Fine mapping of a Phytophthora-resistance locus RpsGZ in soybean using Genotyping-by-Sequencing

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Abstract

Background Phytophthora root rot (PRR), caused by *Phytophthora sojae* is one of the most important soil-borne diseases in many soybean-production regions in the world. Identification of resistant gene(s) and incorporating them into elite varieties are an effective way for breeding to prevent soybean from being harmed by this disease. A valuable mapping population of 228 F8:11 recombinant inbred lines (RILs) derived from a cross of resistant cultivar Guizao1 and susceptible cultivar BRSMG68 and a high-density genetic linkage map with an average distance of 0.81 centimorgan (cM) between adjacent bin markers in this population were used to map and explore the candidate genes. **Results** In this study, the PRR resistance in Guizao1 was controlled by a single Mendelian locus, and was fine mapped to a 460.702-kb genomic region on chromosome 3 that harbours 20 genes, including 8 disease resistance (R)-like genes in the reference Williams 82 genome. These 8 candidate genes potentially involved in programmed cell death, cell death, apoptosis and ADP binding by adopting Gene Ontology (GO) enrichment analysis. **Conclusions** These findings of fine mapping of a novel *Rps* locus will serve as a basis for cloning, transferring of resistant genes and breeding of *P. sojae* resistant soybean cultivars through marker-assisted selection.

Background

Phytophthora root rot (PRR), caused by *Phytophthora Sojae*, is one of the most important soil-borne diseases in many soybean-production regions in the world, causes significant soybean production losses [1].

The soybean resistance to *P. sojae* is mainly controlled by two mechanisms, namely, complete or partial resistance genes [2, 3]. The former is related to a single dominant gene resistance [4–19] in which *P. sojae* interacts with *Rps* genes in a gene-for-gene system preventing disease development in plants [20], and the latter involves multiple genes and limits damage to the plant [3, 21].

To our knowledge, more than 24 *Rps* genes/alleles on 9 different soybean chromosomes had been identified and mapped, among which *Rps1* (containing five alleles *Rps1a*, *Rps1b*, *Rps1c*, *Rps1d* and *Rps1k*), *Rps7*, *Rps9*, *RpsYu25*, *RpsYD29*, *RpsUN1*, *RpsHN*, *RpsHC18* and an unnamed *Rps* gene in soybean Wascshiroge and E00003 were mapped on chromosome 3 [7, 10, 14, 22–30]. *Rps3* (containing three alleles *Rps3a*, *Rps3b* and *Rps3c*) and *RpsSN10* were on chromosome 13, which was linked with *Rps8* [6, 22, 31, 32]. *Rps2* and *RpsUN2* were found on chromosome 16 [22, 28]. Additionally, *Rps4*, *Rps5*, *Rps6*, *Rps12* and *RpsJS* were located on chromosome 18 [16, 19, 22, 33, 34], *Rps8*, *RpsYB30*, *RpsZS18*, *RpsSu*, *Rps10* and *Rps11* were located on chromosome 8, 19, 2, 10, 17 and 7, respectively [6, 8, 18, 35–38]. Among the identified *Rps* genes on chromosome 3, *Rps1k* was mapped to a 125-kb region and cloned, and had an NBS-LRR structure that was typical of resistance protein [24, 39]. *RpsYD29* was mapped to a 204.8-kb region, and two nucleotide-binding site and leucine-rich repeat (NBS-LRR) type genes *Glyma03g04030.1* and *Glyma03g04080.1* were identified [27]. Moreover, *RpsQ* was fine mapped to a 118-kb region [13].

Recently, along with the progress of massively parallel DNA sequencing platforms, whole-genome sequencing (WGS) has become primary strategy for next-generation sequencing (NGS) technologies in SNP discovery and genotyping large populations. These methods include re-sequencing, genotyping by-sequencing (GBS) [40], specific length amplified fragment sequencing (SLAF-seq) [41], restriction site-associated DNA tag sequencing (RAD-seq) [42], and 2b-RAD [43]. NGS technologies have been widely utilized in soybean, wheat, sunflower and other crops to develop SNP markers and for mapping resistance gene/QTL [35, 44–48]. A dominant soybean phytophthora root rot resistance gene was mapped using a high-density soybean genetic map, comprising 3469 recombination bin markers using RAD-seq technology on 196 F_{7:8} RILs [49].

