BMC Plant Biology

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Genome-wide identification of Brassinosteroid insensitive 1-associated receptor kinase 1 genes and expression analysis in response to pathogen infection in cucumber (*Cucumis sativus* L.)

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Abstract

Background *BAK1* (Brassinosteroid insensitive 1-associated receptor kinase 1) plays an important role in disease resistance in plants. However, the function of *BAK1* family in cucumber and the decisive genes for disease-resistance remain elusive.

Results Here, we identified 27 *CsBAK1s* in cucumber, and classified them into five subgroups based on phylogenetic analysis and gene structure. *CsBAK1s* in the same subgroup shared the similar motifs, but different gene structures. Cis-elements analysis revealed that *CsBAK1s* might respond to various stress and growth regulation. Three segmentally duplicated pairwise genes were identified in cucumber. In addition, Ka/Ks analysis indicated that *CsBAK1s* were under positive selection during evolution. Tissue expression profile showed that most *CsBAK1s* in Subgroup II and IV showed constitutive expression, members in other subgroups showed tissue-specific expression. To further explore whether *CsBAK1s* were involved in the resistance to pathogens, the expression patterns of *CsBAK1s* to five pathogens (gummy stem blight, powdery mildew, downy mildew, grey mildew, and fusarium wilt) reveled that different *CsBAK1s* had specific roles in different pathogen infections. The expression of *CsBAK1-14* was induced/repressed significantly by five pathogens, *CsBAK1-14* might play an important role in disease resistance in cucumber.

Conclusions 27 *BAK1* genes were identified in cucumber from a full perspective, which have important functions in pathogen infection. Our study provided a theoretical basis to further clarify the function of *BAK1s* to disease resistance in cucumber.

Keywords *Cucumis sativus* L., *BAK1*, Gene family, Expression pattern, Pathogen infection

Introduction

BAK1 (Brassinosteroid insensitive 1-associated receptor kinase 1), also known as SERK3 (Somatic embryogenesis receptor-like kinase 3), involves in plant growth and development via brassinosteroid (BR)-mediated signaling [1–3]. BAK1 is a co-receptor of brassinosteroid-insensitive1 (BRI1) that senses and transmits BR signal, and is necessary for BR signal transduction to response to

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various external environment signals [3, 4]. Moreover, as one of the major components both in pattern recognition receptor (PRR)-triggered immunity (PTI) and effector-triggered immunity (ETI), BAK1 also play a major role in plant innate immunity [5]. As a LRRs-RLK (Leucine-rich repeats receptor-like kinase) and co-receptor for different LRR-type PRRs (Pattern recognition receptors), BAK1 forms a receptor complex with multiple PRRs and involved in multiple PTI signaling pathways to regulate plant immunity [5]. BAK1 interacts with FLS2/EFR to mediate plant innate immune response [6, 7]. BAK1 could be involved in a BR-independent cell-death pathway negatively by interacting with BAK1-interacting receptor-like kinase 2 (BIR2) [8–11]. In addition, BAK1 participates in nucleotide-binding domain and leucine-rich repeat (NLR)-mediated effective defense responses as a negative regulator and is required for ETI [12]. ETI response often induce hypersensitive response (HR) and cause cell death in plants [13]. BAK1 participates in NLR-mediated effective defense responses as a negative regulator. BAK1 regulates ETI activation as a target for many effectors and acts upstream of certain NLRs to inhibit *R* gene expression [14]. In turn, NLRs could guard BAK1 and other SERKs, and when BAK1-mediated PTI pathway is countered by pathogen, NLRs are activated and initiate autoimmune response by ETI response.

BAK1 belongs to SERK subfamily widely distributed in plants [5], and regulates cell death with SERK4 [14]. In *Arabidopsis*, 5 SERKs were reported [15], but only one *BAK1* gene, *AT4G33430*, was identified as a specific interactor for BRI1 [16], and has been reported to play a positive role in plant immunity [17]. BAK1 interacted with an atypical LRR-RLK BIR2 to negatively regulate PAMP-triggered immunity [10]. The depletion of BAK1 in *Arabidopsis* showed significant cell death and ADR1 (Activated disease resistance 1) acted as a sensor for NLRs to trigger ETI [18]. In rice, 2 SERKs were found [19] and the homolog of *Arabidopsis thaliana BAK1* gene, *OsBAK1*, positively regulate resistance to rice blast fungus, *Magnaporthe grisea* [20] and *Magnaporthe oryzae* [21], and bacterium pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99 [22]. Cotton has two BAK1 orthologs, and GhBAK1 mediates resistance to *Verticillium* wilt. Silencing *GhBAK1* by Virus-induced gene silencing showed *Verticillium* wilt susceptibility, induced cell death and ROS production [23]. In Grapevine, heterologous *VqSERK3*/*BAK1* expression in *Arabidopsis* bak1-4 mutant lines showed increased powdery mildew resistance, reduced cell death, and strengthened stomatal immunity [24]. In wheat, 12 SERKs showed different roles in plant growth and development [25]. 9 SERKs in *Medicago truncatula* and 17 in *Glycine max* were identified [26]. However, BAK1 and it's the closest paralog BAK1-LIKE 1 (BKK1) usually play a redundant role in plant immunity and BR signaling pathways [27–29]. These findings suggest that BAK1 plays an important role in plants disease resistance.

Cucumber (*Cucumis sativus* L.) has an important economic value and is main vegetable produce worldwide. However, various diseases cause severe quality damage and yield decline in cucurbits production, practically gummy stem blight (GSB), powdery mildew (PM), downy mildew (DM), grey mildew (GM), fusarium wilt (FW) and others. Therefore, breeding and development of cultivar with broad-spectrum resistance (BSR) is the most important strategy to cucumber production. However, the function of *BAK1* in cucumber for disease-resistance remain elusive. In this study, we identified a total of 27 *BAK1* genes in cucumber, and divided them into five subgroups. Phylogenetic, gene structures, motif compositions, synteny, gene duplication and cis-elements were analyzed. In addition, the expression patterns of *CsBAK1s* between susceptible and resistant lines were analyzed after inoculation with five different pathogens and speculated the potential functions of *CsBAK1* gene family for pathogens infection. This study provided a theoretical foundation to further clarify the function of *BAK1s* to disease resistance in cucumber.

Results

Identification and characterization of cucumber *BAK1* gene member

We identified a total of twenty-seven *BAK1* genes in cucumber by an HMM model for Pkinase domain (PF00069) and BLASTP (Additional file 2: Table S1). All *CsBAK1s* were mapped onto seven chromosomes (Fig. 1), and named *CsBAK1-1* to *CsBAK1-27* based on their position on the *Cucumis sativus* genome (Chinese Long v3) (http://cucurbitgenomics.org/ftp/genome/cucumber/Chinese_long/). As shown in Fig. 1, twenty-seven *CsBAK1s* were distributed on all seven chromosomes, of which Chr.1 had four members, Chr.2, 3, and 4 each had two members, Chr.5 and 6 each had three members, and Chr.7 had ten members. The twenty-seven CsBAK1 protein sequence lengths ranged from 992 (CsBAK1-5) to 156 (CsBAK1-27) amino acids (aa), the molecular weights (MVs) ranged from 17,422.90 Da (CsBAK1-27) to 112,297.85 Da (CsBAK1-4), and isoelectric points (pI) varied from 4.81 (CsBAK1-12) to 9.23 (CsBAK1-17) (Additional file 1: Table S1). Subcellular characterization predicted cell membrane localization for ten proteins, nucleus localization for seven proteins, cell wall localization for six proteins, cytoplasm localization for three proteins, and cell membrane/nucleus localization for one protein. 11 of 27 CsBAK1s were characterized as unstable and most of CsBAK1s were hydrophilic proteins. All

