Genome-Wide Survey of Leucine-Rich Repeat Receptor-Like Protein Kinase Genes and CRISPR/Cas9-Targeted Mutagenesis BnBRI1 in Brassica napus

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The leucine-rich repeat receptor-like protein kinase (LRR-RLK) family represents the largest group of RLKs in plants and plays vital roles in plant growth, development and the responses to environmental stress. Although LRR-RLK families have been identified in many species, they have not yet been reported in B. napus. In this study, a total of 444 BnLRR-RLK genes were identified in the genome of Brassica napus cultivar “Zhongshuang 11” (ZS11), and classified into 22 subfamilies based on phylogenetic relationships and genome-wide analyses. Conserved motifs and gene structures were shared within but not between subfamilies. The 444 BnLRR-RLK genes were asymmetrically distributed on 19 chromosomes and exhibited specific expression profiles in different tissues and in response to stress. We identified six BnBRI1 homologs and obtained partial knockouts via CRISPR/Cas9 technology, generating semi-dwarf lines without decreased yield compared with controls. This study provides comprehensive insight of the LRR-RLK family in B. napus. Additionally, the semi-dwarf lines expand the “ideotype” germplasm resources and accelerate the breeding process for B. napus.

Keywords: Brassica napus, LRR-RLK, CRISPR/Cas9, BnBRI1, semi-dwarf

INTRODUCTION

Receptor-like protein kinases (RLKs) serve as receptors for signaling transduction pathways that regulate many biological process (Clark et al., 1997; Li, 2010; Xun et al., 2020; Lu et al., 2021). Leucine-rich repeat receptor-like protein kinases (LRR-RLKs) comprise one of the largest groups of RLKs (Liu et al., 2017). There are 200–300 LRR-RLKs in Arabidopsis, tomato, rice, potato, and maize, respectively (Song et al., 2015; Wei et al., 2015; Bettembourg et al., 2017; Sun J. et al., 2017; Li et al., 2018). LRR-RLKs usually contain an extracellular, tandemly organized LRR domain (20–30 amino acid residues), a single-pass transmembrane domain, and functional protein kinase domain (Mishra et al., 2021).
LRR-RLKs are highly conserved, widely distributed and play vital roles in plant growth, development and stress responses. For instance, SERK3/BAK1 function in the plant immunity, growth, and cell death (Zhou et al., 2019). HSL3 function in regulating plant stomatal closure and the drought stress response through modulate hydrogen peroxide homeostasis (Liu et al., 2020). AtPXK1 functions in signal transduction pathways that respond to temperature fluctuations (Jung et al., 2015). XIPI/CEPRI and CEPR2 are involved in the regulation of lateral root growth in Arabidopsis (Dimitrov and Tax, 2018). OsDOCS1 plays critical roles in plant root cap development (Bettembourg et al., 2017). OsSTLKL regulates salt stress tolerance, potentially by regulating the ROS scavenging system, Na⁺/K⁺ ratio and MAPK signaling pathway (Lin et al., 2020).

The LRR-RLK BRII encodes a receptor serine/threonine kinase and has an extracellular domain that contains 25 leucine-rich repeats. BRII and BAK1 interact and regulate brassinosteroid signaling in Arabidopsis, and BRII can phosphorylate BAK1. Arabidopsis overexpressing BRII are highly sensitive to brassinosteroid and have higher brassinosteroid binding activity (Nam and Li, 2002). BRII is an important plasma-membrane receptor for plant steroids, as shown by immunoblotting and brassinolide is an important plasma-membrane receptor for plant brassinosteroid binding activity (Nam and Li, 2002). BRII

Phylogenetic Trees, Conserved Motif, and Gene Structure Analysis BnLRR-RLKs

Multiple sequence alignment of the full-length BnLRR-RLK proteins were performed with the FFT-NS-I method of the MAFFT software (Katoh et al., 2019). Fasttree software was used as the maximum likelihood method for constructing phylogenetic trees (Price et al., 2009). The EvolView software was used to visualize the phylogenetic tree (Zhang et al., 2012). Conserved motifs (the maximum is set to 10) were predicted using the MEME v5.2.0 with the maximum number of motifs and the optimum width of each motif falls between 10 and 200 residues (Bailey et al., 2009). The structures of the BnLRR-RLK genes were displayed based on GFF annotation files by TBtools software (Chen et al., 2020).
Chromosomal Location, Gene Duplication, and Genomic Synteny of BnLRR-RLKs

