Fine mapping and candidate gene analysis of gummy stem blight resistance in cucumber stem

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Abstract

Key message Two candidate genes (Csa6G046210 and Csa6G046240) were identified by fine-mapping gsb-s6.2 for gummy stem blight resistance in cucumber stem.

Abstract Gummy stem blight (GSB) is a serious fungal disease caused by Didymella bryoniae, that affects cucumber yield and quality worldwide. However, no GSB-resistant genes have been identified in cucumber cultivars. In this study, the wild cucumber accession ‘PI 183967’ was used as a source of resistance to GSB in adult stems. An F₂ population was mapped using resistant line ‘LM189’ and susceptible line ‘LM6’ derived from a cross between ‘PI 183967’ and ‘931’. By developing InDel and SNP markers, the gsb-s6.2 QTL on Chr. 6 was fine-mapped to a 34 kb interval harboring six genes. Gene Expression analysis after inoculation showed that two candidate genes (Csa6G046210 and Csa6G046240) were induced and differentially expressed between the resistant and susceptible parents, and may be involved in disease defense. Sequence alignment showed that Csa6G046210 encodes a multiple myeloma tumor-associated protein, and it harbored two nonsynonymous SNPs and one InDel in the third and the fourth exons, and two InDels in the TATA-box of the basal promoter region. Csa6G046240 encodes a MYB transcription factor with six variants in the AP2/ERF and MYB motifs in the promoter. These two candidate genes lay the foundation for revealing the mechanism of GSB resistance and may be useful for marker-assisted selection in cucumber disease-resistant breeding.

Introduction

Breeding for plant disease resistance has become one of the main objectives of vegetable improvement programs. Cucumber is an important vegetable crop worldwide. In 2020, cucumber was grown on 2.3 million hectares with a global production of 91.3 million metric tons (FAOSTAT). Gummy stem blight (GSB) is a serious fungal disease in cucurbits production with a general incidence of 20% that typically causes 20–35% reductions in yield, but losses can be as high as 80% (Kothera et al. 2003; Li 2007; Liu et al. 2017). GSB is caused by Didymella bryoniae which is both seed- and soil-borne. It infects tissues, throughout the entire growth period by forming watery-like spots, leading to severe yield losses and quality decline. GSB was first identified more than 130 years ago, and it has been detected in every region where cucurbits are grown (Chester 1891; Chiu et al. 1949). However, there is no effective method to prevent GSB, making it important to identify GSB resistance genes for the development of GSB-resistant cultivars.
There have been active efforts to identify genetic markers linked to resistance in cucurbits. In melon (Cucumis melo L.), five loci for GSB resistance have been identified, four of which show single inheritance and the other, single recessive inheritance (Zuniga et al. 1999; Wako et al. 2002; Frantz et al. 2004). In watermelon, a pair of recessive genes (db/dh) were identified that offered some GSB resistance (Norton 1979; Gusmini et al. 2005). Using Bulked Segregant RNA-Seq and competitive allele-specific PCR (KASP), two candidate genes, Cla001017 (Nucleotide-binding site-leucine-rich repeat resistance protein) and Cla001019 (pathogenesis-related protein), were identified as being associated with GSB resistance in watermelon (Ren et al. 2020).

In cucumber, there are several reports of the genetic and molecular mechanisms of GSB resistance. To date, nine loci associated with GSB resistance in leaves at the seedling stage, and five loci associated with GSB resistance in stems at the adult stage have been identified in C. hystrix (Chen et al. 1995) and C. sativus var. hardwickii (Liu et al. 2017; Zhang et al. 2017). Analysis of F2 populations developed with C. hystrix as a source of resistance led to the identification of two QTLs related to GSB resistance in seedling leaves—one on Chr. 4 within a 12 cM interval, and another on Chr. 6 within a 11 cM interval (Lou et al. 2013). Using another RIL (Recombinant inbred line) population derived from C. hystrix, Zhang et al. (2018) identified a major QTL on Chr.1 and considered Csa1G654870 (Ribonuclease 3-like protein) as candidate gene. Another set of RILs developed using C. sativus var. hardwickii (‘PI 183,967’) as the resistant parent crossed with susceptible cucumber line ‘931’, led to the identification of several loci related to GSB resistance in seedling leaves and adult stems. GSB resistance in seedling leaves was associated with six QTLs across four chromosomes, with gsb5.1 on Chr. 5 within a 0.5 cM interval considered as the major QTL (Liu et al. 2017). Five QTLs were detected on three chromosomes for GSB resistance on adult stems, with a major QTL gsb-s6.2 on Chr.6 within a 6.8 cM interval (Zhang et al. 2017).

