A giant chimeric NLR gene confers extreme broad-spectrum resistance to a plant pathogen

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Title: A giant chimeric NLR gene confers extreme broad-spectrum resistance to a plant pathogen

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Abstract:

Phytophthora root and stem rot (PRSR) caused by *Phytophthora sojae* is the most destructive soybean soil-borne disease worldwide. Discovery of genes conferring broad-spectrum resistance to the pathogen is an urgent need to prevent the outbreak of the disease. Here we show that soybean *Rps11* is a 27.7-kb nucleotide-binding site-leucine-rich repeat (NBS-LRR or NLR) gene conferring extreme broad-spectrum resistance to the pathogen. *Rps11* is located in a genomic region harboring a cluster of unusually large NLR genes belonging to a single evolutionary lineage that is distinct from all other lineages in the soybean genome, and was derived from rounds of intergenic and intragenic unequal recombination. Such recombination events have resulted in promoter fusion and expansion of the LRR domain that presumably explains such broadness of the resistance spectrum. The NLR gene cluster exhibits drastic structural diversification among phylogenetically representative varieties, including gene copy number variation ranging from five to 23 copies, and absence of allelic copies of *Rps11* (i.e., *rps11*) in any of the non-*Rps11*-donor varieties examined. Our study thus exemplifies innovative evolution of NLR genes and NLR gene clusters and will accelerate the deployment of *Rps11* for soybean protection.

Main

Phytophthora is a genus of plant-damaging oomycetes with more than 170 identified species¹, many of which are plant pathogens of considerable economic importance such as *Phytophthora infestans* that caused the Irish potato famine and subsequent diaspora², and *Phytophthora sojae* that is responsible for annual yield losses estimated at ~\$200 million in the United States and 1-2 billion worldwide^{3,4}. In general, plant diseases caused by Phytophthora are difficult to control

chemically⁵, thus deployment of resistant varieties is an effective and environmentally friendly strategy to manage Phytophthora diseases⁶. In recent decades, a few dozen resistance-to-*P. sojae* (Rps) loci/alleles have been mapped to a number of genomic regions enriched with NLR genes (soybase.org); nevertheless, many of those loci/alleles have become ineffective to newly evolved races/isolates of the pathogen⁷, and few Rps loci/alleles have been isolated and/or functionally validated. Therefore, effective introgression/pyramiding of specific Rps genes into elite soybean cultivars for sustained resistance remains challenging.

The *Rps11* locus alone is responsible for the extreme broad-spectrum resistance

We have recently identified an Rps locus, designated *Rps11*, in a soybean landrace, PI 594527, which carries resistance to 12 *P. sojae* races/isolates examined⁸ (Extended Data Fig. 1). Based on the responses of 209 F_{2:3} families derived from a cross between PI 594527 and a susceptible variety Williams to *P. sojae* race 1, *Rps11* was initially mapped to a 348-kb genomic region on chromosome 7⁸, with 12 sequencing gaps of unknown sizes according to the Williams 82 reference genome assembly v2.0 (soybase.org). Nevertheless, the initial mapping only indicates that *Rps11* confers resistance to race 1. To evaluate the *Rps11* resistance, we inoculated a subset of the F_{2:3} families, including 14 families of the *Rps11/Rps11* (PI 594527) genotype and 14 families of the *rps11/rps11* (Williams) genotype with 14 *P. sojae* races/isolates separately, and observed perfect genotype-phenotype associations (Supplemental Table1), suggesting that the *Rps11* locus alone is responsible for the broad spectrum of resistance carried by PI 594527. We then evaluated F₅ recombinant inbred lines (RIL) derived from several F₂ homozygotes (*Rps11/Rps11*) with 158 additional *P. sojae* isolates collected from soybean fields across Indiana and found that those RILs carry resistance to 127 (80%) isolates (Supplemental Table 2).

