Conserved Markers Order in Quantitative Trait Loci Confers Resistance against Black Root Rot Disease in Cotton

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Background and objective: Black root rot, incited by the soil borne fungus Thielaviopsis basicola, can cause substantial yield loss and reduced fiber quality in cotton (Gossypium spp.). The isolation of candidate resistant genes in tetraploid genome AADD cotton species (2n=4x=52) remains challenging in the absence of research of black root rot resistance on progenitor DD genome diploid cotton (G. raimondii). In this study, by exploiting Phytozome database, a comparative map of the black root rot-resistance quantitative trait loci in DD genome was constructed. Materials and methods: Simple sequence repeats markers associated with these three quantitative trait loci in the AA genome were used as “anchored-probes” frameworks for establishing relationships between the two cotton genomes AA and DD. Results: Our results showed that there was colinearity between the genetic map of simple sequence repeats markers on AA genome and the physical map of these simple sequence repeats markers on DD genome. It was suggested that the syntenic loci on chromosome 2, 7 and 11 on DD genome could correspond the black root rot resistance. Conclusion: This study could serve as a fundamental step in isolating and introducing the resistance gene against black root rot into elite cotton cultivars.

Keywords: Comparative Mapping; Phytozome; Resistance Gene; Simple Sequence Repeats; Quantitative Trait Loci.

Diseases present a significant impact to cotton (Gossypium spp.) cultivation. It is estimated that annual cotton yield loss due to this disease is approximately 60% of potential production (Rothrock, 1997, Blasingame, 2005). Black root rot (BRR) is a seedling disease caused by Thielaviopsis basicola, a soil- borne pathogenic fungal with a broad infection spectrum of crops. Since its first reported case on cotton in Arizona in 1922 (King and Presley, 1942), it has become one of the significant threats in cotton industry.

Despite the main commercial tetraploid cotton genome AADD species grown worldwide are G. barbadense and G. hirsutum, they lack resistance to BRR. As a result, tremendous efforts have been made toward developing BRR resistance germplasm, yet commercial germplasm has not been available. Nevertheless, BRR partial resistance has been demonstrated in several studies conducted in AA genome G. arboreum (variance PI1415) and G. herbaceum (variance A20) (Wheeler et al., 1999, Wheeler et al., 2000).

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Most recently, by employing crossbreeding from these two cultivars, followed by genetic analysis with simple sequence repeats (SSR) markers, Niu et al. (2008) detected three quantitative trait loci (QTL) BRR5.1, BRR9.1, BRR13.1, conferring BRR resistance. Since DD genome is the progenitor of the AADD genome, this could indicate that DD genome possibly harbor R genes for disease resistance. However, research on isolating R genes on DD genome, particularly against Thielavopsis basicola, still is in embryonic stage.

The importance of comparative mapping is the establishment of the syntenic relationships between genomes from different species (Kliebenstein et al., 2001, Murphy et al., 2001, Schmidt, 2002). Mountain of evidences have accumulated in comparative mapping analysis in many species of great economic importance, such as Pinaceae, soybean (Glycine max), barrel medic (Medicago truncatula), cabbage (Brassica oleracea), potato (Solanum tuberosum), and Arabidopsis thaliana (Babula et al., 2003, Gebhardt et al., 2003, Grant et al., 2000, Lukens et al., 2003, Zhu et al., 2003) By using a standard set of frequently applied markers such as SSR and RFLP, comparative mapping assists the translation and transferring the information from one genomic map to another, such as verification of QTL, obtaining better knowledge of genome evolution, and identification of candidate genes underlying QTL (Duran et al., 2009). Specifically, the idea of transferring map information to improve disease resistance has been conducted in coffee (Psilanthus). Molecular markers were used to isolate the new resistance genes which were subsequently introduced novel more robust sources into commercially elite coffee varieties (Hendre et al., 2011).

In this study, by utilizing Phytozome database, we reported there was a correlation between the genetic map in AA genome and physical map in DD genome. A comparative map was constructed, revealing the conserved order of SSR markers from the genetic mapping results in diploid AA genome from Niu et al. (2008) (Niu et al., 2008) and in DD genome. These results will shed new lights in understanding of shared synteny of QTL conferring black root rot disease between two diploid genomes in cotton.

**MATERIALS AND METHODS**

**Materials**

The study was carried out at Department of Plant and Soil Science, Texas Tech University, USA from January 2014 to May 2014.

**Cottongen**

Cottongen is an online mapping database for cotton (Yu et al., 2014). Cotton Gen contains information on genomic, genetics, breeding, and molecular genetic markers. It also incorporates genomic sequences of different cotton genomes, markers, and traits. Additionally, various platform such as BLAST, JBrowse, MapViewer, Primer3 are also included in the website.

**Phytozome**

Phytozome has been developed in 2008 to serve as a connective hub for plant genome analysis. Besides enabling users to compare every plant gene at the level of sequences, Phytozome also provides access to plant genomics, such as 25 genomes (including cotton), gene and homologous sequences (Goodstein et al., 2012).

**Methods**

**Retrieve the Sequence of Mapped SSR Markers on AA Genome in Cottongen Database**

- Go to the CottonGen website (https://www.cottongen.org/). Along the Tools Quick Start, go to ‘Search Markers’ (Figure 1).
- In the ‘Marker Name’ section, click on ‘contains’ in the first box and then type the name of the marker in the second box (Figure 2). Use the marker name in the publication of Niu et al. (2008)6, page 1318, Figure 3.
- In the ‘Marker Type’ section, click on ‘SSR’. Then hit ‘Search’.
- In the resulting search table, click any of the records that showed in the table.
- In the ‘Marker Overview’, click on the ‘Source Sequence’ to get the sequence of the markers (Figure 3). Copy the sequence of the marker in Notepad program of Microsoft Windows.

