

Supplementary Information for

Tetep genome sequencing and identification of rice blast-resistant genes
in the genome

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Supplementary Information Text

SI Materials and Methods

DNA and RNA preparation and sequencing

The genomic DNA was extracted from young (~ 1 month old) Tetep leaves (multiple seeds) using the Cetyltrimethyl Ammonium Bromide (CTAB) method (1). To obtain high-molecular-weight DNA suitable for PacBio sequencing, we replaced the vortex steps by inversion to avoid shearing of the DNA. RNA was extracted from two-week old leaves (two replications) of Tetep with TaKaRa MiniBEST Plant RNA Extraction Kit.

For PacBio sequencing, five libraries (20kb templates) were built using DNA Template Prep Kit 3.0 and DNA/Polymerase Binding Kit. All libraries were sequenced on a PacBio RS II platform with P6-C4 chemistry at BGI, Shenzhen, China, yielding 50 SMRT cells. For Illumina sequencing, two libraries with insert sizes around 500bp and 350bp were sequenced on Hiseq 2000 (2×100 bp) and Hiseq 4000 (2×150 bp) platforms, respectively (*SI Appendix*, Table S1). Genomic DNA of MH63, IR24 and nine of their derivatives through crosses with Tetep were sequenced on a Hiseq 2000 platform, with ~500bp insert size and the 2×100 bp read format. The Tetep RNA samples were sequenced in the 2×150 bp format on the Illumina Hiseq 4000 platform, each with ~120M clean reads.

De novo assembly

A Tetep genome draft was built using PacBio RSII subreads (*SI Appendix*, Fig. S1), which were extracted from the raw h5 file using bash5tools.py bundled with SMRT Analysis Software v2.3.0 (<http://www.pacb.com/products-and-services/analytical-software/smrt-analysis/>). Subreads less than 500bp long or with a read score less than 0.8 were filtered out, and the remaining subreads were assembled using the Canu assembler v1.4 (2). Canu was run with default parameters except with “genomeSize=449m ovsMemory=40g-100g”. The assembled contigs were polished by Quiver (bundled with the SMRT Analysis Software); the polishing step used all PacBio data to correct sequencing errors in assembled contigs.

The polished contigs were then anchored to twelve chromosomes according to the *Oryza sativa* ssp. *indica* genome MH63 (3). The MH63 genome was constructed by combining the bacterial artificial chromosome (BAC)-by-BAC approach and the Illumina whole genome shotgun (WGS) data, which could thus be used as a high-quality reference genome. We first searched for the best matches between the Tetep contigs and the MH63 genome using NCBI blastn with a e-value $< 1e-10$. The hits were then fed to Chromosomer (4), a reference-based genome arrangement tool, to produce the Tetep draft chromosomes. Chromosomer was run with the gap size set to 20kb, equal to the insert size of the PacBio library, and the ratio threshold set to 1.1. The ordering procedure linked contigs into chromosomes with padding gaps (“Ns”), which were then filled with all raw PacBio subreads using PBJelly (5). Finally, we ran a second round of Quiver to polish those filled gaps.

The assembled genome is smaller than that estimated by flow cytometry (6). However, the assembled size is similar to those estimated through the Kmer-based approach (*SI Appendix*, Table S13), suggesting that our assembly is complete. This is also reflected by a high portion (98.0%) of the 248 core eukaryotic genes present in our Tetep genome draft (*SI Appendix*, Table S3).

Error estimation

We used the Illumina sequencing reads to estimate the error rate in the assembled Tetep genome. The short Illumina reads were first mapped to the assembled Tetep genome with BWA-MEM 0.7.10-r789 (7). The mapped results were then processed with Picard (version 1.114) MarkDuplicates to remove non-biological PCR duplicates (8). Then, we called homozygous SNPs and indels (1~3bp) using GATK (version 3.7) HaplotypeCaller with option “-stand_call_conf 30.0”. As the DNA sources for PacBio and Illumina sequencing were nearly identical (from the same seed pool), those homozygous variants called from Illumina reads most likely resulted from uncorrected sequencing errors in the assembled genome. The heterozygous variants were mainly caused by mapping errors in duplicate regions and were thus discarded. The error rate was then estimated as the number of homozygous variants / the assembled genome size. The estimated error rate was $< 2 \times 10^{-6}$ for base errors and $< 1 \times 10^{-5}$ for indel errors.

Whole genome alignment and variant identification between Tetep and the other three rice genomes

The Tetep genome was aligned to the Nipponbare, MH63 and R498 genomes using MUMmer version 3.1 (9) with options “--maxmatch -c 200 -l 100”. The results were filtered using “delta-filter -1” to get one-to-one alignments. SNPs and small indels were detected using “show-snps -ClrT”, while large indels and structural variants were identified through the “show-diff” function. The average genome diversity was estimated as “Number of SNPs / Number of aligned bases”.

Prediction of *NLR* genes and identification of *NLR* pairs

Protein-coding genes were first identified using a two-pass MAKER-P pipeline (10), which collected evidence from homologous expressed sequence tags (EST data, retrieved on April 6th, 2016 from NCBI dbEST using “*Oryza sativa*” as the keyword), homologous protein sequences (retrieved on May 27th, 2016 from UniProt using “*Oryza sativa*” as the keyword) and local transcriptome sequencing data. The first pass of MAKER-P uses a trained SNAP (11) model based on the gff file generated by CEGMA, while the second pass uses a re-trained SNAP model and a trained AUGUSTUS (12) gene model based on the first pass. The local RNA-seq data were used as evidence after being assembled using both Cufflinks (13) (genome-guided assembly, first mapped to the Tetep genome using STAR (14) aligner) and Trinity (15) (genome-free transcriptome *do novo* assembly).

For each annotation set, the NB-ARC domain was predicted using hmmscan

(HMMER version 3.1b2) against the Pfam database version 30.0 (16). The LRR domains were predicted with NLR-parser (version 1.0) by searching for motifs 9, 11 and 19 (17). We found that the evidence-based pipeline captured very few *NLRs* (*SI Appendix*, Table S4). One possible explanation for this could be that the MAKER-P pipeline relies heavily on homology- or expression-based evidence, while such evidence is mostly lacking for *NLRs* because *NLRs* are generally expressed at low levels and are highly polymorphic. To generate a comprehensive Tetep *NLR* set, we applied an *ab initio* gene-finding program Fgenesh (18) for gene prediction. Fgenesh was run by choosing the “*Oryza sativa* Indica Group” as the organism-specific gene-finding parameters. Before prediction, we masked the repeat sequences using RepeatMasker (19) with option “-species rice” against RepBase (version 20170127). About 49% of the genome sequence was masked during this process (*SI Appendix*, Table S14). *NLRs* identified from the Fgenesh gene set recovered around 120 more *NLRs* which were lost in the MAKER-P set (*SI Appendix*, Table S4).

To test whether most *NLRs* in Tetep were properly predicted, we directly identified all putative *NLR* regions from the whole genome using NLR-annotator (17). The genomic regions containing putative *NLRs* (hereafter referred as “*NLR* regions”) were predicted by dividing the whole genome into 20kb sliding windows with a step size of 5kb. Through the Nipponbare genome, we estimated a coefficient of annotated *NLRs* compared to all putative *NLR* regions as “*NLRs* per Mb / *NLR* regions per Mb”. Under the assumption that the coefficient is the same in the Tetep genome, the number of all possible Tetep *NLRs* was calculated as “Effective genome size * *NLR* regions per Mb * coefficient”.

This strategy does not rely on any prior-annotated genes, so it is an unbiased predictor of the number of *NLRs* that are actually present in a genome (17). The NLR-annotator identified 519, 504, 514 and 531 putative *NLR* regions in Tetep, Nipponbare, MH63, and R498, respectively (*SI Appendix*, Table S4). Around 95.0% of predicted regions overlapped with those annotated ones in Tetep, Nipponbare and MH63, suggesting a high consistency (*SI Appendix*, Table S4). The remaining non-overlapping regions likely corresponded to regions with pseudogenes. The overlapping rate was lowest in R498 genome (85.3%), suggesting that fewer *NLR* genes annotated (i.e. those genes that were not predicted in the original gene set as we used the same *NLR* prediction pipelines for all genomes) in the R498 genome. Therefore, we concluded that our prediction captured nearly all *NLRs* in the three genomes. Even if we take the number of *NLRs* in Nipponbare as a baseline (i.e. 100% *NLRs* identified), we should have missed fewer than 32 *NLRs* (~7.0% of identified *NLRs*) in the Tetep genome. This not only implies that the *NLR* genes we discovered in the Tetep genome were comprehensive, but also suggests that we made a fair comparison among the four different rice genomes. For the R498 genome, the identified putative *NLR* regions was similar to the other three genomes.

Homologs of each Tetep *NLR* gene in Nipponbare, MH63, and R498 were identified by OrthoFinder (20) (version 2.2.7, default parameters) using their NB-ARC protein sequences. Each homologous pair (i.e. Tetep vs Nipponbare, Tetep vs MH63, or Tetep vs R498) was then aligned by coding sequence using MUSCLE

(21), and the nucleotide diversity (p-distance) between each homolog pair was calculated using MEGA-CC (22).

For phylogenetic analysis, the nucleotide sequences were first aligned by Clustalw2 (23). Their protein sequences were used as a guidance to avoid non-3 multiply gaps introduced in the final alignments. Phylogenetic trees were constructed using FastTree (24) (version 2.1.10) with options “-nt -gtr” (1,000 bootstrap replicates by default). FastTree was chosen in this study as it can rapidly infer maximum-likelihood (ML) phylogenies for huge alignments, and is robust to inaccurate alignments from the large number of divergent *NLRs*. It also produces better bootstrap-supported trees than other tools like RAxML (25) in all test runs. The phylogenetic trees were constructed using both the nucleotide sequences of the full coding region and the nucleotide sequences of the NB-ARC domains. Since both results were highly consistent with each other topologically, only the results using full coding sequences were presented in the paper as they give clearer distinguishable clades than using NB-ARC domains alone.

There are currently several known cases for which two *NLR* genes are required to effect the resistance function, with one protein in the pair acting in effector recognition (i.e. directly binding AVR effectors, known as a sensor) and the other acting in signaling activation (i.e. releasing the switch to initiate signaling, known as a helper). Since all pairs for which functions are known are present in the head-to-head arrangement in a genome, we searched for head-to-head *NLR* genes in the Tetep, Nipponbare, MH63 and R498 genomes. The candidate *NLR* pairs were first identified through searching for *NLR* genes near each other (enclosing no more than 2 non-*NLR* genes) in five different genomes, i.e. the Tetep, Nipponbare, MH63, R498 and *B. distachyon* genomes. Most candidate *NLR* pairs were found with the head-to-head arrangement, but some could be changed due to genomic rearrangement events. Therefore, the *B. distachyon* genome was chosen as an outgroup to infer the ancestral state. An *NLR* pair would be retained if itself or any of its homologous pairs in *B. distachyon* or any other rice genomes followed the head-to-head arrangement. The identified *NLR* pairs were then used to construct phylogenetic trees (Fig. 4 and *SI Appendix*, Fig. S3), and were further confirmed by our manual inspection of the phylogenetic tree to make sure that the two members of a pair were not from a recent duplication of a single *NLR* after splitting of the ancestors of a cultivar. In this study, a sensor (helper) was identified by its homologous relationship with sensor (helper) members of known *NLR* pairs. If this definition could not be made, we checked whether it carries an extra domain that might recognize pathogens (i.e., a sensor if yes, but a helper if not). Some examples of extra domains are WRKY, RATX, NOI, and TRX, which could act as a decoy of pathogen effectors (26).

Large-scale cloning, transformation and testing of *NLR* genes

Multiple PCR primer pairs were designed for each *NLR* gene based on the Tetep genome sequence (*SI Appendix*, Dataset S4). The products of long-range PCRs with the native promoter and terminator were inserted into the binary vector pCAMBIA1300. To improve the cloning efficiency, we introduced a new restriction

site, ASCI, into the multiple cloning sites (MCS) of pCAMBIA1300. For some genes originally harboring the ASCI restriction site, we used XbaI, SalI, EcoR I, BamHI, KpnI or XhoI as the alternatives. After validation by Sanger sequencing, each clone was transformed into two blast-susceptible cultivars, TP309 and Shin2, using the *Agrobacterium* strain EHA105. Transgenic lines (T₀) were first confirmed by PCR and Sanger sequencing to ensure the presence of desired transgenic DNA fragments, and then grown in the field to obtain seeds (T₁ and T₂) for further testing. Approximately 93 *NLRs* were sequenced with 5 or more reactions, yielding longer assembled sequences, and were used in comparison with annotated *NLRs*. The assembled Sanger sequences have been deposited in the Figshare database (<https://doi.org/10.6084/m9.figshare.7775810.v1>).

The 12 pathogen strains used in this study were chosen in consideration of their geographic origin, sequence diversity and sporulation efficiency from 61 field strains collected from various geographic areas of China during 2008-2009 and characterized by Huang et al. (27) (*SI Appendix*, Fig. S2). The leaf blast resistance was evaluated in T₁-T₂ plants (3-weeks old seedlings) after spraying the blast spore suspension (5×10^5 spores/ml) and placing in the dark for 24 h (100% humidity, 26°C), followed by 12-h light/12-h dark (90% relative humidity) for 6-7 days until disease spots appeared clearly.

For each test, the number of tested blast strains was usually less than 12, for three reasons: 1) Hard-to-control sporulation efficiency of a blast strain. Our definition of a successful plant infection included a blast spore suspension with 5×10^5 spores/ml. Although the experimental environments were strictly managed, the sporulation efficiency sometimes varied across strains, possibly due to subtle environmental disturbances. This led to fewer than 12 strains with sufficient inoculum concentration for testing. 2) Loss of proper controls, especially the susceptible controls, in some cases. In order to make sure the testing results were trustable, we required the resistant (Tetep) and susceptible controls (TP309 and Shin2 wild-type) to behave properly when treated under the same conditions. However, several controls occasionally behaved unexpectedly, e.g. if the susceptible control displayed resistance to a certain pathogen strain, possibly caused by some unnoticeable environmental influences, then those testing results for the strain would become invalid. 3) To provide reliable results, we required consistent testing outcomes across three independent replicates. As each replicate had varied pathogen strains available for testing, the final number of successful tests became even fewer.

For each gene, 8-10 independent transformants were selected. We conducted a pilot survey on transformants of *Pi37* and 20 other randomly selected *NLRs* and found that the resistance phenotypes were highly consistent across independent transformants of those genes. Therefore, to reduce the workload in managing each of those transformants independently, which could be extremely labor-intensive, we did not further distinguish between independent transformants in subsequent experiments once we obtained the true positive transgenic lines. Such a strategy greatly increased the speed of the large-scale testing. In addition, we made a trade-off by only considering those most reliable resistant transformants (equivalent to a quantitative

scale of 0-2 on the 0-9 scale of standard evaluation system of rice (28)) as resistant (*R*) to further simplify the procedure. The binary vector pCAMBIA1300 can be purchased from Addgene (<http://www.addgene.org>). The modified plasmids as well as seeds for transgenic analyses are available upon request.

Pedigree tracing of *NLR* genes in Tetep descendants

The selected resistance rice pedigree contains three parental lines, four intermediate lines, and five elite descendants (Fig. 3A). The intermediate lines IR1544 and 1318 confer strong resistance to blast disease. Wanhui88 (WH88) and Duoxi-1-hao (DX1) are excellent restorer lines with moderate resistance to blast disease. All five elite lines, i.e. Chenghui047 (CH047), Mianhui2009 (MH2009), Neihui2539 (NH2539), Shuhui527 (SH527) and Zhonghui8006 (ZH8006), were bred with the aim to improve their yield and quality while sustaining part of the resistance from their resistant donors. MH2009 displayed strong blast resistance, at level 4~5 (6~8 for the MH63 control) to leaf blast and level 3~5 (7~9 for the MH63 control) for neck blast (tested in 1996, 1997, 1999, 2001 by Institute of Plant Protection, Sichuan Academy of Agricultural Sciences) (29). SH527 also confers strong resistance to blast, showing a resistance level of 0~3 (tested by Sichuan Agricultural University during 1996~1998) (30). CH047 showed very strong blast resistance, i.e. at level 4 (9 for the MH63 control) to leaf blast and 0 (2 for the MH63 control) to neck blast (tested during 1997~1998 by Institute of Plant Protection, Sichuan Academy of Agricultural Sciences) (31). NH2539 is an excellent restorer line for rice breeding and has good blast resistance in production (32), while ZH8006 has been less studied for its resistance in the literature.

Sequencing reads of all related cultivars from the selected Tetep pedigree were mapped to the Tetep genome using the same pipeline described above. To test whether an *NLR* gene in Tetep was passed to its descendants, we divided the whole genome into 10kb windows and calculated nucleotide diversities between each cultivar and Tetep. For each window, a cultivar was considered to have a Tetep source if the cultivar was much more similar to Tetep (nucleotide diversity $\leq 0.016\%$, the median of nucleotide diversities across all Tetep's derivatives) than to any of its other parents (nucleotide diversity $\geq 0.025\%$, the median of nucleotide diversities across all non-Tetep parents). The *NLR* genes residing in the inherited windows were then regarded as derived from Tetep. Venn tool on <http://bioinformatics.psb.ugent.be/webtools/Venn/> was used to create the Venn diagrams.

Generation of knockout mutants of paired *NLRs*

To generate knockout mutants for those sensor and helper *NLRs*, unique single-guide RNA (sgRNA) spacer sequences were designed to target the coding parts before or within the region encoding the NB-ARC domain of a target *NLR*. Each spacer's complementary oligonucleotides were inserted into the BsaI restriction site of sgRNAs. Each sgRNA was then incorporated into the Cas9 vector using the Gateway

recombination method (33) and transformed into the *japonica* cultivar Wuyungeng24. The transformed lines were verified using PCR and Sanger sequencing when the transgenic plants were 20~30 days old.

Among all designed *NLRs*, only 78 of the 264 transformed plants were successfully edited, suggesting an overall editing rate of ~29.6% (*SI Appendix*, Table S10, rate calculated as “edited plants” / “all transformed plants verified”). Eight non-*NLR* genes were arbitrarily selected and edited with the same procedures in Wuyungeng24 which served as controls (*SI Appendix*, Table S10). This editing rate was noticeably lower than that for the non-*NLR* genes surveyed (~67.5%), indicating a fitness reduction in some failed cases possibly due to autoimmunity. Such a fitness cost was also suggested by phenotypic changes and unsuccessful cases in our large-scale *NLR* gene cloning and transformation experiments (*SI Appendix*, Fig. S4 and Table S11).

Potential off-target sites were predicted using Cas-OFFinder (34) for 18 Cas9 sgRNAs from those edited plants with “NRG” string to match both the canonical NGG and non-canonical NAG PAM (protospacer adjacent motif) (35). No sgRNAs had predicted off-target sites with fewer than 2-nt mismatches, and only 4 sgRNAs had predicted off-target sites with 2-nt or more mismatches, suggesting that the designed spacers were highly specific. To further assess possible off-target effects, we arbitrarily selected 9 of 18 edited plants for whole genome sequencing and identified the mutations in each plant using a pipeline described before (36). The targeted mutations were confirmed for all 9 sequenced plants, and no novel mutations were found in predicted off-target sites even when up to 3-nt mismatched were allowed, suggesting a very low off-targeting rate. This was consistent with the high specificity and low off-targeting rate of the CRISPR/Cas9 system reported before (37).

To further confirm the resistance function of the paired *NLRs*, we tried to transform each pair as a unit into a susceptible cultivar. Due to the technical difficulty in cloning long genes, e.g. >20kb, only two *NLR* pairs, *chr11.fgenesH1896/chr11.fgenesH1897* (*Os11g39310/Os11g39320*) and *chr11.fgenesH2445/chr11.fgenesH2446* (*Os11g45970/Os11g45980*), were successfully cloned from Tetep and separately transformed into the susceptible TP309 rice cultivar. The two transgenic plants either conferred strong or enhanced resistance to rice blast strains. For example, when testing neck blast, which is considered the most destructive phase of the blast disease (38), the transgenic TP309 plants with a single transformed helper *NLR chr11.fgenesH2446* could only confer resistance to one of the five tested blast strains, whereas the transgenic lines with the pair of *chr11.fgenesH2445/chr11.fgenesH2446* could confer resistance to all 11 tested blast strains (Fig. 4B, Two-sided Fisher's Exact Test, $P = 0.00275$). Moreover, in line with a higher influence when a helper *NLR* was present alone, the transgenic plants of the two helpers, *tig00011732.fgenesH48* and *chr11.fgenesH2446*, displayed more frequent lethal phenotypes (4 of 20 and 5 of 20 individuals died during growth) than the transgenic plant of the sensor *tig00011732.fgenesH49* (only 1 of 20 individuals died during its growth, one-sided exact binomial test, $P = 0.00013$).

Design of a PCR marker set for verifying Tetep-derived *NLRs*

Candidate PCR primer pairs were designed using Primer3 (39) on all Tetep *NLRs* including 10kb upstream and 10kb downstream flanking regions. The candidate primer sequences were then mapped to the Tetep genome using Blastn (E-value = 10) and only unique primers (i.e., primers that hit only themselves) were selected.

The PCR primer pairs were designed to amplify nearly every *NLR* gene in the Tetep genome. For each single or paired *NLR* (a total of 43 identified *NLR* pairs), one or two primer pairs were designed (*SI Appendix*, Fig. S6A); ~ 60 *NLRs* only have one pair of primers because no other unique primer pairs could be designed (*SI Appendix*, Fig. S6, Datasets S1 to S3). For a *NLR* cluster (defined as a cluster of three or more *NLRs* with the distance between two adjacent *NLRs* < 300kb; a total of 23 clusters containing a total of 133 *NLRs*), usually three or four pairs of primers were designed to target the first and last genes plus one to target a middle region (*SI Appendix*, Fig. S6A).

We designed PCR primer pairs to amplify regions of 800 – 10,000 bp that are clearly distinct between Tetep and Nipponbare (representative for *japonica* rice cultivars) or 9311/MH63/R498 (representative for *indica* rice cultivars). For example, we designed 121 PCR primer pairs (*SI Appendix*, Fig. S6B and Dataset S1) each of which includes an indel (200~700 bp) between Tetep and Nipponbare or 9311/MH63/R498 (*SI Appendix*, Dataset S2), so that the PCR products could be immediately distinguished by their band sizes in gel electrophoresis (*SI Appendix*, Fig. S7A). However, as such indel markers are not abundant in *NLR* regions, the majority of our PCR markers made use of the presence of variant nucleotides between Tetep and other cultivars (*SI Appendix*, Dataset S3), that is, they are so-called allelic or allele-specific primers (*SI Appendix*, Fig. S6B). An allelic PCR primer pair could be verified by the presence/absence of a PCR band of the right size in the electrophoresis gel (*SI Appendix*, Fig. S6B and 7B). These candidate markers in Tetep were checked in the four other genomes, i.e. Nipponbare, 9311, MH63 and R498, using Blast search (E-value = 10). Each primer pair that had no hits in at least one of the four genomes was selected. The remaining PCR markers were selected by screening primer pairs that had Blast hits in other genomes but contained SNPs or small indels (≥ 2 bp) compared to the corresponding Tetep regions (*SI Appendix*, Dataset S2). These PCR markers can usually also serve as allele-specific markers. If they cannot be distinguished by PCR bands, they can be checked by Sanger sequencing, although it may require more than one sequencing reaction. In total, we designed 1,909 PCR primer pairs.