In this study, we found that the cultivar Guizao1 had a broad-spectrum resistance, which may carry some *Rps* genes or alleles. The objectives of our project were to characterize the inheritance of the *Rps* gene(s) and fine-map the candidate gene(s) in the resistant cultivar Guizao1 using a high-density genetic linkage map, comprising 3715 recombination bin markers using RAD-seq technology on 228 F₈ RILs derived from a cross of Guizao1 × BRSMG68.

Result

Phenotype reaction of the parents to *P. sojae* isolates

To investigate the phenotype of Guizao1 and BRSMG68, six isolates of *P. sojae* were used to test the reactions on the soybean genetic differentials (Table 1). The inoculation results showed that BRSMG68 shared the same reaction for SSSSSS with Williams, indicating that BRSMG68 did not contain disease-resistant genes. Guizao1 (RSRSSS), Chapman (RRRRSR), L85–3059 (RSRSSR) and Harosoy (RSSSSS) were PRR-resistant cultivars to PNJ4 strain, while other varieties were PRR-susceptible cultivars to PNJ4 strain (Table 1). Furthermore, Guizao1 was also PRR-resistant to PNJ1 strain but PRR-susceptible to Pm28, PNJ3, Pm14 and P6497 strains different from that of Chapman, L85–3059 and Harosoy. The inoculation results suggest that Guizao1 may contain new *Rps* genes or resistance locus.

Genetic analysis of resistance to *P. sojae* PNJ4

Among the 228 F_{8:11} RILs from the cross of Guizao1 × BRSMG68, 113 RILs were homozygous resistant and 115 RILs homozygous susceptible, with the segregation ratio fitting with the genotypic Mendelian ratio of 1R:1S ($\chi^2 = 0.004$, $P = 0.95$, Table 2). These results indicated that the resistance gene in Guizao1 was controlled by a single locus, temporarily designated as *RpsGZ*.

Fine mapping RpsGZ by high-throughput genome-wide resequencing

Based on the high-density map constructed with bins as markers and using CIM with WinQTLCart for PMD resistance gene localization, only one PMD resistance locus was detected on chromosome 3 in Guizao1 (Fig. 1), with a LOD value of 88.3, which explained 81.8% of the phenotypic variance. In the high-

density linkage map, *RpsGZ* was placed in bin31 through six recombinant monoclonal lines (Fig. 2). This placed *RpsGZ* in a region between 4,022,530 and 4483231 bp on GlymaWm82.a1.v1.1, in appropriately 460,702 bp. A BLAST search showed 20 annotated genes based on this assembly (Table 3; <http://www.soybase.org>). The putative functions of these predicted genes were annotated by BLAST searches against the TAIR protein datasets, and 8 R-like genes (*Glyma03g04080*, *Glyma03g04100*, *Glyma03g04121*, *Glyma03g04140*, *Glyma03g04180*, *Glyma03g04200*, *Glyma03g04260* and *Glyma03g04300*) were found to contain Nucleotide-binding site (NBS) -leucine-rich repeat (LRR) domains, which are important for plant disease resistance genes.

Gene Ontology (GO) enrichment analysis of the candidate genes

The AgriGO toolkit was used to perform gene ontology (GO) analysis [50, 51]. Among the 20 genes in the region close to *RpsGZ* detected in this study, 13 genes were found to have at least one GO annotation. These genes were predicted to be involved in some biological processes and molecular function (Fig. 3; Additional file: Table S1), including programmed cell death, cell death, apoptosis, cellular process, ADP binding, ribonucleotide binding, adenyl nucleotide binding, purine ribonucleotide binding, ATP binding and nucleoside binding. In biological process, the GO terms such as “programmed cell death” and “apoptosis” were significantly enriched (Fig. 3a). In molecular function, the GO terms “ATP binding” and “ADP binding” were significantly enriched (Fig. 3b). The 8 R-like candidate genes potentially involved in programmed cell death, cell death, apoptosis and ADP binding. The results suggest that death signalling may provide a potential mechanism for protecting the host cell and inhibiting the spread of pathogen.

Discussion

Soybean is one of the most important crops in China. There are a large number of soybean accessions in China, of which many PRR resistance cultivars/lines were identified in previous study [10, 13, 14, 52–56]. In the present study, Guizao1 was a PRR-resistance cultivar to *P. sojae* PNJ4 and PNJ1, and distinct from that of other soybean cultivars tested (Table 1). Genetic analyses of the resistance to *P. sojae* PNJ4 in Guizao1 was controlled by a single locus.

