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 Thielaviopsisbasicola, 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 sequences 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.

Key Words: *Comparative mapping; Resistance gene; Phytozome; Simple sequence repeats; Quantitative trait loci*

INTRODUCTION

Diseases present a significant impact to cotton (Gossypium spp.) cultivation. It is estimated that annual cotton yield loss due to disease is approximately 60% of potential production [1-3]. Black root rot (BRR) is a seedling disease caused by Thielaviopsisbasicola, a soil-borne pathogen fungal with a broad infection spectrum of crops. Since its first reported case on cotton in Arizona in 1922, 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. arboretum* (variance PI1415) and *G. herbaceum* (variance A20) [4,5]. Most recently, by employing crossbreeding from these two cultivars, followed by genetic analysis with simple sequence repeats (SSR) markers, Niu et al., 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 *Thielavopsisbasicola*, still is in embryonic stage [6].

The importance of comparative mapping is the establishment of the syntenic relationships between genomes from different species [7-10]. 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 [11-15]. 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. 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 [16,17].

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, revealingthe conserved order of SSR markers from the genetic mapping results in diploid AA genome from Niu et al., 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

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

CottonGen: CottonGen is an online mapping database for cotton [18]. 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 [19].

Retrieve the sequence of mapped SSR markers on AA genome in CottonGen database

Go to the CottonGen website. Along the Tools Quick Start, go to 'Search Markers' (Figure 1).

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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. (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.

Figure 1) The CottonGen website entry display.

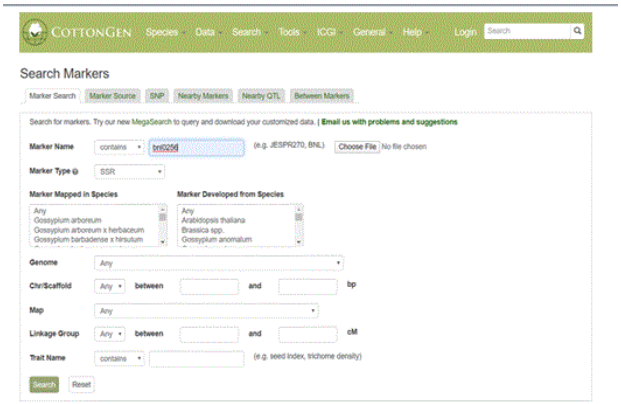

Figure 2) CottonGen SSR marker entry display.

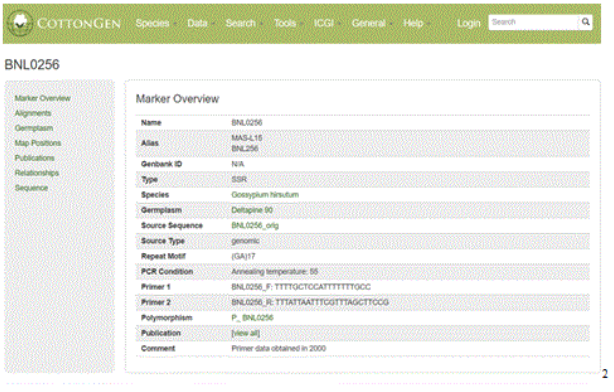

Figure 3) CottonGen representation of the selected SSR marker.

Anchor A genome-derived SSR markers on D genome by Phytozome

Go the thePhytozome website. Along the top menu header, go to 'Species' and choose '*Gossypiumraimondii* v2.1' (Figure 4).

In the new resulting page, along the menu under the title '*Gossypiumraimondii* v2.1 (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 sequenc'. 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).

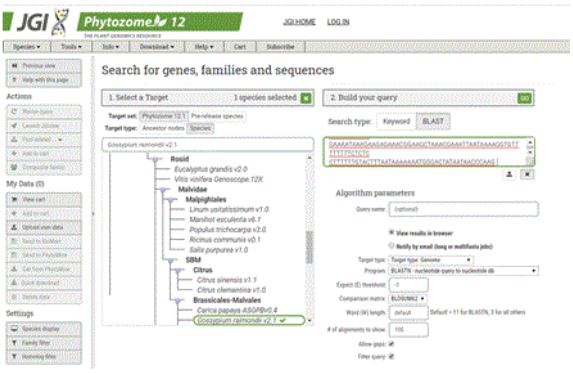

Figure 4) Phytozome website entry display.

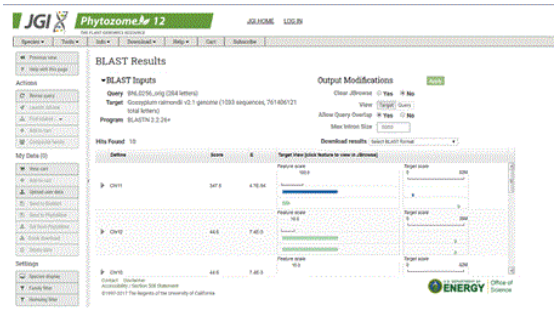

Figure 5) Phytozome representation of BLAST results.