We selected 320 primer pairs that target *NLR* regions in Tetep, tested them in Nipponbare and 9311, and found 282 successful pairs (*SI Appendix*, Dataset S1), yielding a success rate of $282/320 = 88.1\%$. The targeted regions include all of the 23 clusters (a total of 133 *NLR* genes) and 43 *NLR* pairs, so that the 320 primer pairs covered 268 *NLR* genes, over half of 455 Tetep *NLRs*. Importantly, only 146 of the 320 selected PCR primer pairs were required to verify all of the 23 *NLR* clusters and 43 *NLR* pairs. Thus, our PCR marker set can serve as a flexible and simple-to-use molecular marker library to rapidly verify every Tetep-derived *NLR* cluster and pair.

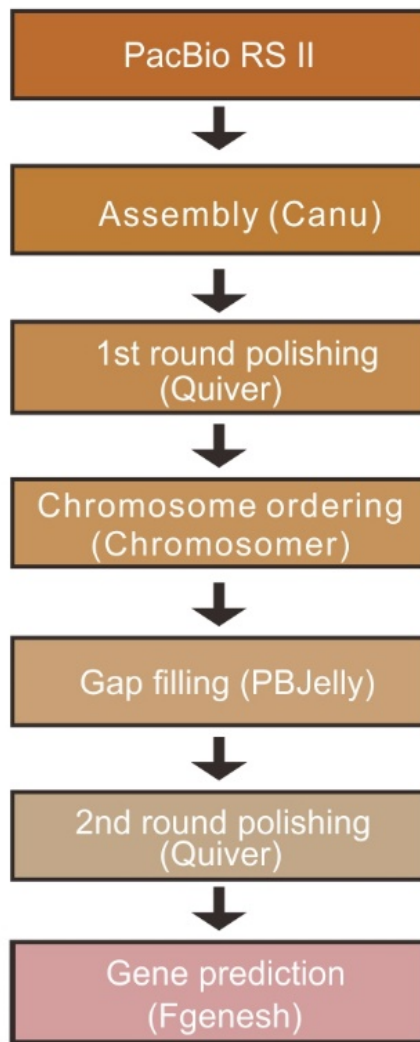


Fig. S1. Assembly workflow of the Tetep genome.

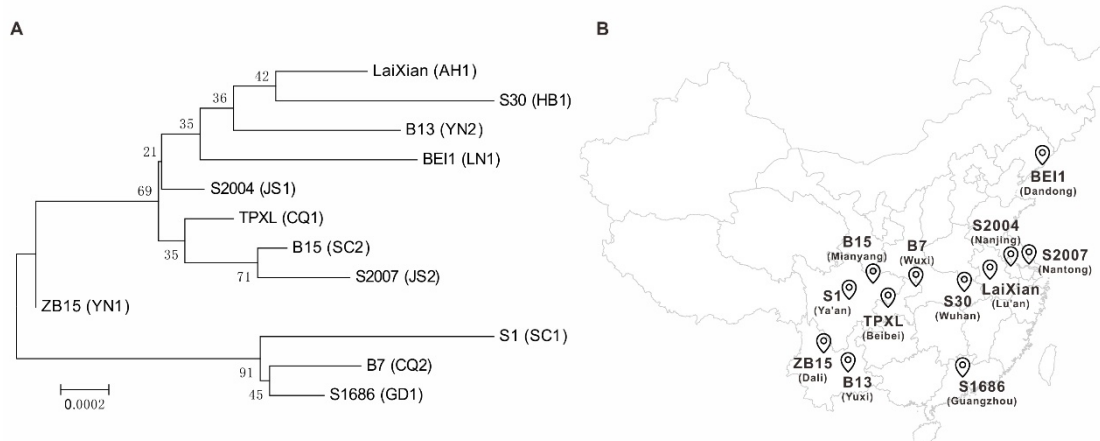
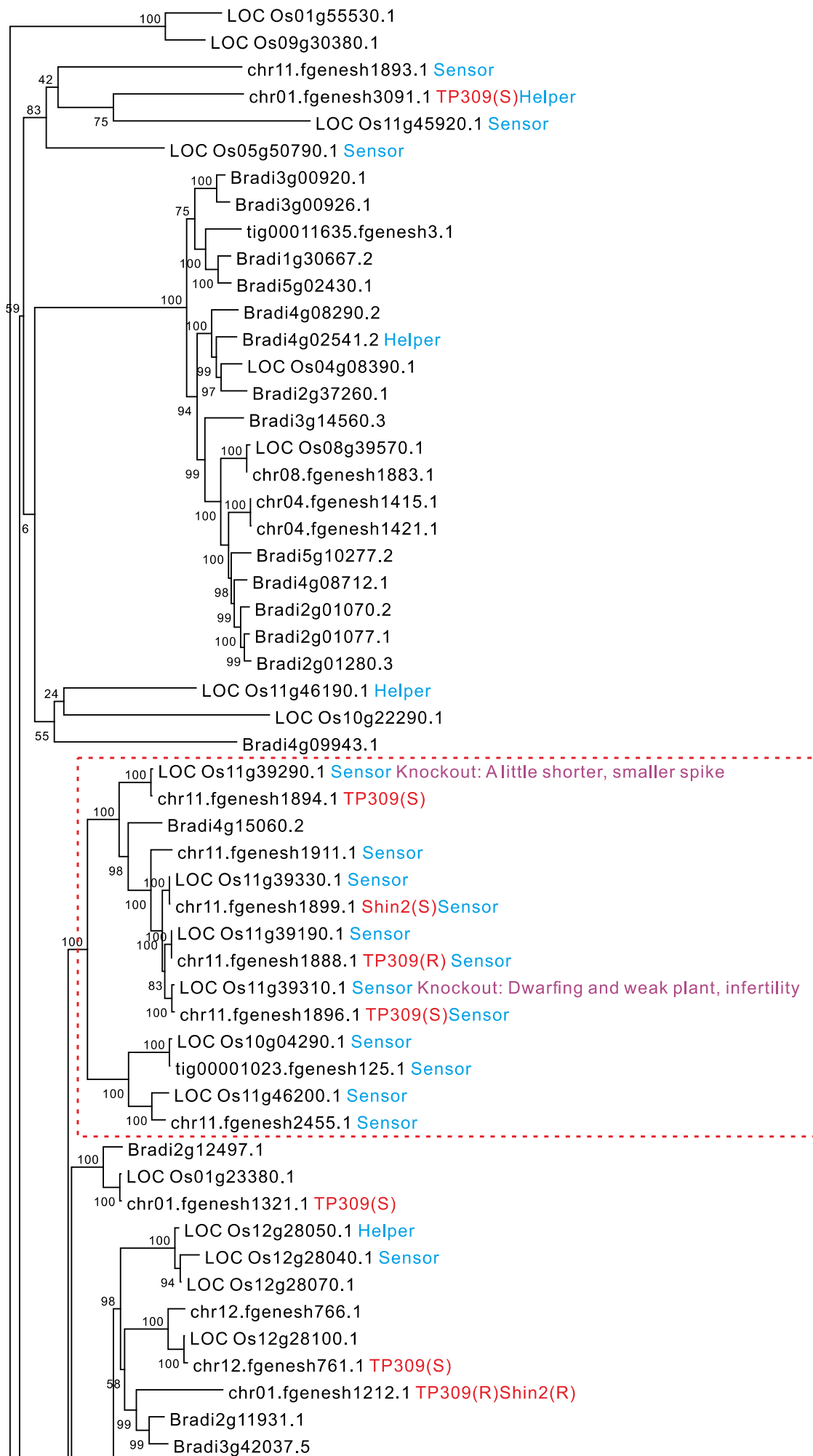
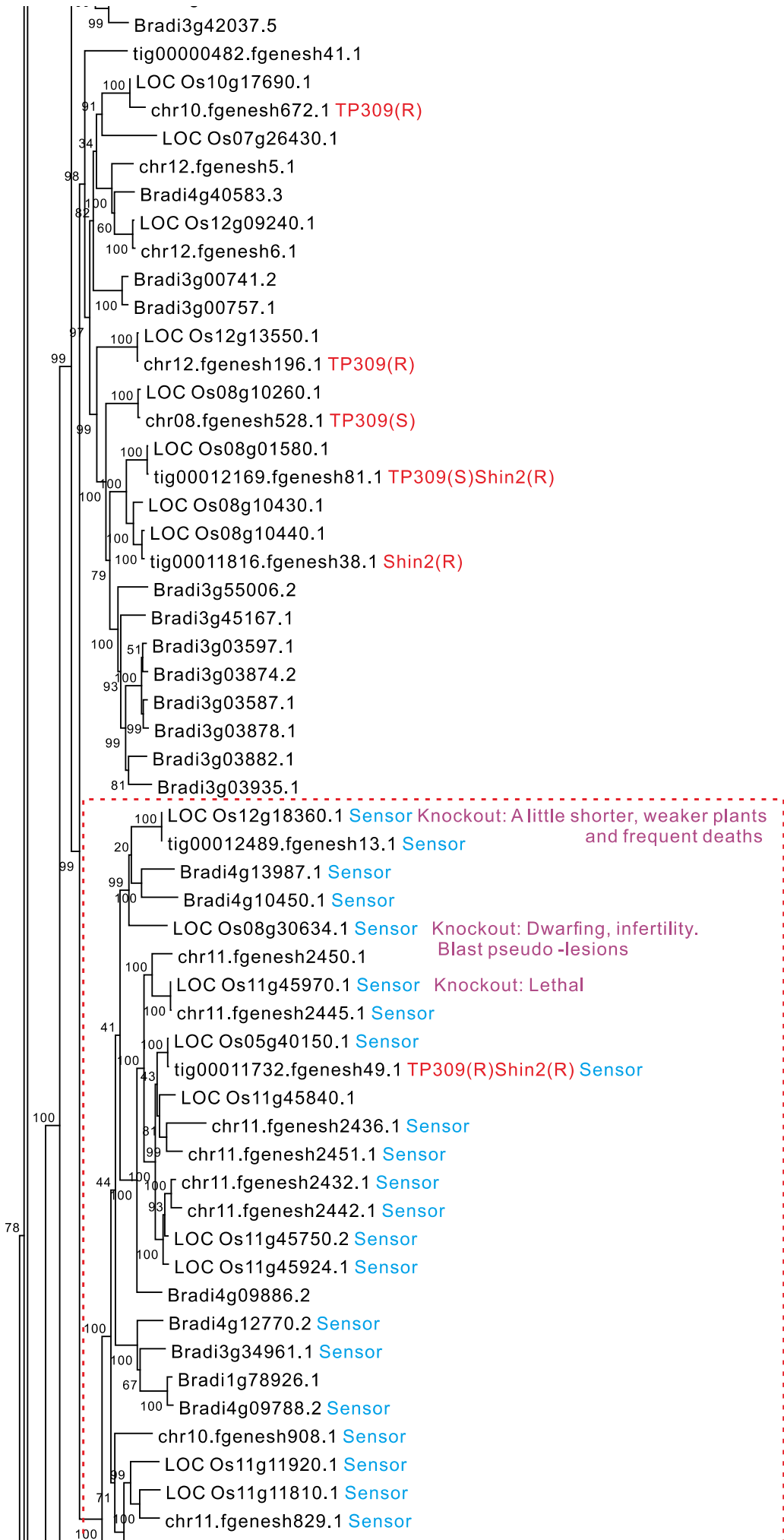
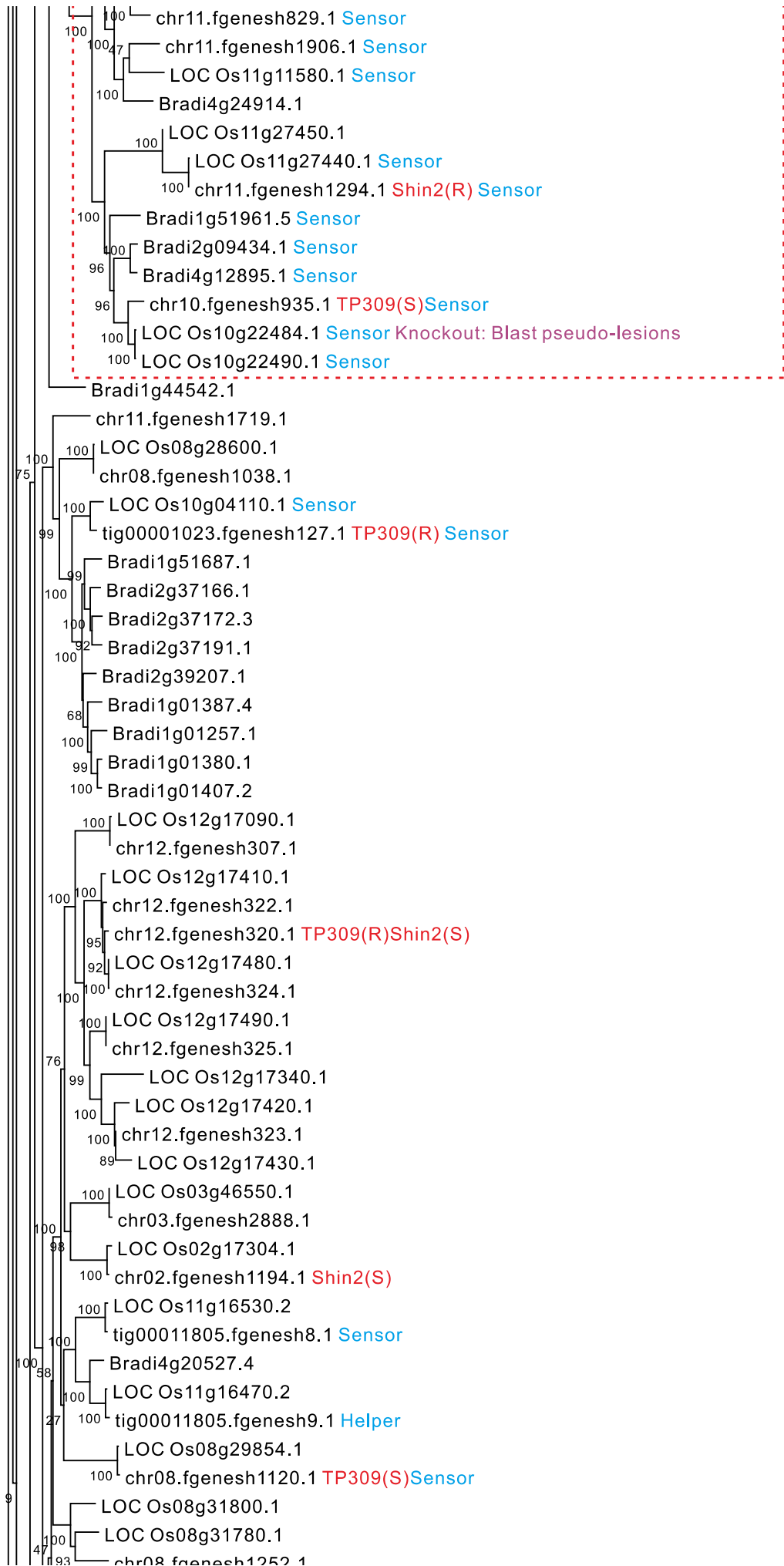
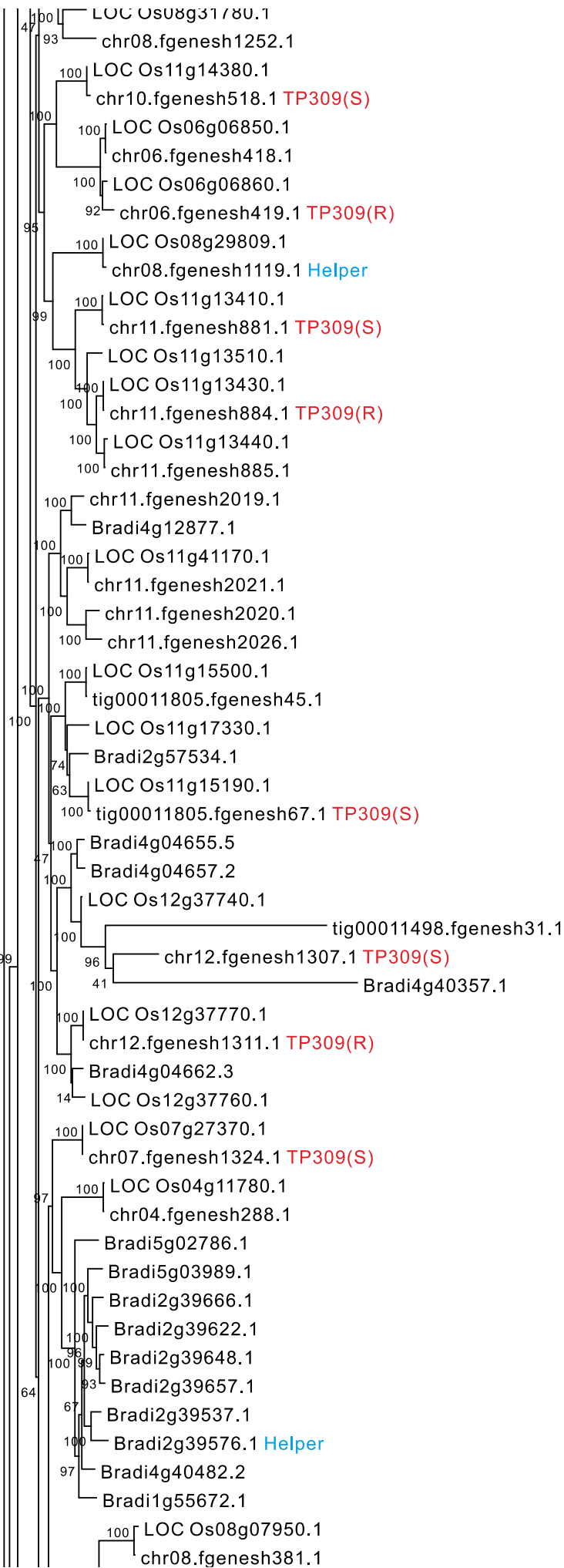


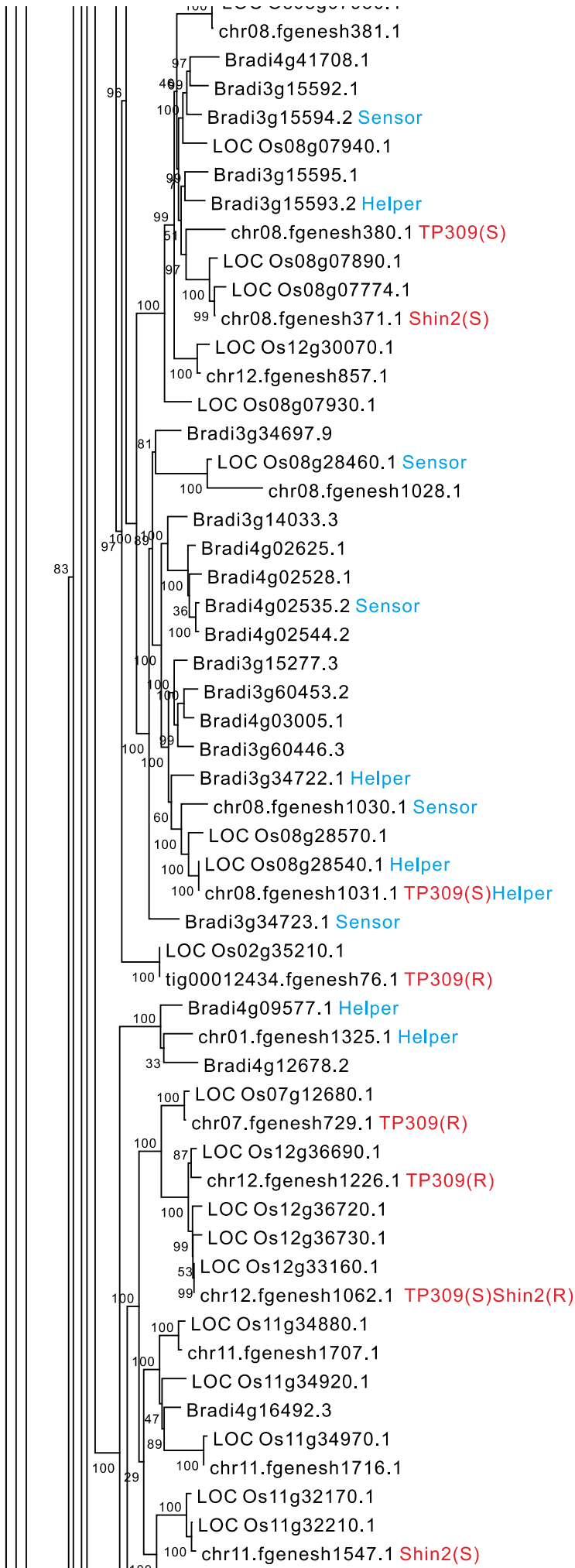
Fig. S2. Phylogenetic tree and geographic locations of the 12 testing blast pathogen isolates. (A) An ML phylogenetic tree adapted from Zhang et al. (2015) (40) where the same strains were used. The corresponding IDs used in Zhang et al. (2015) were given in parenthesis. (B) The geographic locations of the 12 testing pathogen strains. The city names where the strains were collected were given in parenthesis.