To more fine map the PRR-resistance locus, *RpsGZ* was mapped in a population based on genotyping through resequencing, resulting in the integration of 54,002 SNPs into 3748 recombination bin units. These markers were then employed to construct a high-density bin linkage map with an average distance of 0.81 cM between adjacent markers. The map had well-distributed linkage distances and higher resolution than did the conventional map, and gene/QTL mapping was thus more accurate and reliable. The position of *RpsGZ* was refined through fine mapping to a 460,702 bp interval between 4,022,530 - 4,483,231 bp on chromosome 3, which was the rich regions of *Rps* gene.

Previous studies showed that 16 known *Rps* genes were identified and mapped to chromosome 3 before *RpsGZ*, including five alleles of *Rps1* (*Rps1a*, *1b*, *1c*, *1d*, *1k*) [23, 24, 39, 57, 58], *Rps7* [23], *RpsYu25* [25], *Rps9* [29], an *Rps* gene in Waseshiroge [26], *RpsYD29* [27], *RpsUN1* [59], an *Rps* gene in soybean E00003 within the *Rps1k* interval [30], *RpsWY* [49], *RpsHN14*, *RpsQ* [13], and *RpsHC18* [10]. Nevertheless, the positional relationships of these *Rps* genes had not been confirmed, and a part of the mapping intervals for these *Rps* genes overlapped. Therefore, whether these genes were allelic or at a new locus need to be confirmed.

In the present study, *RpsGZ* was a distinct gene from the *Rps1* alleles because five differentials carrying *Rps1* (*1a*, *1b*, *1c*, *1d* and *1k*) were PRR-susceptible to *P. sojae* PNJ4, though the candidate region of *RpsGZ* partly overlaps with the regions of the *Rps1* and *Rps1k*. Cultivar Wayao (*RpsWY*) was susceptible to *P. sojae* PNJ4 and Guizao1 was resistant to *P. sojae* PNJ4 [49], and these two mapping parents had different resistance reaction, suggesting that *RpsGZ* may be different from *RpsWY*. When compared with the nucleotide position of *Rps* genes mapped to chromosome 3 (Table 4) according to the Glyma1.0 soybean gene annotation database (<http://soybase.org/>), the position information of *RpsGZ* suggests that *RpsGZ* is distinct from *Rps1a*, *Rps1b*, *Rps1c*, *Rps1d*, *Rps9*, *RpsQ*, *RpsYu25* and *RpsHC18*.

In addition, *Rps7* was mapped to a 15,457.9 kb genomic region (3,910,260–19,368,212 bp) flanked by SSR markers Satt009 and Satt125 [23]. The *Rps* gene in Waseshiroge was located between Satt009 and T003044871 and may reside in the nucleotide region between 3,910,260 and 4,486,048 bp of Williams 82 genome [26]. The *Rps* gene in cv. E00003 was positioned within interval 4,475,877 to 4,563,799 bp [30]. *RpsHN* was mapped to a 278.7 kb genomic region flanked by SSR markers SSRSOYN–25 and SSRSOYN–44 and may reside at the nucleotide position 4,227,863 and 4,506,526 bp [14]. *RpsUN1* was defined to the region at 4,001,862 - 4,362,217 bp flanked by the two SSR markers BARCSOYSSR_03_0233 and BARCSOYSSR_03_0246 [28]. *RpsGZ* and the *Rps7*, *RpsHN*, *RpsUN1*, and *Rps* gene from Waseshiroge or E00003 may be tightly linked genes, different alleles of the same gene, or identical alleles of the same gene. However, further confirmation is needed. Moreover, if the sources of resistance mentioned above carry different resistance genes, a pyramiding effect of different resistance genes may increase the resistance of soybean cultivars to *P. sojae*.

NBS-LRR genes are the vast family of plant disease resistance genes [60], and local tandem duplication of NBS genes has created many homogenous clustered loci identified in each legume genome studied [61]. Meziadi et al. suggested that NBS-LRR proteins were encoded by one of the largest and most variable multigene families and were often organized into complex clusters of tightly linked genes in plants [62]. In soybean, 319 putative NBS-LRR genes and 175 disease-resistance QTLs were found, with 36 NBS-LRR genes clustered on chromosome 3, and most of the NBS-LRR genes were located at the front end of chromosome 3 [63]. The 16 identified *Rps* genes were all mapped to the regions between 2,916,450 - 9,758,181 bp on chromosome 3. Among the 20 genes in the region close to *RpsGZ* detected in this study, 8 tightly linked candidate resistance genes contain a NBS-LRR domain. Most of the R-like genes had the same functional description, including iapoptosis, signal transduction, ADP binding, defense response, systemic acquired resistance, salicylic acid biosynthetic process and other biological

and metabolic processes, suggesting that these 8 R-like genes may be involved in defence mechanisms against this disease.