***Rps11* is pinpointed through gap-free sequence-based fine mapping with a sizable population**

Because the *rps11* region in Williams 82, which is identical to the corresponding region in Williams, had not been fully assembled, and also because this region is enriched with NLR genes and thus thought to be highly labile, it was difficult to design markers for fine mapping. In order to clone *Rps11*, we sequenced and assembled the PI 594527 genome into 43 scaffolds with a scaffold N50 of 26.4 Mb (see details in Method). In the course of PI 594527 sequencing, Chu *et al.* developed a high-quality Williams 82 genome assembly⁹ (dubbed v3.0), which reveals a number of assembly errors in the *rps11* region in the Williams 82 assembly v2.0 (Extended Data Fig. 2). To eliminate potential inaccuracy in the initial *Rps11* mapping result caused by those assembly errors, we annotated and compared the *Rps11/rps11* regions harboring the entire NLR clusters from the PI 594527 genome assembly and the Williams 82 genome assembly v3.0. The *Rps11* region from PI 594527 is composed of 12 NLR genes (dubbed R1-R12) (Fig. 1a). Of these, R1, R4, R6, R7, R9, R11, and R12 are intact and predicted to encode large NLR proteins containing 2,315-2,463 amino acids; R2, R3, R5 and R8 are truncated, with 2 to 4 exons lost at their 3' ends; R10 carries a 1.4-kb insertion in its first exon (Extended Data Fig. 3a). Only R1, R4, R6, R9 and R12 are expressed in stems inoculated with race 1 as revealed by RNA-seq (Extended Data Fig. 3b). The corresponding region from the Williams 82 assembly v3.0 is composed of eight NLR genes (dubbed r1-r8) (Fig. 1a and Extended Data Fig. 4). Only a small proportion of the region is shared between PI 594527 and Williams 82 as syntenic blocks, which allowed us to design markers for fine mapping (Fig. 1a).

Using two boundary markers, SSR286 and SSR320, in the conserved regions adjacent to the NLR clusters, we genotyped 17,050 F₄ plants derived from heterozygous (*Rps11/rps11*) F₃ plants of the initial mapping population and identified 43 recombinants between the two markers (Extended Data Fig. 5). These recombinants were then genotyped with eight additional markers distributed in the syntenic blocks between the boundary markers and inoculated with *P. sojae* race 1, and eventually defined the *Rps11* locus to a 151-kb region of PI 594527, harboring four NLR genes (R5, R6, R7 and R8) (Fig. 1b and Extended Data Fig. 5). Of the four genes, only R6 is expressed in inoculated stems and thus considered as the candidate gene of *Rps11* (Fig. 1c and Extended Data Fig. 5). Consistently, only recombinants in which the expression of R6 was detected are resistant to race 1 (Fig. 1c and Extended Data Fig. 6). R6 is also expressed in other tissues (Fig. 1d) and is predicted to encode an NLR protein of 2,463 amino acids. The genomic DNA of R6, excluding its regulatory sequences, is 14.1-kb, but it possesses a 13.1-kb 5' untranslated region (UTR) as determined by 5' Rapid amplification of cDNA ends (5'RACE) (Fig. 1e) and a 0.5-kb 3'UTR, totaling 27.7 kb (Fig. 1f).

The candidate gene for *Rps11* is functionally validated through complementation test

To functionally validate the candidacy of R6 for *Rps11*, we expressed the coding sequence (CDS) of R6 using a ubiquitin promoter in an elite soybean variety 93Y21, which is susceptible to *P. sojae* races 25, 31 and OH12108-06-03. As expected, the T₂ progeny with the R6-transgene showed resistance to these three races at levels similar to those shown by a homozygous F₅ RIL (*Rps11/Rps11*) derived from the cross between PI 594527 and Williams (Fig. 2a-b, and Extended Data Fig. 7). By contrast, the non-transgenic 93Y21 (Control) was susceptible to these same races (Fig. 2a-b and Extended Data Fig. 7). As expected, the expression of the R6-transgene was detected

in those T₂ progenies (Fig. 2c), and its expression level in each of the two transformation events is correlated with the level of resistance as indicated by the survival rate of inoculated seedlings (Extended Data Fig. 8). Together, these observations confirm that R6 in PI 594527 is *Rps11*.