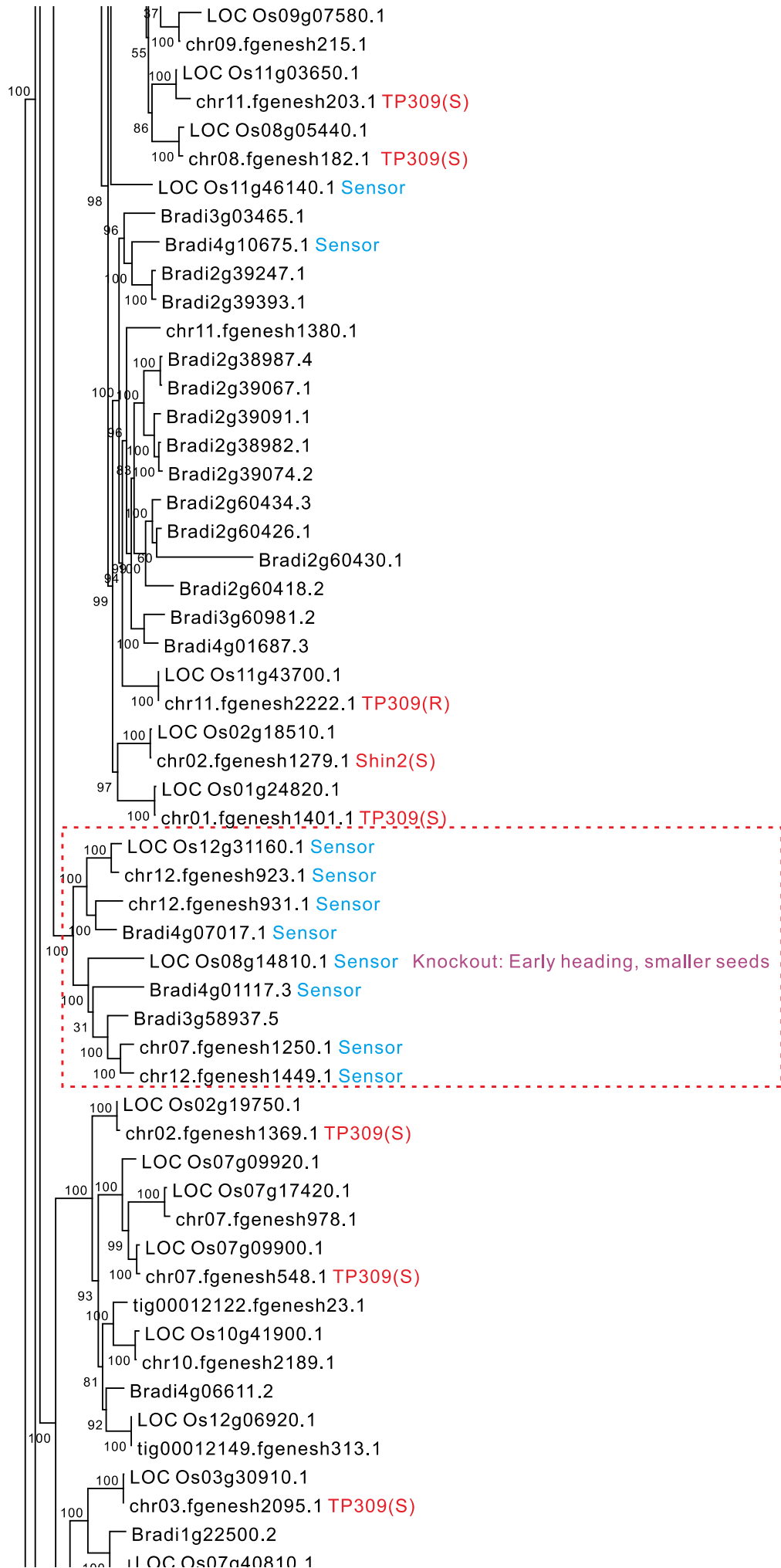


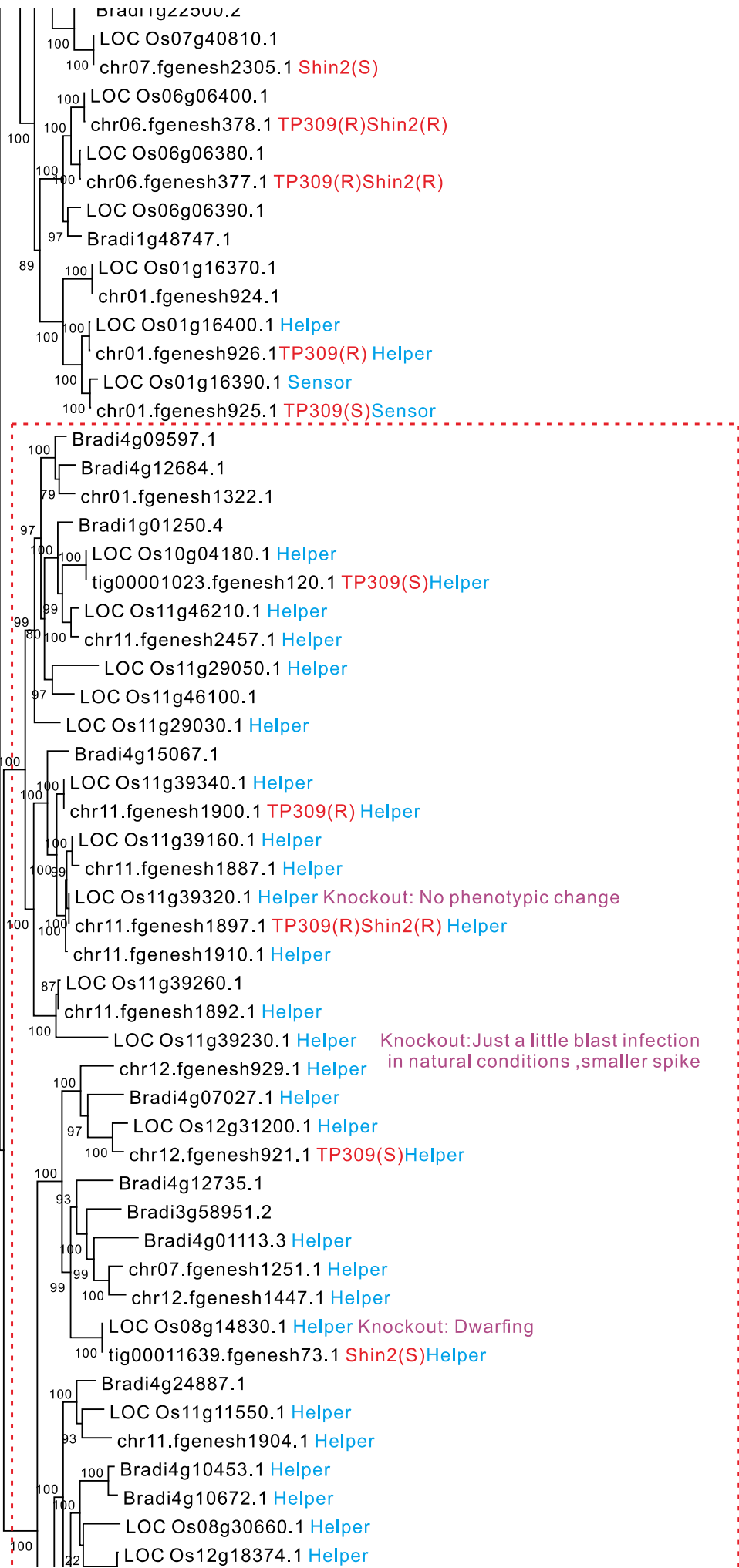




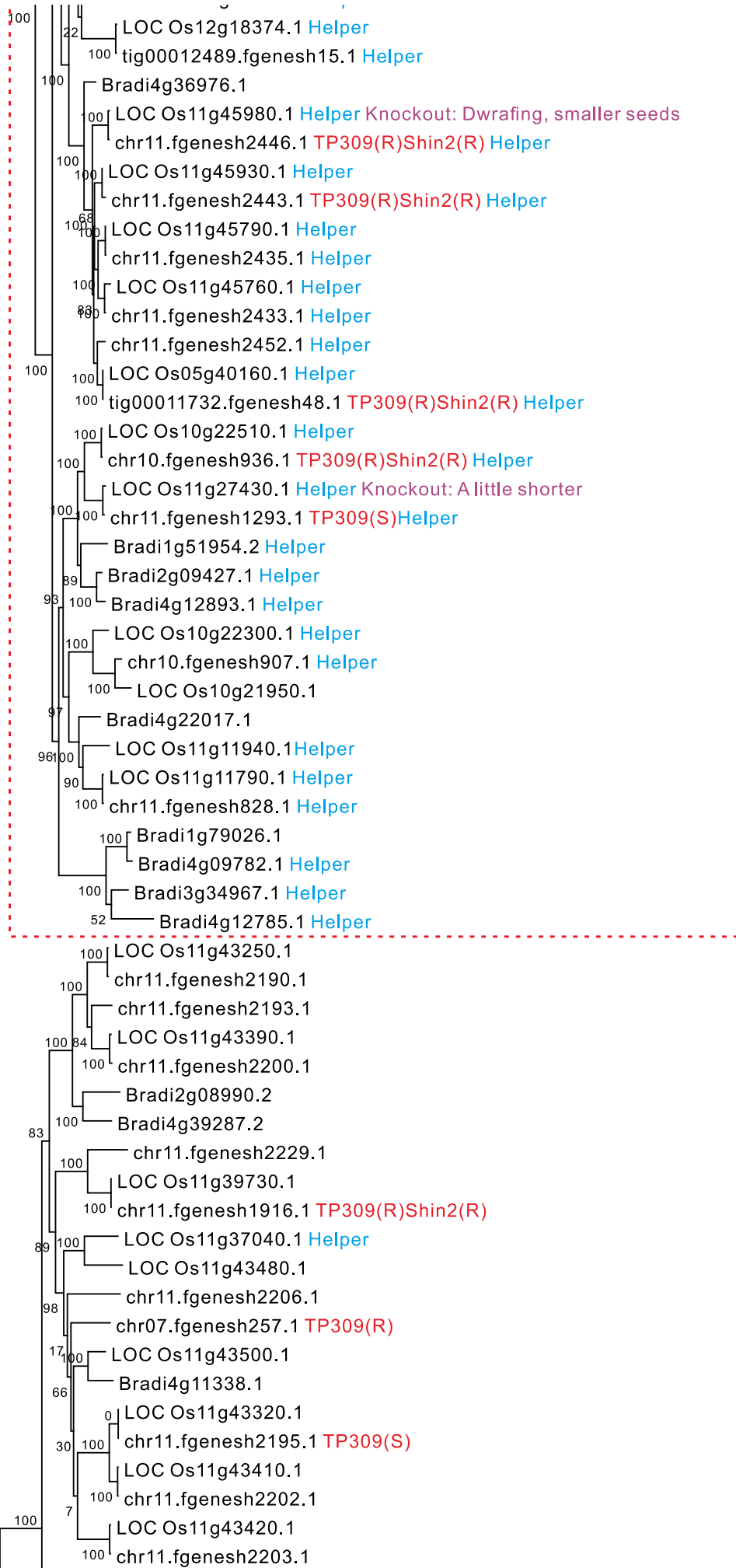


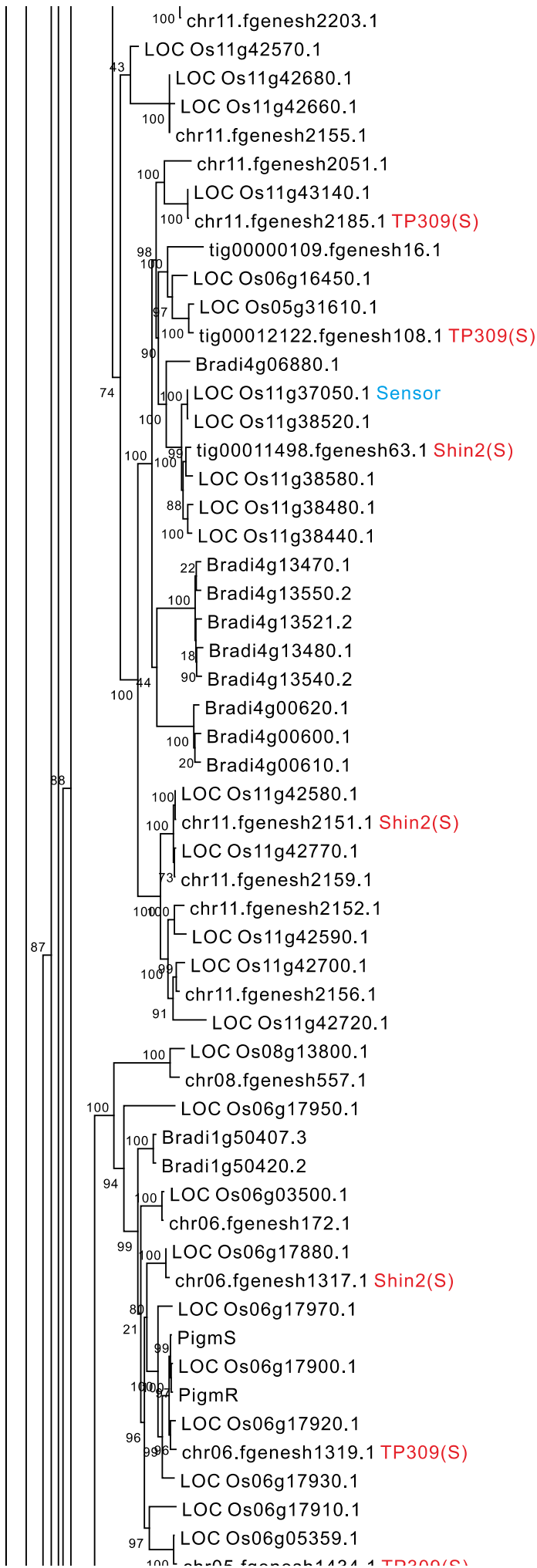


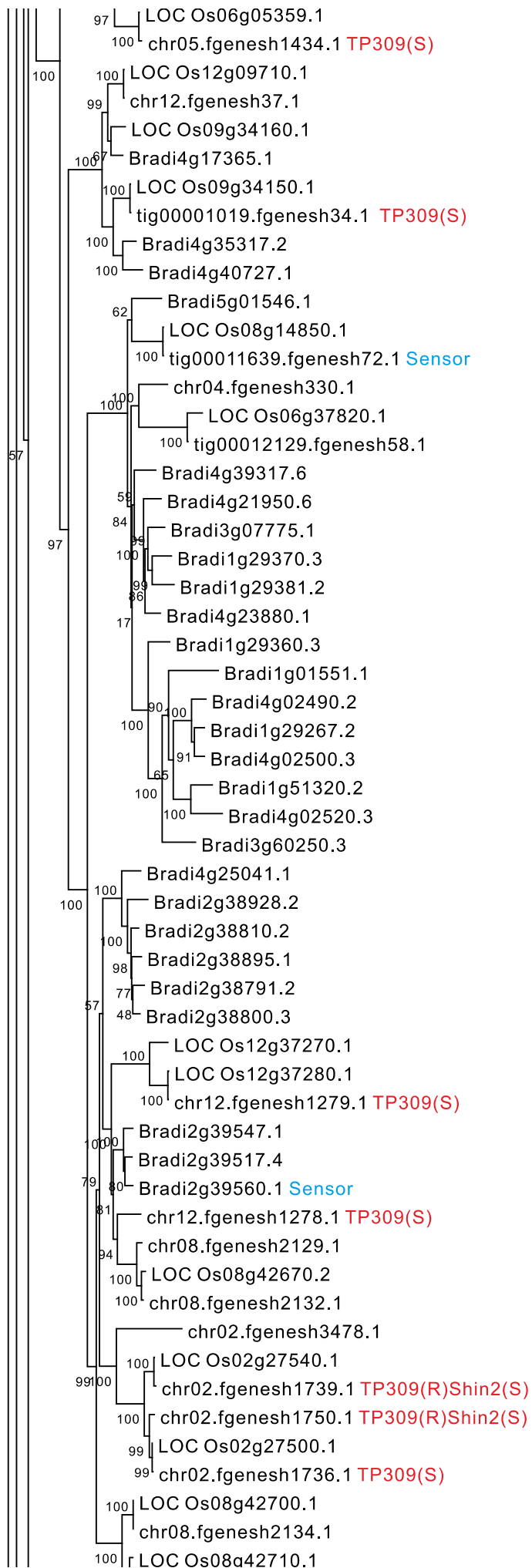


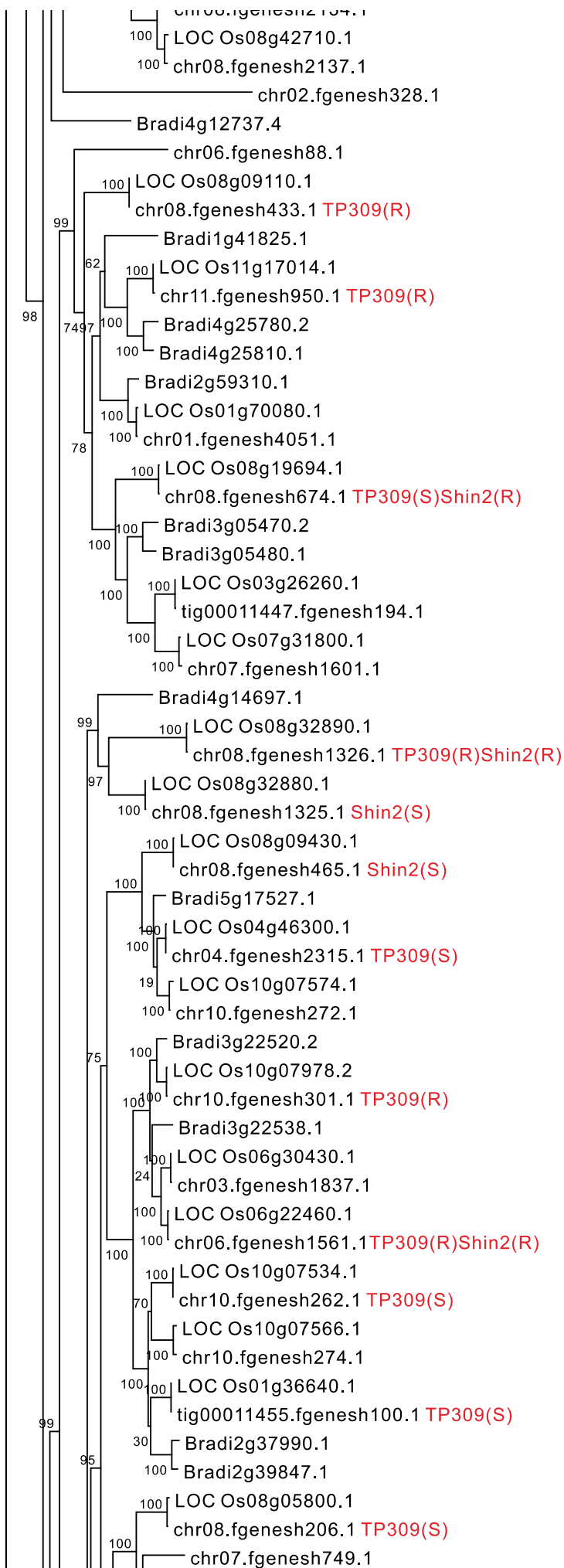


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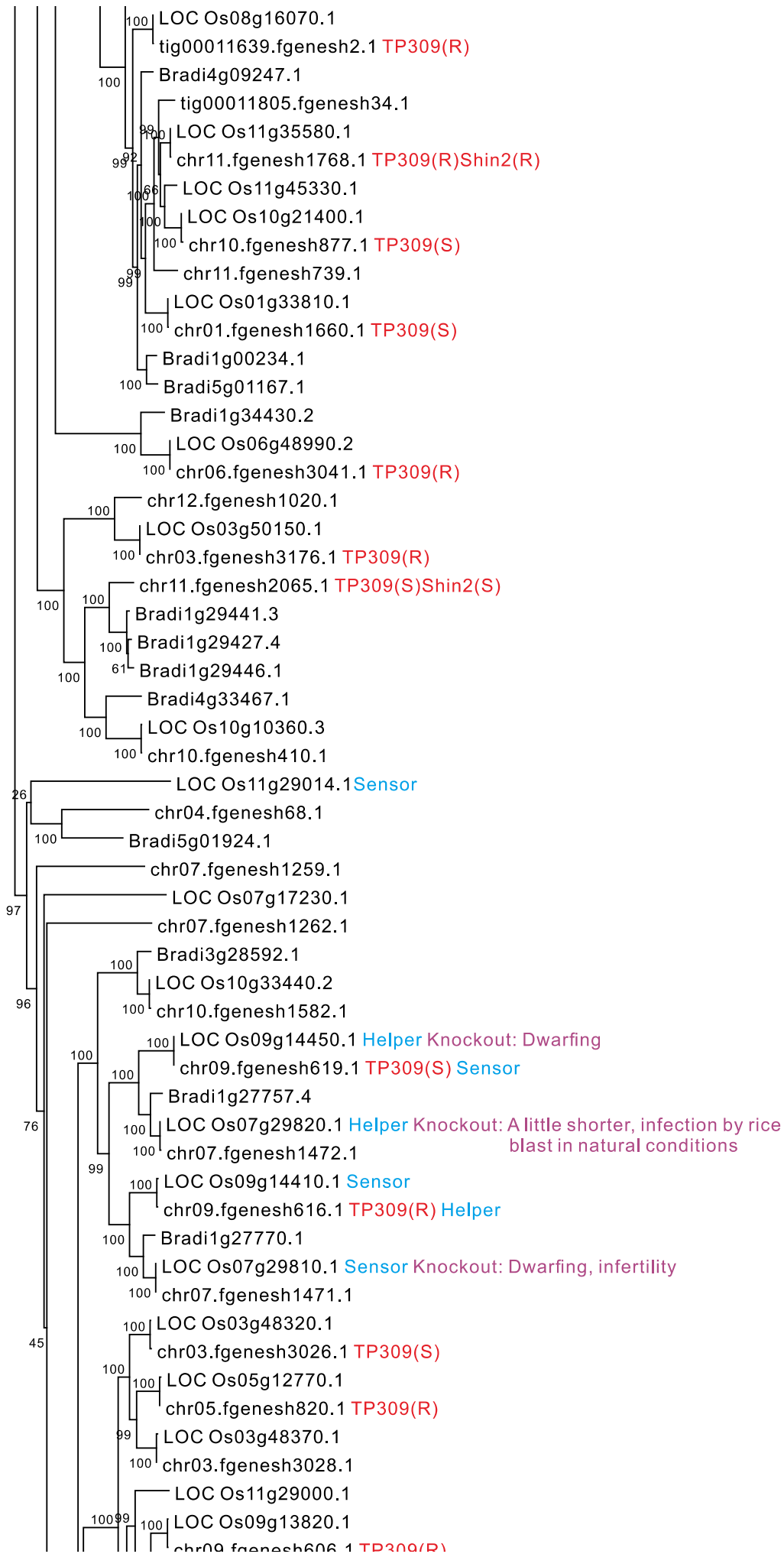


