QTL Mapping of Web Blotch Resistance in Peanut by High-throughput Genome-wide Sequencing

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Abstract

Background: Web blotch is one of the most important foliar diseases worldwide in peanut (*Arachis hypogaea* L.). The identification of quantitative trait loci (QTLs) for peanut web blotch resistance represents the basis for gene mining and the application of molecular breeding technologies.

Results: In this study, a peanut recombinant inbred line (RIL) population was used to map QTLs for web blotch resistance by high-throughput genome-wide sequencing. Frequency distribution of disease grade and disease index in five environments indicated wide phenotypic variation in response to web blotch among RILs. A high-density genetic map was constructed, containing 3,634 bin markers distributed on 20 peanut linkage groups (LGs) with an average genetic distance of 0.5 cM. In total, eight QTLs were detected for peanut web blotch resistance in at least three environments, explaining from 2.8 to 15.1% of phenotypic variance. The two major QTLs *qWBRA04* and *qWBRA14* were detected in all five environments and were linked to 41 candidate genes encoding nucleotide-binding site leucine-rich repeat (NBS-LRR) or other proteins related to disease resistances.

Conclusions: The results of this study provide a basis for breeding peanut cultivars with web blotch resistance.

Background

Peanut web blotch, also called muddy spot or net blotch [1], caused by Phoma araehidieola Marasas, Pauer & Boerema [2], is one of the most important foliar diseases in peanut (Arachis hypogaea L.). Peanut web blotch was first reported in Texas (U.S) in 1970s and then, in the early 1980s, it was found in the major peanut growing areas of Shandong and Liaoning Provinces of China [3, 4]. Subsequently, it was discovered in Shanxi Province [5] and Henan Province [6]. The web blotch disease can occur during the whole peanut growing period and can cause significant yield losses, usually around 10%~20% but with peaks of more than 50%. Therefore, it has been regarded as one of the most urgent issues to be addressed in some peanut growing areas [7, 8].

Although the web blotch disease has considerable economic importance in peanut farming systems, previous researches on this disease are relatively few compared with those on other main peanut foliar diseases. Zhang et al. (2019) presented the draft genome sequence of a Phoma araehidieola isolate named Wb2 and indicated that the draft genome of Wb2 was about 34.11 Mb and contained 37,330 open reading frames (ORFs), with G + C content 49.23% [9]. Smith et al. (1979) reported that Virginia and runner market-type peanut cultivars are more resistant to web blotch than the Spanish market-type cultivars [10]. Zhang et al. (2011) showed that resistance to web blotch was controlled by three major genes and several minor genes, and identified one quantitative trait locus (QTL) located on linkage group 7 [11]. At present, researches on this disease mainly focus on the classification status of the pathogen asexual generation, the pathogen molecular biology, chemical control and epidemic rules [12–16].

With advances in genomic sequencing technologies and the availability of diploid and tetraploid genome assemblies [17, 18], high-throughput genome-wide sequencing has become a primary strategy in peanut to identify single nucleotide polymorphisms (SNP) markers linked to resistance genes and QTLs. For example, four bacterial wilt resistance QTLs were identified on chromosome B02 using a RIL population and 2,187 SNP markers [19]. A major QTL for resistance to late leaf spot, on chromosome B05, and two major QTLs for resistance to early leaf spot, on chromosomes A03 and B04, were mapped using a high-density genetic map comprising 2,753 SNP markers and a F_9 RIL population of 192 individual lines [20]. Additionally, 62 QTLs for 14 yield-related traits were detected on 12 chromosomes across three environments using a high-density genetic map including 2,636 recombination bin markers and a F_6 RIL population of 242 lines [21].

In the present study, high-throughput genome-wide sequencing technology was used to obtain SNP markers, and a SNP-based genetic linkage map was constructed to identify the QTLs for resistance to peanut web blotch. The results of this study will help to better understand the mechanisms of interaction between P. araehidieola and peanut and to develop resistant cultivars.

Results

Phenotypic analysis

Wide phenotypic variation in response to peanut web blotch was observed among RILs under all the five environments tested in this study (Fig. 1). The distribution of the disease scale recorded during the years 2007 and 2008 was shown in Fig. 1A, whereas the distribution of the disease index recorded during the years 2012 and 2018 (both in the field and indoor) was shown in Fig. 1B-D.