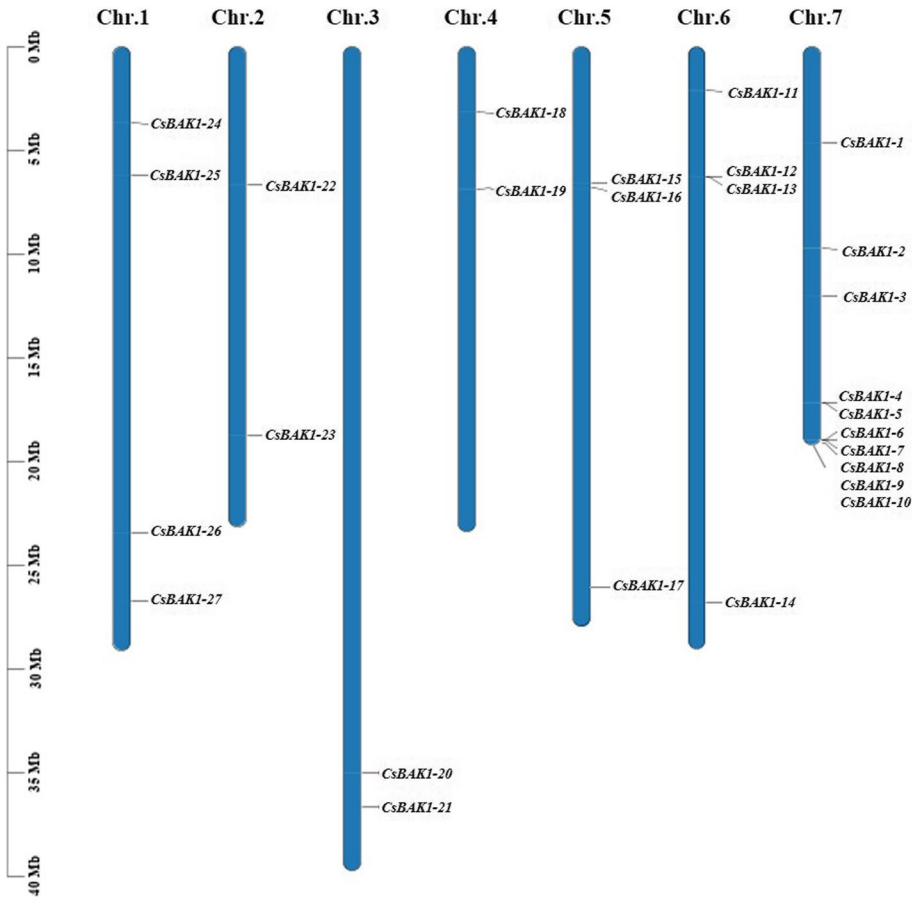

Fig. 1 Chromosomal location of *CsBAK1* genes in cucumber. Blue bars represent the chromosomes. Chromosome numbers are shown at the top of the bar. *CsBAK1* genes are labeled at the right of the chromosomes

the data collected suggested a high variability among the CsBAK1s. The present study provided a comprehensive analysis of candidate *CsBAK1s*.

Phylogenetic analysis and classification of CsBAK1s

In order to thoroughly investigate the BAK1 in plants, 83 BAK1s from 54 different species with 27 CsBAK1s protein sequences were used to conduct a neighbor-joining phylogenetic tree (Additional file 2: Table S2). As shown in Fig. 2, all BAK1s were divided into four groups. Most of CsBAK1 members were classified into Group II and clustered with BAK1s from *Oryza sativa* and *Arabidopsis*. Interestingly, only CsBAK1-3 was classified into Group II, and closely related to *Cucurbitaceae* and other dicotyledonous species, such as *Glycine max*, *Citrus clementina*, and *Medicago truncatula*, etc. Most of CsBAK1s were clustered with other species in Group I other than *Cucurbitaceae*, which implied a relatively high synteny between Group I across various species, and Group II in *Cucurbitaceae* might occurred genetic divergence.

CsBAK1 structure and motif composition

In order to reveal the evolution of cucumber BAK1 family precisely, 27 CsBAK1s were further classified into five subgroups based on conserved amino acid sequence (Fig. 3a). The exon–intron structure of *CsBAK1s* showed that the number of exons ranged from 2 to 16. The same subgroup didn't show similar gene structure, and exhibited a diversity of exon/intron structure (Fig. 3b).

Motif analysis identified ten conserved motifs of CsBAK1 proteins (Fig. 3c and Additional file 4: Table S4). Eight CsBAK1s in Subgroup II contained one specific Motif 2. CsBAK1 proteins in Subgroup III had longer amino acid sequences, but less motifs than those in the other subgroups. They only contained two motifs, Motif 1 and Motif 9. CsBAK1s in Subgroup IV had the most variable number of motifs. CsBAK1-24 and CsBAK1-17 had one motif (Motif 5), CsBAK1-10 and CsBAK1-27 had two motifs, CsBAK1-23 had three motifs, CsBAK1-2 had four motifs, and CsBAK1-21 had five motifs. All CsBAK1s in Subgroup V had Motif 8, five of six CsBAK1s had Motif 1 and Motif 3, except CsBAK1-14. CsBAK1s in

