveinal chlorosis that leads to necrosis, but there is little or no epinasty and any dead leaves usually remain attached to the plant [4]. The main factors affecting the virulence of *Verticillium* wilt in cotton are the *V. dahliae* pathotype and the inoculum density of the fungus [5].

To date, the most effective and feasible way to control *Verticillium* wilt disease is the development of cotton cultivars with resistance to the pathogen using conventional breeding and transgenic technologies [6–9]. There are approximately 50 species in the *Gossypium* genus, of which four are cultivated, including two allotetraploids (*Gossypium hirsutum* and *Gossypium barbadense*) and two diploids (*Gossypium herbaceum* and *Gossypium arboreum*) [10,11]. *G. hirsutum*, also known as upland cotton, is the most widely planted of the four cultivated *Gossypium* spp., and has been the subject of most genetic studies and breeding efforts. It produces more than 95% of the annual cotton crop worldwide (National Cotton Council, http://www.cotton.org/, 2006), but most of the commercial cultivars of the species are susceptible or only tolerant to *Verticillium* wilt. *G. barbadense*, another important cultivated species of cotton, is characterized by its extra-long-staple cotton compared to upland cotton. Of the four cultivated cotton species, *G. barbadense* is the most resistant to *Verticillium* wilt. For this reason, breeders have tried to introgress resistance gene(s) from *G. barbadense* to *G. hirsutum*. However, linkage drag between the resistance and undesired agronomic traits and distortion in segregation of the interspecific hybrid has severely hampered the exploitation of these lines. As a result, little progress has been made toward the selective breeding of cotton for resistance to *Verticillium* wilt, and the needs of the cotton industry are far from being achieved [2].

Quantitative trait loci (QTL)/genes resistant to *Verticillium* wilt have been detected in *G. barbadense* and *G. hirsutum* cultivars. A random amplified polymorphic DNA marker linked with a resistance gene at a distance of 12.4 cM was identified. This marker was associated with a phenotypic variance (PV) of 12.1% [12]. Two QTL clusters with high contributions were detected on chromosome (Chr.) D7 and Chr.D9 by composite interval mapping [13]. With the use of an F_2 population (from a cross between a *G. barbadense* cultivar and a *G. hirsutum* cultivar) and a single isolate of *V. dahliae*, three large-effect QTL (CM12, STS1, and BNL3147-2) conferring resistance to *Verticillium* wilt were detected on Chr.A11 [14]. Several QTL showing resistance to the disease have been also detected in various studies [4,15,16]. However, differences in markers, isolates, and developmental stages among these studies and the unavailability of chromosome tagging data make comparisons of results obtained from these studies difficult.

Chromosomal segment introgression lines (CSILs) carrying introgressed chromosomal segments in the same genetic background offer great advantages for studying the genetic functions of chromosomal segments. Moreover, CSILs are a unique system for mapping purposes, thanks to the marked reduction in confounding interactive effects between segregating loci in the genetic background, such as the ones that occur in other types of mapping populations [17]. We developed a CSIL population using the cotton genetic standard *G. hirsutum* cv. TM-1 as the recipient parent and the long-staple cotton *G. barbadense* cv. Hai 7124 as the donor parent, and employed our 330 simple sequence repeat (SSR) anchored markers for molecular marker-assisted selection (MAS) in the BC_5S_{1-4} and BC_4S_{1-3} generations. The CSIL population comprised 174 lines containing 298 introgressed segments, of which 86 lines (49.4%) contained a single introgressed segment. The introgressed segments covered a total length of 2948.7 cM (with an average length of 16.7 cM), representing 83.3% of the cotton genome [18]. In the present study, we used these CSILs to identity QTL affecting resistance to *Verticillium* wilt. Our major objectives were to conduct genome wide screening of chromosome regions containing resistance gene(s), identify the genetic mechanisms of tetraploid cotton resistance to *Verticillium* wilt, and find markers linked to QTL conferring resistance to multiple *V. dahliae* isolates during the seedling stage in order to facilitate improved cotton breeding programs.

2. Materials and methods

2.1. Pant materials

G. hirsutum cv. TM-1, the genetic standard upland cotton, was obtained from the Southern Plains Agricultural Research Center, USDA-ARS, College Station, Texas, U.S.A. [19]. *G. barbadense* cv. Hai 7124, grown extensively in China, is the offspring of an individual plant selected during earlier studies of inherited resistance to *V. dahliae* in our laboratory [20,21]. *G. hirsutum* cv. Junmian 1 is distributed widely in Xinjiang municipality and is highly sensitive to *Verticillium* wilt, and was selected as a control. One set of CSILs was developed using MAS in the genetic standard *G. hirsutum* cv. TM-1 background (the recipient parent) and the *G. barbadense* cv. Hai 7124 (the donor parent) which is resistant to *Verticillium* wilt.

2.2. Inoculation and phenotyping of CSILs in the greenhouse

Two defoliating *V. dahliae* isolates found commonly in the Yangtze River cotton-growing region of China, V991 and V07DF2, were selected to represent isolates with strong and extrastrong virulence. The defoliating isolate D8092, from the Yellow River cotton-growing region, was selected to represent isolates of intermediate virulence. *V. dahliae* isolates were grown on potato dextrose agar plates at 25 °C for 10–14 d. Inocula for experiments were prepared by spreading a conidial suspension on agar plates that were then incubated at 25 °C for 6–7 d. Conidia were then collected and diluted to 1×10^7 cells mL^{-1}.

The 166 CSILs were grown in paper cups of 7.3 × 5.1 × 8.3 cm in the greenhouse at Nanjing Agriculture University (Nanjing, China) from 2009 to 2011. These CSILs were planted in a