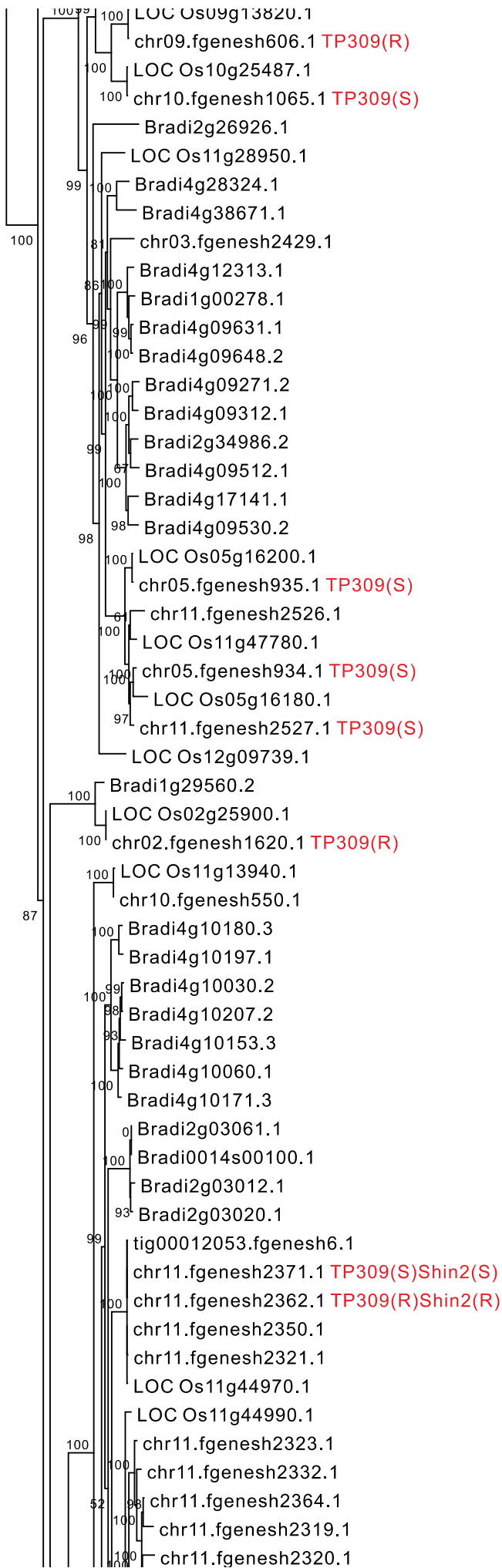




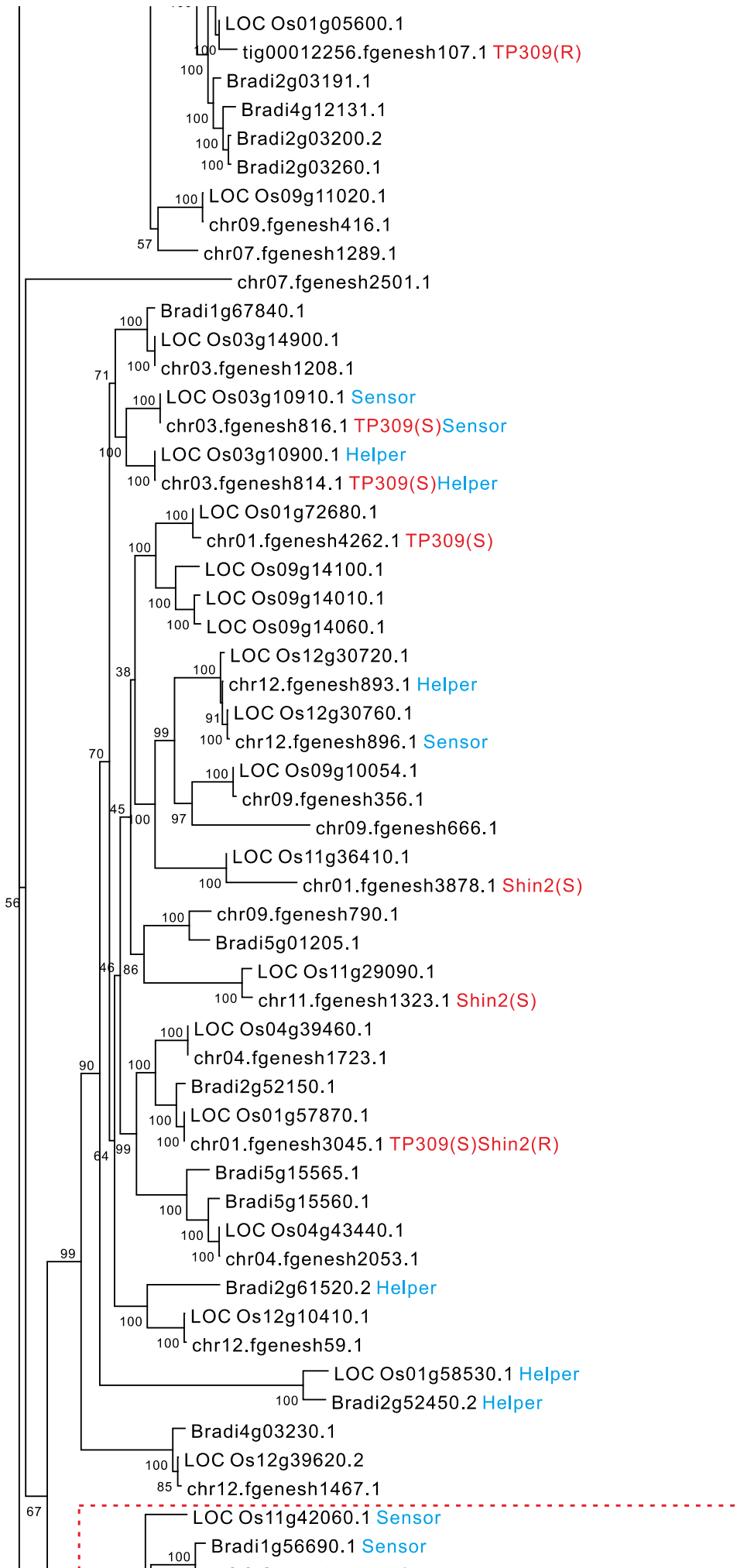


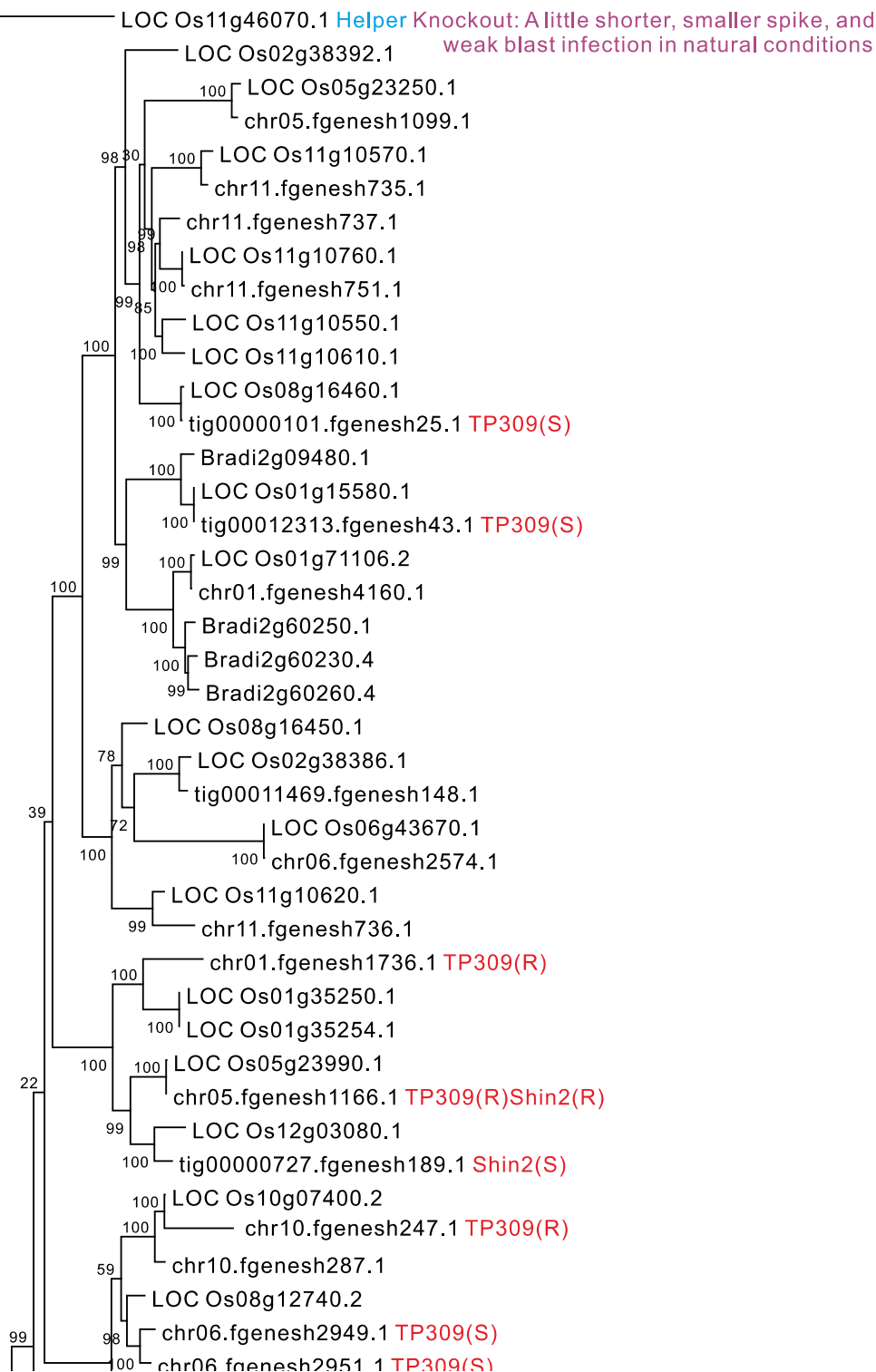
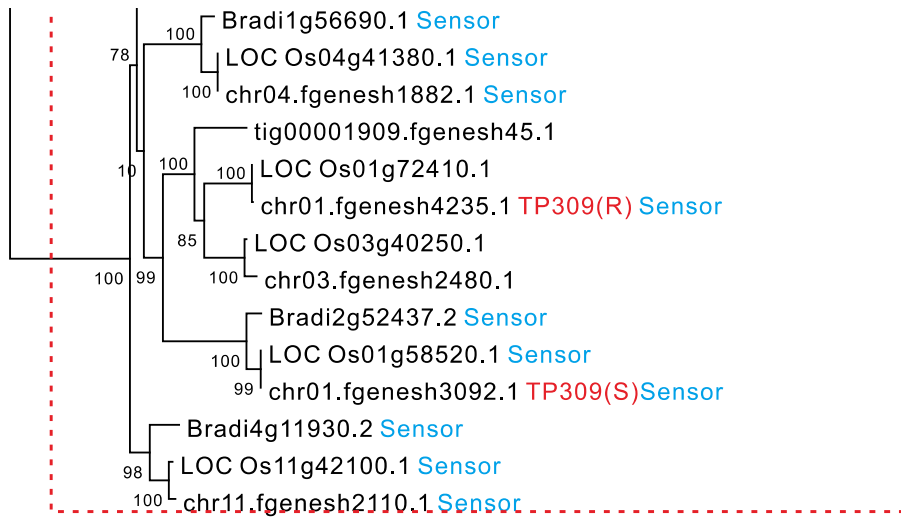