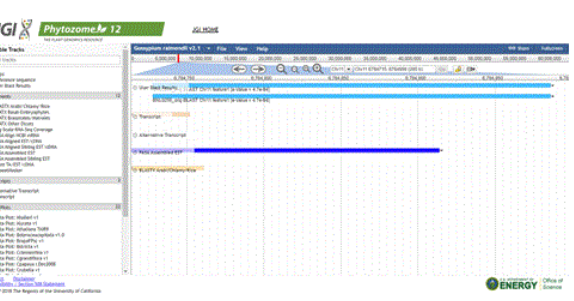

Figure 6) Phytozome representation of chromosomal position of SSR marker.

RESULTS AND DISCUSSION

We showed here that after anchoring the SSR markers from the results of Niu et al., 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 SRR markers inversions, especially in the chromosomal regions on DD genome which corresponds to the linkage group A [9]. This observation has been made by Rong et al., [20]. 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. 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 [21]. Overall, this result confirmed the accuracy of the genetic map in previous study by Niu et al., [6].

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

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

Linkage group number	SSR markers on A genome (appear in order)	Hypothetical synteny order on D genome	SSR markers on D genome (appear in order)	Chromosome number on D genome	First position on D genome	Last position on D genome
	NAU0921	MGHES27	MGHES27		5509175	5509752
	MGHES41	TMA18	BNL0256		6784715	6784998
	BNL3895	BNL0256	NAU1041		9892984	9898779
LGA9	NAU1041	NAU1041	MGHES41	11	11011950	11014308
	BNL0256	BNL3895	NAU0921		17797360	17798057
	TMA18	MGHES41	BNL3895		23404926	23405317
	MGHES27	NAU0921	TMA18		57111815	57112593
	BNL3442	BNL3442	BNL3442		3320225	3320674
LGA13	BNL1034	BNL1034	BNL1034	7	5461060	5461360
	NAU0760	NAU0760	NAU0760		6856140	6856464
	BNL2589	BNL2589	BNL2589		6986385	6986892
	BNL3147	BNL3147	BNL3147		7340897	7341396
	BNL1681	BNL1681	BNL1681		14808675	14808963
	BNL4094	BNL4094	BNL4094		19878990	19879398
	BNL2632	BNL2632	BNL2632		24142720	24143238
	NAU1063	NAU1063	BNL0625		28319473	28319761
	BNL0625	BNL0625	NAU1063		36248258	36250575
	BNL1408	BNL1408	BNL1408		43869105	43869532
	BNL1066	BNL1066	BNL0836		52098774	52099213
	BNL1231	BNL1231	BNL1066		54550357	54551604
	MGHES16	MGHES16	BNL1231		57124813	57125015
	CIR196	CIR196	MGHES16		58185366	58186970
	BNL0836	BNL0836	CIR196		58205755	58206145
	BNL1683	CIR114	BNL3580		7879824	7880305
	MGHES10	BNL3580	CIR241		7879830	7880229
	BNL2646	CIR241	CIR114		8053239	8053760
	BNL3791	BNL1667	BNL1667		9774273	9774659
	CIR049	BNL3888	BNL3888		11188791	11189262
	CIR089	BNL3090	BNL3090		13608472	13608924
LGA5	BNL3090	CIR089	CIR089	2	16149735	16149932
	BNL3888	CIR049	CIR049		16247068	16247503
	BNL1667	BNL3791	MGHES10		24401561	24403232
	CIR241	BNL2646	BNL3791		32038546	32038941
	BNL3580	MGHES10	BNL2646		43358571	43358990
	CIR114	BNL1683	BNL1693		59691568	59691832

monophyletic 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). The polyploidization and subsequent independent evolution resulted in the formation of six tetraploid species: G. *hirsutum* (AD), G. *barbadense* (AD), G.*tomentosum* (AD), G. *mustelinum* (AD), G. *darwinii* (AD) and G. *ekmanianum* (AD) [6].

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) [4,5]. Most recently, by employing crossbreeding from these two cultivars, followed by genetic analysis with SSR markers, Niu et al., detected three QTL, BRR5.1, BRR9.1, BRR13.1, conferring BRR resistance [6].

SSR markers, also known for their informative, versatile, and readily detectable properties, have been extensively utilized in saturation of the large and complex genomes [22-26]. 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. However, these researches mainly focused on commercial tetraploid cotton Gossypiumhirsutum, Gossypiumbarbadense 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 3320225 to position 58206145, chromosome 11 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.

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.

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.

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