Chromosome location information of the BnLRR-RLK genes was extracted from the GFF file, and plotted by the MapChart version 3.0 software (Voorrips, 2002). In order to accurately analyze BnLRR-RLK duplication events, the e-value of e^{-100} was used to align all of the protein sequences in ZS11 with the BLASTP program (Camacho et al., 2009). The duplication pattern of these genes were detected with the default parameters of the MCScanX software, and divided into tandem duplication and segmental duplication (Krzywinski et al., 2009). Similarly, the e-value of e^{-100} was used to align all of the protein sequences between ZS11 and Arabidopsis with the BLASTP program, and all syntenic blocks were mapped with JCVI software (Tang et al., 2008).

Expression Patterns of BnLRR-RLKs

RNA-seq data from 12 tissues (root, stem, leaf, flower, silique, sepal, pistil, stamen, ovule, pericarp, wilting pistil, and bloosomy pistil) of ZS11 were downloaded from the NCBI database (project ID: PRJNA394926) (Sun F. M. et al., 2017). RNA-seq data of ZS11 leaf under abiotic stress, including dehydration, salt, ABA and cold stress treatments, were downloaded from the NGDC database (project ID: CRA001775) (Zhang et al., 2019). These transcriptome data were mapped to the ZS11 reference genome database (project ID: CRA001775) (Zhang et al., 2019). These transcriptome data were mapped to the ZS11 reference genome using HISAT2 software (Kim et al., 2015). The TPM values (Transcripts Per Million) were calculated by FeatureCounts R package and the heatmaps were presented using TBtools software (Liao et al., 2014; Chen et al., 2018).

CRISPR/Cas9 Vector Construction and Transformation

To construct the CRISPR/Cas9 recombinant plasmid, a common target sequence of six BnBRII1 genes was designed (Supplementary Table 1), and this target dsDNA was generated via direct annealing of two oligonucleotides primers, and assembled into the pHE401 vector (Xing et al., 2014). The resulting pHE401-BRII1 constructs were transformed into the agrobacterium tumefaciens strain GV3101 by electroporation and subsequently transformed into the selfing line K407 of B. napus using the method described previously (Bhalla and Singh, 2008).

Genomic DNA extracted from T3 plants was used for PCR amplification with genotyping primers (Supplementary Table 1), the PCR products were conducted by sanger sequencing, and cloned into a pEASY-Blunt cloning vector (TransGen Biotech, Beijing, China), positive single clones were sequenced to identify mutations (Aoke Biotech, Beijing, China).

Analysis of Seedlings Hypocotyl Length and Growth Response to Brassinozole

To analyze the hypocotyl variation of the gene-edited mutants, the seeds were sown in humus soil under 16 h light/8 h dark photoperiod at 22°C, and the hypocotyl length was measured after 6 days. To verify the response to Brassinozole (BRZ), the seeds of mutants were sterilized (75% alcohol for 30 s, 15% NaClO for 8 min, rinse 5 times with sterile water) and bunch planting at 1/2 strength Murashige and Skoog solid medium (1/2 MS), which contained 0 and 1,000 nM BRZ, respectively (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). After 5 days of darkness cultivation at 22°C, the hypocotyl length was measured. Each treatment was repeated three times.

Quantitative Real-Time PCR Analysis

To detect the expression level of BnBRII1, total RNA was extracted from seedlings of the L18, L24 and WT by RNAiso Plus kit (TaKaRa, Dalian, China). One microgram of RNA was reverse-transcribed to cDNA using One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). qRT-PCR was performed using a SYBR Green Master Mix kit (TaKaRa, Dalian, China). The BnUBC21 gene was used as an internal reference, the primer pairs are listed in Supplementary Table 1. Each sample contains 3 biological replicates and 3 technical replicates.

