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Synteny of Cotton SSR Markers Genomes Paves the Way for Resistance Against Black Root Rot Disease in Cotton

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Abstract

Background: Black root rot disease is documented for substantial reducing cotton yield and fiber quality. 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. In this study, by exploiting Phytozome database, a comparative map of the black root rot-resistance quantitative trait loci in DD genome was constructed.

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 findings showed that there was conserved orders among mapped simple sequence repeats markers on AA genome and the physical map of these simple sequence repeats markers on DD genome.

Conclusion: It was suggested that the syntenic loci on chromosome 2, 7 and 11 on DD genome could harbor the resistance gene against the black root rot disease. This study could serve as a fundamental step in isolating and introducing the resistance gene against black root rot into elite cotton cultivars.

Introduction

Diseases exhibit an adverse impact on cotton (*Gossypium* spp.) production. The yield loss is projected at approximately 60% of the annual potential production [1,2]. Black root rot (BRR) is a seedling disease caused by *Thielaviopsis basicola*, a soil-borne pathogen fungal with a broad infection spectrum of crops. Since its first reported case on cotton in Arizona in 1922 [3], it has become one of the significant threats in cotton industry.

Because of the susceptibility to BRR of the two commercially important tetraploid cotton genome AADD species *G. barbadense* and *G. hirsutum*, tremendous efforts have been made toward developing BRR resistance germplasm. However, BRR partial resistance has only been demonstrated in several studies conducted in uncommercial AA genome specie *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, three quantitative trait loci (QTL) BRR5.1, BRR9.1, BRR13.1 were demonstrated to improve BRR resistance [6]. Nevertheless, there is a limited number of reports on how cotton DD genome, which is the progenitor of the cotton genome AADD species, confer BRR tolerance.

The importance of comparative mapping is the establishment of the syntenic relationships between genomes from different species [7-9]. 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* [10-14] 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 [15]. 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].

The purpose of this study is to physically map the published SSRs from three QTL conferring BRR resistance on AA genome to DD genome in cotton. By utilizing CottonGen and Phytozome database, our findings suggested that there was a correlation between the genetic map in AA genome and physical map in DD genome. A comparative map was constructed, illustrating the conserved order of SSR markers from the genetic mapping results in diploid AA genome and in DD genome [6]. These results will shed new lights in understanding of shared synteny of QTL conferring black root rot disease between two diploid genomes in cotton, which could also pave the way to isolate the resistance gene against BRR in DD genome.

Methods

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 [17]. CottonGen contains information on genomic, genetics, breeding, and molecular genetic markers. It also incorporates genomic sequences of different cotton genomes, markers, and traits. Additionally, various platforms such as BLAST, JBrowse, MapViewer, Primer3 are also included on the website.

Retrieving AA genome-derived SSR markers sequence

1. Go to the CottonGen website (https://www.cottongen.org/). Along the Tools Quick Start, go to 'Search Markers' (Figure 1).
2. 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.* [6], page 1318, Figure 3.
3. In the 'Marker Type' section, click on 'SSR'. Then hit 'Search'.
4. 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.

Phytozome

Since its development from 2008, Phytozome has become a connective platform for much research on plant genome. Besides its easily and friendly accessible database, which contains 25 plant genomes including cotton, Phytozome is also equipped with tools for comparative analysis so that scientists can compare every plant genes at the various level of sequences [18].

Localizing AA genome derived SSR markers to DD genome

1. Go the Phytozome website (https://phytozome.jgi.doe.gov/pz/portal.html#). Along the top menu header, go to 'Species' and choose '*Gossypium raimondii* v2.1' (Figure 4).

2. In the new resulting page, along the menu under the title '*Gossypium raimondii* v2.1 (Cotton), click on 'BLAST search' (Figure 5).
3. 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'.
4. 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 1: The CottonGen website entry display