Sequencing, SNP and bin markers discovery

A whole-genome resequencing strategy was applied to construct paired-end libraries for the parental lines and their 212 RIL progenies. The length of DNA fragments in the libraries was about 350bp. Approximately, 490 Gb of clean data (Q20>96%) were produced, resulting from 6,285 million reads, each with a length of 150 bp. In total, 600 million reads were generated for each of the two parents, whereas reads generated for each of the RILs varied from 22.37 to 24.83 million (Supplementary Table S1). Coverage rate, mapped reads rate, sequence depth and other results from alignment to the reference genome are shown in Supplementary Table S1. In particular, the coverage rate associated with the two parents Zheng8903 and Yuhua4 were 98.51% and 99.05%, respectively, whereas it ranged from 53% to 63.63% in the RIL population (Table S1). The sequencing depth was 35.23× for both parents, whereas it ranged from 1.31× to 1.46× for the RIL population. Originally, 636,831 SNPs were called from the 214 samples using the GATK protocol. Then, 556,615 SNPs were retained after filtering for low quality loci in the two parents, due to missing values, heterozygosis, depth < 10 and GQ < 20. Finally, 138,039 SNPs which were homozygous and polymorphic between two parents were used for further analyses.

Construction of physical recombination maps and high density genetic linkage map

To avoid errors caused by low coverage associated with RIL sequencing, a sliding window with 15 consecutive SNPs was used to find the more accurate recombined breakpoints. The physical recombination map of 212 RILs was constructed based on the recombination map of each progeny (Supplementary Figure S1). After that, all chromosomes of the 212 RILs were aligned and compared for the minimal of 100-kb intervals. As a result, a total of 3,634 bin markers for the 212 lines were obtained in this way, and the genotypes and physical locations of the bins are given in Supplemental Table S2.

The obtained 3,634 bin markers were used to construct a genetic linkage map by the software JoinMap®v5.0 [22]. Twenty linkage groups were generated and assigned to the 20 chromosomes of the cultivated peanut according to the physical positions. The total genome length was 1,817.91 cM and the marker density across the 20 linkage groups ranged from 0.39 to 0.66 cM with an average of 0.50 cM (Table 1). The LG16 had the lowest marker number (129) and the lowest genetic length (54.58 cM), while LG3 had the highest marker number (277) and the highest genetic length (135.61 cM) (Table 1 and Fig. 2). More than 97.5% of the inter-markers distance was lower than 3 cM. The highest inter-marker distance (16.06 cM) was associated with LG16 (Table 1).

QTL mapping and candidate genes prediction for peanut web blotch resistance

QTL mapping of peanut web blotch resistance was performed with MapQTL® v6.0 [23], using phenotypic data collected across five environments. Eight QTLs associated with peanut web blotch resistance, located in eight different LGs, were confirmed in at least two environments, explaining from 2.8 to 15.1% of phenotypic variation and displaying LOD values ranging from 1.32 to 7.45 (Table 2). Two QTLs (*qWBRA04* and *qWBRA14*) located on LG04 and LG14 were significantly associated with resistance in all the five testing environments in this study (Table 2, Fig. 3 and Fig. 4) and explained more than 10% of phenotypic variation, indicating they are probably the major QTLs with stable expression. Except for *qWBRA13* and *qWBRA05*, which were detected in four and two environments respectively, the other four QTLs *qWBRA03*, *qWBRA16*, *qWBRA17*, *qWBRA19* were detected in three environments (Table 2). Absolute values displayed by the additive effect parameter ranged from 0.11 to 8.29, and were negative for the QTLs on LG4, LG5, LG13, LG14, LG19 (indicating that the favorable allele originates from the resistant parent Zheng8903), and positive for the QTLs on LG3, LG16 and LG17 (indicating that the favorable allele originates from the susceptible parent Yuhua4).