Although several loci with resistance to GSB have been detected in different studies, none of them overlap and none have unambiguously identified GSB-resistance-related genes in cucumber. In addition, due to the narrow genetic diversity of cucumber, it is difficult to obtain cultivars with resistant genotypes. Thus far, resistant loci have only been found in wild cucumber relatives, and in a wild perennial congener of cucumber (Cucumis hystrix Chakr.) (Lou et al. 2013; Liu et al. 2017; Zhang et al. 2017). Identifying multiple sources of GSB resistance in wild cucumber resources will be needed to develop robust disease resistance in commercial cucumber.

In our previous study, a major locus gsb-s6.2 for GSB resistance in adult stems was identified based on two homozygous lines, i.e., ‘LM189’ (GSB resistant) and ‘LM6’ (GSB sensitive) derived from a cross of C. sativus var. hardwickii (PI 183967) and ‘931’ (Zhang et al. 2017). In this study, we fine-mapped gsb-s6.2 using an F2;3 population derived from ‘LM189’ and ‘LM6’. Candidate genes in the 34 kb interval were further assessed by qRT-PCR. Our study will provide the basis for determining the molecular mechanisms underlying GSB resistance and promote breeding for this trait in cucumber.

Materials and methods

Plant materials

The QTL mapping used 160 F9 recombinant inbred lines (RILs) derived from a cross between the wild GSB-resistant cucumber accession PI 183967 (DI = 27.09), and the cultivated GSB-susceptible accession ‘931’ (DI = 96.43) (Zhang et al. 2017). ‘931’ is a Northern China type inbred line from Shandong province called ‘Xintaimici’. In order to exclude the effect of other minor QTLs, two homozygous lines (a resistant line ‘LM189’ with a DI of 6.32 and a susceptible line ‘LM6’ with a DI of 79.95) were selected to construct 1000 F2 and 97 F2;3 segregating populations for fine mapping. All materials were grown in fall 2021 at the Shouguang farm of the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences. All plants were arranged with complete randomized blocks design with three replications.

Phenotype and assessment for GSB resistance

The specific strain Didymella bryoniae from Vegetable disease prevention and control innovation team (Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences) was used for inoculation. When pycnidia formed on cucumber fruit for 7–10 days, spores were collected from fruit and suspended in sterile water with 10⁶ spores/mL used for inoculation.

When the plants reached the adult stage, which was about 75 days after planting, the healthy and robust stems at the bottom 1/3 of the plants were pruned to 15 cm and placed into a container covered with filter paper. Then, all stems were inoculated evenly with sprayer and sealed for moisturizing cultivation. Five plants for each line were evaluated in one replicate and one test was conducted with three replicates. Plants started to show obvious symptom 3 days after inoculation, and resistance to the disease was recorded for each plant when we observed apparent symptom 5 days post-inoculation. The disease rating scale of each stem was divided into 5 grades followed by Zhang et al. (2017), based on the percentage of the stem surface showing

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infection: Grade 0 = no symptoms, Grade 1 = 0–25%, Grade 2 = 25–50%, Grade 3 = 50–75%, and Grade 4 > 75% (Fig. 1).

A Disease index (DI) was calculated using the following formula:

\[ DI(\%) = \frac{\sum (\text{Number of plants with disease rating} \times \text{Disease rating})}{\text{Highest disease rating} \times \text{Total number of plants}} \times 100 \]

The DI score was used to classify GSB resistance as follows: Plants with a DI ≤ 15% were considered to be ‘highly resistant’, those with a DI between 15 and 35% were classified as ‘resistant’, a DI of 35–55% were ‘medium resistance’, a DI between 55 and 80% meant plants were ‘susceptible’ and a DI > 80% indicated ‘highly susceptible’ to GSB (Zhang et al. 2017).