***Rps11* possesses a chimeric structure and lacks allelic copies in the soybean pangenome**

In the PI 594527 genome assembly, a total of 512 NLR genes were annotated (Supplemental Table 3 and Supplemental Table 4). Apart from the large NLR genes in the *Rps11* region, there are six additional large NLR genes in the genome – one located on chromosome 16, and five clustered in a region on chromosome 18. Compared with a typical NLR gene, the large genes were enlarged by tandem duplications of the LRR domain (Fig. 3a). We found that all these large genes are phylogenetically grouped into a single clade (Fig. 3b) and share similar structures (Extended Data Fig. 3a and Extended Data Fig. 4), suggesting that they share a single origin. Comparative analysis reveals that these three genomic regions hosting the large NLR genes were derived from two soybean whole genome duplication (WGD) events¹⁰. Dot plot and phylogenetic analyses suggest that the 12 NLR genes in the *Rps11* region were likely derived from a single copy R5, after the second WGD event occurred ~13 million years ago, through rounds of tandem duplication events, each involving one or two adjacent NLR genes (Fig. 3c-e). Apparently, the tandem duplications were also accompanied by removal of NLR genes from the region, leading to the absence of allelic copies of most NLR genes between PI 594527 and Williams 82/Williams.

Subsequently, we annotated 296 NLR genes in the corresponding regions from the high-quality genome assemblies of 28 soybean accessions chosen to construct a pan-genome¹¹⁻¹⁴, and observed a striking copy number variation of NLR genes among these varieties, ranging from 5 to 23 copies

(Fig. 4 and Supplemental Table 5). Such variation is primarily caused by unequal recombination between different NLR genes, resulting in segmental duplications, deletions, and inversions (Fig. 4, Box 1 and Box 2). The variation appears to be associated with the phylogenetic relationships among the accessions revealed by the SNP data within the *Rps11* region (Fig. 4). Inter-genomic comparison demonstrates that *Rps11* is a unique gene in PI 594527 without an allelic copy in the other accessions (Extended Data Fig. 9). Nevertheless, the ~13.1-kb 5'UTR of *Rps11* was found to match the putative 5'UTRs of two adjacent NLR genes in W05, suggesting that the large 5'UTR of *Rps11* was formed by fusion of the two 5'UTRs in PI 594527 after its divergence from W05 (Fig. 4, Box 3). In addition, the presence/absence of the conserved transcription start region (TSR) is likely responsible for the drastic expression variations of the NLR genes in the *Rps11*/*rps11* region across the diverse soybean genomes (Fig. 4, Box 4, and Supplemental Table 5).

Discussion

We isolated *Rps11* conferring an extremely broad spectrum of PRSR resistance in soybean using a gap-free sequence-based fine mapping and cloning approach. Given such a magnitude of structural and gene copy number variations of the NLR gene cluster and the lack of allelic copies of the *Rps11* locus in the soybean pangenome, the sizable mapping population and the high quality of genome assemblies of both parental lines have been essential for the success. As few NLR clusters harboring known resistance loci have been fully sequenced and compared at the population level, this study sheds light on the evolutionary plasticity and consequences of resistance genes such as gain, loss, and reinforcement of resistance. Previously, two small NLR genes have been reported to be responsible for Rps resistance conferred by the *Rps1-k* locus in Williams 82^{15,16}. Intriguingly, neither of the two genes can be found in any versions/sources of the Williams 82

genome assembly including unassembled contigs (soybase.org; 9). Thus, the candidate gene(s) of *Rps1-k* remains to be validated. In addition, *Rps1-k* has become ineffective to many of newly evolved *P. sojae* races (8). Therefore, the source of resistance conferred by *Rps11* is highly desirable. The LRR domains in NLR genes are involved in determining the plant's ability to recognize specific pathogen effectors^{17,18}, but whether the expanded LRR domain in *Rps11* is responsible for such a broad resistance spectrum remains to be tested. This gene appears to be a suitable model for investigating molecular mechanisms underlying the extreme broadness of resistance spectrum and for developing strategies such as such as engineering of NLR genes for resistance enhancement. This study also provides *Rps11*-based unique markers for precise selection of the gene and will accelerate the deployment of the gene for soybean protection