To identify candidate genes for peanut web blotch resistance, coding sequences in the genomic region associated with the QTLs *qWBRA04* and *qWBRA14* were examined for predicted function, according to the *Arachis hypogaea* cv. Tifrunner reference genome annotation database [18]. A total of 41 candidate genes were identified with a putative role in disease resistance (Table 3). In detail, the region of *qWBRA04*, spanning a linkage interval of 1.10 cM, corresponds to a physical interval of ~86kb and contains four nucleotide binding site-leucine rich repeat (NBS-LRR) genes. The genes *Arahy.Q7VTCQ* and *Arahy.9YX67Z* contain coiled-coil (CC) domains and the genes *Arahy.SK6LYR* and *Arahy.1RZ0PJ* contain Toll-interleukin receptor (TIR) domain (Table 3). The region of *qWBRA14*, spanning 0.48 cM, physically corresponds to ~2.8 Mb and contains 37 genes encoding disease resistance protein. Among them, 19 genes contain TIR

domains and one gene contains a CC domain, whereas the remaining 17 genes encode other proteins with a putative role in disease resistance, such as a *bZIP* transcription factor and a *WRKY* transcription factor-like protein.

Discussion

A high density genetic linkage map with 3,634 bin markers was constructed based on high-throughput whole-genome sequencing and a sliding window strategy. The genetic map was overall consistent with the peanut physical genome assembly, except for some translocation occurring between LG3 and LG13 and between LG6 and LG16. A total of 277 bin markers were distributed on LG3, while the physical positions of the last 70 markers were on chromosome A13. And there were 47 markers originating from chromosome A03 were mapped on the tail of LG13. Moreover, 43 bin markers from chromosome A16 were inserted in the LG6. The reason for such seeming translocation might be that there were some assembly errors in the reference genome which were illustrated in Peanutbase (https://www.peanutbase.org/peanut_genome _v1 _v2) [24]. The genetic order of the markers on LG1, LG4, LG5, LG10, LG12 and LG18 was fully consistent with their physical orders and a few markers on the other LGs were inversed with the adjacent markers.

In total, eight novel QTLs distributed on eight chromosomes were identified for peanut web blotch resistance, which were confirmed in at least two of the experimental trials. Three of them were major QTLs, as they were associated with PVE > 10%, whereas the other five were minor QTLs, in accordance with the predicted results of Zhang et al. (2011). Except for *qWBRA03*, which was detected only in naturally conditions, the other seven QTLs were detected both in the natural and artificial inoculation conditions. The two major QTLs *qWBRA04* and *qWBRA14* were stably detected across all the five testing environments in this study, and thus might be of great potential in breeding resistant peanut varieties.

In the present study, we identified 56 candidate disease resistant genes in the target region of eight QTLs (Table 3 and 4), of which 41 were linked with the two major and stable QTLs *qWBRA04* and *qWBRA14* (Table 3), while the other 15 were associated with the other six QTL intervals (Table 4). The 41 candidate genes covered by the intervals of *qWBRA04* and *qWBRA14* included 21 TIR-NBS-LRR and 3 CC-NBS-LRR, which are the two well-known *R* gene types [25]. Also, there were two candidate genes encoded LRR and NB-ARC (nucleotide-binding domain shared with APAF-1, various R-proteins and CED-4) domain, which also encoded resistance genes [26]. The rest genes located in the target region encoding proteins contain disease resistance response related domains but could not assigned to the well-known R-gene types for eight of them, and the other seven with indirect functions for resistances or downstream of resistant signaling pathways (Table 3). Especially, the *qWBRA14* covers a region of ~2.8 Mb and contained 37 genes encoding disease resistance protein, so the two flanking markers can be used to develop breeder friendly marker KASP (competitive allele specific PCR) and validated using peanut germplasm. In the future study, we plan to further expand the population size to more precisely map the resistance-related QTLs and clone the resistant gene.

Conclusion

In this study, eight QTLs for peanut web blotch resistance were detected and two major QTLs qWBRA04 and qWBRA04 were linked to 41 candidate genes encoding nucleotide-binding site leucine-rich repeat (NBS-LRR) or other proteins related to disease resistance, which may shed some insights on understanding web blotch resistance and facilitate the development of resistant peanut cultivars.

Methods

Plant materials

A RIL population including 212 F_{12} lines derived from the parental lines Zheng8903 and Yuhua4 was used in this study. The RIL population was constructed by the Peanut Research Laboratory of Industrial Crops Research Institute, Henan Academy of Agricultural Sciences in 2004 [11]. The female parent Zheng8903 with the pedigree '79-266//71-31/Chico' is a breeding line showing high level of resistance to peanut web blotch, originating and preserving in our lab [27]. The male parent Yuhua4 is a variety released in 1991 by the Henan Academy of Agricultural Science [28], showing high level of susceptibility to web blotch [27].