Genomic DNA extraction and genotyping

Genomic DNA was extracted from the cotyledon of the two parentals and F2 plants following the modified CTAB protocol (Saghai-Maroof 1984), and was used for PCR amplification and genotyping. The 20 μL PCR reaction mixture contained 2 μL DNA template (50–100 ng/μl), 10 μL 2× Phanta Max Master Mix, 1 μL forward primer (2 μM), 1 μL reverse primer (2 μM) and 6 μL ddH2O. The PCR amplification program was as follows: 95 °C for 3 min; 35 cycles of 95 °C denaturation for 15 s, 58 °C annealing for 15 s and 72 °C extension for 1 min/kb; a final 72 °C extension for 5 min. PCR products were separated by 1% (w/v) agarose gel electrophoresis and then sequenced by Sangon Biotech (Shanghai) (https://www.sangon.com/). Sequence alignment was performed using the online software Multiple sequence alignment (http://multalin.toulouse.inra.fr/multalin/multalin.html). For SSR markers, PCR products were separated using non-denaturing polyacrylamide gel electrophoresis.

Genetic linkage map construction and QTL mapping

To develop the markers used for QTL mapping, as well as specific locus amplified fragment sequencing (SLAF-seq) data from the RILs (Unpublished) and the whole genome sequence of ‘PI 183,967’ and ‘931’, seven SLAF markers and two SSR markers in proximity to the gsbs-6.2 locus on Chr.6 were selected and used for QTL mapping (Table S1). The QTL IciMapping v.3.1 software (http://www.isb Breeding.net) was used to construct a high-density genetic linkage map for gsbs-6.2. Combined with the DI scores of GSB-resistance at the adult stage in our previous study (Zhang et al. 2017), inclusive composite interval mapping (ICIM) was used for QTL mapping and analysis.

Segregation population and fine mapping of gsbs-6.2

To reduce the interval around the gsbs-6.2 locus, an F2 population was produced by first crossing the resistant ‘LM189’ with the susceptible ‘LM6’, and selfing the resultant offspring to produce an F2. The F2 recombinants were screened by the flanking markers (SSR04083 and SSR02940) developed in previous study (Zhang et al. 2017), and selfed to produce an F2:3 population. The F2:3 lines were screened for resistance after pathogen inoculation and phenotype collection. Based on resequencing data from publicly available sequence data NCBI Short Read Archive (SRA) (http://www.ncbi.nlm.nih.gov/sra?term=SRA056480) (Shang et al. 2014), SNP/InDel variations were identified in the gsbs-6.2 interval between ‘PI 183,967’ and ‘931’ and designed SNP/InDel markers used for fine mapping (Table S2). Using the cucumber reference genome 9930_v2 (http://cucurbignomics.org/organism/2) (Huang et al. 2009), the sequences of the candidate genes within the target interval and their putative function were identified. All primers used in this study were listed at Table S2.

qRT-PCR analysis of candidate genes in the target interval

The parents of RILs, ‘PI 183,967’ and ‘931’, were used to detect the expression of candidate genes. When the second true leaf fully opened, all plants were inoculated with Didymella bryoniae. True leaves were sampled from the two parents at 0, 12, 48, and 96 h post-inoculation (hpi) and were immediately frozen in liquid nitrogen for RNA extraction. Three biological replicates consisting of three plants were analyzed for each sample. Total RNAs were extracted using TransZol Up Plus RNA Kit (TransGen Biotech). The quality of RNA was determined by electrophoresis of a 1% (w/v)
agarose gel. First-strand cDNA was synthesized from 1 μg total RNA using the TransScript First-Strand cDNA Synthesis SuperMix Kit (TransGen, China). qRT-PCR was performed by SYBR green Super Mix and CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Gene-specific primers used for qRT-PCR were designed using Primer3.0 (https://primer3.ut.ee/), and were listed in Table S3. The expression level of CsaActin (Csa2G301530) was used as the internal control, the relative expression levels of candidate genes were calculated according to the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001), and three independent biological replicates were used for each sample.

Results

Saturating and QTL mapping of gsb-s6.2

In our previous study, we identified the gsb-s6.2 locus that was associated with GSB resistance in adult cucumber. Within this region, seven SNPs and two SSRs were identified between markers SSR04083 and SSR16020, which differentiated ‘PI 183,967’ from the ‘931’ sequence. These markers were then used to genotype the 160 RILs derived from crossing these genotypes, and combined with the GSB resistance DI in the RILs, we identified gsb-s6.2 located between MarkerG19776 and MarkerG20046 (Fig. 2). The QTL had a LOD score of 4.49, and it explained 22.40% of the observed phenotypic variation. Importantly, the area flanking gsb-s6.2 was reduced from 1.9 to 1.0 Mb.