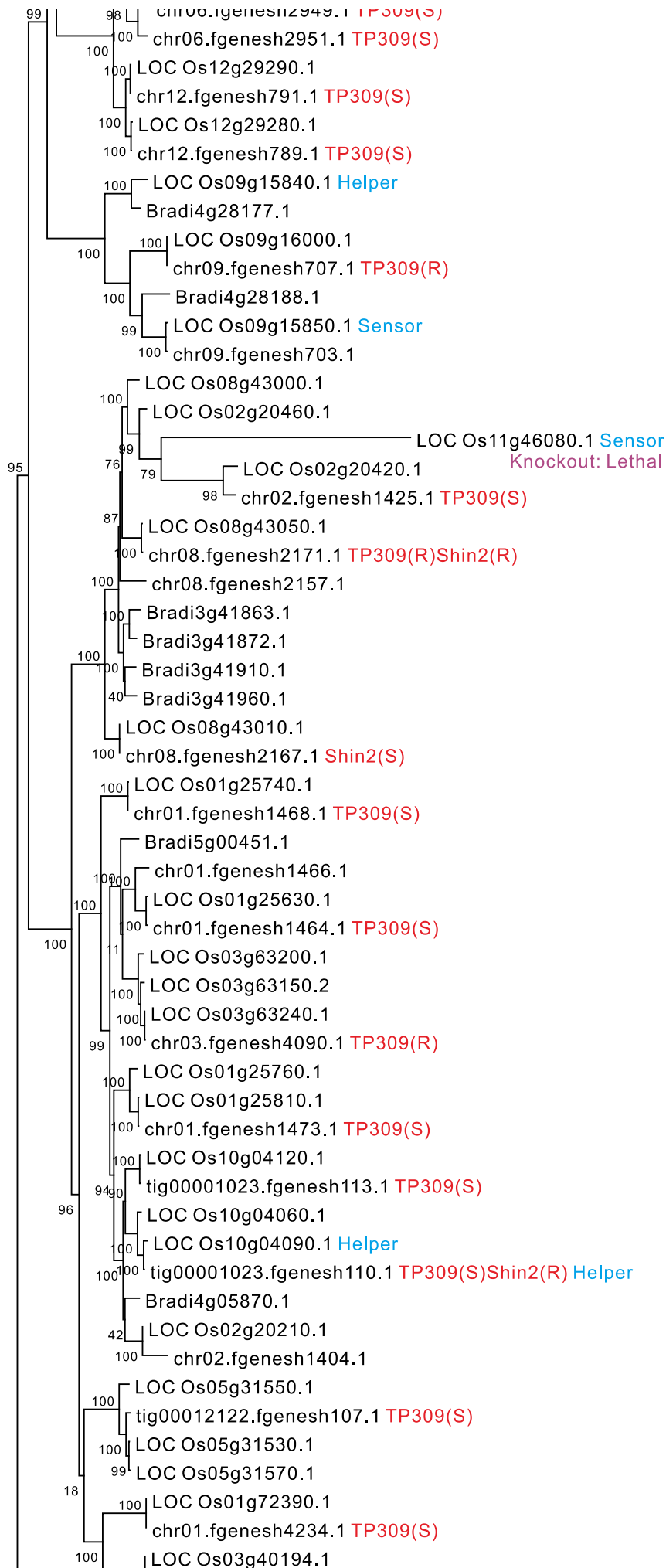


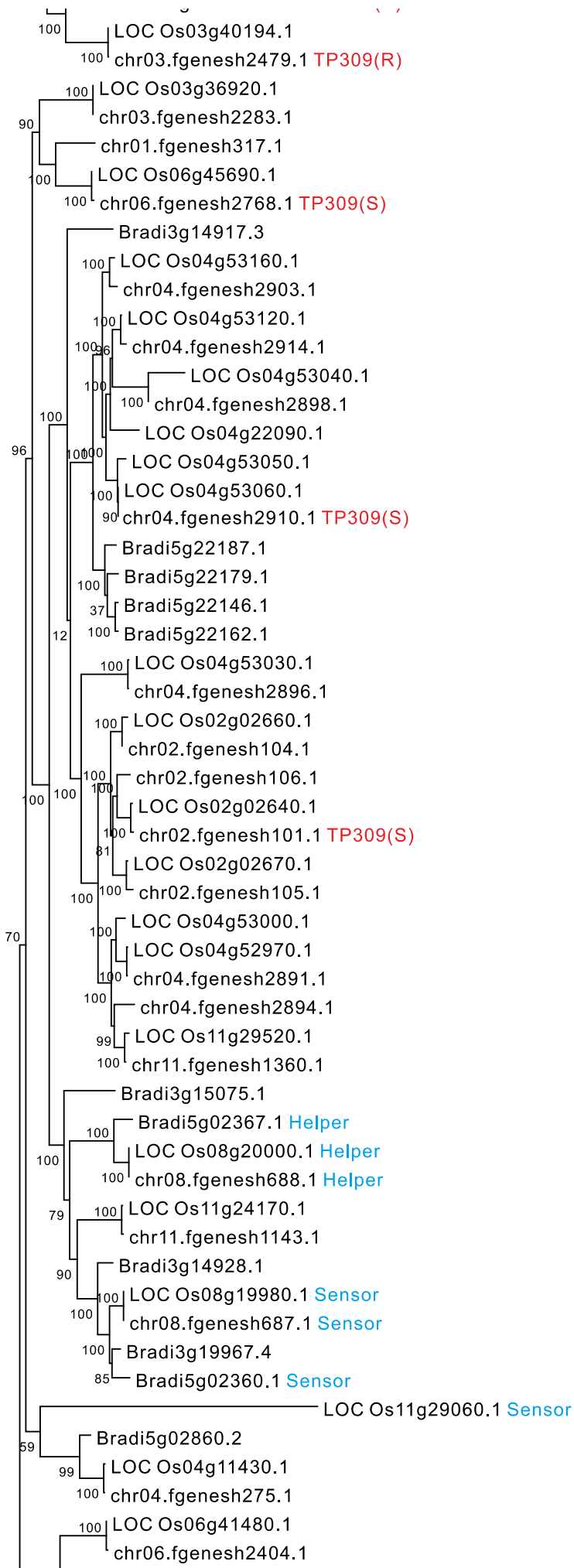


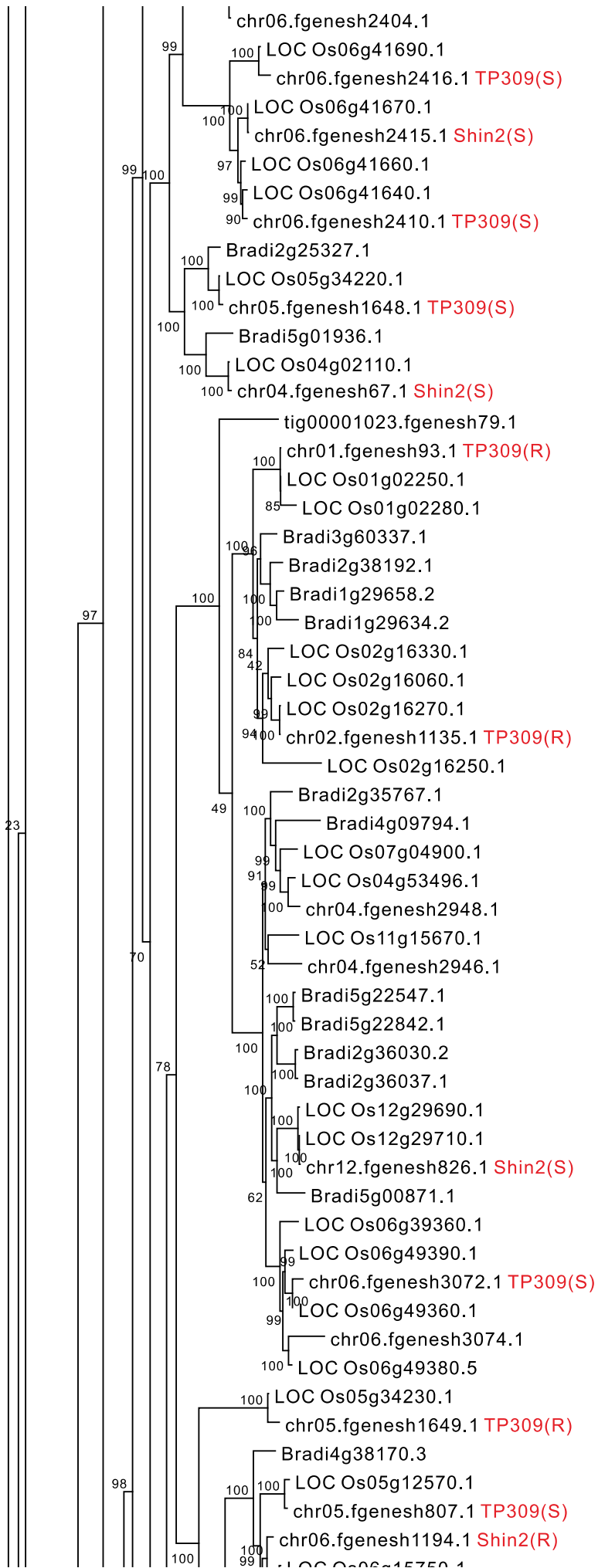


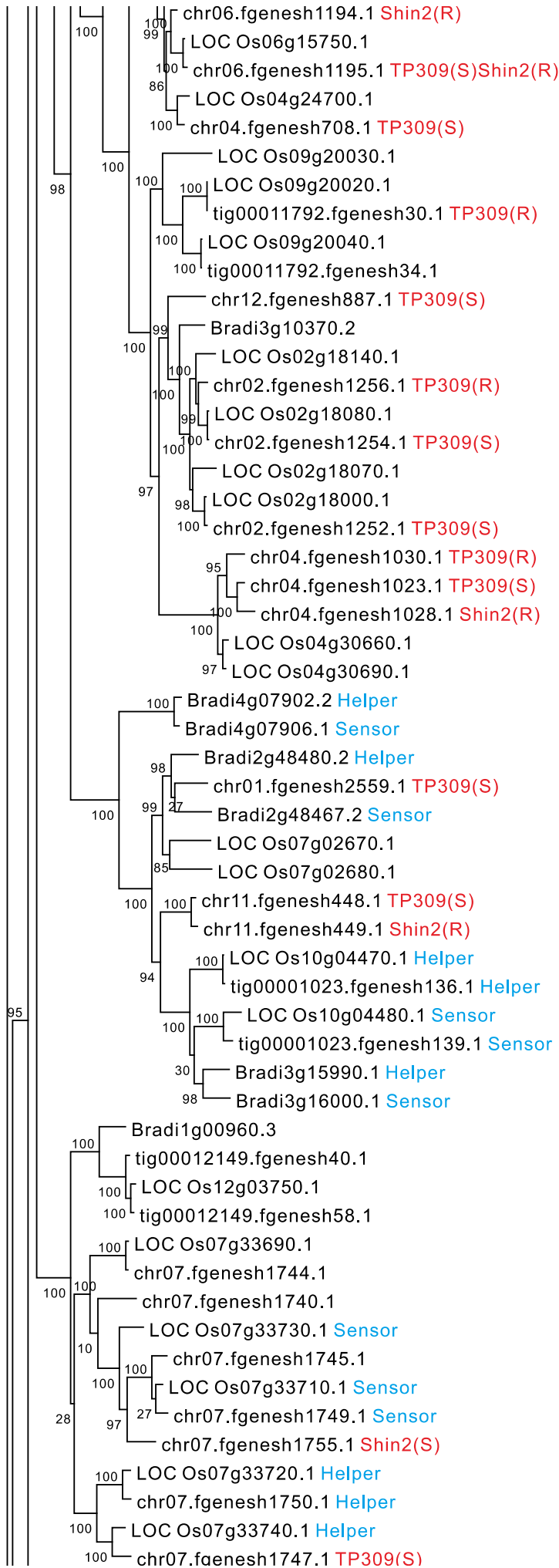


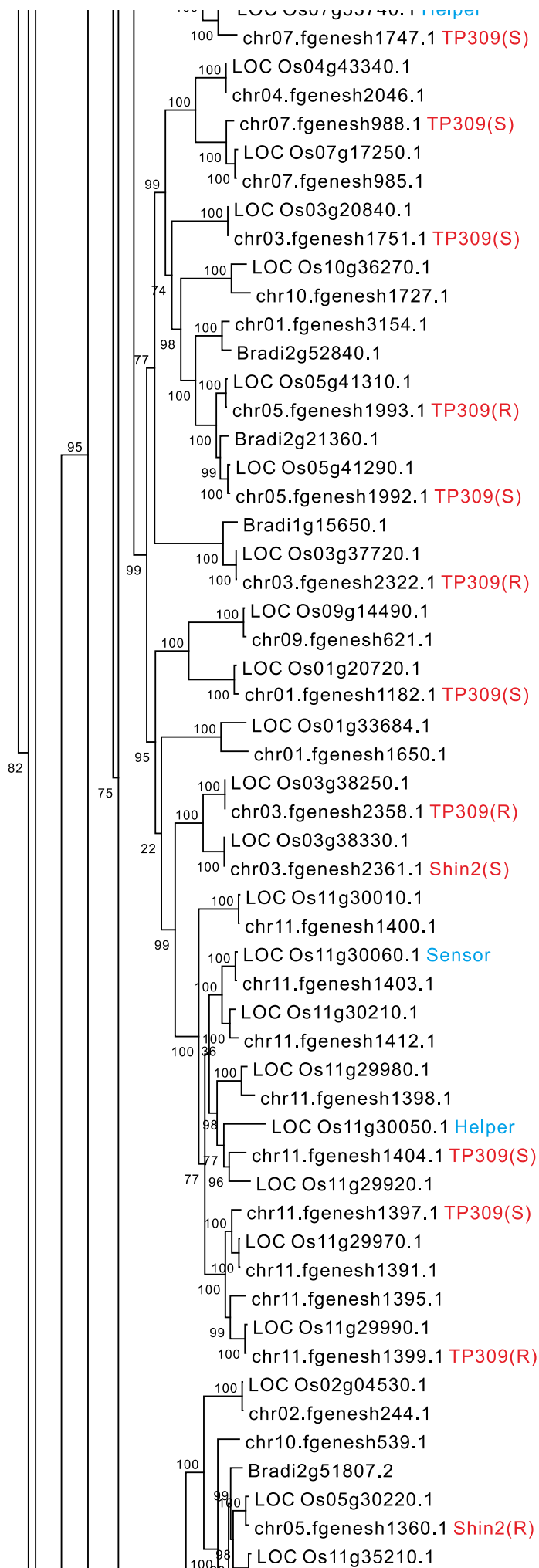


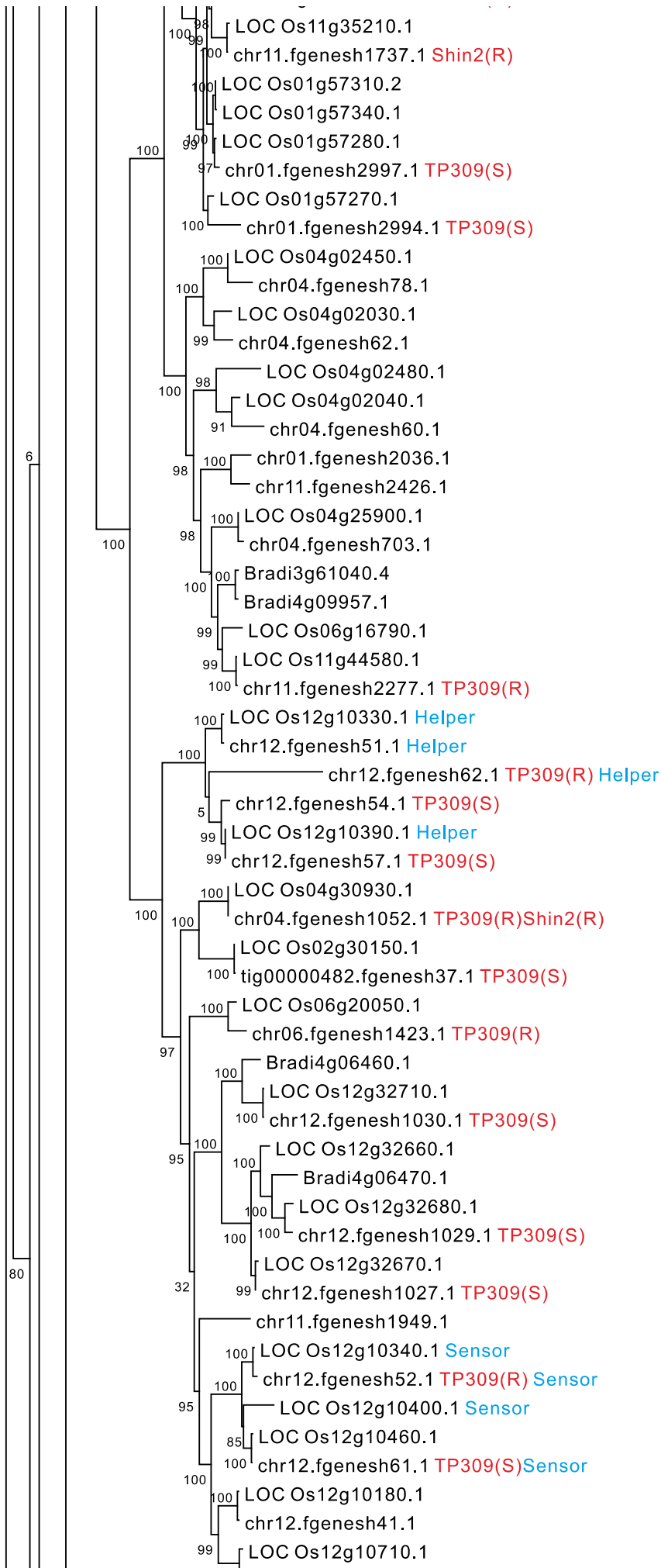












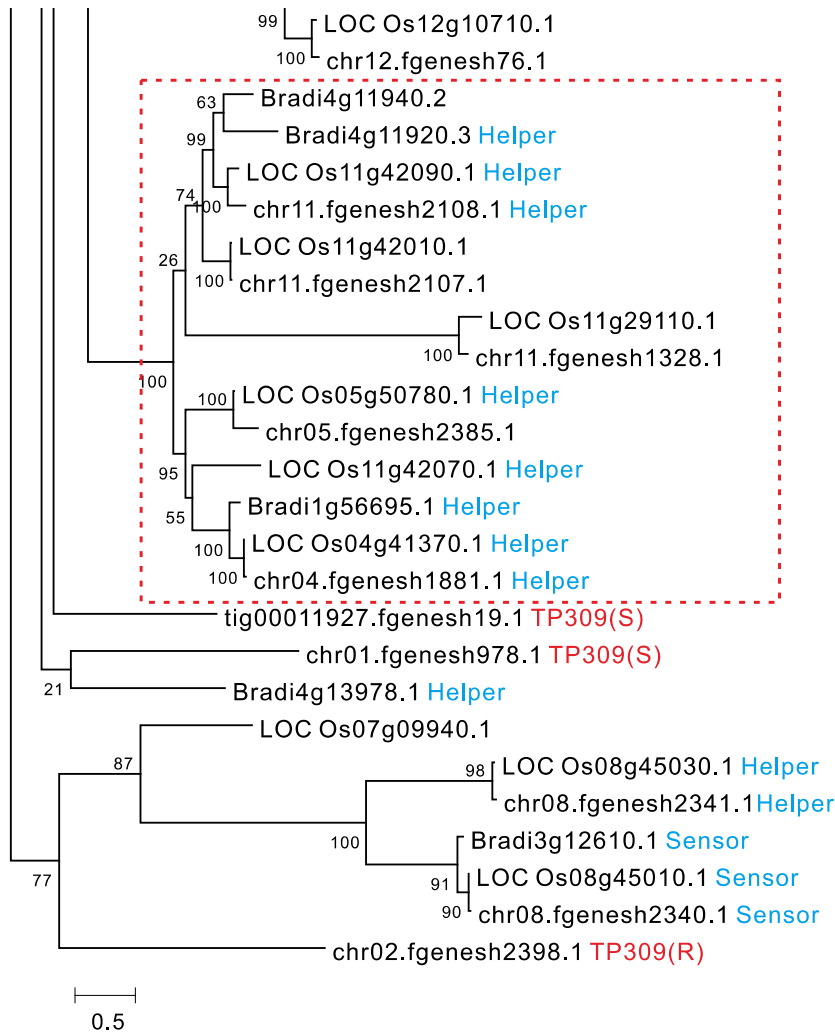


Fig. S3. Phylogenetic tree of all *NLRs* identified in Tetep (*.fgenes*), Nipponbare (LOC_Os*) and *B. distachyon* (Brad*). This tree was constructed using the protein sequences of NB-ARC domains from all identified *NLRs* by the ML (Maximum Likelihood) method (1000 bootstrap replicates). Those paired-*NLR* clades shown in Fig. 4 and 6 are shown in red dashed rectangles. A tested *NLR* is presented as TP309 (R) [Shin2 (R)], if it is resistant to at least one tested blast pathogen isolate when transformed into TP309 (Shin2) or as TP309 (S) [Shin2 (S)], if it is susceptible to all tested blast pathogen isolates when transformed into TP309 (Shin2). A short description of the phenotypic changes in CRISPR/Cas9 mutants are also given for each investigated *NLR*. A sensor (helper) was classified by its homologous relationship with sensor (helper) members of known *NLR* pairs. If this definition could not be made, we checked whether it carries an extra domain that might recognize pathogens, e.g. WRKY, RATX, NOI, and TRX, which could act as a decoy of pathogen effectors. The *NLR* will be classified as a sensor if yes, but a helper if not.

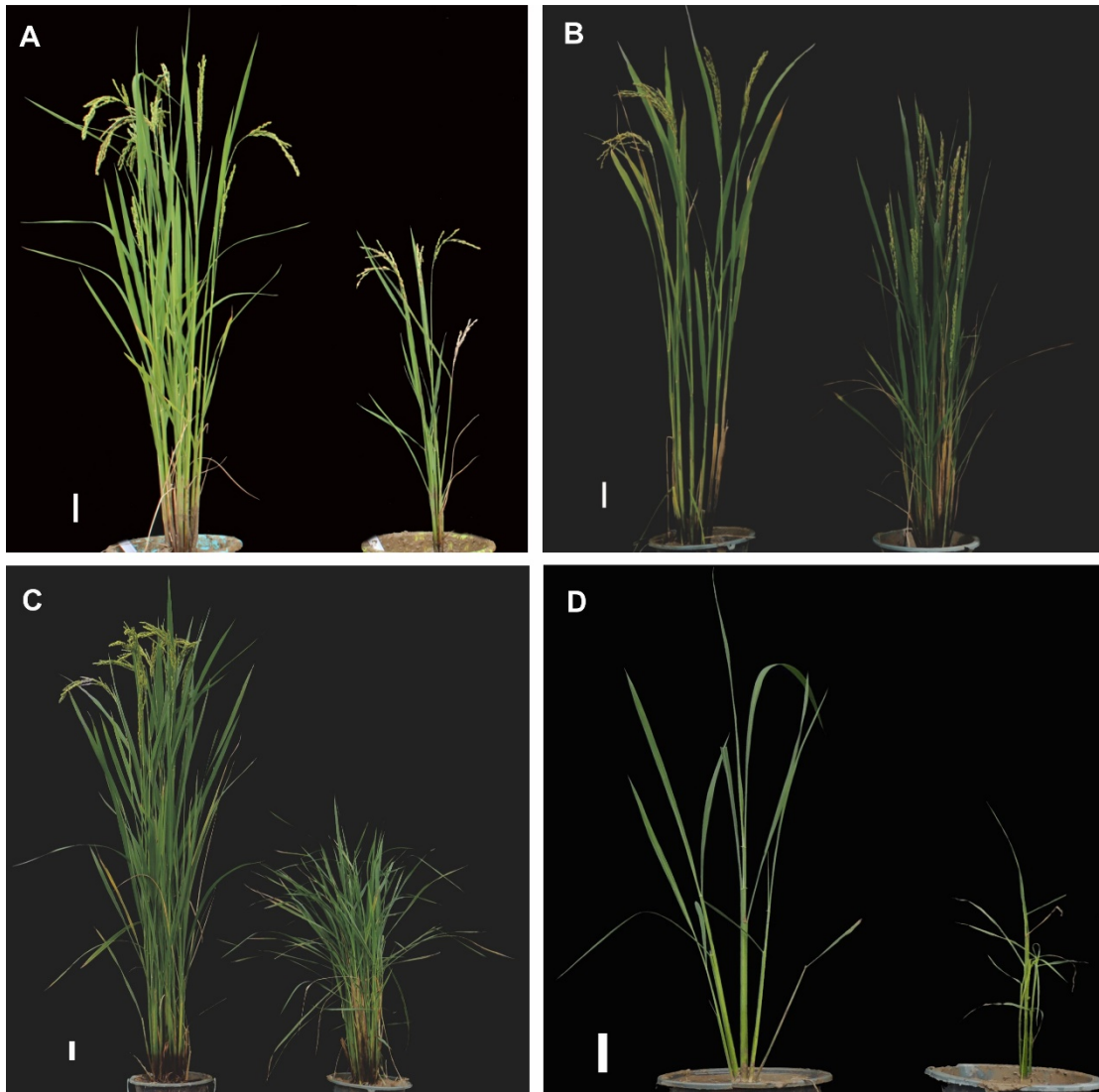


Fig. S4. Phenotypes of Tetep *NLR* transformants. (A) TP309 transformant of *chr11.fgenes2446*. Left: wild type; right: T₁ transformant. The transgenic lines displayed shortened statures compared to WT. ~80 days old plants. (B) TP309 transformant of a highly resistant *NLR chr11.fgenes2443*. Left: wild type; right: T₁ transformant. ~90 days old plants. The transgenic lines displayed dwarfism and generally had a low seed-setting rate compared to WT. Few seeds could be successfully germinated from these transformants. (C) TP309 transformant of *chr10.fgenes935*. Left: wild type; right: T₀ transformant. The transgenic lines displayed dwarf phenotypes and low seed-setting rates. 70 days old plants. (D) Shin2 transformant of *chr11.fgenes829 (RGA5)*. Left: wild type; right: T₀ transformant. The transgenic lines displayed retarded growth compared to WT. 19 days old plants. No transgenic lines were obtained for its helper counterpart *chr11.fgenes828 (RGA4)* after all transformation trails. Scale bars = 5 cm.

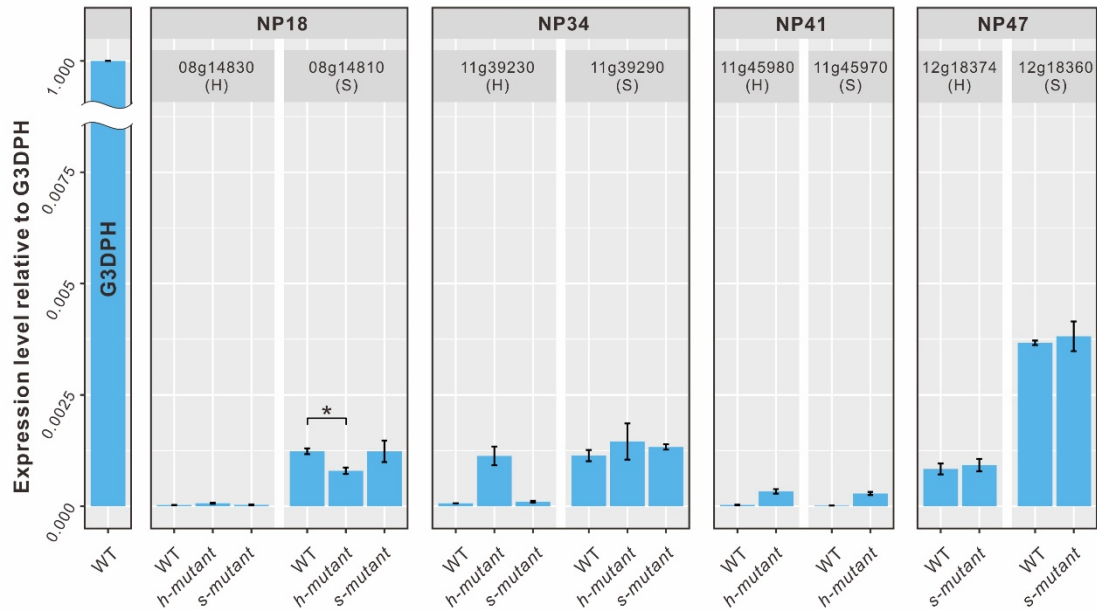


Fig. S5. Expression level changes in paired *NLRs* after knockout. Four *NLR* pairs (a black-box for each pair) were selected for expression analyses using RT-qPCR. The expression levels of two members [H (S) indicates that this gene is a helper (sensor)] from a pair were measured in the wild-type (WT) plants, its helper mutants (*h-mutant*) and its sensor mutants (*s-mutant*). For pair NP41, the sensor mutant was unavailable, so only the WT and helper mutant plants were measured. Similarly, only the WT and sensor mutant plants were measured in pair NP47 as no helper mutants were obtained. All measured genes displayed low expression levels as reported before (40, 41). Although several genes seemed to show altered expression levels in their helper mutants, the changes were not statistically significant. No significant changes in gene expression levels were observed in all sensor mutants. Therefore, the functional changes of those *NLRs* were likely to be due to changes at the protein level but not at the transcription level. RNA samples were collected from fresh leaves of around 21-day-old plants. The y-axis represents the expression level relative to the endogenous gene control G3DPH ($2^{-\Delta CT}$). Bars represent mean \pm SD (n=3). The unpaired t-test was used to calculate the statistical significance between plants. Significance level after Bonferroni correction for multiple testing: * $P < 0.004$, ** $P < 0.0008$, *** $P < 8 \times 10^{-5}$.

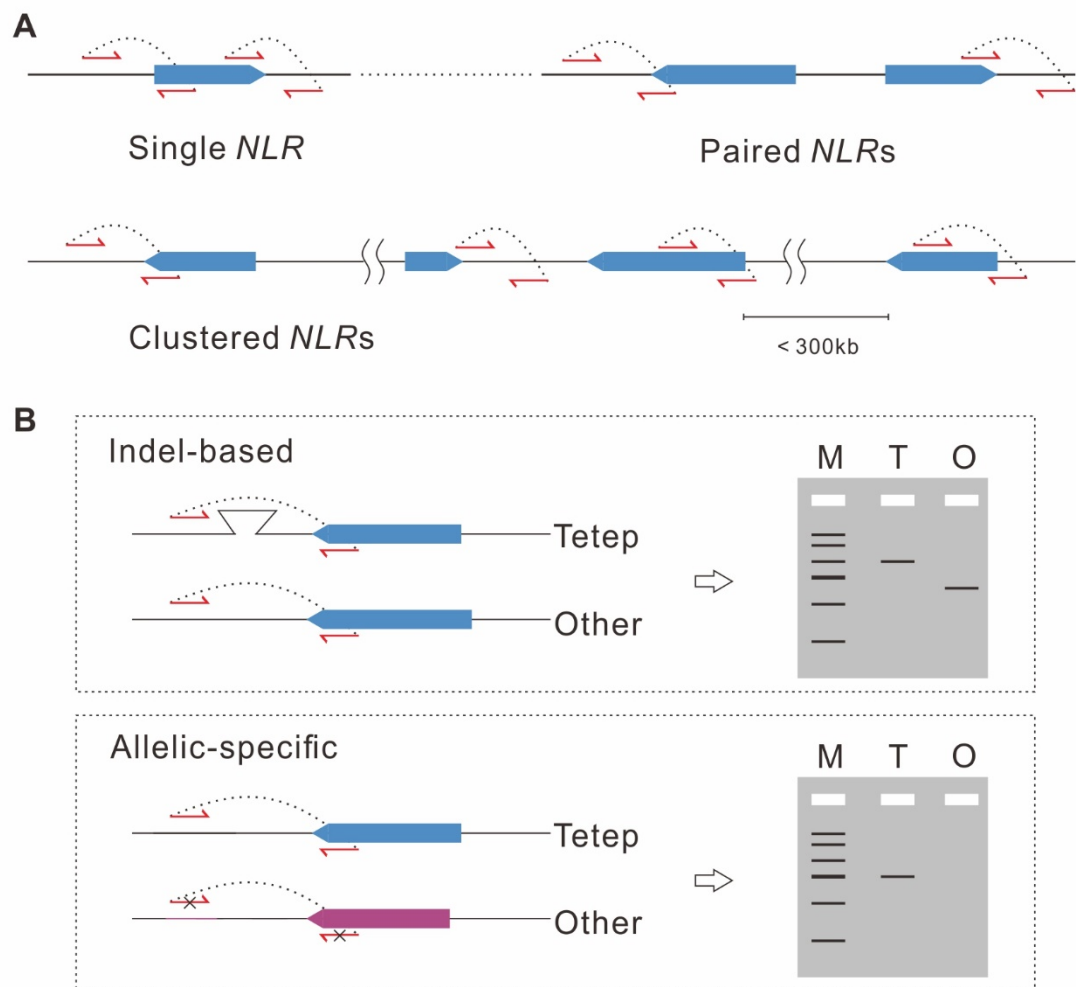
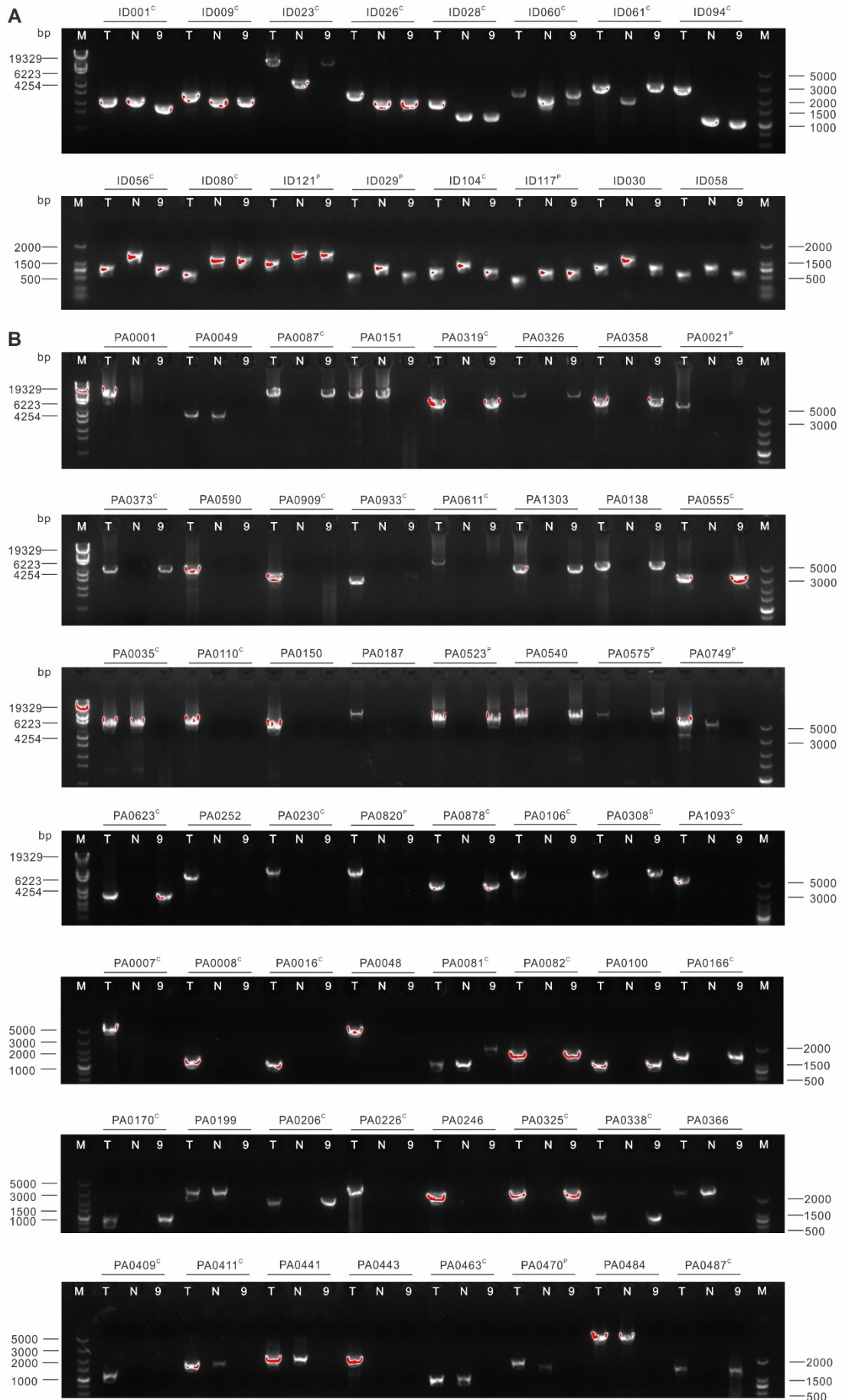