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Figures and Legends

Fig. 1 | Map-based cloning of the *Rps11* locus. **a**, Comparison of the NLR gene clusters between Williams 82 and PI 594527. Black boxes represent NLR genes and light-blue shades represent syntenic blocks between two genomes. **b**, Physical locations of the markers used for fine mapping of the *Rps11* locus. The black line represents the genome region in the *Rps11* region on chromosome 7. The name of each marker used for mapping were labeled above the line. Numbers under the line indicate the number of recombinants at each marker. Dashed lines show the position of the markers within the NLR gene cluster. The final mapping boundaries are highlighted by orange dashed lines. **c**, Expression analyses in key recombinants. Green/light-green boxes

219 represent the five expressed NLR gene from PI 594527, black boxes represent the non-expressed
 220 NLR genes from PI 594527. Grey boxes represent the NLR genes from Williams 82. Dashed lines
 221 represent unknown genotype. The phenotype of each key recombinant is indicated as resistant or
 222 susceptible at the right side of each recombinant type. **d**, Expression profile of *Rps11* in different
 223 tissues. The error bars represent standard errors. **e**, 5' Rapid amplification of cDNA ends (5'RACE)
 224 performed for the 5 expressed NLR genes (R1, R4, R6, R9 and R12). x axis represents the distance
 225 upstream of the first exon. Brown bars represents the 5' RACE reads mapped to each NLR gene.
 226 Red lines/shades show the promoter regions sharing sequence similarity. Arrows at the right side
 227 indicate the direction of the NLR genes. **f**, Gene model of *Rps11* (R6). The vertical arrow points
 228 to the transcription start region (TSR). Orange color indicates the region encoding the NBS
 229 domain; Light-blue color indicates the regions encoding the LRR domains; Grey color indicates
 230 the region without a domain detected. Open boxes represent UTRs.
 231

Fig. 2 | Complementation test results. **a**, Photographic illustration of the resistance in two independent transgenic lines. In each transgenic event, three homozygous T2 families, a non-transgenic line (Control), and a F₅ RIL (*Rps11/Rps11*) were inoculated. Scale bars = 2.5 cm. **b**, Statistics of the resistance test of homozygous T2 families compared with non-transgenic lines (Control). The Y-axis is the survival rate after inoculation. The error bars represent standard errors. **c**, Relative expression of the transgene (R6) compared to endo-reference gene in transgenic lines and non-transgenic lines. The error bars represent standard errors.

Fig. 3 | Evolutionary history of the giant NLR genes in the *Rps11* locus. **a**, Comparison of protein sequences between a giant NLR gene and a typical NLR gene. Lines represent the alignments between two protein sequences. Orange color highlights the NB-ARC domain region. Light-blue color highlights the LRR domain regions. **b**, Phylogenetic tree of all the NLR genes in PI 594527 built using the conserved NB-ARC domain region. Giant NLR genes and typically sized NLR genes are labeled. **c**, Dot plot of sequence comparison within the *Rps11* region in PI 594527. Light-green color highlights the segmental duplication of R1-R2, R3-R4 and R11-R12 (Duplication 1, D1). Light-orange color highlights the segmental duplication of R7, R8 and R10 (Duplication 2, D2). **d**, Phylogenetic relationship of all the NLR genes underlying *Rps11* and the NLR gene, Chr16.R1, from its whole-genome duplication region, constructed using transcript sequence. Background colors highlight the groups produced by each duplication event. **e**, Illustration of the evolutionary history of the giant NLR gene cluster in the *Rps11* region. Green boxes represent NLR genes and black boxes represent predicted non-NLR genes.