Fine mapping of gsb-s6.2

To fine map gsb-s6.2, two homozygous lines, ‘LM189’ (GSB-resistant, DI = 6.32) and ‘LM6’ (GSB-susceptible, DI = 79.95), were selected from the 160 RIL populations and were used to construct segregation populations. Ninety-seven recombinants were screened from 1,000 F2 individuals by the flanking SSR markers SSR04083 and SSR02940. Three markers (gsb6.2–22, gsb6.2–6 and gsb6.2–12) were used to detect recombinants genotypes in the primary region, and five, six and three recombination events were identified, respectively (Fig. 3a). Combined with the phenotyping of the F2:3 population, gsb-s6.2 was mapped to a 425 kb genomic region between markers gsb6.2–22 and gsb6.2–6 (Fig. 3a). Using resequencing data of ‘PI 183,967’ and ‘931’, we developed one SNP and six InDel markers within the 425 kb region, restricting the gsb-s6.2 locus to a 34 kb interval between gsb6.2-reIndel3 and gsb6.2-reSNP2. Examination of the 9930_v2 reference genome (Huang et al. 2009), showed that there were six predicted genes in this 34 kb region (Table 1, Fig. 3b).

Candidate gene analysis of gsb-s6.2

To determine which of the candidate genes may underlie the observed variation in GSB response, the expression patterns of the six genes, in ‘PI 183,967’ relative to ‘931’ were analyzed by qRT-PCR after infection with Didymella bryoniae. Ideal candidates would differ in expression between the parentals and be responsive to fungal inoculation. Csa6G046220 was expressed at very low levels and was barely detectable in the samples assessed, and was therefore not considered further. Likewise, when expression was compared after inoculation, expression of Csa6G045200 and Csa6G046200 (Fig. 4a, b) were significantly down- and upregulated respectively, but importantly, show similar expression patterns in the susceptible and resistant parents, and were therefore not ideal candidates. The remaining genes showed differences in response between the parentals. In the GSB-susceptible parent, Csa6G046210 parent was sharply upregulated at 12 hpi, and then downregulated at 48 hpi, but was unaffected in the GSB-resistant parent after inoculation (Fig. 4c). The expression level of Csa6G046230 was higher in the GSB-resistant parent.
than the GSB-susceptible parent, at two of the three time-points examined (Fig. 4d). Csa6G046240 exhibited a sharp increase in expression at 12 hpi and a significant drop in the remaining hpi examined, in both parentals (Fig. 4e). These results suggested that Csa6G046240 had the most dramatic expression pattern changes in response to the pathogen infection and that Csa6G046210 had the most distinct expression pattern between the GSB-susceptible and the GSB-resistant parents. We, therefore, speculated that Csa6G046210 and Csa6G046240 might be associated with GSB resistance in cucumber, and may be involved in the interaction between the GSB pathogen and cucumber biological processes.

**Table 1** Six predicted genes in the 34 kb fine mapped interval of gsb-s6.2 on Chr.6 using the cultivar ‘9930’_v2 reference genome

| Gene         | Position (bp)          | Functional annotation                             |
|--------------|------------------------|---------------------------------------------------|
| Csa6G045200  | 3,673,353–3,676,699    | Vesicle-associated membrane protein               |
| Csa6G046200  | 3,678,586–3,685,961    | AMME syndrome candidate gene                      |
| Csa6G046210  | 3,688,921–3,692,134    | Multiple myeloma tumor-associated protein         |
| Csa6G046220  | 3,694,050–3,694,353    | 1-Deoxy-D-xylulose-5-phosphate synthase           |
| Csa6G046230  | 3,694,379–3,696,056    | ATPase inhibitor                                   |
| Csa6G046240  | 3,698,010–3,700,011    | MYB transcription factor                          |

**Nucleotide polymorphisms in the candidate genes**

To determine which, if any, of the candidate genes are likely to be associated with variation in GSB resistance, we determined the sequence of their open reading frame in both parents and sought to identify polymorphisms. Only one of the six candidates, i.e., Csa6G046210, was polymorphic, including three nonsynonymous variants (two SNPs which resulted in a Thr92Arg and Val200Glu substitutions, and one 21 bp InDel which led to the absence of seven amino acids in one parent (Fig. 5).