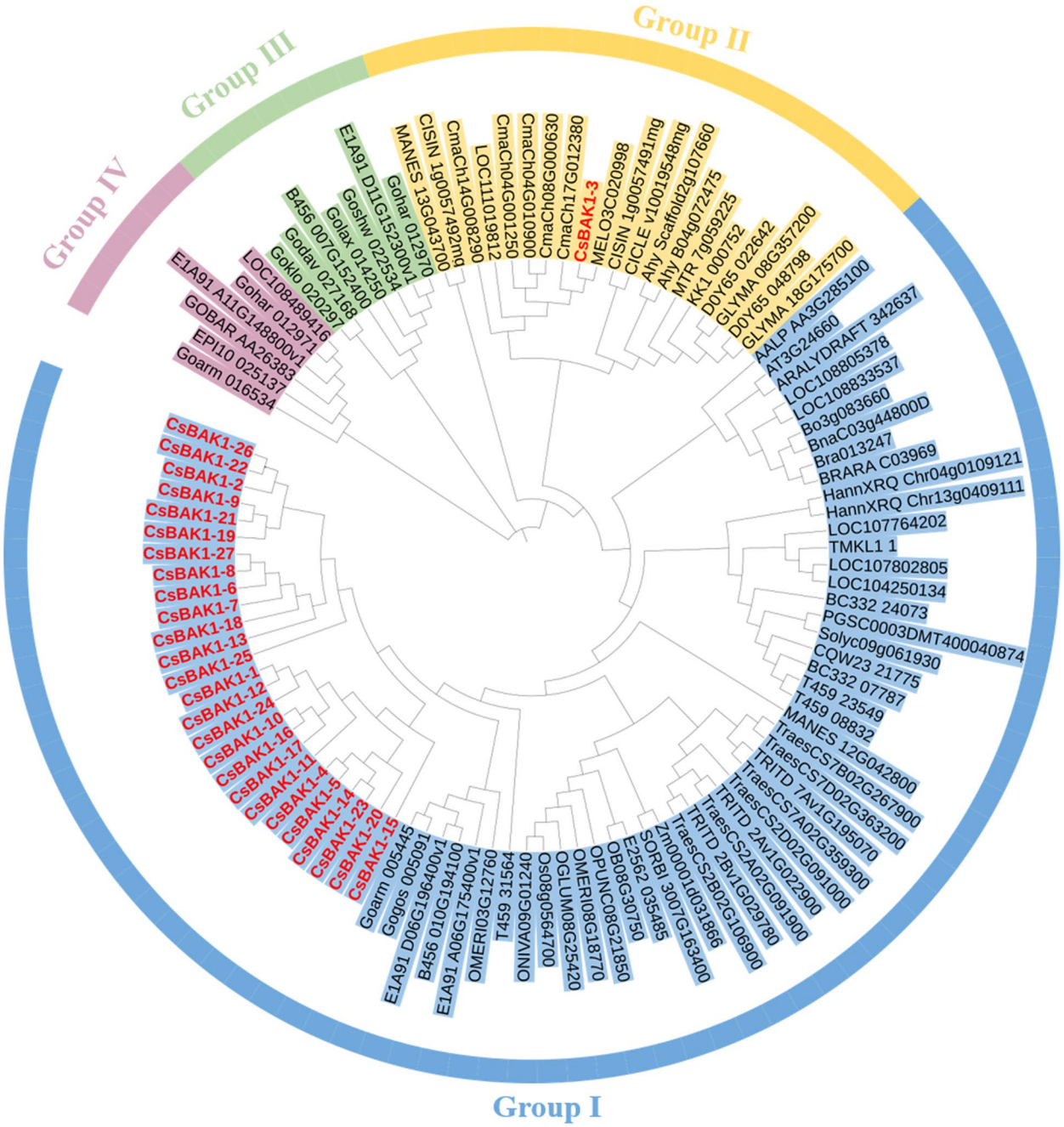

Fig. 2 Phylogenetic analysis of BAK1 proteins from different species. BAK1 proteins were firstly aligned using the ClustalW, and the phylogenetic tree was then constructed using iTOL. A total of 1,000 bootstrap replications were applied. Red letters represented CsBAK1s in cucumber

the same subgroup shared the similar motif composition, indicating that members in the same subgroup might have similar biological functions.

Synteny and gene duplication analysis of *CsBAK1s*

To further invested the evolution and extension of *CsBAK1* gene family, genome-wide synteny analysis identified three segmentally duplicated pairwise genes, including *CsBAK1-8*/*CsBAK1-20*, *CsBAK1-14*/*CsBAK1-25*, and *CsBAK1-17*/*CsBAK1-24* (Fig. 4a). Among these gene pairs, *CsBAK1-8*/*CsBAK1-20* and *CsBAK1-17*/*CsBAK1-24* were clustered together and had similar gene structures. These results indicated that *CsBAK1s* duplication mainly result from segmental

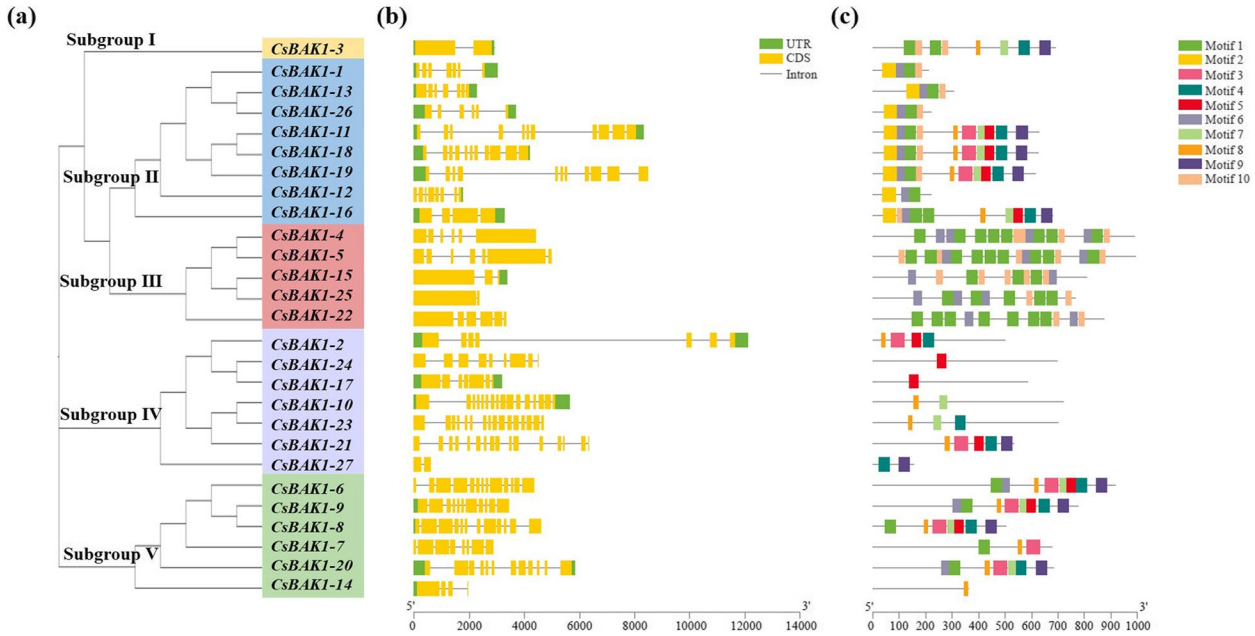

Fig. 3 Gene structure and conserved motif analysis of CsBAK1 gene family. **a** Phylogenetic analysis of CsBAK1s. **b** Gene structure of CsBAK1s. Green boxes, yellow boxes and gray lines represent untranslated regions, exons and introns respectively. **c** Motif composition of CsBAK1s. Different motifs are shown in different colors, and their detailed information is provided in Table S4

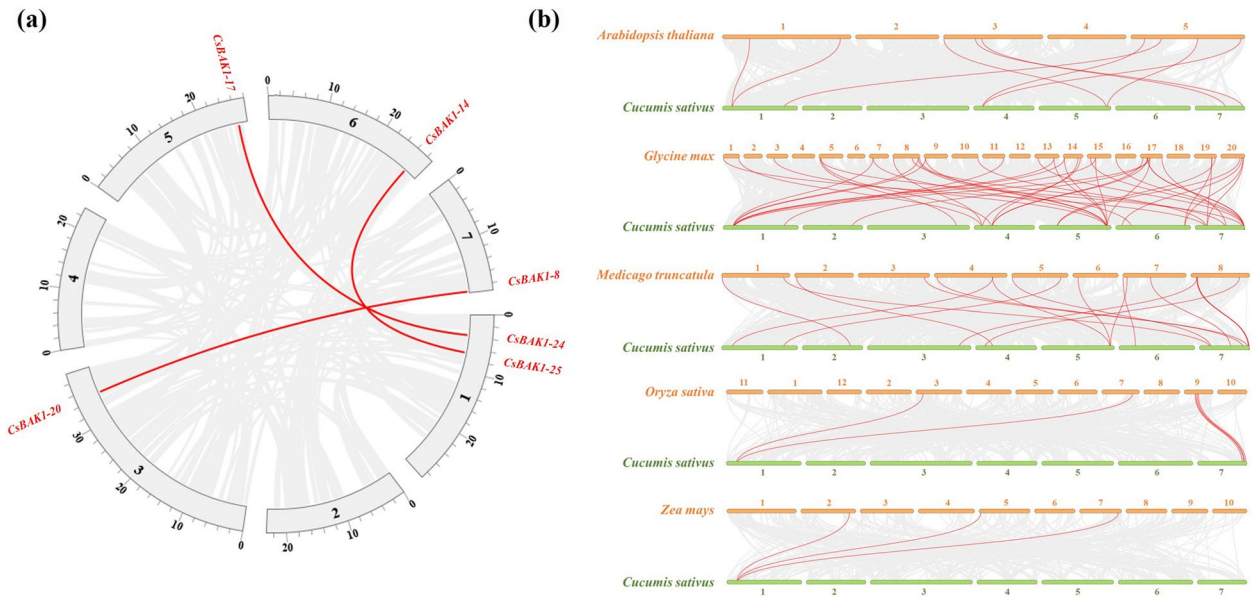

Fig. 4 Duplication and collinearity analysis of *BAK1s*. **a** Duplications of *BAK1s* in cucumber. Segmental duplication genes are connected with red lines. **b** Synteny analysis of *BAK1s* between *Cucumis sativus* and *Arabidopsis thaliana*, *Glycine max*, *Medicago truncatula*, *Oryza sativa*, and *Zea mays*