Fig. S6. Design of PCR primers to verify Tetep-derived *NLRs*. (A) Multiple PCR primer pairs designed to amplify nearly every *NLR* gene in the Tetep genome. For single and paired *NLR* genes, we design two primer pairs for each gene to amplify two regions in the gene; however, in a number of cases only one pair can be designed because no suitable second pair can be found. For a *NLR* cluster, we design two primer pairs for the first *NLR* gene, one primer pair for the upstream region and one primer pair for the 3' region of the middle *NLR* gene, and one primer pair for the 3' end of the last *NLR* gene. (B) Design of indel-based and allele-specific primer pairs. A primer pair is designed to include an indel in the Tetep genome relative to one or more of the other four genomes if such an indel can be found. If no indel can be found, then we design an allele-specific primer pair that can amplify the targeted region in Tetep but not in one or more of the other genomes, denoted by "Other" in the figure. Notations: M: standard DNA marker, T: Tetep, O: one of Nipponbare, R498, 9311 and MH63.



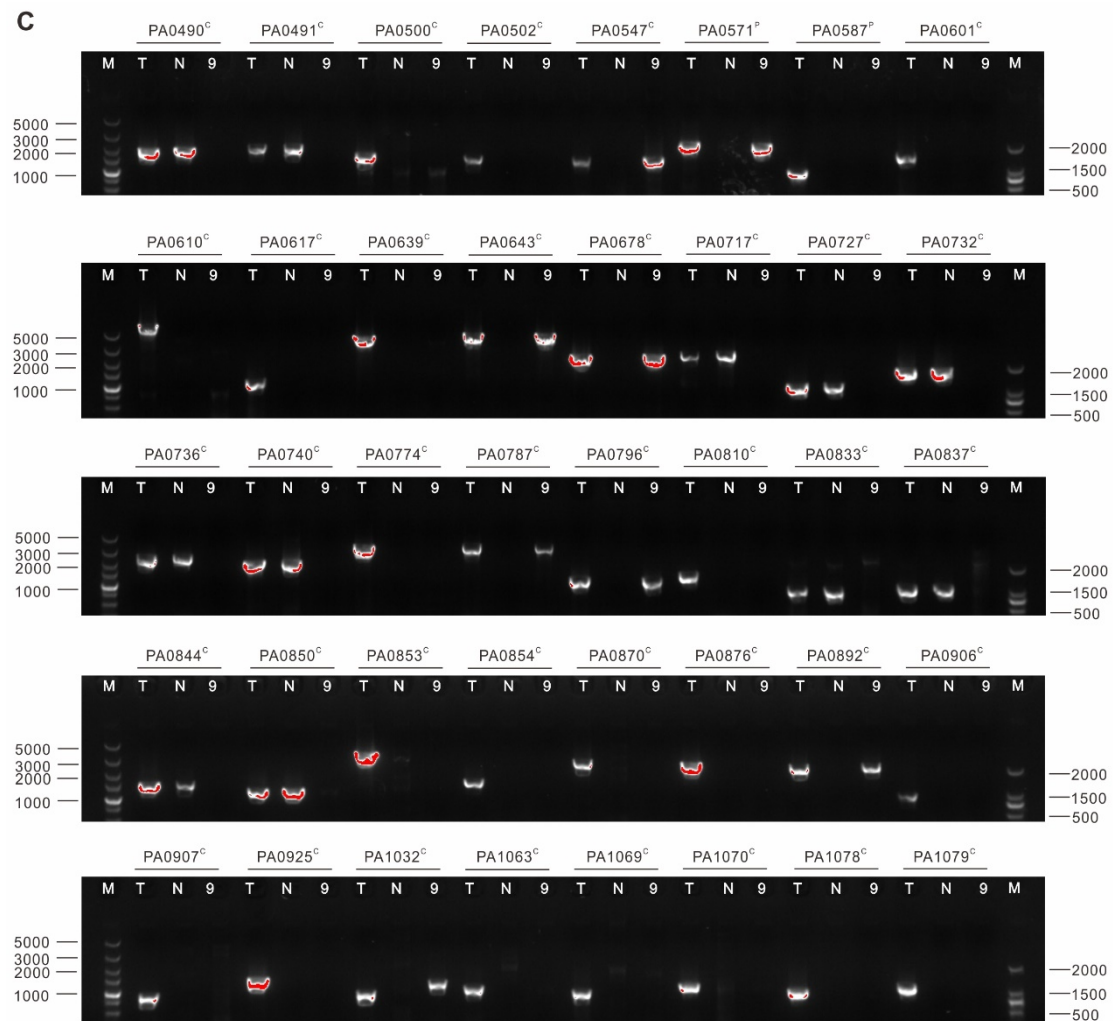


Fig. S7. Verification of PCR markers designed to amplify Tetep-derived *NLRs*. (A) Verification results of 16 indel-based PCR primer pairs. These results indicate that indel-based PCR markers can be used to distinguish Tetep (T) from Nipponbare (N) or 9311 (9) by band size in gel electrophoresis. The full list of verified indel-based PCR markers and amplification results are given in Dataset S1. All predicted indels between Tetep and each of four other genomes within the *NLR* regions are given in Dataset S2. (B, C) Verification results of 96 allelic-specific PCR primer pairs. These results indicate that allelic-specific PCR markers can be used to distinguish Tetep from Nipponbare and 9311 by the presence or absence of bands in gel electrophoresis. The full list of verified allelic-specific PCR markers and amplification results are given in Dataset S1. PCR markers designed for *NLR* clusters are marked by superscript “C” while PCR markers designed for paired *NLRs* are marked by superscript “P”. Standard DNA marker (M), leftmost: λ -*EcoT14* I digest, and rightmost: DL 5,000.

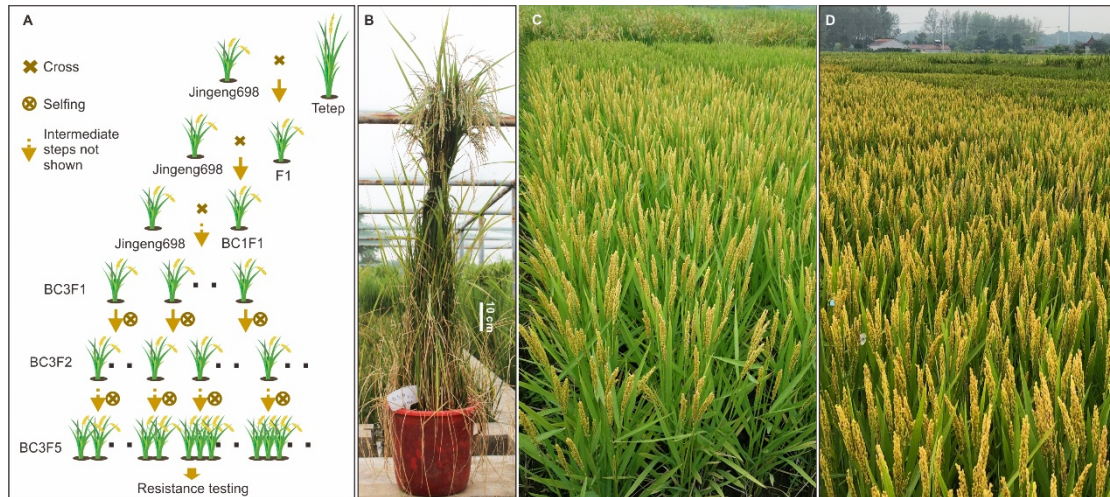


Fig. S8. Experimental crosses between Tetep and a blast-susceptible *japonica* cultivar Jingeng698. (A) Detailed crossing procedures. Multiple crossing steps were adopted to obtain fertile progeny with beneficial yield traits from Jingeng698 as well as improved resistance from Tetep. During these steps, the progeny were selected if they 1) carry the *japonica*-derived S5 locus (42) in order to overcome the hybrid sterility between *indica* and *japonica* rice; 2) have favored phenotypes similar as Jingeng698; and 3) contain the Tetep-derived “tig00011732.fgenes48 / tig00011732.fgenes49” NLR block. The progeny at BC3F5 generation (each from independent BC3F1 generation) were used for resistance testing. BC, backcross. (B) Phenotypes of rice Tetep. Tetep is poor in yield traits with a tall stature (~150 cm). Photo taken in Aug 30, 2017, ~130 days old. (C) Field photo of rice Jingeng698. Jingeng698 carries good yield traits but is susceptible blast disease. (D) Cross progeny (BC3F5 generation) with improved resistance. The progeny have improved resistance to blast, while carry the yield traits from Jingeng698. Photos (C, D) taken in Sep 6, 2018, ~150 days old.

Table S1. Sequencing statistics of the Tetep genome.

Platform	Insert size (bp)	No. of reads*	Average read length (bp)	N50** (bp)	Total Data (Gb)	Coverage depth**
Genomic PacBio (RS II)	20,000	7,774,683	8,532	11,763	66.3	147.7
Genomic Illumina (Hiseq 2000 PE)	500	93,480,914	100	100	9.3	20.8
Genomic Illumina (Hiseq 4000 PE)	350	161,737,412	150	150	24.3	54.0
RNA-seq (Illumina Hiseq 4000 PE)	300	120,777,542	150	150	18.1	-
RNA-seq (Illumina Hiseq 4000 PE)	300	125,590,036	150	150	18.8	-

* For genomic PacBio data, “reads” here stand for subreads, i.e. reads partitioned from polymerase read after removing adapters;

** N50 was calculated based on the 449 Mb genome size estimated by Mahesh et al, 2016 (6).

Table S2. Assembly metrics at each assembly stage.

Assembly stage	No. of Contigs	Average contig length	N50	Total size excluding gaps (Mb)
Canu assembly	1,119	357.1 kb	799.3 kb	399.7
1st round polishing	1,119	357.3 kb	799.6 kb	399.9
Chromosome anchoring	12 chrs* + 357 contigs	1.12 Mb	28.1 Mb	399.8
Gap filling	12 chrs + 356 contigs	1.13 Mb	28.1 Mb	401.7
2nd round polishing	12 chrs + 356 contigs	1.13 Mb	28.1 Mb	401.8

*chromosomes

Table S3. Statistics of the completeness of the Tetep genome based on the presence of the 248 core eukaryotic genes (CEGs)

	No. of CEGs	Tetep		Nipponbare		MH63		R498	
		CEGs Present*	Completeness** (%)	CEGs Present	Completeness (%)	CEGs Present	Completeness (%)	CEGs Present	Completeness (%)
Group 1	66	62	93.94	65	98.48	60	90.91	65	98.48
Group 2	56	55	98.21	54	96.43	52	92.86	55	98.21
Group 3	61	61	100	61	100	60	98.36	61	100
Group 4	65	65	100	65	100	64	98.46	65	100
All Groups	248	243	97.98	245	98.79	236	95.16	246	99.19

*Number of CEGs present in genome; **Percentage of CEGs present in genome

Table S4. Comparison of annotated *NLRs* in different rice genomes.

	Tetep		Nipponbare	MH63	R498	
	MAKER-P	Fgenesh	MSU v7	RS1	IGDBv3***	
Annotated	Effective Genome Size (Mbp)	402	373	360	391	
	No. of non-TE Coding Genes	37,054	41,937	39,049	37,324	37,549
	Average CDS length*	1,213	1,049	1,064	1,116	1,087
	<i>NLRs</i> annotated	327	455	473	455	409
	<i>NLRs</i> annotated per Mbp	0.81	1.13	1.27	1.26	1.03
Estimated**	All <i>NLR</i> regions predicted	519	504	514	531	
	<i>NLR</i> regions predicted per Mbp	1.29	1.35	1.43	1.36	
	Overlapped regions with annotated	498	476	493	453	
	% of overlapped regions	96.0%	94.4%	95.9%	85.3%	

*Only the representative or longest transcripts were considered if the annotation set contained alternative spliced isoforms; **Estimated putative *NLR* regions by NLR-annotator; ***Data downloaded from <http://www.mbkbase.org/R498/>

Table S5. Nucleotide diversity between Tetep *NLRs* and orthologous Nipponbare, MH63, and R498 *NLRs*.

	Tetep	Nipponbare	MH63	R498
NLRs with orthologues ^a	Count	360	335	332
	Mean diversity of nearest orthologues ^b	0.0596	0.0422	0.0483
NLRs without orthologues	BLASTP Identity & Coverage \geq 50%	78	88	104
	BLASTP Identity & Coverage < 50%	17	32	19
Genomic average diversity ^c		0.0084	0.004	0.0041

^a Orthologues were identified using OrthoFinder based on protein sequences of NB-ARC domain;

^b The nucleotide diversity (p-distance) was calculated for all orthologue gene pairs using the aligned coding sequences of NB-ARC domain. For each Tetep *NLR* gene, only its nearest orthologue in Nipponbare or in MH63 or in R498 was calculated here;

^c Genomic average diversity was estimated as “Number of substitutions / Number of aligned bases” on the whole genome alignment.

Table S6. Tetep *NLRs* tested in two susceptible cultivars Shin2 and TP309 using 12 blast pathogen strains. R stands for “Resistant”, S for “Susceptible” and N for “Not assessed”. The results are given in the order of Shin2/TP309, e.g. R/S to S2007 means this gene conferred resistance to blast strain S2007 when transformed to Shin2 but was susceptible to the same strain when transformed to TP309. Single “N” stands for no results for both cultivars, i.e. equivalent to “N/N”.

ID*	Gene ID	S2007	BEI1	TPXL	S30	S2004	ZB15	S1	B7	B13	S1686	B15	LaiXian
001	chr12.fgenes1062	R/S	R/S	R/N	R/S	R/S	R/N	R/N	R/N	R/N	R/S	R/N	R/N
002	chr06.fgenes1195	N/S	S/S	R/S	R/S	R/S	R/S	R/N	R/S	R/N	S/S	R/S	R/N
003	chr06.fgenes377	N	R/R	N	R/R	R/S	R/S	R/N	R/N	R/N	S/N	R/N	S/N
004 ^{ab}	chr11.fgenes2443(H)	N/R	R/S	S/R	S/R	S/R	S/N	S/R	S/N	S/N	N/R	S/R	N
005	chr06.fgenes378	R/R	R/S	R/S	R/S	S/S	S/R	R/S	S/S	R/N	N/S	S/S	S/S
006 ^a	chr04.fgenes1052	R/R	R/S	R/N	S/R	S/R	R/R	N	N	N	N	N	N
007	chr11.fgenes1916	S/R	R/R	R/S	R/S	R/R	N	N/S	N	N	N/R	N/S	N
008	chr05.fgenes1166	S/R	S/S	R/R	R/S	S/N	N/S	R/S	R/R	R/S	N/S	N	N
009	chr03.fgenes4090	N/R	N/R	N	N/R	N/R	N	N	N	N	N/R	N	N
010 ^{ab}	tig00011732.fgenes48(H)	S/R	R/S	S/R	N/S	S/S	N/R	N/S	N/S	N/S	N	N/S	R/S
011 ^a	chr11.fgenes2349	S/R	S/S	S/S	S/R	S/S	S/R	R/R	R/S	S/S	S/S	S/S	S/S
012 ^a	chr11.fgenes2222	N/R	N	N/S	N/S	N/R	N	N/R	N	N	N/R	N	N
013	chr02.fgenes1135	N/R	N/S	N/R	N/S	N/R	N	N/S	N	N	N/R	N	N
014	chr06.fgenes1561	N/R	R/N	N/S	S/S	R/S	N	N	N	N	S/S	S/N	R/N
015 ^b	chr11.fgenes1897(H)	N	R/S	N/S	S/S	R/S	N/S	N	N/S	N	S/N	S/R	R/N
016 ^{ab}	tig00011732.fgenes49(S)	S/S	S/S	S/R	S/S	S/S	S/R	N	S/R	N	N	R/S	S/S
017 ^{ab}	chr11.fgenes2446(H)	N/S	S/S	S/S	R/R	N/S	N	R/S	R/R	R/S	N/S	N	N/S
018	chr08.fgenes1326	R/S	S/S	S/R	S/S	R/S	N/S	N/S	N/S	N/S	R/S	S/N	S/S
019 ^a	chr11.fgenes2397	S/S	S/R	S/R	S/S	N/S	N/S	N/R	N/R	N/S	N/S	N/S	N/S
020 ^a	chr12.fgenes1311	N/R	N/R	N	N/R	N/S	N	N	N	N	N	N	N

021	chr01.fgenes1736	N	N/R	N	N/S	N/S	N/R	N	N	N/R	N	N	N
022^{ab}	chr10.fgenes936(H)	S/R	R/S	S/N	S/R	S/N	N	N	N	N	N	N	N
023	chr08.fgenes337	N/R	N/R	N	N/S	N/R	N/S	N	N	N	N/S	N	N
024^a	chr11.fgenes2359	N/R	N/R	N	N/S	N/S	N/S	N	N	N	N/R	N	N
025^a	chr07.fgenes257	N/R	N/R	N/S	N/R	N/S	N/S	N	N	N	N	N	N/S
026^b	chr09.fgenes616(H)	N/R	N/R	N/R	N/S	N/S	N	N/S	N	N	N/S	N	N
027	chr08.fgenes433	N/R	N/S	N/R	N/S	N/S	N	N	N	N	N/S	N/R	N/S
028	chr01.fgenes1212	S/S	S/S	N/R	S/R	N/S	N	S/S	N	S/S	R/S	N	N/S
029	chr02.fgenes1750	S/R	S/S	N/R	S/S	S/S	S/S	S/N	N/S	N	N	N/R	N
030^a	chr05.fgenes1360	S/N	S/N	R/N	S/N	S/N	S/N	N	R/N	N	N	R/N	S/N
031^a	chr12.fgenes320	N/R	S/S	S/R	S/S	S/S	S/R	N	S/N	S/N	N	S/N	N/S
032^a	chr11.fgenes2362	N/R	R/S	S/R	S/S	S/S	S/N	S/N	S/N	S/S	N	S/S	N/S
033^b	chr11.fgenes1294(S)	R/N	S/N	S/N	S/N	S/N	R/N	S/N	S/N	R/N	S/N	S/N	S/N
034	chr04.fgenes1028	R/N	R/N	N	S/N	S/N	N	N	N	N	N	N	N
035^a	chr03.fgenes2322	N/S	N/R	N	N	N/S	N/R	N	N	N	N	N	N
036^a	chr05.fgenes1993	N/S	N/R	N	N/R	N	N	N	N	N	N/S	N	N
037^b	chr12.fgenes52(S)	N/R	N/S	N	N/R	N/S	N	N	N	N	N	N	N
038	chr02.fgenes1620	N/R	N/S	N/S	N/R	N/S	N	N	N	N	N	N	N
039	chr06.fgenes419	N/R	N/S	N	N/R	N/S	N	N	N	N	N/S	N	N
040	chr07.fgenes465	S/S	N	S/S	R/S	S/S	N	N	N	S/R	N	N	N/S
041	chr08.fgenes2171	N	S/S	R/R	S/S	N	N	R/R	S/S	S/S	N	N	N
042	chr11.fgenes884	N/R	N/S	N/R	N/S	N/S	N	N	N	N	N/S	N	N
043^b	tig00001023.fgenes127(S)	N/S	N/R	N/R	N/S	N/S	N	N	N	N	N/S	N	N
044	chr11.fgenes1399	N/S	N/S	N/R	N/S	N/S	N/R	N/S	N	N	N	N	N
045	tig00012256.fgenes107	N/S	N/S	N/S	N/S	N/R	N/R	N	N	N	N	N	N/S