The cis-regulatory elements upstream of the start ATG contain binding sites for transcription factors and other regulatory molecules, and are essential to transcription (Wittkopf et al. 2012). Differential expression of functional genes may also be due to variation in promoter sequences, leading to phenotypic variation (Carroll et al. 2008; Wittkopf...
et al. 2012; Dong et al. 2014). We further examined if there could be sequence variation in the promoter sequences of Csa6G046210 (Fig. S2), which led to differences in expression between the two parents. Intriguingly, two InDels (a 31 bp and a 48 bp deletion) in the TATA-box, which is part of the core promoter element that defines the transcription start-site, were of interest.

Within the 2 kb upstream regulatory sequence of Csa6G046240, we identified 13 SNPs or InDels that differentiated the parentals (Fig. 6). We used PlantPAN3.0 (http://plantpan.tips.ncku.edu.tw/) to predict if these SNPs and InDels occurred within putative cis-elements. Polymorphisms were detected in three different AP2/ERF motifs, and four different MYB motifs within the promoter.
Phylogenetic analysis of Csa6G046210 and Csa6G046240

Csa6G046210 encodes a tumor-associated protein, which has been functionally characterized in humans, but with no known function in plants. The putative Csa6G046210 polypeptide has an MMtag domain at the N-terminus, and may contain nuclear localization signals (Fig. S3a). A Basic Local Alignment Search Tool (BLAST) analysis against NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) indicated that the MMtag domain are conserved across different species, and the protein encoded by Csa6G046210 is similar to that in other cucurbits (Fig. S4a). Protein–protein interaction databases and functional protein association networks (3659. XP_004140907.1) showed that proteins with the MMtag domain could interact with protein possessing a WD40 repeat domain (Fig. 5a, Table S4).

Csa6G046240 encodes a transcription factor DIVARI-CATA, an orthologue of the AtDIV2 gene in Arabidopsis, which has roles in salt stress and ABA signaling according to the description from GenBank records. BLAST search analysis indicated that the protein encoded by Csa6G046240 clustered with other Cucurbitaceae DIVARICATA-like proteins, and shows high similarity to MYB-like proteins in the Leguminosae (Fig. S4b), suggesting that DIVs had similar function in dicotyledons. DIV and DIV-like proteins belong to the R-R-type clade of the MYB family (Fang et al. 2018). Csa6G046240 contains the SANT superfamily domain and the MYB-DNA-binding domain against the GenBank Conserved Domain Database v3.12 (www.ncbi.nlm.nih.gov/cdd/) (Fig. S3b). The predicted protein interaction network by STRING (https://cn.string-db.org) showed that several heat shock 70 proteins (HSP70) potentially interact with CsDIV (Fig. S5b, Table S5).

Discussion

Gummy stem blight is one of the major diseases in cucumber (Liu et al. 2017; Zhang et al. 2017). Identifying candidate genes related to GSB resistance would enhance cucumber breeding and reduce crop losses due to this disease. However, the genetic mechanism of GSB resistance in cucumber is complicated and controversial. Furthermore, genetic sources of resistant are scarce in cucumber cultivars, and most germplasm used for GSB resistance in cucumber are wild cucumbers, including Cucumis hystrix and C. sativus var. hardwickii. The accession PI 183,967 (C. sativus var. hardwickii) is the progenitor of cultivated cucumber and carries valuable genes for resistance to GSB both in leaves at the seedling stage, and in stems at the adult stage (Liu et al. 2017; Zhang et al. 2017). Therefore, dissecting the genetic loci and determining mechanisms for GSB resistance in PI 183,967, would allow for a translational breeding pipeline for disease resistance in cucumber cultivars.

Most studies on QTL mapping of GSB resistance are focused on seedling leaves, and few look at the stems of adult plants. In our previous study, we identified loci associated with GSB resistance on adult stem derived from PI 183,967, and showed that it was a quantitative trait controlled by three major genes. QTL mapping identified five QTLs on Chr. 1, 3 and 6 using SSR markers. QTL gsb-s6.2 had the highest phenotypic variation of 22.7% and was regarded as the major QTL with a physical distance of 1.9 Mb (Zhang et al. 2017). QTL mapping is an effective way to elucidate quantitative traits in plants. However, genes associated with resistance in PI 183,967 have not been identified.

