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

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Research Article

Keywords: Cucumber, Gummy stem blight (GSB), Stem resistance, Fine-mapping, Candidate genes

Posted Date: April 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1567368/v1

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 F_2 population was mapped using resistant line 'LM116' and susceptible line 'LM34' 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 to GSB resistance, and may be useful for marker-assisted selection in cucumber disease-resistant breeding.

Key Message

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

1 Introduction

Breeding for plant disease resistance has become one of the main objectives of vegetable improvement programs. Gummy stem blight (GSB) is a serious fungal disease in cucurbits production 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 dominant inheritance and the other, single recessive inheritance (Zuniga et al., 1999; Wako et al., 2001; Frantz et al., 2004). In watermelon, a pair of recessive genes (*db*/*db*) were identified that offered some GSB resistance (Gusmini et al., 1979; Norton, 1979; Gusmini et al., 2005). Using Bulked Segregant RNA-Seq and Kompetitive 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 F_2 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 populations 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 183967') 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., 'LM116' (GSB resistant) and 'LM34' (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 $F_{2:3}$ population derived from 'LM116' and 'LM34'. 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.

2 Materials And Methods

2.1 Plant Materials

The QTL mapping used 160 recombinant inbred lines (RILs) derived from a cross between the wild GSB-resistant cucumber accession PI 183967, and the cultivated GSB-susceptible accession '931' (Zhang et al., 2017). In order to exclude the effect of other minor QTLs, two homozygous lines (a resistant line 'LM116' and a susceptible line 'LM34') were selected to construct 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.

2.2 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 into sterile water with 10^6 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. Resistance to the disease was recorded 3–5 days after inoculation for each plant. 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 infection: Grade 0 = no symptoms, Grade 1 = 0–25%, Grade 2 = 25–50%, Grade 3 = 50–75%, and Grade 4 > 75%. A Disease index (DI) was calculated using the following formula:

$$\mathrm{DI}\ (\%) = 100\times\frac{\Sigma(\text{Number of plants with disease rating}\times\text{Disease rating})}{\text{Highest disease rating}\times\text{Total number of plants}}$$

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%-35% were classified as ‘resistant’, a DI of 35%-55% were ‘medium resistance’, a DI between 55–80% meant plants were ‘susceptible’ and a DI > 80% indicated ‘highly susceptible’ to GSB (Zhang et al., 2017).

2.3 Genomic DNA extraction and genotyping

Genomic DNA was extracted from the cotyledon of the two parentals and F_2 plants following the modified CTAB protocol (Saghai-Maroof, Soliman et al. 1984), and was used for PCR amplification and genotyping. The 20 μL PCR reaction mixture contained 2 μL DNA template (50–100 ng/ul), 10 μL 2 × Phanta Max Master Mix, 1 μL forward primer (2 μM), 1 μL reverse primer (2 μM) and 6 μL ddH_2O. 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.

2.4 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 183967’ and ‘931’,

seven SLAF markers and two SSR markers in proximity to the *gsb-s6.2* locus on Chr.6 were selected and used for QTL mapping (Table S1). The QTL IciMapping v.3.1 software (http://www.isbreeding.net) was used to construct a high-density genetic linkage map for *gsb-s6.2*. 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.

2.5 Segregation population and fine mapping of *gsb-s6.2*

To reduce the interval around the *gsb-s6.2* locus, an F_2 population was produced by first crossing the resistant 'LM116' with the susceptible 'LM34', and selfing the resultant offspring to produce an F_2. The F_2 recombinants were screened by the flanking markers (SSR04083 and SSR02940) developed in previous study (Zhang et al., 2017), and selfed to produce an $F_{2:3}$ population. The $F_{2:3}$ 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 *gsb-s6.2* interval between 'PI 183967' and '931' and designed SNP/InDel markers used for fine mapping (Table S2). Using the cucumber reference genome 9930_v2 (http://cucurbitgenomics.org/organism/2), 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.

2.6 qRT-PCR analysis of candidate genes in the target interval

The parents of RILs, 'PI 183967' 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 hours 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 *CsActin* (*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.