duplication other than tandem duplication. Five comparative syntenic maps of *CsBAK1s* with five species were used to investigate of syntenic blocks, including two monocots (*Oryza sativa* and *Zea mays*) and three dicots (*Arabidopsis thaliana*, *Glycine max*, and *Medicago truncatula*) (Fig. 4b). The orthologous pairs between two monocots (*Oryza sativa* and *Zea mays*) were 6 and 3, between three dicots (*Arabidopsis thaliana*, *Glycine max*, and *Medicago truncatula*) were 9, 43, and 18, which showed that dicots may have more syntenic conservation

than monocots. In addition, Ka/Ks analysis of the three orthologous gene pairs showed that the values of all three gene pairs were less than 1 (Additional file 5: Table S5), and indicated that *CsBAK1s* were under positive selection during evolution.

Identification of *cis*-regulatory elements in the promoters of *CsBAK1s*

Cis-regulatory elements (CREs) at promoter (2,000 bp regulatory region upstream of the ATG) were used to detect the potential regulatory mechanism of *CsBAK1s* (Table S6). Phytohormone-responsive (129), abiotic and biotic stress (105), and growth development (6) elements were identified on the promoter regions of *CsBAK1s* (Fig. 5 and Additional file 6: Table S6). Phytohormone responsive elements mainly included abscisic acid (45), gibberellin (28), auxin (10), salicylic acid (13) and methyl jasmonate (MeJA) (33). Among stress-related elements, defense and stress (16), wound (3), anoxic (68), drought (11) and low temperature (7) were identified. Furthermore, we also detected growth development elements, such as circadian response (6). These results showed that *CsBAK1s* might be regulated by various cis-regulatory elements in the promoters during stress responses.

Expression patterns in different tissues of *CsBAK1s*

RNA-seq data of 26 *CsBAK1* genes based on the public database (PRJNA80169) was used to analyze tissue-specific expression patterns in different tissues of Chinese Long (Root, stem, leaf, female flower, male flower, ovary, and tendril), except *CsBAK1-27* had no data. Four *CsBAK1s* in Subgroup II (*CsBAK1-1*, *CsBAK1-11*, *CsBAK1-13*, and *CsBAK1-26*) and two genes in Subgroup IV (*CsBAK1-2* and *CsBAK1-10*) had significantly higher expressions in all seven tissues (Fig. 6 and Additional file 7: Table S7), other members in Subgroup II (except *CsBAK1-12*) and Subgroup IV (except *CsBAK1-21*) showed relatively higher expressions in all seven tissues than that in other subgroups, which indicated that these *CsBAK1s* in Subgroup II and IV might have vital roles in cucumber growth development. *CsBAK1s* in Subgroup I, Subgroup III, and Subgroup V had relatively higher expressions in some specific tissues. *CsBAK1-3* in Subgroup I had relatively higher expressions in leaf, root, ovary and stem, except for female and male flowers. *CsBAK1-15* in Subgroup III had higher expressions in ovary and female, *CsBAK1-8* and *CsBAK1-14* in Subgroup V had relatively higher expressions in root, which suggested that these members had important regulatory functions in specific tissues.

Fig. 5 Predicted cis-elements in the promoter regions of *CsBAK1s*. Different colors represent different cis-elements. The bottom scale bar indicates the length of promoter

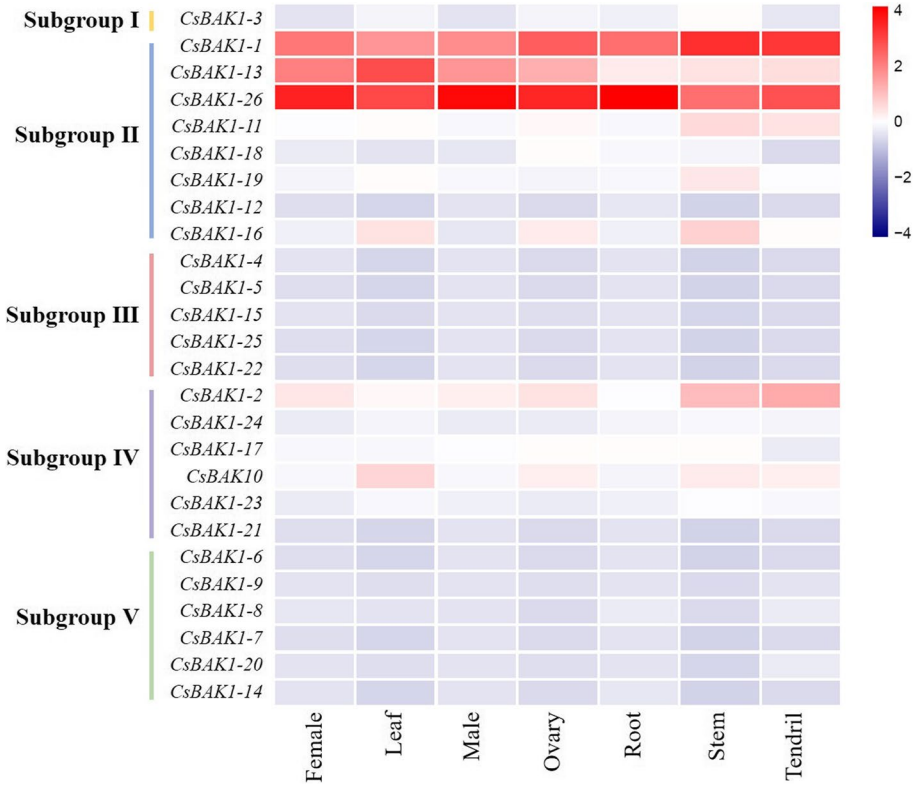

Fig. 6 Expression profiles of *CsBAK1s* in different tissues. The expression levels of *CsBAK1s* gene family were displayed based on $\log_2$RPKM values

Expression analysis of *CsBAK1s* in response to different pathogens

To thoroughly understand the possible functions of *CsBAK1s* in response to cucumber diseases, five pathogens (GSB, PM, DM, GM, and FW) were used to analyze expression patterns of *CsBAK1s*. Most of *CsBAK1s* showed significantly different expression patterns to response to different pathogens infection (Fig. 7 and Additional file 8: Table S8).