In this study, we fine-mapped and delimited our previously identified gsb-s6.2 locus to a 34 kb genomic region containing six candidate genes (Csa6G045200, Csa6G046200, Csa6G046210, Csa6G046220, Csa6G046230 and Csa6G046240). These genes have different functional annotations, encoding vesicle-associated membrane proteins, an AMMECR1 protein family, tumor-associated proteins, phosphate synthases, ATPase inhibitors, and MYB transcription factors, respectively (Table 1). Based on expression analysis and sequence alignment, we identified Csa6G046210 and Csa6G046240 were the main candidate genes underlying gsb-s6.2.

Csa6G046210 encodes a tumor-associated protein. We identified two nonsynonymous SNPs and one 21 bp InDel, which resulted in two amino acids alteration (Thr92Arg...
and Val200Glu) and the Indel of seven amino acids (Fig. 4). Csa6G046210 has a conserved MMtag domain and may contain nuclear localization signals, so it might act as a signal molecule in the nucleus. MMTag domain-containing protein similar to human multiple myeloma tumor-associated protein 2 (hMMTAG2). Homologs of Csa6G046210 in Arabidopsis, AT3G52220, encode kinase phosphorylation protein and contain phosphorylation sites for several protein kinases. Protein phosphorylation is an important mechanism regulating plant immunity. In Arabidopsis, protein phosphates (TOPPs) were identified as the key regulators of plant immunity (Liu et al. 2013). In N. benthamiana, protein phosphatase 1 catalytic (PP1c) could form holoenzymes with the Phytophthora infestans RXLR effector Pi04314 to promote late blight disease (Boevink et al. 2016). Protein–protein interaction showed that the MMTag domain could interact with the WD40 repeat domain. WD-repeat proteins are involved in a variety of regulatory processes ranging from signal transduction and transcription regulation to cell cycle control and apoptosis. In wheat (Triticum aestivum L.), numerous TaWD40s were involved in response to biotic stresses and mainly expressed at the early stage of pathogen infection (Hu et al. 2018). Similar to a candidate gene for resistance to spotted wilt virus in tomato (Qi et al. 2022), Csa6G046210 showed a distinct expression pattern between two parents at 12 and 96 hpi ($P < 0.05$), but no significant difference at 48 hpi, we speculated that the expression pattern may be consistent with the infection process (Fig. 4c). Therefore, we inspected its regulatory regions and found two significant InDels which caused deletions in two TATA-box elements (Fig. S2). As the core promoter element, the TATA box recruits the basal transcription machinery for transcription initiation. Further, a recent study found that the TATA box could influence promoter strength (Jores et al. 2021). We speculate that Csa6G046210 may play a role in GSB resistance and has a response to pathogen by regulating gene expression, however, we cannot exclude the changes in protein. More functional experiments need to be done and to verify the functional mechanism of Csa6G046210. The variations of Csa6G046210 could be validated using natural population and used for marker-assisted selection of GSB resistance in cucumber breeding.

Csa6G046240 encodes a transcription factor DIVARICATA and showed the most significantly increased expression after inoculation among the candidate genes in the QTL interval. qRT-PCR analysis showed that Csa6G046240 was the most significantly up-regulated in both parental lines, but its expression levels were noticeably higher in GSB-susceptible parent ‘931’ compared to the GSB-resistant parent ‘PI 183,967’ (Fig. 4e). We were unable to identify nonsynonymous variants in the Csa6G046240 coding sequences between two parents. Therefore, we speculated that Csa6G046240 may contain mutations in the promoter cis-regulatory elements. Many mutations in the promoter regions were discovered (Fig. 5), including three variants in AP2/ERF motifs, and another three variants were in MYB motifs. The AP2/ERF and MYB motifs might be part of the GSB resistance response to pathogen infection, by activating gene expression. The homolog in Arabidopsis encodes an R-R type MYB protein, which plays a negative regulatory role in plant salt stress response and is essential for ABA signal transduction according to the description from GenBank records (AT5G04760). MYB transcription factors are widely distributed in plants, and interact with various transcription factors and play an important role in biotic and abiotic stresses (Ambawat et al. 2013). After pathogen infection, AtMYB30 triggers programmed cell death and a hypersensitive response by regulating hormone levels and specific gene expression (Daniel et al. 1999; Raffaele et al. 2006; Raffaele et al. 2008; Li et al. 2009). In our study, Heat shock proteins 70 (HSP70) are predicted to interact with CsDIV. Heat shock proteins are known to protect plants against abiotic and biotic stresses by maintaining protein homeostasis (Tutar et al. 2010; Zhang et al. 2015; Vierling, 1991; Sun et al. 2002; Wang et al. 2004). Thus, as a transcription factor, Csa6G046240 might modulate pathogen infection response by regulating downstream signaling pathways.