Fig. 4 | Diversification of the NLR gene cluster across 30 diverse soybean genomes. The phylogenetic tree on the left side was built using SNPs data with the *Rps11* region. Orange color highlights the four major haplotype groups. Each black box represents an NLR gene. Grey shades represent syntenic blocks among genomes. Light-blue highlight inversion events. Green highlight tandem duplication events. Light-red highlight potential unequal recombination events. Box 1 and Box 2 exemplify a deletion and a duplication event induced by unequal recombination,

respectively. The name of each genome was labeled at right side of each cluster and the numbers in parentheses are the total copy number of NLR genes in each genome at the *Rps11* corresponding region. Box 3 shows the sequence comparison of the promoter regions of *Rps11* and that of W05.r5 and W05.r6. Light-blue shades represent syntenic blocks. Box 4 shows the comparison of the expression levels of NLR genes with TSR (TSR⁺) and without TSR (TSR⁻) in the *Rps11*/*rps11* region across all the genomes. Red asterisks indicate the significance and *P* value is shown above the plot.

Methods

Plant materials and resistance evaluation

The mapping populations were generated from an initial cross between PI 594527 and Williams. In total, 17,050 F₃ plants derived from heterozygous F₂ individuals were screened for identification of recombinants. Only the recombinants with one side heterozygous and the other side homozygous Williams genotype were used for fine mapping, because the two expected phenotypes could be accurately distinguished. About 30 seedlings from each line were inoculated with Race 1 of *P. sojae* using a protocol previously described^{3,19}. Lines with less than 25% of progenies surviving after inoculation were classified as susceptible; recombinants with 25-75% of progenies surviving were classified as segregating, lines with more than 75% of progenies surviving after inoculation were classified as completely resistant.

Genotyping the recombinants.

SSR markers and insertion/deletion markers (InDel) were identified and designed based on re-sequencing data of the two parental lines. Marker_176kb was a dominant marker that could only

be amplified from the donor line. Only the markers with a unique hit at the *Rps11* region were used for fine mapping. Kompetitive allele specific PCR (KASP) makers were also used to identify and genotype the recombinants. All makers used in this study are listed in Supplemental Table 6.

Long and short read genome sequencing

Long-read data was generated using the Pacific BioSciences (Menlo Park, CA, USA) Sequel platform at Corteva Agriscience™ (Johnston, IA, USA). Eight SMRT cells were performed with 10-hr movies and v6 chemistry. Raw subreads were filtered to a minimum of 12 kb generating 77× genome coverage. The raw subread N50 length was 28.9 kb. Linked short-read data were generated by sequencing 10X Genomics (Pleasanton, California) Chromium libraries at Corteva Agriscience™ on the Illumina (San Diego, California) HiSeq2500 platform in a PE151 configuration. The coverage depth and mean molecule length for the Chromium library were 45.2× and 93.8 kb, respectively.

Genome assembly and sequence polishing

Canu ²⁰ v1.8 (<https://github.com/marbl/canu>) was used to self-correct the raw subreads and to assemble the corrected reads into contigs. The following changes were made to the default parameters: correctedErrorRate=0.065, corMhapSensitivity=normal, and ovlMerDistinct=0.99. A minimum contig length of 30 kb was applied. Additional sequence polishing was performed by aligning raw PacBio subreads to the contig assembly using pbmm2 v0.12.0 (<https://github.com/PacificBiosciences/pbmm2>) and applying the Arrow algorithm from the Genomic Consensus package v2.3.2 (<https://github.com/PacificBiosciences/GenomicConsensus>)

to identify and correct remaining consensus errors in the contigs. These tools were acquired from pbbioconda (<https://github.com/PacificBiosciences/pbbioconda>). The consensus sequence accuracy was further enhanced by complementing the long read contig assembly with Chromium linked short-reads. Chromium datasets were aligned to contigs using Long Ranger v2.2.2. The sequence assembly polishing tool Pilon²¹ v1.22 (<https://github.com/broadinstitute/pilon>) was used to correct individual base errors and small indels from the Chromium data aligned to the contigs using the “--fix bases --minmq 30” parameters.