Statistical Analysis

All data were analyzed with One-way analysis of variance (ANOVA) of SPSS 26 software. GraphPad prism (version 8.0.2) was used for drawing graphs. Each experiment was repeated at least three times, and significant differences are indicated at the significance levels of P ≤ 0.05.

RESULTS

Identification of Leucine-Rich Repeat Receptor-Like Protein Kinase Gene Families in Brassica napus

We detected a total of 444 BnLRR-RLK genes in the genome of B. napus cultivar ZS11, which is nearly double the number of LRR-RLK genes in Arabidopsis (Table 1). Specifically, we uncovered 215 LRR-RLK genes in the A subgenome and 225 LRR-RLK genes in the C subgenome. We renamed all of the BnLRR-RLK genes on the basis of chromosomal localization. Gene position and other details are presented in Supplementary Table 2. We found that BnLRR-RLK protein lengths were between 459 and 1,303 amino acids (aa). In addition, the protein molecular weights (MV) ranged from 51.31 to 143.19 kDa, and the isoelectric point (pI) from 4.77 to 10.27.

Phylogenetic Relationship, Conserved Motif, and Gene Structure Analysis

To evaluate the evolutionary relationships between LRR-RLK genes in B. napus and A. thaliana, we constructed a phylogenetic tree using the 444 BnLRR-RLK full-length proteins together with 225 AtLRR-RLK proteins. The 444 BnLRR-RLKs were subdivided into 22 groups (I-XV) (Figure 1). The largest group, LRR-RLK-III, contained 99 genes. In contrast with A. thaliana, no genes were identified in group I-2. There were at least twice as many LRR-RLK genes in each subgroup of B. napus vs. A. thaliana, except for groups I-1, VI-2, VIII-1, and XV, which had a similar number of LRR-RLK genes in B. napus and A. thaliana.
These data suggest that allopolyploidization contributed to the expansion of LRR-RLKs in B. napus, but that not every subfamily has an increased the number of genes.

In addition, we analyzed conserved motifs and gene structures to define BnLRR-RLK family characteristics. Using MEME tools, we identified 10 putative motifs (Supplementary Table 3). We found that the type, number, and arrangement of the N-terminus determined the classification of different subfamilies, and expanded the variety and quantity of LRR-RLKs (Supplementary Figure 1). The N-termini of these proteins were enriched for many different tandem repeat motifs, such as motif 1, motif 3 and motif 4. Intriguingly, motif 2 and motif 5 were often found at the C-terminus.

The gene structure characteristics revealed that different subfamilies shared different intron-exon distribution, whereas members within the same subfamily had a similar gene structure, suggesting a similar evolution within subgroups but not between subgroups, based on GFF annotation files (Supplementary Figure 1). The large number of intron-exons in subgroups I-1, II, XIII, V, and VI-2, indicate a complex gene structure, whereas other subfamilies contained few intron-exons and showed simpler gene structures.

**The Distribution, Gene Duplication, and Genomic Synteny**

Knowledge of the chromosome distribution of gene family members is essential for studying family member duplication and collinearity. We found that the 444 BnLRR-RLK genes were widely distributed on 19 chromosomes, and the distribution was extremely uneven, with about 40 genes on chromosome 3C and 13 genes on chromosomes 4A and 8A, respectively (Supplementary Figure 2). We did not observe any preference for the distribution of LRR-RLK genes on each chromosome, with the exception that there were no LRR-RLK genes on the upper arm of chromosome 4A.

Gene duplication is a major mechanism underlying the expansion of gene family members. Based on BLAST and MCScanX software, we identified a total of 6 tandem duplication events and 395 segmental duplications in the ZS11 genome (Figure 2 and Supplementary Tables 4, 5). Only 9 events were detected within the same chromosome, whereas 386 segmental duplications occurred across chromosomes, which suggests that segmental duplication events across chromosomes plays a key role in LRR-RLKs expansion. Further analysis showed that 51 duplication events occurred on the AA subgenome, 55 events on the CC subgenome, whereas 289 events occurred across AA/CC subgenomes. Considering that B. napus (AACC genome) derived from a spontaneous hybridization between B. rapa (AA genome) and B. oleracea (CC genome), and contains the complete diploid chromosome sets of both parental genomes. Therefore, we infer that allopolyploidization plays a significant role in the expansion of the LRR-RLKs in B. napus.