LM99 showed significant GSB-resistance than LM90 after inoculation GSB pathogen in seedling at 12 hpi, therefore, transcriptome sequencing was conducted at 0 and 12 hpi. A total of 156.08 Gb Clean Data was obtained. After quality control, high-quality reads (Q30 > 92.40%) were used to align with Cucumber (Chinese Long) v3 Genome, and alignment efficiency was from 87.81% to 95.89%. Most of *CsBAK1s* were downregulated significantly (Log_2|(-Foldchange)| > 1) in resistant line (LM99) after inoculation GSB, only four *CsBAK1s* (*CsBAK1-12*, *CsBAK1-14*, *CsBAK1-21*, *CsBAK1-22*) were upregulated in resistant line. Four *CsBAK1s* (*CsBAK1-5*, *CsBAK1-12*, *CsBAK1-14*, *CsBAK1-15*) were up-regulated, and six *CsBAK1s* (*CsBAK1-7*, *CsBAK1-8*, *CsBAK1-16*, *CsBAK1-18*, *CsBAK1-20*, and *CsBAK1-21*) were down-regulated in susceptible line LM90. Four genes (*CsBAK1-8*, *CsBAK1-16*, *CsBAK1-18*, and *CsBAK1-20*) were down-regulated both in LM99 and LM90, two *CsBAK1s* (*CsBAK1-12* and *CsBAK1-14*) were up-regulated both in LM99 and LM90. Ten *CsBAK1s* (*CsBAK1-1*, *CsBAK1-2*, *CsBAK1-3*, *CsBAK1-4*, *CsBAK1-10*, *CsBAK1-11*, *CsBAK1-13*, *CsBAK1-19*, *CsBAK1-23*, *CsBAK1-24*, and *CsBAK1-26*) and one gene (*CsBAK1-22*) were down- and up-regulated merely in LM99, respectively. *CsBAK1-21* showed different expression pattern between two lines (Fig. 7). In order to verify the accuracy of RNA-seq data, the expression pattern of 15 genes were examined by qRT-PCR, which showed that there was significantly correlation with RNA-Seq and qRT-PCR ($r^2 = 0.9093$) (Additional file 10: Figure S1; Additional file 9: Table S9).

For PM, nine genes were up-regulated and four *CsBAK1s* were down-regulated in susceptible line (D8), eight and five *CsBAK1s* were up- and down-regulated in resistant line (SSL508-28), respectively. In particular, the expression of *CsBAK1-9*, *CsBAK1-11* and *CsBAK1-14* showed opposite patterns in two lines. For DM, eleven *CsBAK1s* were up-regulated and one gene was down-regulated in susceptible line (Vias), fifteen *CsBAK1s* were up-regulated and two *CsBAK1s* were down-regulated in resistant line (PI88). In addition, three *CsBAK1s* in Subgroup V (*CsBAK1-8*, *CsBAK1-9*, and *CsBAK1-14*)

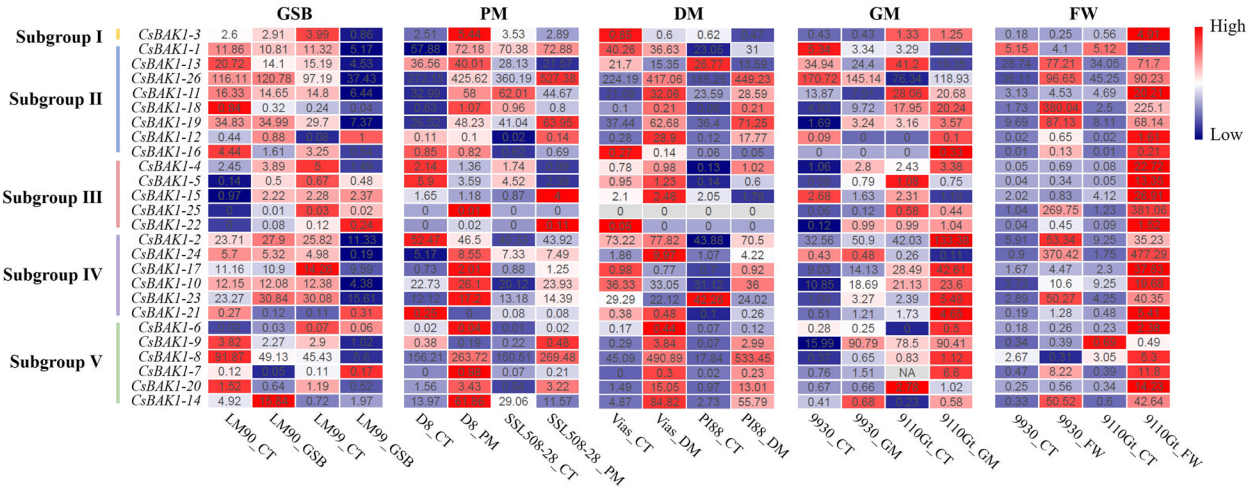

Fig. 7 Expression profiles of *CsBAK1s* after inoculation with different pathogens. GSB: gummy stem blight; PM: powdery mildew; DM: downy mildew; GM: grey mildew; FW: fusarium wilt. LM90: GSB susceptible line; LM99: GSB resistant line; D8: PM susceptible recurrent parent line; SSL508-28: PM resistant segment substitution line; Vlas: DM susceptible line; PI88: DM resistant line. 9930: GM and FW susceptible line; 9110Gt: GM and FW resistant line. Numerical values indicated expression level of log_2RPKM values for GSB, PM, and DM, and the relative expression level for GM and FW by qRT-PCR. Normalized *z*-score values were used to generate color-coded heatmap. *CsBAK1-27* had no expression in any situation

showed dramatically up-regulated expression level after inoculation DM both in two lines. For GM, twelve and four *CsBAK1s* were up- and down-regulated in susceptible line (9930) respectively, nine and four *CsBAK1s* were up- and down-regulated in resistant line (9110Gt) respectively. For FW, nineteen and two *CsBAK1s* were up- and down-regulated in susceptible line (9930) respectively, twenty-four up-regulated *CsBAK1s* and none down-regulated gene were detected in resistant line (9110Gt). Notably, the expression level of four *CsBAK1s* were significantly up-regulated both in two lines after inoculation FW. These results showed that different *CsBAK1s* responded to specific pathogens, and differential expression *CsBAK1s* between resistant and susceptible lines might have decisive roles in disease resistance. Interesting, *CsBAK1-14* had significant responses to all pathogens, which was up-regulated after pathogens infection in susceptible and resistant lines, except that down-regulated in resistant line after inoculation PM.

Discussion

There are two innate immunity systems to respond to pathogen infection in plants, PTI and ETI [30]. The coreceptor-like protein kinase BAK1 forms a receptor complex with multiple pattern recognition receptors (PRRs) in multiple PTI signaling pathways [5], but also acts upstream of NLRs to inhibits *R* gene expression in ETI [14]. The members of *BAK1* genes have been identified in *Arabidopsis* [10, 16–18], *Oryza sativa* [20–22], *Gossypium* [23], and *Vitis vinifera* [24], and so on. However, the systematic identification of *BAK1* gene members in cucumber has not been reported. In this study, we conducted comprehensive genome wide analyses of *BAK1s* in cucumber, and 27 *CsBAK1s* were identified in our study (Fig. 1 and Additional file 1: Table S1).