Creating genome maps

Genome maps were generated in the Bionano Saphyr platform at Corteva Agriscience™ using the Direct Label and Stain (DLS) approach²². Nuclear DNA was isolated from leaf tissue using a modified version of the Bionano Prep™ Plant Tissue DNA Isolation protocol (<https://Bionanogenomics.com/wp-content/uploads/2017/01/30068-Bionano-Prep-Plant-Tissue-DNA-Isolation-Protocol.pdf>) that did not include a gradient centrifugation step. DLE-1–labeled molecule data were filtered to create a dataset with a molecule N50 of 441 kb and 267× coverage. The resulting genome maps were filtered to remove coverage and length outliers. The final genome map assembly consisted of 43 maps with a genome map N50 of 26.7 Mb and a total map length of 985 Mb. This dataset was assembled via the Bionano Genomics Access software platform (Solve3.2.2_08222018) with the configuration file optArguments_nonhaplotype_noES_noCut_DLE1_saphyr.xml.

Hybrid scaffolding of genome maps with sequence contigs.

Hybrid scaffolds were generated from the polished contigs and the Bionano genome maps using the Bionano Genomics Access software (Solve3.3_10252018) and the DLE-1 configuration file hybridScaffold_DLE1_config.xml. In addition to auto-conflict resolution performed by the software, manual curation was performed to resolve overlapping and embedded contigs by providing additional “Conflict resolutions” and re-running the hybrid scaffolding. In the final product, the assembly had 43 hybrid scaffolds (Scaffold N50= 26.4 Mb, Total scaffold length= 978.1 Mb) with 229 leftover contigs that were not scaffolded with a combined length of 21.3 Mb.

Building chromosome-scale pseudomolecules

A reference-based approach was feasible to create chromosome-scale pseudomolecules using the Glycine max Wm82.a2.v1 reference assembly (<https://phytozome.jgi.doe.gov/>), because only an average of 2.15 scaffolds per chromosome needed to be placed. To map hybrid scaffolds to the reference, each scaffold was chunked into 100-bp fragments and then aligned to the reference genome using minimap2²³ v2.10 (<https://github.com/lh3/minimap2>). Then, a custom script was used to determine the chromosome position and orientation for each scaffold based on the alignment of each “chunked scaffold cloud”. All scaffolds were able to be placed using this method. Leftover unscaffolded contigs were concatenated with 100-bp N-gaps and assigned to Chr00.

NLR gene annotation and expression analysis

NLR genes were annotated using NLR-Annotator²⁴. RNA samples were extracted from mixed stem tissues from seedlings of each key recombinants using RNeasy Plant Mini Kit (Cat No.

74904, Qiagen) and were treated with RNase-Free DNase Set (Cat No. 79254, Qiagen) to remove DNA. RNA-seq was performed by Purdue Genomic Core Facility (<https://www.purdue.edu/hla/sites/genomics/>). RNA-seq data were mapped to the genome of the donor line using STAR²⁵ and expression was calculated based on the number of reads mapped to each NLR gene.