To investigate the evolution of the LRR-RLKs, we analyzed their synteny between B. napus and A. Thaliana at the whole genome level. A total of 374 gene pairs were detected between the two genomes (Supplementary Figure 3 and Supplementary Table 6). Most AtLRR-RLK genes have multiple orthologous genes in B. napus. For instance, AtBRI1 (At4G39400) corresponds to 6 BnBRI1 genes, indicating that BnBRI1 genes expanded in rapeseed. However, the 29 AtLRR-RLKs have only one collinear BnLRR-RLK gene.

**Expression Profiles of BnLRR-RLKs in Different Tissues**

The specific expression of genes in different tissues sheds light on gene function. In order to explore the function of the BnLRR-RLKs, we investigated their expression profiles in 12 different tissues, using public available RNA-seq data. We detected the expression of 426 BnLRR-RLKs in at least one tissue, whereas 18 genes were not expressed in any of the tissues analyzed (Figure 3 and Supplementary Table 7). We divided the 426 genes into two groups: group 1 displayed high expression levels in different tissues, whereas group 2 displayed low expression in tissues. For instance, BnLRR-RLK-6A-15 was highly expressed in ovule, BnLRR-RLK-8A-12 and BnLRR-RLK-8C-6 were highly expressed in root. These results reflected that the expression of LRR-RLKs vary in different tissues, suggesting that BnLRR-RLKs play functionally diverse roles in tissue development.
Expression Profiles of BnLRR-RLKs in Response to Abiotic Stresses

LRR-RLK genes play a major role in abiotic stress responses. To explore the expression of LRR-RLK genes in response to abiotic stress, we examined their expression patterns under four abiotic stresses (dehydration, salt, abscisic acid (ABA), cold) using the published transcriptome data of B. napus. A total of 132 genes were extracted from the expression matrix based on STDEV value > 2 among different treatments (Figure 4 and Supplementary Table 8). These genes clustered into two groups.

Group 1 contained 41 genes with significantly different expression patterns under different stress treatments. For example, BnLRR-RLK-4A-8, BnLRR-RLK-3C-3, and BnLRR-RLK-3A-8 were all up-regulated after 4 h-salt or 4 h-cold treatment, suggesting they may be involved in the related stress response. Interestingly, some genes displayed opposing expression profiles under different treatments. For instance, BnLRR-RLK-9A-6 was sharply down-regulated after 1 h-dehydration, but up-regulated after 4 h-ABA treatment. These data suggest that the responses to dehydration and ABA stress have opposing molecular mechanisms.

Group 2 contained 91 genes with low expression levels under each stress treatment. Unusually, part of the genes exhibited sharply changes in expression levels after a specific stress treatment, for instance, BnLRR-RLK-4C-17 and BnLRR-RLK-5A-10 were significantly up-regulated after 4 h-cold treatment, BnLRR-RLK-7A-5 and BnLRR-RLK-8A-2 were up-regulated after 4 h-ABA treatment, whereas BnLRR-RLK-1A-4 and BnLRR-RLK-3C-11 were strikingly down-regulated under dehydration treatment. The results provided useful information for studying the function of LRR-RLKs in response to abiotic stress.