Phylogenetic analysis showed that 27 *CsBAK1s* were classified into two groups (Fig. 2). 26 of 27 *CsBAK1s* were clustered into Group I, and only *CsBAK1-3* was classed into Group II. However, the potential functions of *BAK1s* in Group II are unclear, and the function of *CsBAK1-3* still needs to be clarified by further works. Genome-wide synteny analysis of *CsBAK1s* showed that the number of *BAK1s* in *G. max* was 43, in *M. truncatula* was 18, in *Arabidopsis* was 9, in *O. sativa* was 6, and in *Z. mays* was 3 (Fig. 4). More *BAK1s* members in dicots than monocots suggested the more extensive duplication events in dicots. 27 *CsBAK1s* were distributed into all chromosomes, and Chr.7 carried more *CsBAK1s* than other chromosomes. A possible explanation was that the same chromosomal segmental duplication caused the clustering of *CsBAK1s* in Chr.7 [31], and a comprehensive analysis for the whole cucumber genome needs to be conducted to verify this hypothesis. 27 *CsBAK1s* were further divided into five subgroups, and *CsBAK1* genes within the same subgroup showed similar gene structures and motifs (Fig. 3), which speculated that *CsBAK1s* might be functional differentiation during evolution and duplication and the same subgroup members might have similar functions. The 8 *CsBAK1s* in Subgroup II were the most homologous to *AtBAK1* (*ATSERK3*) involved in pathogen resistance through regulation of PTI signaling pathway and NLR-dependent cell death [5, 27, 32],

which indicated that *CsBAK1* members in Subgroup II might involve in BAK1-mediated immune signaling in cucumber.

The interaction between plants and pathogens were mediated by complex mechanisms, and the autoimmunity to pathogen infection in cucumber cannot be regulated by a single gene/pathway, but the synergy of many genes. Cis-elements identified phytohormone responsive elements, stress-related elements, and growth development elements in the promoter regions of *CsBAK1s* (Fig. 5 and Additional file 7: Table S6), which revealed that *CsBAK1s* might be regulated by various stress responses and growth regulation. There were a relatively high occurrence of defense and stress responsive elements in the promoters of Subgroup III (4 of 5), indicating that *CsBAK1s* in Subgroup III were more likely to be involved in the defense response, except for *CsBAK1-4*. Moreover, the expression patterns after different pathogens infection showed that *CsBAK1-25* in Subgroup III almost had no response to four pathogens (GSB, PM, DM, and GM), but had significant response to FW (Fig. 7). This unique expression pattern gave us a potential information that different cis-elements in the promoters of *CsBAK1s* responded to different pathogen infections.

Tissue expression patterns often reflect the gene functions [33]. Expression profile of *CsBAK1s* showed that most of members in Subgroup II and IV had constitutively expression in cucumber, and others in Subgroup I, Subgroup III, and Subgroup IV were dominantly expressed in specific tissues, which probably due to functional divergences among different subgroups. Tissue specific expressions of *CsBAK1s* might have important roles in growth development. *CsBAK1* members in different subgroups had preferential expressions to specific tissues, and different members in the same subgroup had similar expression patterns, which probably due to functional divergences among different subgroups. Some members had no expressions or very low expressions, that may due to functional redundancy (Fig. 6 and Additional file 7: Table S7).

BAK1 could participate in PTI pathway by recognizing PRRs as a coreceptor for many PAMPs, but also regulates ETI activation as a target for many effectors [13]. To further detect the response to different pathogens for *CsBAK1s*, we analyzed expression patterns after GSB, PM, DM, GM, and FW infection. The expression levels of most *CsBAK1s* were changed after pathogen infection, indicating that *CsBAK1* gene family had a non-negligible role in cucumber disease resistance. Notably, the expression patterns of *CsBAK1s* were discrepant in different pathogens infection, which implied that *CsBAK1* gene family has various functions to response for different cucumber diseases. Previous studies showed that BAK1 involved in cell death and innate immunity by independent signaling pathways, and loss function of BAK1 did not lead to cell death [5, 34]. In this study, most of *CsBAK1s* were down-regulated in the resistant line after GSB infection, and *CsBAK1-8* and *CsBAK1-14* in Subgroup V had dramatic changes after GSB infection both in susceptible and resistant lines (Fig. 7), but the expression levels were significantly higher in susceptible line than that in resistant line, these two genes might be involved in GSB infection in cucumber. In our study, *CsBAK1s* were significantly down-regulated in resistant line after GSB infection, and might be involved in GSB resistance negatively. In contrast, most of *CsBAK1s* were up-regulated by PM, DM, GM, and FW infection. RNA-seq data showed that *CsBAK1-14* had significantly opposite expression patterns between resistant and susceptible lines, up-regulated in susceptible line (D8) but down-regulated expression in resistant line (SSL508-28) after PM infection, implying *CsBAK1-14* might has a vital role in PM resistant. For DM, we found that four of six genes in Subgroup V were up-regulated significantly to response for DM infection both in two lines, *CsBAK1* gene family in Subgroup V might play important roles in DM infection. For GM and FW, most of *CsBAK1s* were up-regulated in resistant lines, which indicated that *CsBAK1s* might be involved in GM and FW resistance positively. Notably, *CsBAK1-14* could have an important role in several pathogen infections, and the overexpression or gene-editing by CRISPR/Cas9 need to be conducted to further clarify its function. In summary, we speculated that *CsBAK1* gene family was an important regulator of disease resistance in cucumber, and different *CsBAK1* genes had diverse and specific functions in response to different pathogens with peculiar and complex ways.

Conclusion

In this study, we characterized and analyzed 27 *BAK1* genes in cucumber from a full perspective. The expression patterns in different tissues showed that *CsBAK1s* might be involved in pathogen infection divergently, and play essential roles in cucumber growth development and specific organ development. Different *CsBAK1s* had specific roles in different pathogen infections, and some specific *CsBAK1s* could have important function in a particular pathogen infection. However, the biological functions and regulatory pathways of *CsBAK1s* in cucumber need to be further clarified. This study provided a theoretical basis to further clarify the function of *CsBAK1s* to different pathogen infection in cucumber.

Materials and methods

Identification of *BAK1* family members in cucumber

The whole cucumber sequence was downloaded from the Cucumber (Chinese Long) v3 Genome (http://cucurbitgenomics.org/ftp/genome/cucumber/Chinese_long/). The Hidden Markov Model (HMM) of Protein kinase domain (PF00069) was download from the Pfam protein family database (http:// pfam.xfam.org/) to search *BAK1* gene members with E value < 0.01 as criterion. Then, BAK1 amino acid sequences in all plants were obtained using keyword "BAK1" from NCBI (https://www.ncbi.nlm.nih.gov/protein), and a BLASTP-algorithm was conducted to search against cucumber genome. Combined with these two methods, the predicted BAK1 protein sequences were considered as cucumber BAK1 (CsBAK1) (Additional file 2: Table S1).

Sequence analysis and structural characterization

Expasy (https://web.expasy.org/protparam/) and Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/#) were used to predict the physicochemical properties and subcellular localization of *CsBAK1s*, respectively. Gene structures of *CsBAK1s* were analyzed and visualized by TBtools [35]. MEME (http://meme-suite.org/tools/meme) was used to analyze the conserved motifs of *CsBAK1s*.

Phylogenetic analysis

The full-length amino acid sequences of CsBAK1 (Additional file 3: Table S2) derived from different plant species were used for phylogenetic analysis. Multiple-sequence alignment was performed using the ClustalW (https://www.genome.jp/tools-bin/clustalw) with the default parameters, and then phylogenetic analysis was conducted using iTOL (https://itol.embl.de/). *CsBAK1s* were classified into different groups according to the phylogenetic tree in other species and cucumber.

Analysis of *Cis*-acting element in *CsBAK1* genes' promoters

The 2,000 bp sequences upstream of *CsBAK1s* were extracted from the cucumber genome database and submitted to PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) to identify Cis-acting elements and transcription factor binding sites.