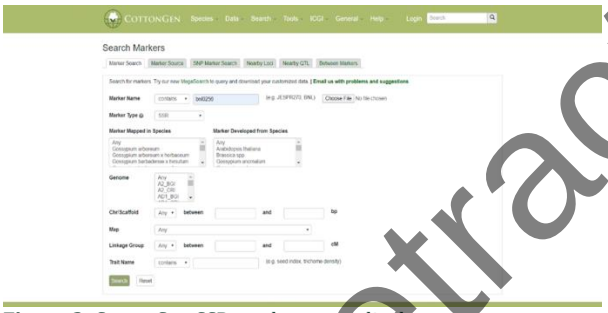

Figure 2: CottonGen SSR marker entry display

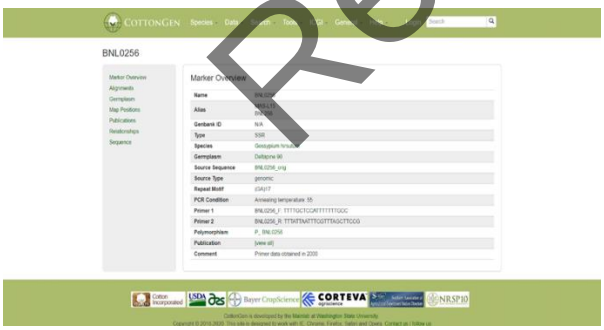

Figure 3: CottonGen representation of the selected SSR marker

Figure 4: Phytozome website entry display

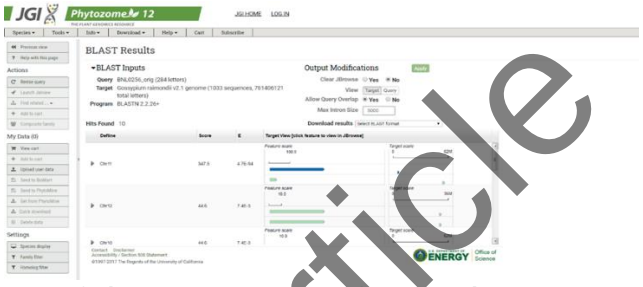

Figure 5: Phytozome representation of BLAST results

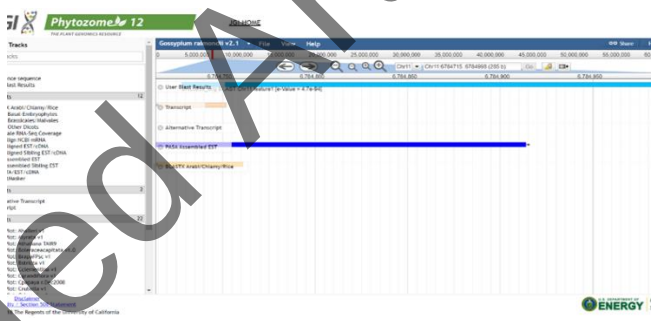

Figure 6: Phytozome representation of chromosomal position of SSR marker

Results

We showed here that after anchoring the SSR markers from the results of Niu *et al.* [6] 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 A9. The same observation was also portrayed in study by Rong *et al.*, [19]. 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 [19]. 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 [20]. Overall, this result confirmed the accuracy of the genetic map in previous study [6].

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
LGA9	NAU0921	MGHES27	MGHES27	11	5509175	5509752
	MGHES41	TMA18	BNL0256		6784715	6784998
	BNL3895	BNL0256	NAU1041		9892984	9898779
	NAU1041	NAU1041	MGHES41		11011950	11014308
	BNL0256	BNL3895	NAU0921		17797360	17798057
	TMA18	MGHES41	BNL3895		23404926	23405317
	MGHES27	NAU0921	TMA18		57111815	57112593
LGA13	BNL3442	BNL3442	BNL3442	7	3320225	3320674
	BNL1034	BNL1034	BNL1034		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
LGA5	BNL1683	CIR114	BNL3580	2	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
	BNL3090	CIR089	CIR089		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

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

Discussion

Evolutionary evidence has suggested that from the origin of a common ancestor, diploid cotton species continued evolving and subsequently dividing into eight current monophyletic groups denoted as A–G, and *K*. A hybridization occurred approximately 1 to 2 million years ago between two diploid cotton species (2n = 2x = 26): *G. raimondii* (D_5) and *G. arboreum* (A_2) or *G. herbaceum* (A_1). This event introduced the emergence of allotetraploid species (2n = 4x = 52) [21,22]. After undergoing the polyploidization and following independent evolution processes, these tetraploid cottons differentiate into six present tetraploid species including *G. hirsutum* $(AD)_1$, *G. barbadense* $(AD)_2$, *G.tomentosum* $(AD)_3$, *G. mustelinum* $(AD)_4$, *G. darwinii* $(AD)_5$ and *G. ekmanianum* $(AD)_6$ [23].

Owing to its information, versatility and easy detection in genetic experiments, SSR markers have been widely employed in QTL mapping and saturating in many plant genomes [24,25]. In cotton, mountain of evidence has been gathered in data mining to discover and characterize new SSR marker to narrow down the QTL regions. The ultimate purpose of this process is to isolate the candidate genes responsible for desired agricultural traits, including disease tolerance [24,26-29]. However, the susceptibility to BRR of two commercial tetraploid cotton species *Gossypium hirsutum*, *Gossypium barbadense* or crosses generated from these two species with other tetraploid species have hindered the development cotton cultivars conferring resistance to this disease. As a result, researches mainly focused on elaborating how cotton diploid genomes contribute to improving BRR resistance.

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. 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.

Conclusively, our study revealed that three QTL regions conferring BRR resistance in AA genome exhibited a significant collinearity with DD genome. While the orders of SSR markers on linkage group A13 on AA genome and on its counterpart region of DD genome are conserved, there were some minor inversions among of SSR markers on linkage group A7 and A5 on two genomic regions 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 resistance genes against BRR in DD genome in the future.

Author Contributions

All authors of this study participated in experimentation, data mining, analysis and drafting of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgment

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