Plasmid Construction and Transformation

To make the over-expression construct for the *Rps11* candidate gene R6, the CDS of R6 was synthesized by Genscript as 3 fragments and assembled with AtUbi3 promoter and Gateway ATT sites by homologous recombination in yeast to make a Gateway entry vector²⁶. This was recombined into a Gateway destination vector by LR reaction using Gateway Technology with Clonase II (25-0749, Invitrogen) for transformation into *Ochrobactrum*. *Ochrobactrum*-mediated soybean embryonic axis transformation was done as previously described (US20180216123A1; WO2020/005933A1; WO2020/092494A1). Mature dry seeds of soybean cultivar 93Y21 were disinfected using chlorine gas and imbibed on semi-solid medium containing 5g/l sucrose and 6 g/l agar at room temperature in the dark. After an overnight incubation, the seed was soaked in distilled water for an additional 3-4 hrs at room temperature in the dark. Intact embryonic axis explants were isolated and transferred to the deep plate with 15 mL of *Ochrobactrum haywardense* H1-8 suspension (OD 0.5 at 600 nm) in infection medium composed of 1/10X Gamborg B5 basal medium, 30 g/L sucrose, 20 mM MES, 0.25 mg/L GA3, 1.67 mg/L BAP, 200 µM acetosyringone and 1 mM dithiothreitol in pH 5.4. The plates were sealed with parafilm (“Parafilm M” VWR Cat#52858), then sonicated (Sonicator-VWR model 50T) for 30 seconds. After sonication, embryonic axis explants were transferred to a single layer of autoclaved sterile

filter paper (VWR#415/Catalog # 28320-020). The plates were sealed with Micropore tape (Catalog # 1530-0, 3M, St. Paul, MN)) and incubated under dim light (5-10 $\mu\text{E}/\text{m}^2/\text{s}$, cool white fluorescent lamps) for 16 hrs at 21°C for 3 days.

After co-cultivation, the base of each embryonic axis was embedded in shoot induction medium (R7100, PhytoTech Labs) containing 30 g/L sucrose, 6 g/L agar and 25 mg/L spectinomycin (S742, PhytoTech Labs) as a selectable agent and 500 mg/L cefotaxime (GoldBio, ST Louis, MO, USA) in pH5.7. Shoot induction was carried out at 26°C with a photoperiod of 18hrs and a light intensity of 40-70 $\mu\text{E}/\text{m}^2/\text{s}$. After 4-6 weeks in selection medium, the spectinomycin-resistant shoots were cut and transferred to ½ strength MS rooting medium (M404, PhytoTech Labs) containing 15 g/L sucrose, agar 6 g/L, 10 mg/L spectinomycin and 250 mg/L cefotaxime for further shoot and root elongations.

Marker-free transgenic soybean plants were generated by the Cre-lox site-specific recombination system using heat shock treatment. For heat shock treatment of soybean, 2-4 cm T0 plantlets with roots in 100 x 25 mm Petri dishes on spectinomycin free-rooting medium were transferred into a Percival incubator (Percival Scientific, Perry, IA, USA) at 45°C, 70 % humidity for 2 hrs in the dark. After the heat shock treatment, T0 plantlets were transferred to moistened Berger BM2 soil (Berger, Saint-Modeste, QC, Canada), and kept enclosed in clear plastic tray boxes in a Percival incubator at 26°C with a 16-hr photoperiod at 250-350 $\mu\text{E}/\text{m}^2/\text{s}$. Leaf punch samples were collected for molecular analyses from newer growth 2 weeks after acclimatization of T0 events. Hardened plantlets were potted in 2-gallon pots containing moistened SunGro 702 and grown to maturity for harvest in a greenhouse. The presence of the construct in the transgenic

plants was confirmed by PCR with primers specific to the cloning vector and expression analysis of R6 in the transgenic plants by qPCR.

Data and materials availability

All the raw sequence data and the genome assembly from this article have been deposited in NCBI database under BioProject PRJNA718574. Novel biological materials described in this publication may be available to the academic community and other not-for-profit institutions solely for non-commercial research purposes upon acceptance and signing of a material transfer agreement between the author's institution and the requestor. In some cases, such materials may originally contain genetic elements described in the manuscript that were obtained from a third party(s), and the authors may not be able to provide materials including third party genetic elements to the requestor because of certain third-party contractual restrictions placed on the author's institution. In such cases, the requester will be required to obtain such materials directly from the third party. The authors and authors' institution do not make any express or implied permission(s) to the requester to make, use, sell, offer for sale, or import third party proprietary materials. Obtaining any such permission(s) will be the sole responsibility of the requestor. In order to protect Corteva Agriscience™ proprietary germplasm, such germplasm will not be made available except at the discretion of Corteva Agriscience™ and then only in accordance with all applicable governmental regulations. Plant germplasm and transgenic material will not be made available except at the discretion of the owner and then only in accordance with all applicable governmental regulations.