Chromosomal locations, gene duplications and selection pressure analysis

The chromosomal locations of *CsBAK1s* were illustrated with TBtools. Gene duplication analysis of *BAK1* was conducted with MCScanX and Circos of Tbtools using the amino acid sequences in *Cucumis sativus*, *Arabidopsis thaliana*, *Zea mays*, *Glycine max*, and *Medicago truncatula*. Genomic information was available at Phytozome (https://phytozome-next.jgi.doe.gov/). The Ka/Ks ratio of duplicated *CsBAK1* genes were calculated to evaluate the selection pressure using KaKs_calculator2.0 (https://sourceforge.net/projects/kakscalculator2/).

The tissues expression profile of *CsBAK1s*

Genome-wide transcriptional data of cucumber were obtained from Cucurbit Expression Atlas (http://cucurbitgenomics.org/rnaseq/cu/3). The expression levels of *CsBAK1s* in different tissues were obtained from transcriptome data PRJNA80169, including leaf, stem, female flower, male flower, ovary, root, and tendril.

Real-time PCR

Total RNAs of all samples were extracted using the RNA simple Total RNA Kit (TIANGEN, Beijing, China) following instruction and then the quality was determined by 1% agarose gel electrophoresis. For reverse transcription, the first-strand cDNA was synthesized using the TransScript First-Strand cDNA Synthesis SuperMix Kit (TransGen, China). For qRT-PCR, gene expressions were examined using cDNA templates by SYBR green super mix and ABI 7900 machine (Bio-Rad, Hercules, CA, USA). Gene-specific primers for the candidate genes were designed using Primer3.0 (https://primer3.ut.ee/). The expression level of *CsActin* (*CsaV3_2G018090*) was set as control for normalization and the relative gene expression was calculated followed the $2^{-\Delta\Delta Ct}$ method [36], and three biological replicates with three technical replicates were used for each gene. All primers used in this study were listed in Additional file 4: Table S3. Heatmap in R package was used to visualize the difference of gene expression.

Expression patterns of *CsBAK1s* in response to different pathogens

To thoroughly understand the functions of *CsBAK1s* to major diseases in cucumber, we analyzed the expression patterns of *CsBAK1s* after inoculation different pathogens, including gummy stem blight (GSB), powdery mildew (PM), and downy mildew (DM), grey mildew (GM), and fusarium wilt (FW).

For GSB, the GSB-resistant cucumber accession 'PI 183967' (*Cucumis sativus* var. *hardiwickii* (Royle) Alef.) and the GSB-susceptible accession '931' (*Cucumis sativus* var. *sativus*), and their 160 recombinant inbred lines were used to analyze GSB resistance. A susceptible RIL (LM90) and a resistant RIL (LM99) with similar genetic backgrounds were selected to GSB pathogen inoculation and further genome-wide transcriptome

analysis in this study. LM90 and LM99 were inoculated with *Didymella bryoniae*. LM90 showed apparent plant diseases at 12 hpi, therefore we chose samples at 0 and 12 hpi to construct RNA-seq library by using Illumina HiSeq 3000. The sequence raw data were submitted to the National Center for Biotechnology Information (NCBI) with accession number of PRJNA1127874.

For PM and DM, the RNA-seq data based on the database was used to clarify the expression patterns of *CsBAK1s*. To clarify the expression patterns of *CsBAK1s* to PM, transcriptome data PRJNA321023 was used to analyze the expression patterns of *CsBAK1s* to PM in SSL508-28 (PM-resistantce) and D8 (PM-susceptibility) at 0 and 48 hpi (http://cucurbitgenomics.org/rnaseq/cu/4). The transcriptome data PRJNA285071 was used to analysis the response to DM in a susceptible (cv. Vlaspik) and resistant (PI 197088) cucumber at 0 and 24 hpi (http://cucurbitgenomics.org/rnaseq/cu/19).

For GM and FW, '9110 Gt' is resistant to GM and FW, and '9930' is susceptible to GM and FW. Therefore, '9110 Gt' and '9930' were used to analyze expression patterns of *CsBAK1s* for GM and FW. All plants were grown in the greenhouse at Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, and were inoculated when the second true leaf was fully opened. Then, all true leaves were collected at 0, 12, 48, and 96 h post inoculation (hpi) and immediately frozen in liquid nitrogen for RNA extraction. Three plants for each sample of three replicates were collected. '9930' (susceptible to GM and FW) showed visible disease symptom quickly at 12 hpi for GM, and at 48 hpi for FW, samples collected at 12 and 48 hpi were used to analyze *CsBAK1s* expression level for GM and FW by qRT-PCR, respectively.

Abbreviations

BAK1	Brassinosteroid insensitive 1-associated receptor kinase 1
BR	Brassinosteroid
BRI	Brassinosteroid-insensitive
SERK3	Somatic embryogenesis receptor-like kinase 3
LRRs-RLK	Leucine-rich repeats receptor-like kinase
PRRs	Pattern recognition receptors
BIR2	BAK1-interacting receptor-like kinase 2
NLR	Nucleotide-binding domain and leucine-rich repeat
ADR1	Activated disease resistance 1
BKK1	BAK1-LIKE 1
GSB	Gummy stem blight
PM	Powdery mildew
DM	Downy mildew
GM	Grey mildew
FW	Fusarium wilt
BSR	Broad-spectrum resistance
CREs	Cis-regulatory elements
PRR	Pattern recognition receptor
PTI	Pattern recognition receptor-triggered immunity
ETI	Effector-triggered immunity
PEPRs	PEP-receptors
HR	Hypersensitive response
HMM	Hidden Markov Model

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05453-2.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.
Supplementary Material 8.
Supplementary Material 9.
Supplementary Material 10.

Acknowledgements
We would like to thank the National Key Research and Development Program of China (2023YFD1201503), the National Natural Science Foundation of China (32202487), the Key-Area Research and Development Program of Shandong Province (2021LZGC016), the National Key Research and Development Program of China (2022YFD1200805), the earmarked fund for Modern Agro-industry Technology Research System (CARS-23), the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2021-IVF) and the Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture, P.R. China.

Authors' contributions
Z.S.P. and M.H. designed the experiments, H.J.N. performed the experiments, analyzed the data, and wrote the manuscript. D.S.Y., G.J.T., G.X.F., and Z.S.P. revised the manuscript. L.X.P. participated partial experiments. All authors read and approved of the manuscript.

Funding
This work was supported by the earmarked fund for Modern Agro-industry Technology Research System (CARS-23), the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (CAAS-ASTIP-IVFCAAS) and the State Key Laboratory of Vegetable Biobreeding, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences.