Experimental trials and phenotyping

All the experimental trials to evaluate response to peanut web blotch were conducted at the research station of the Henan Academy of Agricultural Science from May to September. Plant materials mentioned above were evaluated following natural infection, in field trials carried out in 2007, 2008 and 2012, and following artificial inoculation, in field and indoor trials carried out in 2018. For field trails, twenty seeds for each genotype were sown in 3 m long and 0.4 m wide plots, according to a complete random block design with two replicates for natural infection and three replicates for artificial inoculation. For natural infection in the year 2007, 2008 and 2012, disease evaluation was carried out before harvest according to the 0-4 scale described in Yuan *et al*. (2004) [8]. For field inoculation in the year 2018, plots were sprayed with an inoculum of 1.6×10^{-3} g/ml at the flowering stage [29] and disease evaluation was carried out 20 days after inoculation according to the 0-9 scale described in Yu (2011) [30]. For indoor inoculation, five plants were inoculated for each line with two replications at the 6 leaves stage. The inoculum concentration was 2×10^{6} conidia/ml and the preparation of conidia suspension described by Zhang (2019) [27] was followed. Two weeks after inoculation, 12 inoculated leaves at the main stem were collected and the lesion area for each was scanned by the Leaf Area Meter (Wanshen LA-S). The indoor classification standard of peanut web blotch was as follows: for scale 0, no lesion detected; scale 1, 0< lesion area≤6%; scale 3, 6%≤lesion area≤25%; scale 5, 25%≤lesion area≤50%; scale 7, 50%≤lesion area≤75%; scale 9, 75%≤lesion area.

Sequencing and genotyping

Genomic DNA of the parents and 212 RILs was extracted from young leaf tissues using the Plant genome DNA extraction kit (TIANGEN) and randomly sheared by sonication. The DNA fragments with the length of 300 bp were recovered by electrophoresis. After ligating DNA fragments with adapters, libraries were paired-end sequenced using the Illumina Hi-seq platform with read length of 150 bp.

After trimming adapters and low quality reads, clean data was used for alignment to the reference genome, allowing SNP identification and genotyping. In detail, the assembly of *Arachis hypogaea* cv. Tifrunner was used as the reference genome [18]. The aln command in the software bwa-0.7.10 was used to align clean data to the reference genome, and unique reads were used for subsequent SNP variation detection by the software GATK3.3.0. The obtained SNP sets of two parents were filtered used the missing values, heterozygosis, depth and GQ value and the homozygous and polymorphic loci were used for the RIL population. The binary alignment mapping (BAM) files obtained in this study have been submitted to the BioProject database at NCBI under the BioProject ID: PRJNA602098.

Linkage map construction and QTL mapping

As the accuracy of call at single SNP loci was low, due to the low coverage chosen for RIL sequencing, a sliding window approach was applied to evaluate a group of consecutive SNPs for genotyping [31]. The genotypes of all chromosomes of the 212 RILs were aligned and compared for the minimal of 100-kb intervals and the adjacent 100-kb intervals with the same genotype across the entire RIL population were recognized as a single recombination bin [31]. From bin markers, linkage groups (LGs) were constructed using JoinMap v5.0 [22], selecting LOD scores from 2 to 10 to identify groups and the regression algorithm to perform ordering within each LG. The final linkage map was drawn using the R package LinkageMapView. QTL mapping was performed using MapQTL v6.0 [23], by selecting multiple QTL mapping (MQM) to detect potential QTLs with the LOD threshold of 2.5in at least one environment.

Abbreviations

NBS-LRR:nucleotide-binding site leucine-rich repeat; ORFs:open reading frames; qWBR:qtl web blotch resistance; WRKY:amino acid domain; KASP:competitive allele specific PCR

Declarations

Acknowledgments

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Authors' contributions

HL and ZS performed the laboratory and field experiments, and wrote the manuscript. LQ, FQ, PD, JX, and ZZ performed the genotype analysis and QTLs analysis. ZW, SL, and MG provide help in field inoculation. LZ and YC provide help in indoor inoculation. SH, BH, ZZ, and WD provide help to design the experiments

and revise the first draft of the paper. XZ conceived and designed the experiments, facilitated the project, and assisted in manuscript preparation. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in the manuscript and its Additional file 1, Additional file 2, and Additional file 3. The BAM files about resequencing data of RILs obtained in this study have been submitted to the BioProject database at NCBI under the BioProject ID: PRJNA602098. The materials used during the current study are available from the corresponding authors.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Supplementary Files Legend

Addition file 1: Table S1. The summary information of the sequencing and alignment results.