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Author contributions

JM, RA, OC, Jesse M, AB and JT designed the research. WW, LC, KF, Jon M, JB, XW, VL, CC, TF, DO, AH, DD, and GC performed the experiments. WW, LC, KF, XW analyzed the data. JM and WW wrote the manuscript.

Competing interests: Patent protection has been and/or may be sought for work disclosed in this manuscript.

Additional information

Supplemental Information is available for this paper.

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Extended Data Fig. 1 | Resistance spectrum of the *Rps11* donor line. The *Rps11* donor line, PI594527 (left), and the susceptible control line, Williams (right), were inoculated with 12 races/isolates of *P. sojae*. Photos were taken 7 days after inoculation. Scale bars = 2.5 cm.

Extended Data Fig. 2 | Sequence comparison between Williams 82 assembly v2.0 and assembly v3.0. Black boxes represent NBS-LRR genes and grey shades represent syntenic blocks between two versions. Red lines/dots represent sequencing gaps in the assembly v2.0.

Extended Data Fig. 3 | Gene models and expression pattern of the NBS-LRR genes in the

***Rps11* region. a,** Gene models and alignments among twelve NBS-LRR genes in PI 594527.

Black boxes represent exons of each NBS-LRR gene. Light-blue shades represent alignments. **b,**

Expression profile of the twelve NBS-LRR genes in the *Rps11* region. y-axis is the expression

level (FPKM) based on RNA-seq data. The error bars represent standard errors.

Extended Data Fig. 4 | Gene models of the eight NBS-LRR genes in the *Rps11* corresponding region in Williams 82. Black boxes represent exons of each NBS-LRR gene. Light-blue shades represent alignments.

Extended Data Fig. 5 | Fine mapping of the *Rps11* locus. a, Physical position of the *Rps11*

locus on chromosome 7. **b**, The markers used for fine mapping. **c**, Genotype and phenotype of

the recombinants. The numbers in parentheses at left side are the number of recombinants with

same haplotype. Green bars represent heterozygous genotype. Orange bars represent Williams

genotype. Phenotype (survival rate after inoculation) of each recombinant type are shown at right

side. H means heterozygous, S means susceptible. Numbers under each dashed line are the

number of recombinants at each marker. **d**, NBS-LRR gene cluster in the *Rps11* region. Green

bars represent expressed NBS-LRR genes. Grey bars represent non-expressed NBS-LRR genes.

Extended Data Fig. 6 | Detection of the expression of the twelve NBS-LRR genes in each key recombinant. The phenotype of each recombinant is labeled. y-axis is expression level (FPKM) based on RNA-seq data.

Extended Data Fig. 7 | Inoculation results of the transgenic lines with race 31 and Race 06-03.

a, Resistance to Race 31. **b**, Resistance to Race 06-03. *y*-axis is the survival rate after inoculation. The error bars represent standard errors.

Extended Data Fig. 8 | Correlation between the expression of R6 (*Rps11*) and the survival rate after inoculation in T2 population. *x*-axes are relative expression of R6, and *y*-axes are survival rate after inoculation with Race 25 (left), Race 31 (middle), Race 06-03 (right).

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Extended Data Fig. 9 | Sequence comparisons between the *Rps11* donor line and 29 diverse soybean genomes in the *Rps11* region. Black boxes represent NBS-LRR genes from PI 594527, grey boxes represent NBS-LRR genes from other soybean genomes, and grey shade represent homologous alignments between genomes.

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