Availability of data and materials
The public transcriptome data used in this study are available at Cucurbit Expression Atlas of PRJNA321023 (http://cucurbitgenomics.org/rnaseq/cu/4) and PRJNA285071 (http://cucurbitgenomics.org/rnaseq/cu/19). The transcriptome sequencing data after GSB inoculation in the current study are deposited at the National Center for Biotechnology Information (NCBI) with accession number of PRJNA1127874, and the other data that supports the findings of this study are available in the supplementary material of this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 9 October 2023 Accepted: 24 July 2024
Published online: 02 August 2024

References

1. Zhang S, Li C, Ren H, Zhao T, Li Q, Wang S, Zhang Y, Xiao F, Wang X. BAK1 mediates light intensity to phosphorylate and activate catalases to regulate plant growth and development. Int J Mol Sci. 2020;21(4):1437.
2. Wang X, Kota U, He K, Blackburn K, Li J, Goshe MB, Huber SC, Clouse SD. Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Dev Cell. 2008;15(2):220–35.
3. He K, Xu S, Li J. BAK1 directly regulates brassinosteroid perception and BRI1 activation. J Integr Plant Biol. 2013;55(12):1264–70.
4. Gou X, Yin H, He K, Du J, Yi J, Xu S, Lin H, Clouse SD, Li J. Genetic evidence for an indispensable role of somatic embryogenesis receptor kinases in brassinosteroid signaling. PLoS Genetics. 2012;8:e1002452.
5. Wu Y, Gao Y, Zhan Y, Kui H, Liu H, Yan L, Kemmerling B, Zhou JM, He K, Li J. Loss of the common immune coreceptor BAK1 leads to NLR-dependent cell death. Proc Natl Acad Sci. 2020;117(43):27044–53.
6. Zipfel C, Kunze G, Chinchilla D, Caniard AC, Jones J, Boller T, Felix G. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell. 2006;125(4):749–60.
7. Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nuernberger T, Jones J, Felix G, Boller T. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature. 2007;448(7152):497–500.
8. Gao M, Wang X, Wang D, Xu F, Ding X, Zhang Z, Bi D, Cheng YT, Chen S, Li X, et al. Regulation of cell death and innate immunity by two receptor-like kinases in Arabidopsis. Cell Host Microbe. 2009;6(1):34–44.
9. Blaum BS, Mazzotta S, Nöldeke ER, Halter T, Madlung J, Kemmerling B, Stehle T. Structure of the pseudokinase domain of BIR2, a regulator of BAK1-mediated immune signaling in Arabidopsis. J Struct Biol. 2014;186(1):112–21.
10. Halter T, Imkampe J, Mazzotta S, Wierzba M, Postel S, Bücherl C, Kiefer C, Stahl M, Chinchilla D, Wang X, et al. The leucine-rich repeat receptor kinase BIR2 is a negative regulator of BAK1 in plant immunity. Curr Biol. 2014;24(2):134–43.
11. Liu F, Zeng M, Sun Y, Chen Z, Chen Z, Wang L, Cui J-R, Zhang F, Lv D, Chen X. BAK1 protects the receptor-like kinase BIR2 from SNIPER2a/b-mediated degradation to promote pattern-triggered immunity in Nicotiana benthamiana. Plant Cell. 2023;35(9):3566–84.
12. Yuan M, Jiang Z, Bi G, Nomura K, Xin XF. Pattern-recognition receptors are required for NLR-mediated plant immunity. Nature. 2021;592(7852):1–5.
13. Wang ZY. Brassinosteroids modulate plant immunity at multiple levels. Proc Natl Acad Sci. 2011;109(1):7–8.
14. Heese A, Hann DR, Gimenez-Ibanez S, Jones A, Kai H, Jia L, Schroeder JI, Peck SC, Rathjen JP. The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc Natl Acad Sci. 2007;104(29):12217–22.
15. Hecht V, Vielle-Calzada JP, Hartog MV, Schmidt ED, Boutilier K, Grossniklaus U, de Vries SC. The Arabidopsis somatic embryogenesis receptor kinASE 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. Plant Physiol. 2001;127(3):803–16.
16. Nam KH, Li J. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell. 2002;110(2):203–12.
17. Lin W, Li B, Lu D, Chen S, Zhu N, He P, Shan L. Tyrosine phosphorylation of protein kinase complex BAK1/BIK1 mediates Arabidopsis innate immunity. Proc Natl Acad Sci. 2014;111(9):3632–7.
18. Dong OX, Tong M, Bonardi V, El Kasmi F, Woloshen V, Wünsch LK, Dangl JL, Li X. TNL-mediated immunity in Arabidopsis requires complex regulation of the redundant ADR1 gene family. The New Phytol. 2016;210(3):960–73.
19. Singla B, Khurana JP, Khurana P. Structural characterization and expression analysis of the SERK/SERL gene family in rice (Oryza sativa). Int J Plant Genomics. 2009;2009:539402.
20. Hu H, Xiong L, Yang Y. Rice SERK1 gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. Planta. 2005;222(1):107–17.
21. Park HS, Ryu HY, Kim BH, Kim SY, Yoon IS, Nam KH. A subset of OsSERK genes, including OsBAK1, affects normal growth and leaf development of rice. Mol Cells. 2011;32(6):561–9.
22. Liao H, Xiao X, Li X, Chen Y, Fu X, Lin D, Niu X, Chen Y, He C. OsBAK1 is involved in rice resistance to Xanthomonas oryzae pv. oryzae PXO99. Plant Biotechnol Rep. 2016;10(2):75–82.
23. Gao X, Li F, Li M, Kianinejad AS, Dever JK, Wheeler TA, Li Z, He P, Shan L. Cotton GhBAK1 mediates Verticillium wilt resistance and cell death. J Integr Plant Biol. 2013;55(7):586–96.
24. Li Y, Jiao C, Wei Z, Chai S, Jia H, Gao M, Allison J, Li Z, Song C-b, Wang X. Analysis of grapevine's somatic embryogenesis receptor kinase (SERK) gene family: VqSERK3/BAK1 overexpression enhances disease resistance. Phytopathology. 2022;112(5):1081–92.
25. Sharma N, Khurana P. Genome-wide identification and analysis of the TaSERK gene family in bread wheat triticum aestivum L. and TaSERK8 overexpression study in rice. J Plant Growth Regul. 2023;42(7):4131–49.
26. Nolan KE, Kurdyukov S, Rose RJ. Characterisation of the legume SERK-NIK gene superfamily including splice variants: Implications for development and defence. BMC Plant Biol. 2011;11(44):1.
27. Roux M, Schwessinger B, Albrecht C, Chinchilla D, Jones A, Holton N, Malinovsky FG, Tör M, de Vries S, Zipfel C. The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. Plant Cell. 2011;23(6):2440–55.
28. Kim BH, Kim SY, Nam KH. Assessing the diverse functions of BAK1 and its homologs in Arabidopsis, beyond BR signaling and PTI responses. Mol Cells. 2013;35(1):7–16.
29. Meng X, Chen X, Mang H, Liu C, Yu X, Gao X, Torii KU, He P, Shan L. Differential function of Arabidopsis SERK family receptor-like kinases in stomatal patterning. Curr Biol. 2015;25(18):2361–72.
30. Jones J, Dangl JL. The plant immune system. Nature. 2006;444(7117):323–9.
31. Monosi B, Wisser RJ, Pennill L, Hulbert SH. Full-genome analysis of resistance gene homologues in rice. Theor Appl Genet. 2004;109(7):1434.
32. Chien CC, Chang CH, Ting HM. A novel lectin receptor kinase gene, AtG-LecRK-I.2, enhances bacterial pathogen resistance through regulation of stomatal immunity in Arabidopsis. Plant Sci. 2024;343:112071.
33. Pan J, Tu J, Sharif R, Qi X, Xu X, Chen X. Study of JASMONATE ZIM-domain gene family to waterlogging stress in Cucumis sativus L. Vegetable Research. 2021;1(1):1–12.
34. Schwessinger B, Roux M, Kadota Y, Ntoukakis V, Sklenar J, Jones A, Zipfel C. Phosphorylation-dependent differential regulation of plant growth, cell death, and innate immunity by the regulatory receptor-like kinase BAK1. PLoS Genet. 2011;7(4):e1002046.
35. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–202.
36. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29(9):e45.

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