Knockout of BnBRI1 Homologs by CRISPR/Cas9 Targeted Mutagenesis

Based on previous research in Arabidopsis and rice, we identified six BRI1 orthologs (renamed as BnBRI1.a~BnBRI1.f), and six BRI1-Like (BRL) genes in B. napus (Figure 5A and Supplementary Table 9). The BnBRI1 and BnBRL genes both belonged to the Xb-1 subfamily, while the BnBRI1.b and the BnBRI1.f had higher homology with AtBRI1. Clustering analysis based on expression between 12 tissues showed that the six BnBRI1 genes clustered into one subclass and six BnBRL genes into another subclass, and the expression levels of BnBRI1 genes were generally higher than the BnBRL genes (Figure 5B). Furthermore, BnBRI1.c, BnBRI1.e, and BnBRI1.f had higher expression in various tissues compared to other BnBRI1 genes. It is noteworthy BnBRI1.f gene was highly expressed in all tissues.

To generate new germplasm resources with a dwarf and optimized plant structure of B. napus, we designed single guide RNAs targeted to a common conserved region of BnBRI1 genes (Figure 5C), inserted these sgRNA into the phSE401 expression vector and transformed into hypocotyls of rapeseed cultivar K407 (Figure 5D). We obtained a total of 130 transgenic positive plants (Figure 5E). Interestingly, more than 34 T0 plants displayed retarded growth with dark green rolling leaves at the stage of seedling growth (Supplementary Figure 4), consistent with phenotypes of previously reported classical bri1 mutants (Huang et al., 2021). About 42 plants (44%) exhibited an extreme dwarf phenotype (plant height 30–60 cm), and 45 plants (47%) showed dwarf phenotypes (61–80 cm), and 9 plants (9%) showed semi-dwarf phenotypes (81–120 cm) (Figure 5F).

Reduced Expression of BnBRI1 Homologs Leads to Semi-Dwarf Phenotypes in Brassica napus

To determine whether the transgenic lines had gene editing events, we selected the T3 generation plants of the semi-dwarf lines L18 and L24 for subsequent experiments (Figure 6A). The sequencing results of the single clones and PCR products near the sgRNA-targeted sites indicated that all target sites were edited in L24, except the BnBRI1.b and BnBRI1.f genes, similarly, all target sites were edited in L18, except the BnBRI1.f gene. The variations between the two lines were single base insertions of A, T or C (Figures 6B,C and Supplementary Figures 5, 6).

Furthermore, qRT-PCR was conducted (Supplementary Figure 7), and showed that the BnBRI1 expression of the L18 and L24 were both reduced significantly. As a result, the hypocotyl length of both mutants were significantly decreased under 1,000 nM, so the L18 and L24 were BRZ sensitive mutants (Supplementary Figures 8B,D). These verified that the both
gene edited lines are indeed related to brassinostroid signaling transduction pathway.

Agronomic traits were closely related to agricultural production. We planted the L18 and L24 lines in a greenhouse, and investigated the major agronomic traits, plant height, effective branch height, secondary branch number, silique number per plant, silique number of main inflorescence, length of silique and yield per plant (Figures 6C–I). We found that almost all traits exhibited significant differences between the control WT and the two gene edited lines. Specifically, compared with the control, L24 had fewer secondary branches, fewer silique number of main inflorescences, fewer siliques in the whole plant, and shorter siliques, resulting in a significantly lower yield than the control. In contrast, when the plant height and the branch length of L18 were significantly diminished compared to the control, the yield per plant was not reduced. Among yield-related traits, although L18 had shorter silique and a lower silique number of main inflorescence, the increased number of secondary branches increased the total number of silique and prevented a decline in yield.
DISCUSSION

LRR-RLKs sense extracellular signals and stimuli. In this study, we identified 444 LRR-RLK genes in the genome of ZS11, approximately twice as many as in A. thaliana. These 444 genes were further divided into 22 subfamilies, with LRR-RLK-III subfamily as the largest, whereas there were no genes in the I-2 subfamily. In general, a typical LRR-RLK consists of an extracellular tandem LRR domain, a transmembrane domain, and an intracellular protein kinase domain (Mishra et al., 2021), however, the I-1 subfamily also contains a malectin-like domain at the N-terminus. This domain is found in a different receptor-like kinase, known as the Catharanthus roseus receptor-like kinase 1-like proteins (CrRLK1Ls) (Franck et al., 2018). These findings highlight the domain similarity and functional complexity of LRR-RLKs family.