046	chr11.fgenes1737	R/N	S/N	S/N	S/N	R/N	N	N	S/N	N	S/N	S/N	S/N
047	tig00011816.fgenes38	N	S/N	S/N	S/N	N	R/N	S/N	S/N	R/N	S/N	N	S/N
048	chr08.fgenes674	N	S/S	N/S	S/S	R/N	N/S	N/S	N/S	N	S/N	S/N	R/N
049^a	tig00012169.fgenes81	N/S	S/S	S/S	S/S	S/S	R/N	N/S	S/N	N	N	R/N	N/S
050	chr11.fgenes1768	S/S	S/S	S/R	S/S	S/S	S/S	R/N	S/N	S/N	N	S/N	S/S
051^a	chr02.fgenes1739	S/S	S/S	S/S	S/R	S/S	S/N	S/S	S/R	S/S	N/S	N	N/S
052^a	chr11.fgenes2338	N/S	S/S	S/R	S/S	S/S	S/R	S/N	S/N	S/N	N	S/N	N/S
053	chr03.fgenes2479	N	N	N	N	N/R	N	N	N	N	N	N	N
054	chr03.fgenes2358	N	N/R	N	N	N/S	N	N	N	N	N	N	N
055	chr01.fgenes93	N	N/R	N	N/S	N/S	N	N	N	N	N	N	N
056	chr09.fgenes707	N/R	N	N	N	N/S	N	N	N	N	N/S	N	N
057^a	chr02.fgenes2398	N/R	N/S	N	N/S	N	N	N	N	N	N	N	N
058^a	chr05.fgenes820	N/R	N	N	N	N/S	N	N	N	N	N/S	N	N
059^a	chr07.fgenes729	N/S	N/S	N	N/R	N	N	N	N	N	N	N	N
060^a	chr09.fgenes343	N/R	N	N	N/S	N	N	N	N	N	N/S	N	N
061	chr02.fgenes1256	N/R	N/S	N/S	N/S	N	N	N	N	N	N	N	N
062	chr06.fgenes1423	N/S	N/R	N	N/S	N/S	N	N	N	N	N	N	N
063	tig00011639.fgenes2	N/R	N	N	N/S	N/S	N	N	N	N	N/S	N	N
064^a	chr06.fgenes3001	N/S	N/S	N	N	N/R	N	N	N	N	N/S	N	N
065^a	chr10.fgenes247	N/S	N	N	N	N/S	N	N	N	N/S	N/R	N	N
066^a	chr10.fgenes301	N/S	N/S	N	N/S	N/R	N	N	N	N	N	N	N
067^a	chr10.fgenes672	N/S	N/R	N	N/S	N/S	N	N	N	N	N	N	N
068^a	chr11.fgenes2277	N/S	N/S	N	N/R	N	N	N	N	N/S	N	N	N
069^a	chr11.fgenes2354	N/S	N	N	N/S	N/R	N	N	N	N	N/S	N	N
070^b	chr01.fgenes926(H)	N/R	N/S	N/S	N/S	N	N	N	N	N	N	N	N

071^b	chr01.fgenesH4235(S)	N/R	N/S	N	N/S	N/S	N	N	N	N	N	N	N
072^b	chr11.fgenesH1888(S)	N/S	N/R	N	N	N/S	N	N	N	N	N/S	N	N
073^b	chr12.fgenesH62(H)	N/S	N/R	N	N/S	N/S	N	N	N	N	N	N	N
074	chr03.fgenesH3176	N/R	N	N/S	N/S	N/S	N	N	N	N	N/S	N	N
075	chr04.fgenesH1030	N/R	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
076	chr12.fgenesH1226	N	N/R	N	N/S	N/S	N	N/S	N	N	N/S	N	N
077	tig00011792.fgenesH30	N/S	N/S	N	N	N/S	N	N/S	N	N	N/R	N	N
078	chr05.fgenesH1649	N/S	N/R	N	N/S	N/S	N	N/S	N	N	N/S	N	N
079	chr06.fgenesH1194	N	S/N	S/N	S/N	N	N	S/N	S/N	R/N	N	N	N
080	tig00000101.fgenesH4	N	R/N	N	S/N	S/N	N	N	N	N	S/N	S/N	S/N
081	tig00012434.fgenesH76	N	N/S	N	N/R	N/S	N	N	N	N	N/S	N/S	N/S
082^a	chr06.fgenesH3041	N/R	N/S	N	N/S	N/S	N	N/S	N	N	N/S	N	N
083^a	chr12.fgenesH196	N/R	N/S	N	N/S	N/S	N	N/S	N	N	N/S	N	N
084	chr01.fgenesH3045	S/S	R/S	S/S	S/S	S/S	N	N/S	N	N	N	N	N/S
085	chr11.fgenesH449	S/N	S/N	R/N	S/N	S/N	N	S/N	N	N	N	N	S/N
086	chr11.fgenesH950	N/S	N/S	N/S	N/S	N/S	N/R	N/S	N	N	N	N	N
087	chr09.fgenesH606	N	N/R	N/S	N/S	N/S	N/S	N	N/S	N/S	N	N/S	N
088^b	tig00001023.fgenesH110(H)	S/S	N/S	S/S	S/S	S/S	N	N/S	N	R/N	N	N	N/S
089	chr08.fgenesH338	S/R	N/S	N/S	S/S	S/S	N/S	S/N	N/S	N	N/S	N/S	S/S
090^b	chr11.fgenesH1900(H)	N/R	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N	N/S	N/S
091	chr01.fgenesH2994	N	N	N	N	N/S	N	N	N	N	N	N	N
092^a	chr12.fgenesH887	N	N	N	N	N/S	N	N	N	N	N	N	N
093^b	chr11.fgenesH1896(S)	N	N	N	N	N/S	N	N	N	N	N	N	N
094	chr03.fgenesH2095	N/S	N	N	N	N/S	N	N	N	N	N	N	N
095	chr07.fgenesH1747	N/S	N	N	N/S	N	N	N	N	N	N	N	N

096	chr08.fgenes371	S/N	N	N	S/N	N	N	N	N	N	N	N	N
097	chr12.fgenes1029	N/S	N	N	N	N/S	N	N	N	N	N	N	N
098	tig00011511.fgenes88	N	N	S/N	N	N	N	S/N	N	N	N	N	N
099^a	chr02.fgenes1252	N/S	N	N	N	N/S	N	N	N	N	N	N	N
100^a	chr09.fgenes330	N/S	N	N	N/S	N	N	N	N	N	N	N	N
101^b	chr01.fgenes3092(S)	N/S	N	N	N/S	N	N	N	N	N	N	N	N
102	chr02.fgenes1425	N/S	N	N	N	N/S	N	N	N	N	N/S	N	N
103	chr04.fgenes708	N/S	N	N	N	N/S	N	N/S	N	N	N	N	N
104	chr04.fgenes1023	N/S	N	N	N/S	N	N	N	N	N	N/S	N	N
105	chr08.fgenes182	N/S	N/S	N	N	N/S	N	N	N	N	N	N	N
106	chr11.fgenes2185	N/S	N/S	N	N/S	N	N	N	N	N	N	N	N
107	chr12.fgenes761	N/S	N/S	N	N/S	N	N	N	N	N	N	N	N
108	chr12.fgenes1278	N/S	N/S	N	N	N/S	N	N	N	N	N	N	N
109	tig00012256.fgenes109	N/S	N	N	N	N/S	N	N	N	N	N/S	N	N
110^a	chr06.fgenes2410	N/S	N	N	N	N/S	N	N	N	N	N/S	N	N
111^b	chr01.fgenes3091(H)	N/S	N	N	N/S	N/S	N	N	N	N	N	N	N
112	chr01.fgenes1401	N	N/S	N	N/S	N/S	N	N	N	N	N	N	N/S
113	chr01.fgenes1660	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
114	chr02.fgenes1254	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
115	chr05.fgenes935	N/S	N	N/S	N/S	N/S	N	N	N	N	N	N	N
116	chr05.fgenes1434	N/S	N/S	N	N/S	N	N	N	N	N	N/S	N	N
117	chr06.fgenes3072	N/S	N	N	N/S	N/S	N	N	N	N	N/S	N	N
118	chr07.fgenes1755	S/N	N	N	S/N	S/N	N	N	N	S/N	N	N	N
119	chr08.fgenes206	N/S	N/S	N	N	N/S	N	N	N	N	N/S	N	N
120	chr08.fgenes380	N/S	N	N	N/S	N/S	N	N	N	N	N/S	N	N

121	chr08.fgenesh465	N	S/N	N	S/N	S/N	N	N	N	N	N	N	S/N
122	chr11.fgenesh203	N	N/S	N	N/S	N/S	N	N	N	N	N	N	N/S
123	chr11.fgenesh825	N/S	N/S	N	N	N/S	N	N/S	N	N	N	N	N
124	chr11.fgenesh881	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
125	chr11.fgenesh2195	N/S	N/S	N	N/S	N	N	N	N	N	N/S	N	N
126	chr12.fgenesh54	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
127	chr12.fgenesh789	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
128	chr12.fgenesh1027	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
129	chr12.fgenesh1279	N	N/S	N	N/S	N/S	N	N	N	N/S	N	N	N
130	tig00000101.fgenesh25	N	N/S	N	N/S	N/S	N	N	N	N	N	N	N/S
131	tig000001019.fgenesh34	N/S	N/S	N	N/S	N	N	N	N	N	N/S	N	N
132 ^a	chr02.fgenesh1736	N/S	N	N	N/S	N/S	N	N	N	N	N/S	N	N
133 ^a	chr05.fgenesh807	N/S	N/S	N/S	N	N/S	N	N	N	N	N	N	N
134 ^a	chr06.fgenesh2416	N	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
135 ^a	chr10.fgenesh877	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
136 ^a	chr12.fgenesh247	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
137 ^a	tig00000482.fgenesh37	N/S	N	N	N/S	N/S	N	N	N	N	N/S	N	N
138 ^b	chr01.fgenesh4234(H)	N	N/S	N/S	N/S	N/S	N	N	N	N	N	N	N
139 ^b	chr03.fgenesh814(H)	N/S	N	N	N/S	N/S	N	N	N	N	N/S	N	N
140 ^b	chr08.fgenesh1120(S)	N/S	N/S	N	N/S	N/S	N	N	N	N	N	N	N
141	chr01.fgenesh1464	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
142	chr01.fgenesh1468	N/S	N	N/S	N/S	N/S	N	N	N	N	N/S	N	N
143	chr01.fgenesh1473	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
144	chr01.fgenesh2559	N/S	N/S	N	N/S	N/S	N	N/S	N	N	N	N	N
145	chr01.fgenesh3878	S/N	N	S/N	S/N	S/N	N	N	N	S/N	N	N	N

146	chr02.fgenes101	N/S	N	N/S	N/S	N/S	N	N	N	N	N/S	N	N
147	chr02.fgenes642	N/S	N/S	N	N/S	N	N/S	N	N	N/S	N	N	N
148	chr03.fgenes3026	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
149	chr04.fgenes67	S/N	S/N	N	S/N	N	N	N	N	S/N	N	N	S/N
150	chr05.fgenes934	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
151	chr05.fgenes1648	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
152	chr07.fgenes548	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
153	chr07.fgenes988	N	N/S	N/S	N/S	N/S	N	N/S	N	N	N	N	N
154	chr07.fgenes1324	N/S	N	N/S	N/S	N/S	N	N	N	N	N/S	N	N
155	chr07.fgenes2305	S/N	S/N	N	S/N	S/N	N	N	N	N	S/N	N	N
156	chr08.fgenes528	N	N	N	N/S	N	N/S	N/S	N	N	N/S	N/S	N
157	chr11.fgenes448	N	N	N	N/S	N/S	N	N	N/S	N	N/S	N	N/S
158	chr11.fgenes842	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
159	chr11.fgenes1323	S/N	N	S/N	N	N	N	S/N	N	N	S/N	S/N	N
160	chr11.fgenes1404	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
161	chr12.fgenes791	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
162	chr12.fgenes826	S/N	S/N	N	S/N	S/N	N	N	N	N	S/N	N	N
163	chr12.fgenes1030	N/S	N	N/S	N/S	N/S	N	N	N	N	N/S	N	N
164	tig00011455.fgenes100	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
165	tig00011498.fgenes63	S/N	S/N	N	N	S/N	N	N	S/N	N	N	S/N	N
166	tig00012053.fgenes10	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
167	tig00012122.fgenes107	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
168	tig00012313.fgenes43	N	N/S	N	N	N/S	N/S	N	N	N	N/S	N/S	N
169 ^a	chr02.fgenes1369	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
170 ^a	chr06.fgenes2951	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N

171 ^a	chr10.fgenesh262	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
172 ^a	chr10.fgenesh1065	N/S	N/S	N	N/S	N/S	N	N/S	N	N	N	N	N
173 ^a	tig00011927.fgenesh19	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
174 ^b	chr11.fgenesh1899(S)	S/N	N	N	N	S/N	S/N	S/N	N	S/N	N	N	N
175 ^b	chr12.fgenesh61(S)	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
176 ^b	tig00001023.fgenesh120(H)	N/S	N/S	N	N/S	N/S	N	N	N	N	N/S	N	N
177	chr02.fgenesh1194	S/N	N	N	S/N	S/N	N	S/N	N	N	S/N	S/N	N
178	chr04.fgenesh542	N/S	N/S	N/S	N/S	N/S	N	N	N	N	N/S	N	N
179	chr06.fgenesh1319	N/S	N/S	N	N/S	N/S	N	N/S	N	N	N/S	N	N
180	chr08.fgenesh343	N	S/N	S/N	S/N	N	S/N	S/N	S/N	N	N	N	N
181	chr08.fgenesh2167	S/N	S/N	S/N	S/N	S/N	N	N	N	N	N	N	S/N
182	chr10.fgenesh518	N/S	N/S	N/S	N/S	N/S	N	N	N	N	N/S	N	N
183	chr11.fgenesh1397	N/S	N/S	N/S	N	N/S	N	N/S	N	N	N/S	N	N
184	chr11.fgenesh2527	N/S	N	N/S	N/S	N/S	N	N	N	N/S	N	N	N/S
185	chr12.fgenesh1307	N/S	N/S	N/S	N	N	N/S	N	N	N/S	N	N/S	N
186	tig00001023.fgenesh113	N/S	N/S	N	N/S	N/S	N	N/S	N	N	N/S	N	N
187	tig00011805.fgenesh67	N/S	N/S	N/S	N/S	N/S	N	N	N	N	N/S	N	N
188 ^a	chr05.fgenesh1992	N/S	N/S	N	N/S	N/S	N	N/S	N	N	N/S	N	N
189 ^a	chr06.fgenesh2415	S/N	S/N	N	S/N	S/N	S/N	N	N	N	N	N	S/N
190 ^a	chr06.fgenesh2949	N/S	N/S	N	N/S	N/S	N/S	N	N	N	N/S	N	N
191 ^{ab}	chr10.fgenesh935(S)	N/S	N/S	N	N/S	N/S	N	N/S	N	N	N/S	N	N
192	chr01.fgenesh978	N	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N	N/S	N
193	chr01.fgenesh1182	N/S	N/S	N	N/S	N	N/S	N/S	N	N/S	N/S	N	N
194	chr01.fgenesh1321	N	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N	N/S	N
195	chr01.fgenesh2997	N	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N	N/S	N

196	chr02.fgenes1279	N	S/N	S/N	S/N	S/N	S/N	N	S/N	N	N	S/N	N
197	chr03.fgenes2361	S/N	S/N	S/N	S/N	S/N	N	S/N	N	N	N	N	S/N
198	chr04.fgenes2910	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N/S	N	N	N
199	chr06.fgenes1317	S/N	S/N	S/N	S/N	S/N	S/N	S/N	N	N	N	N	N
200	chr12.fgenes57	N/S	N/S	N	N/S	N/S	N/S	N/S	N	N	N/S	N	N
201	tig00012122.fgenes108	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N	N/S	N	N
202 ^a	chr11.fgenes2151	S/N	S/N	S/N	S/N	S/N	N	S/N	N	N	N	N	S/N
203 ^b	chr01.fgenes925(S)	N/S	N/S	N	N/S	N	N/S	N/S	N	N/S	N/S	N	N
204 ^b	chr09.fgenes619(S)	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N/S	N	N	N
205 ^b	chr11.fgenes1293(H)	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N	N	N	N/S
206 ^b	tig00011639.fgenes73(H)	N	S/N	S/N	S/N	S/N	S/N	N	S/N	N	N	S/N	N
207	chr01.fgenes4262	N	N/S	N/S	N/S	N/S	N/S	N	N/S	N/S	N	N/S	N
208	chr04.fgenes2315	N	N/S	N/S	N/S	N/S	N/S	N	N/S	N/S	N	N/S	N
209	chr08.fgenes1325	N	S/N	S/N	S/N	S/N	S/N	S/N	S/N	N	N	S/N	N
210 ^a	chr03.fgenes1751	N	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N	N	N/S	N
211 ^a	chr11.fgenes1547	N	S/N	S/N	S/N	S/N	S/N	N	S/N	S/N	N	S/N	N
212 ^b	chr08.fgenes1031(H)	N	N/S	N/S	N/S	N/S	N/S	N	N/S	N/S	N	N/S	N
213 ^b	chr12.fgenes921(H)	N	N/S	N/S	N/S	N/S	N/S	N	N/S	N/S	N	N/S	N
214	chr11.fgenes1894	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N/S	N/S	N	N/S
215	tig00000727.fgenes189	S/N	S/N	S/N	S/N	S/N	S/N	S/N	S/N	S/N	N	N	N
216 ^a	chr06.fgenes2768	N/S	N/S	N/S	N/S	N/S	N	N/S	N	N/S	N/S	N	N/S
217	tig00011498.fgenes17	N	S/N	S/N	S/N	S/N	S/N	S/N	S/N	S/N	N	S/N	S/N
218	chr11.fgenes2065	N	S/S	S/N	S/S	S/S	S/N	S/N	S/N	S/N	S/N	S/N	S/S
219 ^a	chr11.fgenes2371	N/S	S/S	S/S	S/S	S/S	S/S	S/S	S/S	N/S	N/S	S/S	N/S

* These IDs are used in Fig. 1; ^a Inherited *NLRs*; ^b Paired *NLRs*, H: Helper, S: Sensor.

Table S7. Tetep *NLRs* tested using nine additional blast pathogen strains. Seven *NLRs* which conferred resistance to ≥ 5 blast isolates were further tested using nine additional pathogen isolates (Y10 to S2005). This table uses the same notations as Table S6.

ID	Gene ID	Y10	B3	S21	S32	Y1	S2	S12-4-2	S33	S2005
001	chr12.fgenes1062	R/N	R/N	R/N	R/N	R/N	R/N	S/N	S/N	S/N
002	chr06.fgenes1195	R/N	R/N	R/N	R/N	R/N	S/N	N	N	N
003	chr06.fgenes377	R/N	R/N	S/N	S/N	S/N	R/R	N	N	N
005	chr06.fgenes378	S/N	N	N	R/N	N	R/S	N	R/N	N
008	chr05.fgenes1166	N	N	N	S/N	N	N/S	N	S/S	N
010	tig00011732.fgenes48	N/S	N	N	N	N	S/S	N	N	N
011	chr11.fgenes2349	S/S	N	N	N	N	S/S	R/S	N	S/R

Table S8. List of *NLR* pairs in Tetep and Nipponbare genomes.

Each member of a pair is suffixed with “-H” if it’s a helper and “-S” if it’s a sensor. The unusual domains are given in the last column.

Genome	Pair ID	Gene ID	Unusual Domains (Descriptions)
Nipponbare	NP1-H	LOC_Os01g16400	-
	NP1-S	LOC_Os01g16390	-
	NP2-H	LOC_Os08g30660	-
	NP2-S	LOC_Os08g30634	-
	NP3-H	LOC_Os11g27430	-
	NP3-S	LOC_Os11g27440	-
	NP4-H	LOC_Os11g45790	-
	NP4-S	LOC_Os11g45920	WRKY63;Syntaxin(membrane integrated Q-SNARE proteins participating in exocytosis)
	NP5-H	LOC_Os12g31200	-
	NP5-S	LOC_Os12g31160	-
	NP6-H	LOC_Os08g45030	-
	NP6-S	LOC_Os08g45010	-
	NP7-H	LOC_Os12g28050	-
	NP7-S	LOC_Os12g28040	-
	NP8-H	LOC_Os08g28540	-
	NP8-S	LOC_Os08g28460	-
	NP9-H	LOC_Os11g29050	-
	NP9-S	LOC_Os11g29060	-
	NP10-H	LOC_Os01g58530	-
	NP10-S	LOC_Os01g58520	-
NP11-H	LOC_Os03g10900	-	
NP11-S	LOC_Os03g10910	-	

	NP12-H	LOC_Os04g41370	-
	NP12-S	LOC_Os04g41380	GAS(microtubule-binding protein)
	NP13-H	LOC_Os05g40160	-
	NP13-S	LOC_Os05g40150	-
	NP14-H	LOC_Os05g50780	-
	NP14-S	LOC_Os05g50790	-
	NP15-H	LOC_Os07g29820	-
	NP15-S	LOC_Os07g29810	-
	NP16-H	LOC_Os07g33720	-
	NP16-S	LOC_Os07g33710	-
	NP17-H	LOC_Os07g33740	-
	NP17-S	LOC_Os07g33730	-
	NP18-H	LOC_Os08g14830	-
	NP18-S	LOC_Os08g14810	-
	NP19-H	LOC_Os08g20000	-
	NP19-S	LOC_Os08g19980	-
	NP20-H	LOC_Os09g14450	-
	NP20-S	LOC_Os09g14410	-
	NP21-H (Pi5-1)	LOC_Os09g15840	-
	NP21-S (Pi5-2)	LOC_Os09g15850	-
	NP22-H	LOC_Os10g04090	-
	NP22-S	LOC_Os10g04110	-
	NP23-H	LOC_Os10g04180	-

	NP23-S	LOC_Os10g04290	-
	NP24-H	LOC_Os10g04470	-
	NP24-S	LOC_Os10g04480	-
	NP25-H	LOC_Os10g22300	-
	NP25-S	LOC_Os10g22484	-
	NP26-H	LOC_Os10g22510	-
	NP26-S	LOC_Os10g22490	-
	NP27-H	LOC_Os11g11550	-
	NP27-S	LOC_Os11g11580	-
	NP28-H (RGA4)	LOC_Os11g11790	-
	NP28-S (RGA5)	LOC_Os11g11810	-
	NP29-H	LOC_Os11g11940	-
	NP29-S	LOC_Os11g11920	NOI(AvrRpt-cleavage, binding of the protein to RCS and AvrB)
	NP30-H	LOC_Os11g29030	-
	NP30-S	LOC_Os11g29014	-
	NP31-H	LOC_Os11g30050	-
	NP31-S	LOC_Os11g30060	-
	NP32-H	LOC_Os11g37040	-
	NP32-S	LOC_Os11g37050	-
	NP33-H	LOC_Os11g39160	-
	NP33-S	LOC_Os11g39190	-
	NP34-H	LOC_Os11g39230	-
	NP34-S	LOC_Os11g39290	CREPT(transcriptional regulators)