Addition file 2: Figure S1. The physical recombination map of 212 RILs on 20 chromosomes. Blue: the genotype of the resistant parent Zheng8903; Red: the genotype of the susceptible parent Yuhua4; Yellow: the heterozygous genotype; RILs were arranged from the top to the bottom and chromosomes were ranked from left to right.

Addition file 3: Table S2. Genotype and position of the bins in the peanut genetic linkage map obtained in this study. The letter A indicates the genotype of the parental line Zheng8903, and the letter B indicates the genotype of the parental line Yuhua4.

Tables

Due to technical limitations, Tables 1 - 4 are only available for download from the Supplementary Files section.

Figures

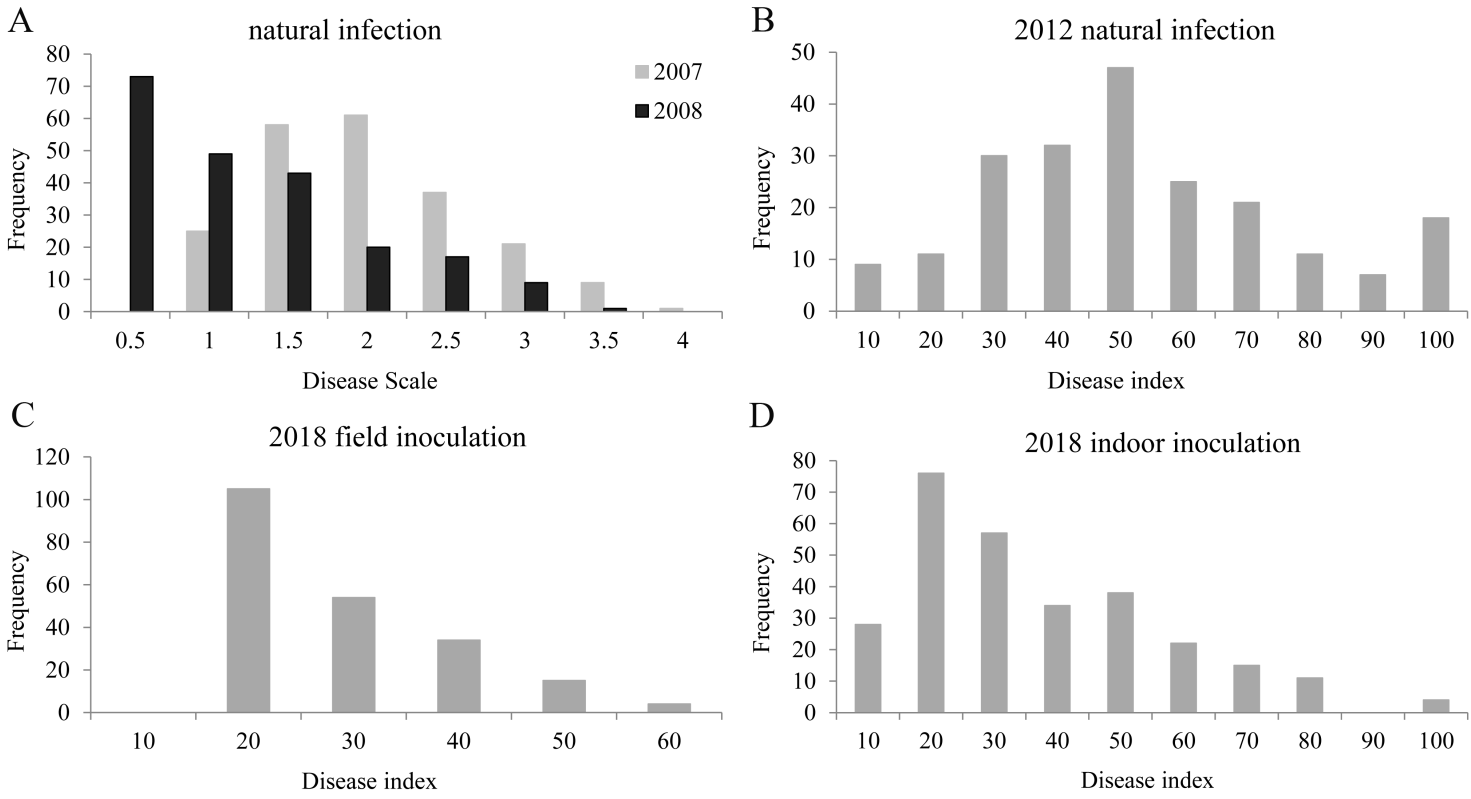

Figure 1

Response of RILs to peanut web blotch in five experimental trials.

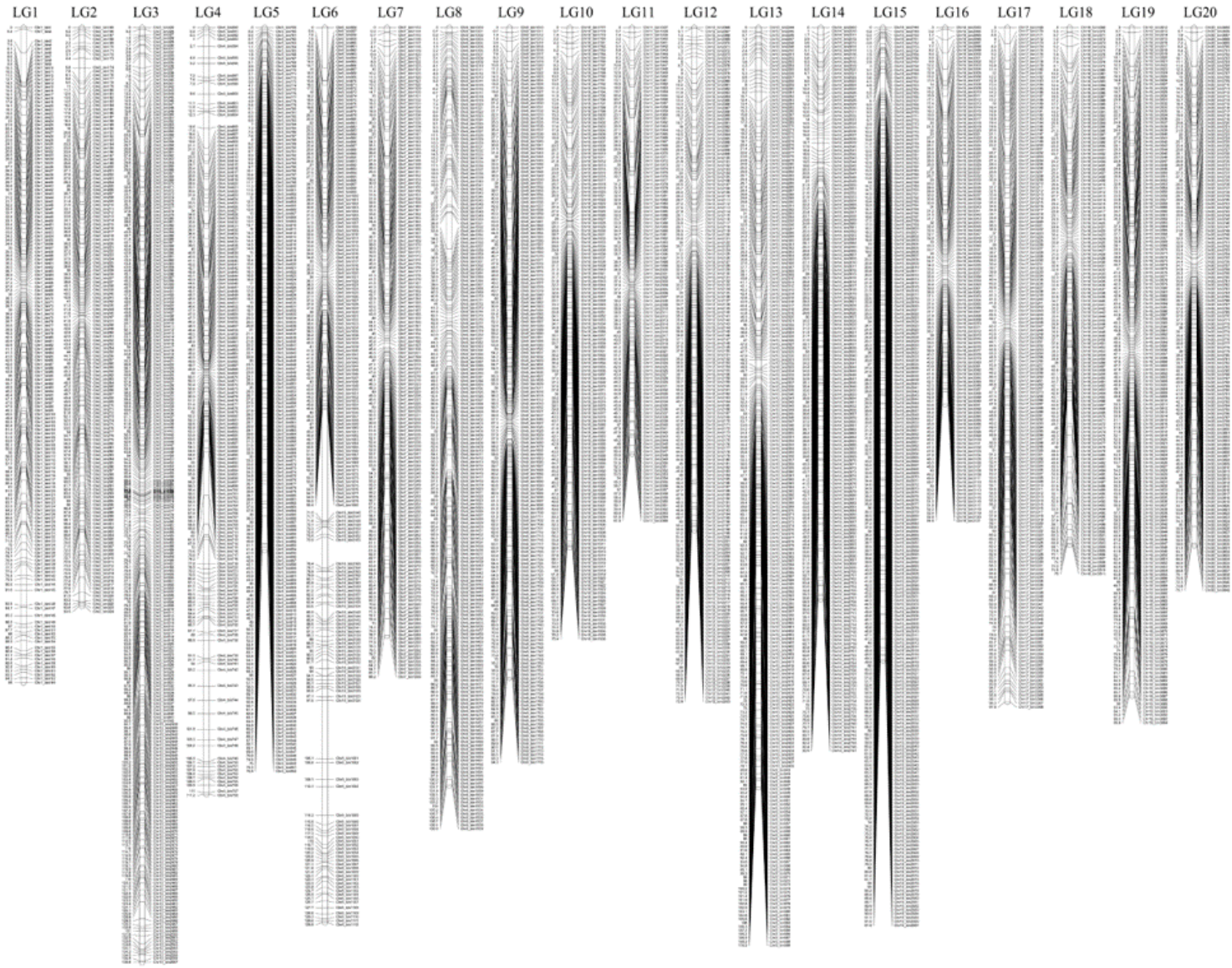

Figure 2

Genetic map obtained from the Zheng8903 × Yuhua4 RIL population.