Gene duplication including tandem, tetraploid, segmental, and transpositional duplication, represents a major mechanism of new gene formation and gene family expansion (Freeling, 2009). Compared with the diploid Arabidopsis, many genes were significantly expanded in the allopolyploidization Brassica napus, likely due to genome polyploidy. Indeed, our research showed that 289 segmental duplications occurred across AA/CC subgenomes. Moreover, 51 and 55 duplication events occurred in AA and CC subgenomes, respectively. These repeated events within each subgenome may relate to their progenitors (Brassica rapa and Brassica oleracea). In addition, 6 tandem duplication events took place on chromosomes A04, A07, C04, C06, C07, and C09, respectively. Thus, at least three types of gene duplications appeared in the BnLRR-RLKs family: tetraploid, segmental and tandem duplication.

Gene expression patterns are closely related to their functions. We examined public RNA sequencing data to determine the
FIGURE 4 | Expression profiles of BnLRR-RLKs under different stresses. Leaf RNA-seq data for ZS11 under abiotic stress, namely dehydration, salt, ABA and cold stress treatments, was downloaded from the NGDC database. Expression level is equal to the mean values and transforms log2 values for normalization. Colors from blue to red represent relative expression levels from low to high.
expression patterns of BnLRR-RLK genes from 12 tissues in ZS11. We found that most BnLRR-RLKs have tissue-specific expression, for instance, BnLRR-RLK-3C-12 and BnLRR-RLK-3A-15 were highly expressed in stamens. Phylogenetic tree clustering suggested that both genes were clustered with a pollen tube related gene, AtPRK4 (At3G20190). Based on these findings we speculate that the two highly expressed genes in stamens are related to pollen tube elongation and fertilization.
We used CRISPR/cas9 technology to edit the six
Yamamuro et al., 2000; Feng et al., 2015; Huang et al., 2021).

2020). Editing the
et al., 2021). Knockout of the two
generated semi-dwarf plants without affecting other traits (Fan
expressed in various tissues; in particular, BnBRI1.f
conservation. Nevertheless, the six
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resistance of
Arabidopsis
SIF
SIF3
SISTRESS INDUCED FACTOR
(AtBARK1) is involved in the BR signaling pathway, suggesting that this gene may
play a role in ABA signaling pathway (Kim et al., 2013). Given
the importance of LRR-RLK family members for plant growth,
development, and stress tolerance, our study of tissue-specific and
abiotic stress expression patterns facilitates the discovery of
LRR-RLK genes with important biological functions and their
underlying regulatory mechanisms.

BR signaling requires the BRI1 and other LRR-RLKs. The
six BnBRI1 genes have similar motif elements and intron-exon
structure, supporting their homology and evolutionary
conservation. Nevertheless, the six BnBRI1 have tissue-specific expression patterns: BnBRI1.c, BnBRI1.e, BnBRI1.f were highly
expressed in various tissues; in particular, BnBRI1.f was highly
expressed in all tested tissues. We propose that the six copies of
BnBRI1 have different functions despite their similarities.

Gene editing technology can be used to improve agronomic
trait in crops and accelerate the breeding process. This technology
was used in rapeseed to knock out the BnaA03.BP gene, which
generated semi-dwarf plants without affecting other traits (Fan
et al., 2021). Knockout of the two BnaMAX1 homologous improved
plant architecture and increased yield (Zheng et al., 2020). Editing the BnD14 gene resulted in a compact architecture and is expected to achieve high-density plants in production (Stanic et al., 2021).

BRI1 mutations caused developmental defects, including
extreme dwarfism and male sterility (Clouse et al., 1996; Yamamuro et al., 2000; Feng et al., 2015; Huang et al., 2021). We used CRISPR/cas9 technology to edit the six BnBRI1 genes in rapeseed, and obtained 130 gene-edited rapeseed plants, some of which exhibited a dwarf or semi-dwarf phenotype. Target identification in the two semi-dwarf strains revealed that all target genes were edited except BnBRI1.b and BnBRI1.f in L24, and BnBRI1.f in L18. Although the BnBRI1.f gene was not edited, we found one nucleotide variation in this gene compared to other five BnBRI1 genes that causes a F689S amino acid change, which probably relates to the phenotypes of L18 and L24. Among yield-related traits, although L18 had shorter silique and decreased silique number of main inflorescence, the increased number of secondary branches led to more silique and ultimately prevented a decline in yield. As a semi-dwarf line with excellent agronomic characteristics, L18 could be used to construct an ideotype plant structure of rapeseed.