Conclusion

We identified and fine mapped a novel *Rps* gene *RpsGZ* that can provide a resistance to *P. sojae* *PNJ4* and *PNJ1* than other known *Rps* genes on chromosome 3. Eight R-like genes may be involved in disease-defense mechanisms. This study provides information regarding the genetic location of the *Rps* resistance locus, which is useful for breeders to apply marker-assisted selection (MAS) in soybean breeding programs to achieve resistance to *P. Sojae*.

Methods

Plant materials

The mapping populations of 228 F_{8:11} recombinant inbred lines (RILs) derived from Guizao1 (P₁, PRR resistance) × BRSMG68 (P₂, PRR susceptible), that were developed with the single seed descent method ADDIN NE.Ref.{676E32F9-D00B–40B2-B9C5-A4830E3C82B7}[64]. The soybean cv. Guizao1 was developed in Guangxi of China. BRSMG68 was introduced from Brazil. TheBoth the soybean cv. Guizao1 and BRSMG68 were obtained from the Guangdong Subcenter of National Center for Soybean Improvement, South China Agricultural University.

To determine which *Rps* gene or *Rps* gene combination was present in Guizao1, a differential set of 143 differential cultivars/genotypes were used. Each cultivar/genotype carries one known single *Rps* gene shown as: Harlon (*Rps1a*), Harosoy13XX (*Rps1b*), Williams79 (*Rps1c*), PI103091 (*Rps1d*), Williams82 (*Rps1k*), L76–988 (*Rps2*), L83–570 (*Rps3a*), PRX146–36 (*Rps3b*), PRX145–48 (*Rps3c*), L85–2352 (*Rps4*), L85–3059 (*Rps5*), Harosoy62XX (*Rps6*) and Harosoy (*Rps7*).. The variety Williams (no known *Rps* gene) was used as a susceptible check to indicate successful inoculation. *Fifteen individual seedlings of each cultivar were also injured using the method of injured hypocotylinoculation with three replications* ADDIN NE.Ref.{48739175–5C80–43CE–9812–7C5BFFDDC469}[25]. All the differential hosts for PRR identification were kindly provided by the National Center for Soybean Improvement, Nanjing Agricultural University.

P. sojae Isolates

Six *P. sojae* isolates (PNJ4, Pm14, Pm28, PNJ1, PNJ3, P6497), which were provided by Prof. Yuanchao Wang and Han Xing at Nanjing Agricultural University, were preserved on V8 juice agar medium (10% V8 vegetable juice, 0.02% CaCO₃ and 1.0% Bacto-agar) [12, 14]. These *P. sojae* isolates were used in the phenotype test of disease resistance to PRR among the Guizao1 and BRSMG68 and 13 differential

cultivars/genotypes. *P. sojae* PNJ4 strain (virulence formula is *1a, 1b, 1c, 1d, 1k, 2, 3b, 3c, 4, 6*) was used to evaluate the RILs population of Guizao1 × BRSMG68.

Evaluation of genetic materials for *Phytophthora* resistance

The method of injured hypocotyl inoculation with slight modification was utilized for disease evaluation in this experiment [12, 25]. For inoculation with *P. sojae*, 15 plants of each hosts for PRR identification, cultivars/genotypes and per line of the RILs population and parents were planted per replication in 11.8-cm-diameter plastic pots filled with vermiculite and kept in a illumination incubator (26/22 °C day/night temperature, 75% relative humidity, 12 h light/12 h dark photoperiod and the average light intensity of ~10000 Lx).

The evaluation test was set up with three biological experiments.