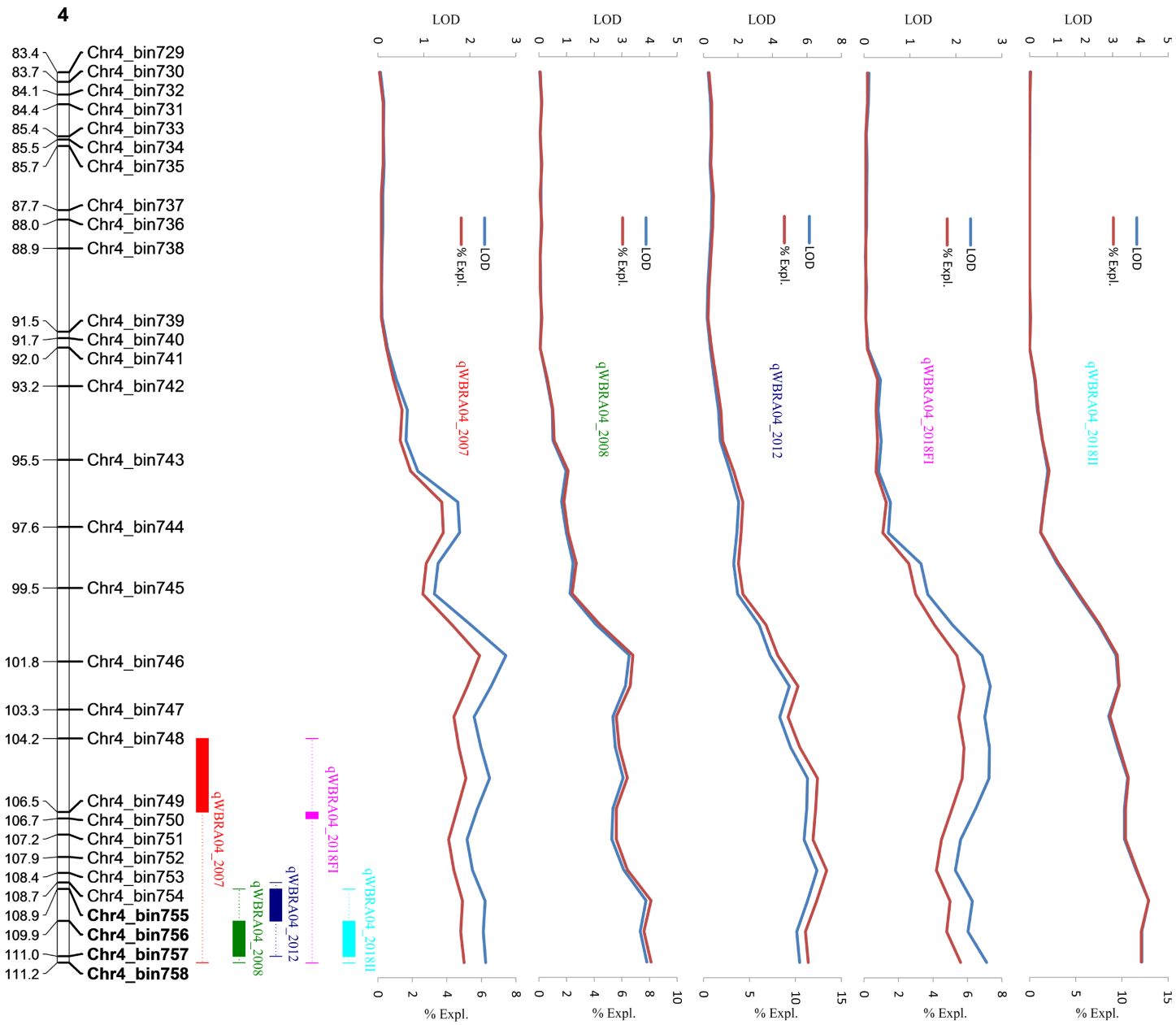

Figure 3

Position, LOD and PVE (%Expl.) curves of the QTL detected on LG4.