To determine the regulatory mechanisms that contribute to the GSB resistance observed in this study, the two candidate genes Csa6G046210 and Csa6G046240 are being cloned and functionally analyzed. These candidate genes will lay the foundation for revealing the mechanism of GSB resistance and may be useful for marker-assisted selection in disease breeding in cucumber.

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Author contributions statement G.X.F. and Z.S.P. designed the experiments, H.J.N. performed the experiments, analyzed the data, and wrote the manuscript. D.S.Y., L.X.P., D.M.B. and Z.S.P. revised the manuscript. M.H participated partial experiments. S.Y.X. provided Didy- mella bryoniae. All authors read and approved the manuscript.

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Data availability All reference data that are not presented in this manuscript are available in the supplemental tables. All materials are available to the public upon request and under material transfer agreement.
Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Ambawat S, Sharma P, Yadav NR, Yadav RC (2013) MYB transcription factor genes as regulators for plant responses: an overview. Physiol Mol Biol Pla 19(3):307–321

Carroll SB (2008) Evo-devo and an expanding evolutionary syntheses: a genetic theory of morphological evolution. Cell 134:25–36

Chester FD (1891) Notes on three new or noteworthy diseases of plants. Bull Torrey Bot Club 18(12):371–374

Chiu WF, Walker JC (1949) Physiology and pathogenicity of the cucurbit black-rot fungus. J Agric Res 78:589–615

Daniel X, Lacomme C, Morel JB, Roby D (1999) A novel myb oncogene homologue in Arabidopsis thaliana related to hypersensitive cell death. Plant J 20(1):57–66

Dong Y, Yang X, Liu J, Wang BH, Liu BL, Wang YZ (2014) Pod shattering resistance associated with domestication is mediated by a NAC gene in soybean. Nat Commun 5:3352

Fang Q, Wang Q, Mao H, Xu J, Wang Y, Hu H, He S, Tu J, Cheng C, Tian G, Wang X, Liu X, Zhang C, Luo K (2018) AtDIV2, an R-type MYB transcription factor of Arabidopsis, negatively regulates salt stress by modulating ABA signaling. Plant Cell Rep 37(11):1499–1511

Frantz J, Jahn M (2004) Five independent loci each control monotenic resistance to gummy stem blight in melon (Cucumis melo L.). Theor Appl Genet 108(6):1033–1038

Gusmin G, Song R, Wehner TC (2005) New sources of resistance to gummy stem blight in watermelon. Crop Sci 45:582–588

Hu R, Xiao J, Gu T, Yu XF, Zhang Y, Chang JL, Yang GX, He GY (2018) Genome-wide identification and analysis of WD40 proteins in wheat (Triticum aestivum L.). BMC Genomics 19:803

Jores T, Tonnies I, Wrightsman T, Buckler ES, Cuperus JT, Fields S, Queitsch C (2021) Synthetic promoter designs enabled by a comprehensive analysis of plant core promoters. Nat Plants 7:842–855

Kothera RT, Keinath AP, Dean RA, Farnham MW (2003) AFLP analysis of a worldwide collection of Didymella bryoniae. Mycol Res 107(3):297–304

Li, Y.: Study on biology characteristics of Didymella bryoniae and screening of resistance germplasm of cucumber [D]. Nanjing Agricultural University (2007)

Li L, Yu XF, Thompson A, Guo M, Yoshida S, Asami T, Chory J, Yin YH (2009) Arabidopsis MYB30 is a direct target of BES1 and cooperates with BES1 to regulate brassinosteroid-induced gene expression. Plant J 58(2):275–286