	NP35-H	LOC_Os11g39320	-
	NP35-S	LOC_Os11g39310	-
	NP36-H	LOC_Os11g39340	-
	NP36-S	LOC_Os11g39330	-
	NP37-H	LOC_Os11g42070	-
	NP37-S	LOC_Os11g42060	-
	NP38-H	LOC_Os11g42090	-
	NP38-S	LOC_Os11g42100	-
	NP39-H	LOC_Os11g45760	-
	NP39-S	LOC_Os11g45750	WRKY125;Syntaxin(membrane integrated Q-SNARE proteins participating in exocytosis);SRP54(signal recognition)
	NP40-H	LOC_Os11g45930	-
	NP40-S	LOC_Os11g45924	WRKY41
	NP41-H	LOC_Os11g45980	-
	NP41-S	LOC_Os11g45970	-
	NP42-H	LOC_Os11g46070	-
	NP42-S	LOC_Os11g46080	-
	NP43-H	LOC_Os11g46190	-
	NP43-S	LOC_Os11g46140	-
	NP44-S (Pik-1)	LOC_Os11g46200	RATX1(Related to ATX1, mediate Avr recognition)
	NP44-H (Pik-2)	LOC_Os11g46210	-
	NP45-H	LOC_Os12g10330	-
	NP45-S	LOC_Os12g10340	-

	NP46-H	LOC_Os12g10390	-
	NP46-S	LOC_Os12g10400	-
	NP47-H (Pi42)	LOC_Os12g18374	-
	NP47-S (Pi-ta)	LOC_Os12g18360	TRX(Thioredoxin, cell-to-cell communication);EN(Endotoxin N, insecticidal toxins, receptor binding)
Tetep	TP1-H	chr01.fgenes1926	-
	TP1-S	chr01.fgenes1925	-
	TP2-H	chr01.fgenes1325	-
	TP2-S	chr01.fgenes1326	-
	TP3-H	chr01.fgenes4234	-
	TP3-S	chr01.fgenes4235	-
	TP4-H	chr04.fgenes1881	-
	TP4-S	chr04.fgenes1882	GAS(microtubule-binding protein)
	TP5-H	chr07.fgenes1251	-
	TP5-S	chr07.fgenes1250	TRX(Thioredoxin, cell-to-cell communication);Glutaredoxin(flower development and Salicylic acid signaling)
	TP6-H	chr07.fgenes1750	-
	TP6-S	chr07.fgenes1749	-
	TP7-H	chr08.fgenes688	-
	TP7-S	chr08.fgenes687	-
	TP8-H	chr08.fgenes1119	-
	TP8-S	chr08.fgenes1120	-
TP9-H	chr09.fgenes619	-	
TP9-S	chr09.fgenes616	-	

	TP10-H	chr10.fgenes907	-
	TP10-S	chr10.fgenes908	-
	TP11-H	chr10.fgenes936	-
	TP11-S	chr10.fgenes935	CG-1(DNA-binding protein)
	TP12-H (RGA4)	chr11.fgenes828	-
	TP12-S (RGA5)	chr11.fgenes829	RATX1(Related to ATX1, mediate Avr recognition)
	TP13-H	chr11.fgenes1293	-
	TP13-S	chr11.fgenes1294	-
	TP14-H	chr11.fgenes1887	-
	TP14-S	chr11.fgenes1888	-
	TP15-H	chr11.fgenes1892	-
	TP15-S	chr11.fgenes1893	-
	TP16-H	chr11.fgenes1897	-
	TP16-S	chr11.fgenes1896	-
	TP17-H	chr11.fgenes1900	-
	TP17-S	chr11.fgenes1899	-
	TP18-H	chr11.fgenes1910	-
	TP18-S	chr11.fgenes1911	-
	TP19-H	chr11.fgenes2433	-
	TP19-S	chr11.fgenes2432	WRKY;FLYWCH(zinc finger domain);Syntaxin(membrane integrated Q-SNARE proteins participating in exocytosis)
	TP20-H	chr11.fgenes2435	-
	TP20-S	chr11.fgenes2436	-

	TP21-H	chr11.fgenes2443	-
	TP21-S	chr11.fgenes2442	WRKY;FLYWCH(zinc finger domain);SRP54(signal recognition)
	TP22-H	chr11.fgenes2446	-
	TP22-S	chr11.fgenes2445	-
	TP23-S	chr11.fgenes2452	-
	TP23-H	chr11.fgenes2451	-
	TP24-S (Pik-1)	chr11.fgenes2455	RATX1(Related to ATX1, mediate Avr recognition)
	TP24-H (Pik-2)	chr11.fgenes2457	-
	TP25-H	chr12.fgenes51	-
	TP25-S	chr12.fgenes52	-
	TP26-H	chr12.fgenes62	-
	TP26-S	chr12.fgenes61	-
	TP27-H	chr12.fgenes921	-
	TP27-S	chr12.fgenes923	-
	TP28-S	tig00001023.fgenes125	-
	TP28-H	tig00001023.fgenes120	-
	TP29-H	tig00011639.fgenes73	-
	TP29-S	tig00011639.fgenes72	-
	TP30-H	tig00011732.fgenes48	-
	TP30-S	tig00011732.fgenes49	-
	TP31-H	tig00011805.fgenes9	-
	TP31-S	tig00011805.fgenes8	-
	TP32-H	tig00012489.fgenes15	-

	(Pi42)		
	TP32-S (Pi-ta)	tig00012489.fgenes13	TRX(Thioredoxin, cell-to-cell communication)
	TP33-H	tig00001023.fgenes110	-
	TP33-S	tig00001023.fgenes127	-
	TP34-H	tig00001023.fgenes136	-
	TP34-S	tig00001023.fgenes139	-
	TP35-H	chr11.fgenes1904	-
	TP35-S	chr11.fgenes1906	SREBP(Sterol-sensing)
	TP36-H	chr11.fgenes2108	-
	TP36-S	chr11.fgenes2110	-
	TP37-H	chr01.fgenes3091	-
	TP37-S	chr01.fgenes3092	-
	TP38-H	chr08.fgenes2341	-
	TP38-S	chr08.fgenes2340	-
	TP39-H	chr03.fgenes814	-
	TP39-S	chr03.fgenes816	-
	TP40-H	chr12.fgenes929	-
	TP40-S	chr12.fgenes931	-
	TP41-H	chr12.fgenes1447	-
	TP41-S	chr12.fgenes1449	TRX(Thioredoxin, cell-to-cell communication)
	TP42-H	chr08.fgenes1031	-
	TP42-S	chr08.fgenes1030	-
	TP43-H	chr12.fgenes893	-
	TP43-S	chr12.fgenes896	-

Table S9. Phenotypes of the sensor or helper mutants of the *NLR* pairs that were knocked out by CRISPR/Cas9. The mutant types and phenotypes were collected from T₁ and confirmed in T₂ and T₃ generations.

Pair ID	Gene ID	Sensor (S) or Helper (H)	Genotypes		Phenotypes of the mutants
			Wild-type	Mutant type	
NP41	Os11g45970	S	TGGAGATCTCGCTTGATGAG	Not Available	Lethal (only a few very weak seedlings observed and eventually died. (3 repeats)
	Os11g45980	H	AGTTCCACCTTTAAGGACAA	AGTTCCACCTTTAAGG A ACAA AGTTCCACCTTTAAGG A ACAA	Dwarfing, smaller seeds, but the other phenotypes were not different from the control. When this allele from Tetep (chr11.fgenesh2446) was transformed into a susceptible cultivar (TP309), the transgenic lines were resistant to a few blast strains, but the transgenic line plants were shorter than the control.
NP15	Os07g29810	S	CTGGTTTACGATACCACCAA	CTG A GTTTACGATACCACCAA CTG T GTTTACGATACCACCAA	Dwarfing, infertility.
				CTGGTTT T ACGATACCACCAA CTGGTTT ACGATACCACCAA	Dwarfing, low setting rate
	Os07g29820	H	TGGTGTAACCAGCTAGAATT	TGG - GTAACCAGCTAGAATT TGG - GTAACCAGCTAGAATT	A little shorter, infection by rice blast in natural conditions.
NP18	Os08g14810	S	CTTGCAAACCATCTCCCAGG	CTTGCAAACCATCT - - CAGG CTTGCAAACCATCT - - CAGG CTTGCAAACCATCT - CCAGG	Early heading, smaller seeds

				CTTGCAAACCATCT - CCAGG	
	Os08g14830	H	ATTGCTGCTGCACCGGCCCT	ATTGCTGCTGCACC -- TCCT ATTGCTGCTGCACC -- TCCT ATTGCTGCTGCACC ---GGT ATTGCTGCTGCACC ---GGT ATTGCTGCTGCACCGG --- T ATTGCTGCTGCACCGG --- T	Dwarfing
NP42	Os11g46080	S	ACGAACGAGACATACCAAGA	Not Available	Lethal (only a few very weak seedlings observed and eventually died. (3 repeats))
	Os11g46070	H	ATTGAAACCTGTGTAGAGAC	ATTGAAACCTGTGT -- AGAC ATTGAAACCTGTGT -- AGAC	A little shorter, smaller spike, and weak blast infection in natural conditions.
NP34	Os11g39290	S	ATCCGGCCTGCAGATACGGC	ATCCGGCCTGCAGATACTGGC ATCCGGCCTGCAGATACTGGC	A little shorter, smaller spike
	Os11g39230	H	GTTAGGTACTCGATGGCAGA	GTTA - GTECTCGATGGCAGA GTTA - GTECTCGATGGCAGA	Just a little blast infection in natural conditions, smaller spike, while the other phenotypes were not different from the control.
NP3	Os11g27440	S	CAGGCAATCAGTTCATCCCT	Not Available	Lethal, could not obtain transgenic seedlings (4 repeats)
	Os11g27430	H	CGATCATCCGGAGCTCGTTC	CGATCATCCGGAG ----- TC CGATCATCCGGAG ----- TC CGATCATCCGGAGCTCGATTC CGATCATCCGGAGCTCGATTC	A little shorter, but the other phenotypes were not different from the control.
NP35	Os11g39310	S	GAAACCTGCTCATCGCGAGC	GAAACCTGCTCATCGCGAGC GA -----(delete 57 bp)	Dwarfing and weak plant, infertility.
	Os11g39320	H	GGCGGCGAGCCCCGCGGGAC	GGCGGCGAGCCCCGCG- GAC	No phenotypic change

				GGCGGCGAGCCCCGCG- GAC	
NP25	Os10g22484	S	CTGTGAAGCACAAATCGGGG	CTGTGAAGCACAAATCGTGGG CTGTGAAGCACAAATCGAGGG CGGGG - GGCGAGGGGCGGGG CGAGC GCGGCGAGGGGCGGGG	Blast pseudo-lesions in leaves of three- and six-week-old seedlings
	Os10g22300	H	GTGAAGACATGCCAACTGCC	Not mutated	
NP4	Os11g45920	S	GGCTACAGCTGGAGGAAGTA	Transformation failed	
	Os11g45790	H	TCCCAGCGGAAGAACATCCC	No suitable primer available	
NP47	Os12g18360 (<i>Pi-ta</i>)	S	GGGAATAGAATGAAAAATCC	GGGAATAGAATGAAAAATCC GGGAATAGAATGAAAAATCC GGG -- TAGAATGAAAAATCC GGG -- TAGAATGAAAAATCC	A little shorter, weaker plants and frequent deaths
	Os12g18374 (<i>Pi42</i>)	H	CGCCTTCACCAAGTGCCTCG	Not mutated	
NP13	Os05g40150	S	GCACGCTTTCTGCAAAGCGT	Not mutated	
	Os05g40160	H	CCGGCGAGGACATCCAAAGT	Not mutated	
NP2	Os08g30634	S	TCCTACCGCTCCAGTCAGGA	TCCTACCGCTCCAGTCAAGGA TCCTACCGCTCCAGTCATGGA	Dwarfing, infertility. Blast pseudo- lesions in leaves of three- and six-week-old seedlings manifesting the lesion mimic phenotype.
	Os08g30660	H	GCTGTCAGGCAGTTGTAAGA	Transformation failed	
NP20	Os09g14410	S	CGGCGCCGGATTGCCGTCGG	Not mutated	
	Os09g14450	H	GACCACGTTCTCGTCTTCCT	GACGCACGTTCTCGTCTTCCT GACTCACGTTCTCGTCTTCCT	Dwarfing
NP23	Os10g04290	S	CCTTATCCCATATCCTCAGG	Not mutated	
	Os10g04180	H	GATCTGATCAGGGGCGTCCG	Not mutated	

NP26	Os10g22490	S	TTGTGCTTCACAGGGAGCCA	Transformation failed
	Os10g22510	H	AAATGCCTTTCCTTCTGATA	Not mutated
NP27	Os11g11580	S	AAAGGTAAGTGATCGAACTT	Transformation failed
	Os11g11550	H	CTCTGTCACTAATATCGTCCG	Transformation failed
NP28	Os11g11810	S	GTATCTTAGTTTATATTCGG	Transformation failed
	Os11g11790	H	CCAAGAAAGCTTGCCCAGAG	Transformation failed
NP29	Os11g11920	S	AGACAGGTCTTCAAACGACA	Transformation failed
	Os11g11940	H	ATCTCGTAGAGGAAGTCTTC	Transformation failed
NP33	Os11g39190	S	ACGAACATCATTACCCCAGT	Transformation failed
	Os11g39160	H	ATTGGCACCGTTGAACCTGT	Transformation failed
NP36	Os11g39330	S	TTCTGGAGGGCAATCGTCCA	Not mutated
	Os11g39340	H	No unique spacer available	Not assessed
NP39	Os11g45750	S	CTCCACCAGCCCTACTTCAG	Not mutated
	Os11g45760	H	No unique spacer available	Not assessed
NP40	Os11g45924	S	TCCACCTGCCATGTTGGCAT	Not mutated
	Os11g45930	H	GGACGACCATCAGCTTTCCA	Transformation failed
NP43	Os11g46140	S	CATGATCAGATTAGCTTTGT	Transformation failed
	Os11g46190	H	No unique spacer available	Not assessed
NP44	Os11g46200	S	GGCCGTATACAGCGTCGCCA	Transformation failed
	Os11g46210	H	TTGTTTTTCCTTGAGGCGAC	Transformation failed
NP5	Os12g31160	S	TCAGCGAACGGACCTGAGAC	Not mutated
	Os12g31200	H	TGATTGTGATTGATGATGTG	Transformation failed

Table S10. Verification of CRISPR/Cas9 edited plants. Eight non-*NLR* genes were arbitrary selected and edited with the same procedures as in Wuyungeng24.

Gene type	Gene ID	No. of verified plants	No. of confirmed plants
Paired <i>NLRs</i>	Os05g40150	14	0
	Os05g40160	18	0
	Os09g14410	7	0
	Os10g04180	8	0
	Os10g04290	9	0
	Os10g22300	9	0
	Os10g22510	10	0
	Os11g39330	7	0
	Os11g45750	11	0
	Os11g45924	9	0
	Os12g18374	9	0
	Os12g31160	6	0
	Os07g29810	11	11
	Os07g29820	3	2
	Os08g14810	6	3
	Os08g14830	2	2
	Os08g30634	10	3
	Os09g14450	10	2
	Os10g22484	21	15
	Os11g27430	8	2
	Os11g39230	10	10
	Os11g39290	6	6
	Os11g39310	16	2
	Os11g39320	9	1
	Os11g45980	8	8
	Os11g46070	12	2
	Os12g18360	15	9
	Os11g27440	lethal	-
	Os11g45970	lethal	-
	Os11g46080	lethal	-
Non-<i>NLR</i> genes	Os01g71000	5	5
	Os02g01440	4	4
	Os05g09520	4	1
	Os05g49680	4	1
	Os05g49780	2	0
	Os06g39640	2	2
	Os07g48980	6	1
	Os10g20160	13	13

Table S11. Tetep NLRs that failed in transformation or to produce seeds after transformation. After co-culturing and screening, around 36 differentiated calli (6 flasks with 6 calli per flask) were obtained for each transformant. Only one callus was obtained for *tig00011805.fgenes8*. The differentiated calli were further cultured to ensure at least 8 rooted plantlets. Some transformants like *chr10.fgenes539*, *chr01.fgenes1470* and *chr11.fgenes2316* could only have fewer than 8 rooted plantlets. No plantlets survived and grew into new plants for the first 5 genes. For the remaining 6 genes, although the transformants could grow into new plants, the mature plants were found to die quickly or eventually infertile, so that no seeds were obtained from those transformants for further resistance testing.

No.	Gene ID	Receptor	Differentiated calli	Rooted plantlets	Regenerated plantlets
1	chr01.fgenes3154	TP309	36	≥ 8	0
2	chr10.fgenes539	TP309	36	7	0
3	chr11.fgenes828 (TP12-H)	TP309	36	≥ 8	0
		Shin2	36	≥ 8	0
4	chr11.fgenes1892 (TP15-H)	TP309	36	≥ 8	0
		Shin2	36	≥ 8	0
5	tig00011805.fgenes8 (TP31-S)	TP309	1	0	0
6	chr01.fgenes1470	TP309	36	5	2
7	chr02.fgenes1404	TP309	36	≥ 8	7
8	chr02.fgenes3478	TP309	36	15	7
9	chr10.fgenes935	TP309	36	16	9
10	chr11.fgenes829 (TP12-S)	Shin2	36	16	10
		TP309	36	≥ 8	5
11	chr11.fgenes2316	TP309	36	7	3

Table S12. Diversities of orthologous *NLR* pairs between Tetep and Nipponbare. Diversity values were calculated using the coding sequences of the orthologous *NLR* pairs (i.e., *NLR* pairs with both orthologous helper and sensor members) between Tetep and Nipponbare.

Orthologous pairs	Diversities between Tetep and Nipponbare	
	Helper	Sensor
TP1-NP1	0.0058	0.0623
TP4-NP12	0.0104	0.0004
TP6-NP16	0.1442	0.1728
TP7-NP19	0.0028	0.0029
TP9-NP20	0.0014	0.0201
TP11-NP26	0.0042	0.1257
TP12-NP28	0.0151	0.3087
TP13-NP3	0.0352	0.0037
TP14-NP33	0.0411	0.0003
TP16-NP35	0.0003	0.0437
TP19-NP39	0.0658	0.0914
TP24-NP44	0.1242	0.2347
TP25-NP45	0.0293	0.0470
TP28-NP23	0.0000	0.0224
TP29-NP18	0.0049	0.0058
TP32-NP47	0.0129	0.0020
TP35-NP27	0.3445	0.3834
TP36-NP38	0.1953	0.0641
TP39-NP11	0.0029	0.0022
Average	0.0548	0.0839

Table S13. Estimation of the Tetep genome size using K-mer analysis of Illumina reads.

Data source	Total number of reads	Average read length	K-mer length	No. of K-mers frequency ≤ 1	Overall depth estimated from K-mer distribution	Estimated Genome size* (Mb)
Illumina PE100	93,480,914	100	19	282,085,268	20	369.2
Illumina PE150	161,737,412	150	19	633,247,290	50	414.3

* Genome size estimated using the methods described in Zhang et al.(43).

Table S14. Repeat contents of the Tetep genome masked by RepeatMasker.

	Number of elements	Length occupied (bp)	Percentage of sequence (%)
Retroelements	77,450	116,988,405	28.14
Non-LTR elements	25,438	10,136,247	2.44
SINEs:	6,997	1,016,674	0.24
LINEs:	9,444	4,600,077	1.11
L1/CIN4	8,997	4,519,496	1.09
LTR elements:	61,009	111,371,654	26.79
Ty1/Copia	10,252	12,978,237	3.12
Gypsy/DIRS1	45,098	96,300,549	23.17
DNA transposons	221,923	62,499,085	15.04
hobo-Activator	28,665	5,662,615	1.36
Tc1-IS630-Pogo	40,978	7,299,510	1.76
Tourist/Harbinger	50,982	10,671,694	2.57
Other	61	7,542	0.00
Unclassified:	54,486	14,817,065	3.56
Total interspersed repeats:		194,304,555	46.74
Small RNA:	4,717	863,927	0.21
Satellites:	751	2,257,457	0.54
Simple repeats:	95,628	4,704,165	1.13
Low complexity:	10,439	521,022	0.13

Dataset S1 (separate file). Verified PCR primer pairs for rapid testing of Tetep-derived *NLRs*. A total of, 282 PCR primer pairs were verified, which covered over half of Tetep *NLRs*, mainly targeting *NLR* clusters and pairs. The indel-based primer pairs (“INS” and “DEL” stand for “insertion” and “deletion” relative to the Tetep genome) distinguish Tetep-derived *NLRs* from those of the other cultivar compared and produce different amplicon sizes (“Larger size” and “Shorter size”). The allelic-specific primer pairs (PA) can only amplify Tetep-derived *NLRs*, so that no PCR band is expected from another genome (denoted as “No Band”). Other primer pairs targeting polymorphic regions (VAR) either serve as allelic primers or could be distinguished by Sanger sequencing (the confirmed variants were marked as “DIFF SEQs”). All 282 primer pairs were successfully verified in either Nipponbare or 9311 or both. Actually, only 2 and 3 primer pairs are required to verify a *NLR* pair (prefix with “TP”) and a cluster (prefix with “CL”), respectively, as shown in Fig. S6. Hence, only half of those primer pairs (marked by “P” for *NLR* pairs, and “C” for *NLR* clusters) were required to verify all *NLR* pairs and clusters. Other primers can verify single *NLRs*, especially those functional ones (denoted by “NLR_R”). “ND” means no difference within the primer regions between Tetep and the other genome.

Dataset S2 (separate file). Predicted medium-sized INDELS between Tetep and each of the four genomes within *NLR* regions. Based on the genome alignments (aligned with "nucmer" from MUMmer with option "--maxmatch -c 200 -l 100"), medium sized (100~1000bp) insertions (INS) or deletions (DEL) were obtained using "dnadiff" from MUMmer with default settings. All coordinates are based on the assembled Tetep genome. For instance, an insertion in "Nipponbare-Tetep" stands for an insertion in Nipponbare compared to Tetep, while a deletion represents a deleted region in Nipponbare compared to Tetep. *NLRs* overlaps or near (≤ 10 kbp) those INDELS are given behind.

Dataset S3 (separate file). Full list of small variant markers between Tetep and each of the four genomes within *NLR* regions. SNPs and small INDELS (<100bp) within or around (≤ 1 kbp) *NLRs* are given based on the comparison results between each of the four genomes with Tetep. All coordinates are based on the assembled Tetep genome.

Dataset S4 (separate file). List of PCR primer pairs and restriction sites used in large-scale cloning of Tetep *NLRs*.

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