**Anchor a Genome-Derived SSR Markers on D genome by Phytozome**

- Go the the Phytozome website (https://phytozome.jgi.doe.gov/pz/portal.html#). Along the top menu header, go to ‘Species’ and choose ‘Gossypium raimondii1’ (Figure 4).
- In the new resulting page, along the menu under the title ‘Gossypium raimondii1 (Cotton), click on
‘BLAST search’ (Figure 5).
• In the second column ‘2. Build your query’, paste the copied marker’s sequence into the box the says ‘Enter a single sequence…’. Then hit ‘Go’.
• The BLAST results page shows the most significant hits. You will choose the first hit with the darkest color arrow bar. In the ‘Target View’ section, Click on that arrow bar in the ‘Feature scale’ column.
• In the close-up viewing mode in JBrowse, copy the information of the chromosome in the first box and the physical position of the marker in that chromosome in the second box (Figure 6).

**RESULTS AND DISCUSSION**

We showed here that after anchoring the SSR markers from the results of Niu *et al.* (2008) on DD genome, there was collinearity between the genetic map of SSR markers associated three QTL conferring BRR on AA genome and the physical position of these SSR markers on DD genome (Table 1). We still observed some minor SSR markers inversions, especially in the chromosomal regions on DD genome which corresponds to a linkage group A9. This observation has been made by Rong *et al.* (2004). These inversions could be explained by the rearrangement of the chromosomal segments during evolution of AA and DD genomes after separating from the first common ancestor (Rong *et al.*, 2004). One more explanation could be the order of SSR markers were calculated based on the recombination frequency which could be utilized to measure the genetic distance between two loci, whereas the physical map was based on the number of nucleotides between two loci (O’Rourke, 2014). Overall, this result confirmed the accuracy of the genetic map in previous study by Niu *et al.* (2008) (Niu *et al.*, 2008).

In this study, we presented a Phytozome-based comparative mapping between two cotton diploid genomes revealing conserved markers order in quantitative trait loci conferring resistance against black root rot disease.

It has been proposed that diploid cotton species may have originated from a common ancestor that subsequently evolved and divided into eight morphologic groups designated as A–G, and K. Approximately 1 to 2 million years ago, the spontaneous hybridization event between two diploid species (2n = 2x = 26): D- genome species closely related to G. raimondii (D5) and A- genome species related to G. arboreum (A2) or G. herbaceum (A1), resulted in the origin of allotetraploid species (2n = 4x = 52) (Wendel, 1989, Wendel *et al.*, 1995). The polyploidization and subsequent independent evolution resulted in the formation of six tetraploid species: G. hirsutum (AD)1, G. barbadense (AD)2, G. tomentosum (AD)3, G. mustelinum (AD)4, G. darwinii (AD)5 and G. ekmanianum (AD)6 (Grover *et al.*, 2015).

![Fig. 1. The Cotton Gen website entry display](Source)https://www.cottongen.org/
Fig. 2. Cotton Gen SSR marker entry display

Fig. 3. Cotton Genre presentation of the selected SSR marker
Despite the main commercial cotton species grown worldwide are G. barbadense and G. hirsutum, they lack resistance to BRR. As a result, tremendous efforts have been made toward developing BRR resistance germplasm, yet commercial germplasm has not been available. Nevertheless, BRR partial resistance has been demonstrated in several studies conducted in diploid AA genome cultivars G. arboretum (variance PI1415) and G. herbaceum (variance A20) (Wheeler et al., 1999, Wheeler et al., 2000). Most recently, by employing crossbreeding from these two cultivars, followed by genetic analysis with SSR markers, Niu et al. (2008) detected three QTL, BRR5.1, BRR9.1, BRR13.1, conferring BRR resistance (Niu et al., 2008).

Fig. 4. Phytozome website entry display

Fig. 5. Phytozome representation of BLAST results
SSR markers, also known for their informative, versatile, and readily detectable properties, have been extensively utilized in saturation of the large and complex genomes (Blenda et al., 2006, Khan et al., 2016). In cotton, a larger body of research has been accumulated in mining and characterizing new SSRs in narrowing down the QTL regions and ultimately isolating the candidate genes responsible for desired traits (Blenda et al., 2006, Kirungu et al., 2018, Tabbasam et al., 2014, Yu et al., 2012, Yu et al., 2011). However, these researches mainly focused on commercial tetraploid cotton Gossypium hirsutum, Gossypium barbadense or crosses generated from these two species with other tetraploid species. We report here a new method that could physically map AA genome-SSR markers in D genome by using Phytozome database. Given the collinearity between regions of AA and DD genomes in this study, we suggested that the syntenic regions on DD genome could also confer the BRR resistance. These regions were on chromosome 2 from position 7879824 to position 59691832, chromosome 7 from position

Table 1. Correlation between the genetic map on AA genome and physical map on DD genome of SSR markers

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from position 5509175 to position 57112593. More research should be done to increase the density of SRR markers in these regions to isolate candidate R-genes.

**CONCLUSION**

Conclusively, our data suggested that there is a collinearity between A genome and D genome. While the orders of SSR markers on linkage group A13 on A genome are conserved on D genome, we observed some minor disorders inversion of SSR markers on D genome compared to their orders on A genome that could be explicable by the rearrangement of the chromosomal segments or recombination frequency. The results from this paper could be further used for fine mapping R genes in D genome in the future.

**Significant Statement**

This study discovered the conserved markers order in quantitative trait loci conferring resistance against black root rot disease in diploid genome cotton. This result can be beneficial in isolating resistance candidate genes in the future.

**ACKNOWLEDGEMENTS**

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