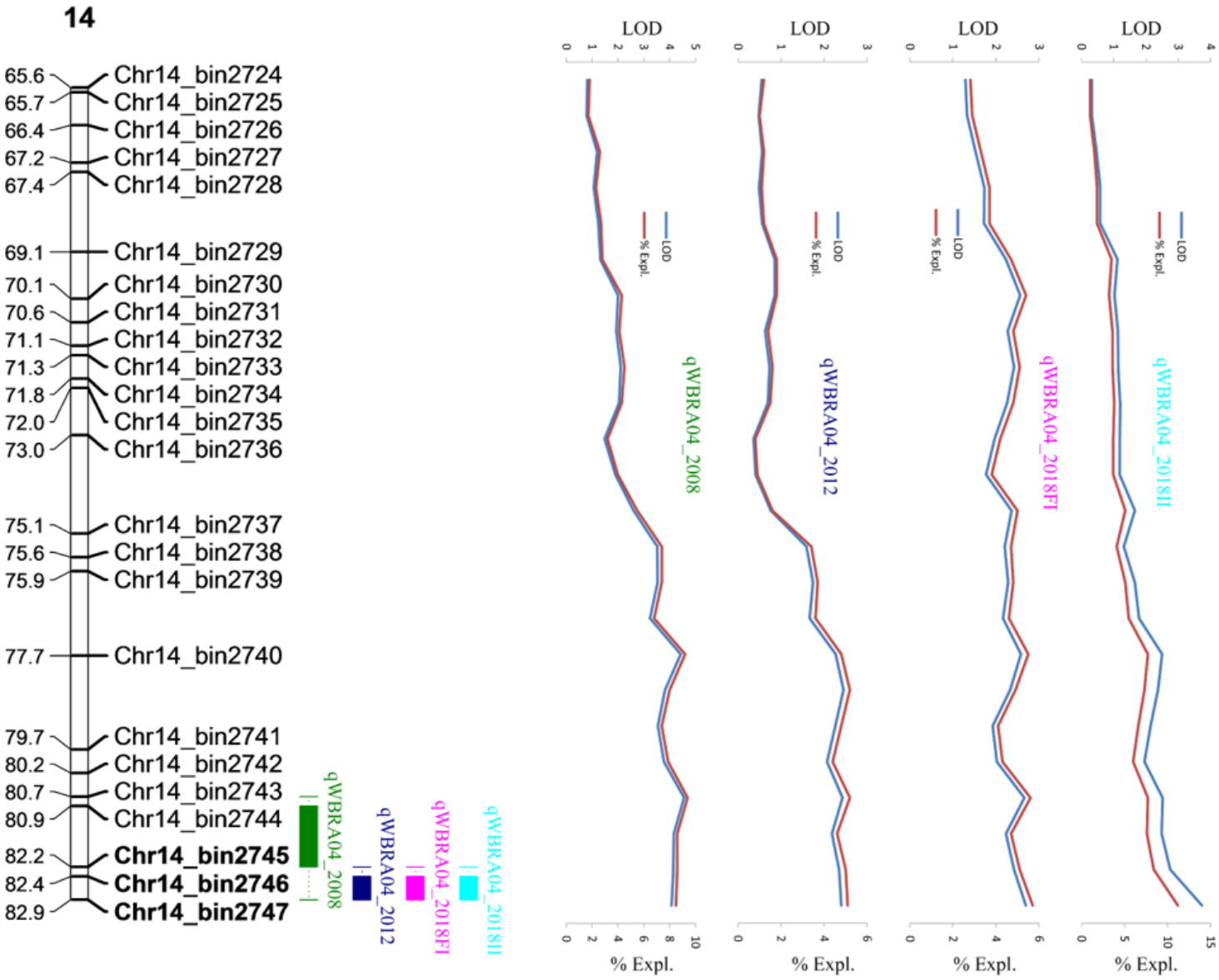

Figure 4

Position, LOD and PVE (%Expl.) curves of the QTL detected on LG14.

Supplementary Files

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- SupplementaryTable2SP.xlsx
- Tables14.docx
- SupplementaryTable1.xlsx
- SupplementaryFigure1SP.docx