Liu J.: Mapping of QTls for resistant to gummy stem blight in Cucumis sativus/hystrix introgression line HH1–8–1–2 [D]. Nanjing Agricultural University (2013)

Liu SL, Shi YX, Miao H, Wang M, Li BJ, Gu XF, Zhang SP (2017) Genetic analysis and QTL mapping of resistance to gummy stem blight in Cucumis sativus seedling stage. Plant Dis 101:1145–1152

Lou LN, Wang HY, Qian CT, Liu J, Bai YL, Chen JF (2013) Genetic mapping of gummy stem blight (Didymella bryoniae) resistance genes in Cucumis sativus/hystrix introgression lines. Euphytica 192:359–369

Norton JD (1979) Inheritance of resistance to gummy stem blight caused by Didymella bryoniae in watermelon. HortScience 14:630–632

Qi SM, Shen YB, Wang XY, Zhang SJ, Li YS, Islam MM, Wang J, Zhao P, Zhan XQ, Zhang F, Liang Y (2022) A new NLR gene for resistance to Tomato spotted wilt virus in tomato (Solanum lycopersicum). Theor Appl Genet 135(5):1493–1509

Raffaele S, Vivas S, Roby D (2006) An essential role for salicylic acid in AtMYB30-mediated control of the hypersensitive cell death program in Arabidopsis. FEBS Lett. 580(14):3498–3504

Raffaele S, Vailleau F, Léger A, Joubès J, Miersch O, Huard C, Blée E, Mongrand S, Domergue F, Roby D (2008) A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in Arabidopsis. Plant Cell 20(3):752–767

Ren RS, Xu JH, Zhang M, Liu G, Yao XF, Zhu LL, Hou Q (2020) Identification and molecular mapping of a gummy stem blight resistance gene in wild watermelon (Citrullus amariss) germplasm PI 189225. Plant Dis 104:16–24

Saghai-Maroo MA (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. P Natl Acad Sci 81(24):8014–8018

Shang Y, Ma Y, Zhou Y, Zhang H, Duan L, Chen H, Zeng J, Zhou Q, Wang S, Gu W, Liu M, Ren J, Gu XF, Zhang SP, Wang Y, Yasukawa K, Bouweester HH, Qi XQ, Zhang ZH, Lucas WL, Huang SW (2014) Biosynthesis, regulation, and domestication of bitterness in cucumber. Science 346:1084–1088

Sun W, Van MM, Verbruggen N (2002) Small heat shock proteins and stress tolerance in plants. Biochem Biophys Acta 1577:1–9

Tutar L, Tutar Y (2010) Heat shock proteins; an overview. Curr Pharm Biotechnol 11:216–222

Vierling E (1991) The roles of heat shock proteins in plants. Plant Mol Biol 42:579–620

Wako T, Sakata Y, Sugiyama M, Ohara T, Ishitoh D, Kojima A (2002) Identification of melon accessions resistant to gummy stem blight and genetic analysis of the resistance using an efficient technique for seedling test. Acta Hort 588:161–164

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9:244–252

Wittkopp PJ, Kalay G (2012) cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence. Nat Rev Genet 13:59–69

Zhang L, Zhao HK, Dong QL, Zhang YG, Wang YM, Li HY, Xing GJ, Li QY, Dong YS (2015) Genome-wide analysis and expression profiling under heat and drought treatments of hsp70 gene family in soybean (Glycine max L.). Front. Plant Sci. 6:773

Zhang SP, Liu SL, Miao H, Shi YX, Wang M, Wang Y, Li BJ, Gu XF (2017) Inheritance and QTL mapping of resistance to gummy stem blight in cucumber stem. Mol Breeding 37:49

Zhang X, Xu J, Li J, Lou QF, Chen JF (2018) QTL Mapping and identification of candidate gene for resistance to gummy stem blight in Cucumis sativus/hystrix introgression line ‘IL77.’ Acta Horticulturae Sinica 45(11):12

Zuniga TL, Jantz JP, Zitter TA, Jahn MK (1999) Monogenic dominant resistance to gummy stem blight in two melon (Cucumis melo) accessions. Plant Dis 83(12):1105–1107

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