CONCLUSION

This study systematically analyzed the LRR-RLKs family in
B. napus. A total of 444 genes were detected in the genome of cultivar ZS11, which were divided into 22 groups based on phylogenetic relationships. These genes were widely and asymmetrically distributed across 19 chromosomes, and exhibited tissue- and stress-specific expression profiles. Moreover, we identified and knocked out BnBRI1 homologs to generate semi-dwarf lines with increased yield. This study lays the foundation to investigate LRR-RLK family gene function, expands the “ideotype” germplasm resources for field breeding, and accelerates the process of rapeseed breeding.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

YFZ and JM designed the study. MS and BL performed the analysis and wrote the manuscript. RA, YTZ, FL, and SHH contributed to genetic transformation of Brassica napus. SWH and CX revised the manuscript. All authors have read and approved the final manuscript version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.865132/full#supplementary-material
**Supplementary Figure 1** | Phylogenetic relationship, conserved motifs and gene structures of BnLRR-RLK proteins. (A) Phylogenetic relationship of BnLRR-RLKs. (B) Conserved motifs of BnLRR-RLKs, boxes of different colors represent different motifs. (C) Gene structure of BnLRR-RLKs. The green and yellow boxes represent the coding sequences (CDSs) and untranslated regions (UTRs), respectively, and black lines represent introns.

**Supplementary Figure 2** | Chromosomal localization of BnLRR-RLK genes across B. napus chromosomes. Words marked in red indicate tandem duplications. The location of each gene was determined based on its physical location on the chromosome.

**Supplementary Figure 3** | The synteny of BnLRR-RLK genes in genomes of Arabidopsis and Brassica napus. The light-gray lines represent all collinear blocks between B. napus and A. thaliana, and light-purple lines represent orthologous relationships between BnLRR-RLKs and AtLRR-RLKs.

**Supplementary Figure 4** | Phenotypes of CRISPR/Cas9 edited BnBRI1 strong mutants at seedling stage. The bRI1 strong mutants showed significant growth retardation with dark green and edge valgus thick leaves, and were eventually lost due to failure to harvest seeds, scale bars, 1 cm.

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Conserved motifs of BnLRR-RLKs, boxes of different colors represent different motifs. (C) Gene structure of BnLRR-RLKs. The green and yellow boxes represent the coding sequences (CDSs) and untranslated regions (UTRs), respectively, and black lines represent introns.

**Supplementary Figure 5** | Sequencing chromatograms of positive single clones in T3 generation. Each single clone was evaluated by Sanger sequencing. The inserted nucleotides are highlighted with yellow colors, pointing by black arrows.

**Supplementary Figure 6** | Sequencing chromatograms of PCR products in T3 generation. Each PCR product was purified and sequenced. The inserted nucleotides are highlighted by black box.

**Supplementary Figure 7** | Expression of BnBRI1 gene in WT, L18 and L24. The BnBRI1 gene was used as an internal control. Error bars represent SE (standard errors) of three independent replicates. Different lowercase letters indicate values significant differences compared to WT at the level P < 0.05.

**Supplementary Figure 8** | Hypocotyl length and response to BRZ in gene edited lines. (A) Phenotypes of the gene edited lines at seedling stage. (B) The gene edited lines under BRZ treatment. (C) Hypocotyl length of seedlings shown in (A). Data are the mean ± SD (n = 15), scale bars, 1 cm. (D) Hypocotyl length of seedlings at different BRZ concentrations shown in (B), scale bars, 1.5 cm. Different letters indicate significant differences based on one-way ANOVA (P < 0.05).
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