To test the phenotypes of the RILs population, the isolate PNJ4 was used to inoculate the parents of Guizao1 and BRSMG68, and 45 individual seedlings per RILs using the method of injured hypocotyl inoculation with slight modification ADDIN NE.Ref.{48739175–5C80–43CE–9812–7C5BFFDDC469}[25]. Briefly, Aafter sowing for seven days, the seedlings were inoculated. A uniform and thinner wound was cut in soybean cotyledon under section 1–2 cm by using single-sided blade sterilized with the outer flame of alcohol lamp. The active edge of the colonyfrom *P. sojae* isolates cultured in the incubator at 25 °C for five days was cut into about 3 mm² block and then embedded in the wound with mycelium surface inside. After inoculation, the seedlings were placed in the culture shelf surrounded by plastic film and kept moisture (90% relative humidity) at 25 °C for 24 hours with spraying steriled water, and then transferred to the illumination incubator (26/22 °C day/night temperature, 75% relative humidity, 12 h light/12 h dark photoperiod and the average light intensity of ~10000 Lx). The evaluation test was set up with three biological experiments. To test the phenotypes of the RILs population inoculated with the isolate PNJ4, the cv. Guizao1 and BRSMG68 were used as the resistance and susceptible checks, and Williams (*rps* gene) was used as the susceptible check to indicate successful inoculation. All the experiments were performed in 2017 and 2018 at South China Agricultural University.

Reactions of all materials were recorded as the percentage of dead seedlings at five days after inoculation. Cultivars for PRR identification were considered resistant if all the seedlings were alive with no expanded lesions. Cultivars were considered susceptible if all the seedlings were dead. The RIL families with 70–100% dead seedlings were considered to be homozygous susceptible (S) ones, while the RIL families with 0–30% dead seedlings were considered to be resistance (R) ones, and the RIL families with 31–69% dead seedlings were considered to be heterozygous resistant (Rs) [12].

Data analysis and candidate gene prediction

A goodness-of-fit to the Mendelian segregation ratio was calculated using Chi-square (X^2) analysis to examine the segregation patterns of the phenotypes.

The linkage map used in this study was constructed previously by Guangdong Subcenter of National Center for Soybean Improvement, South China Agricultural University. This map included 3748 bins and was 3031.9 cM in length, with an average distance of 0.81 cM between adjacent markers on 20 chromosomes. Composite interval mapping (CIM) was used to detect gene/QTL associated PRR resistance using phenotypic data via WinQTLCart 2.5 software (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>). The LOD thresholds for gene/QTL significance were determined by a test (1,000 replications) with a genome-wide scope at the 5% level of significance. Gene mapping results were compared to the SoyBase and the predicted genes in the target regions were analyzed according to the annotation soybean reference genome (Wm82.a1.v1.1) in Soybase (<http://www.soybase.org/>). Functional predictions of genes were manually confirmed by the blastp function in NCBI (<http://www.ebi.ac.uk/Tools/sss/ncbiblast/>) and TAIR protein datasets (<http://www.arabidopsis.org/>).

Availability of data and materials

The data sets supporting the results of this study are included in the manuscript. Soybean seeds are available from the Guangdong Subcenter of the National Center for Soybean Improvement, PR China.

Abbreviations

bp: Base pair; CIM: Composite interval mapping; cM: Centimorgan; cv.: cultivar; GO: Gene ontology; MAS: Molecular assisted selection; NGS: Next-generation sequencing; PPR: Phytophthora root rot; *P. sojae*: *Phytophthora sojae*; RIL: Recombinant inbred line; SNP: Single nucleotide polymorphism

Declarations

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The data sets supporting the results of this study are included in the manuscript. Soybean seeds are available from the Guangdong Subcenter of the National Center for Soybean Improvement, PR China.

Authors' contributions

BJ and YC performed the experiments and analysed the data. YC and ML generated the RILs populations. ZC provided guidance on the molecular analysis. ZJ, RM and YY conducted phenotypic screenings. QX analysed the data of genome-wide sequencing to produce the final linkage and bin maps. HN planned the research, supervised the project and prepared the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Figures

Figure 1

Results of RpsGZ locus analysis using CIM method in the F8:11 RILs. The LOD value distribution on the whole genome of the RIL population from a cross of Guizao1 × BRSMG68. RpsGZ was amplified at the site of bin31 on chromosome 3, which explained 81.8% of the phenotypic variance.

Figure 2

Fine mapping of the RpsGZ locus. Recombinant inbred lines with recombination near the RpsGZ locus were shown with blue and red bars representing homozygous genotypes from BRSMG68 and Guizao1, respectively. Line 120, 289 and Line 303 were PRR-susceptible plants (S). Line 77, 313 and 382 were PRR-resistant plants (R).

Figure 3

Gene ontology (GO) enrichment analysis of the candidate genes of RpsGZ locus. AgriGO (<http://bioinfo.cau.edu.cn/agriGO/>) was used to analyze the candidate genes of RpsGZ locus, and significantly enriched GO categories in biological process (a), and molecular function (b) were shown in orange and yellow boxes.

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