3 Results

3.1 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 183967' 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. 1). 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 Mb to 1.0 Mb.

3.2 Fine mapping of *gsb-s6.2*

To fine map *gsb-s6.2*, two homozygous lines, 'LM116' (GSB-resistant) and 'LM34' (GSB-susceptible), were selected from the 160 RILs populations were used to construct segregation populations. Ninety-seven recombinants were screened from 1,000 F_2 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. 2a). Combined with the phenotyping of the $F_{2:3}$ population, *gsb-s6.2* was mapped to a 425 kb genomic region between markers gsb6.2-22 and gsb6.2-6 (Fig. 2a). Using resequencing data of 'PI 183967' 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, showed that there were six predicted genes in this 34 kb region (Table 1, Fig. 2b).

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

3.3 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 183967' 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. 3a, 3b) 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. 3c). The expression level of *Csa6G046230* was higher in the GSB-resistant parent than the GSB-susceptible parent, at two of the three timepoints examined (Fig. 3d). *Csa6G046240* exhibited a sharp increase in expression at 12 hpi and a significant drop in the remaining hpi examined, in both parentals (Fig. 3e). These results suggest 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.

3.4 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. 4).

The cis-regulatory elements upstream of the start ATG contain binding sites for transcription factors and other regulatory molecules, and are essential to transcription (Wittkopp 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; Wittkopp et al., 2012; Dong et al., 2014). We further examined if there could be sequence variation in the promoter sequences of *Csa6G046210* (Fig. S1), 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. 5). We used PlantPAN3.0 (http://plantpan.itps.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.

3.5 Phylogenetic analysis of *Csa6G046210* and *Csa6G046240*

Csa6G046210 encodes an AMMECR1 protein, which has been functionally characterized in humans, but with no known function in plants. The putative AMMECR1 polypeptide has an MMtag domain at the N-terminus, and may contain nuclear localization signals (Fig. S2a). 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. S3a). 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. 4a, Table S4).

Csa6G046240 encodes a transcription factor DIVARICATA, 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. S3b), 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 super family domain and the MYB-DNA-binding domain against the GenBank Conserved Domain Database v3.12 (www.ncbi.nlm.nih.gov/cdd/) (Fig. S2b). 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. S4b, Table S5).

4 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 183967 (*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 183967, 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 183967, 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 183967 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 2). Based on expression analysis and sequence alignment, we identified *Csa6G046210* and *Csa6G046240* were the main candidate genes underlying *gsb-s6.2*.

Csa6G046210 encodes an AMMECR1 family 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. 3). AMMECR1 has a conserved MMtag domain and may contain nuclear localization signals, so it might act as a signal molecule in the nucleus. AMMECR1 is one of the important genes of the contiguous gene deletion syndrome AMME in mammals (Thomas et al., 2010). Homologs of AMMECR1 in Arabidopsis, *AT3G52220*, encodes kinase phosphorylation protein and contains phosphorylation sites for several protein kinases. But its function in plants is not clear. 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). *Csa6G046210* showed a distinct expression pattern between two parents ($P < 0.05$). Therefore, we inspected its regulatory regions and found two significant InDels which caused deletions in two TATA-box elements (Fig. S1). 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 of protein. More functional experiments need to be done, and to verify the functional mechanism of *Csa6G046210*.

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 183967' (Fig. 3e). We were unable to identify nonsynonymous variants in the *Csa6G046240* coding sequences between two parents. Therefore, we speculated that *Csa6G046240* maybe contain mutations in the promoter cis-regulatory elements. Many mutations in the promoter regions were discovered (Fig. 4), 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 homologous gene in Arabidopsis encodes an R-R type MYB protein, which has 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 to GSB resistance and may be useful for marker-assisted selection in disease breeding in cucumber.

Declarations

Acknowledgements 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 Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture and Rural Affairs, P. R. China.

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 *Didymella bryoniae*. All authors read and approved of the manuscript.

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

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.

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Figures

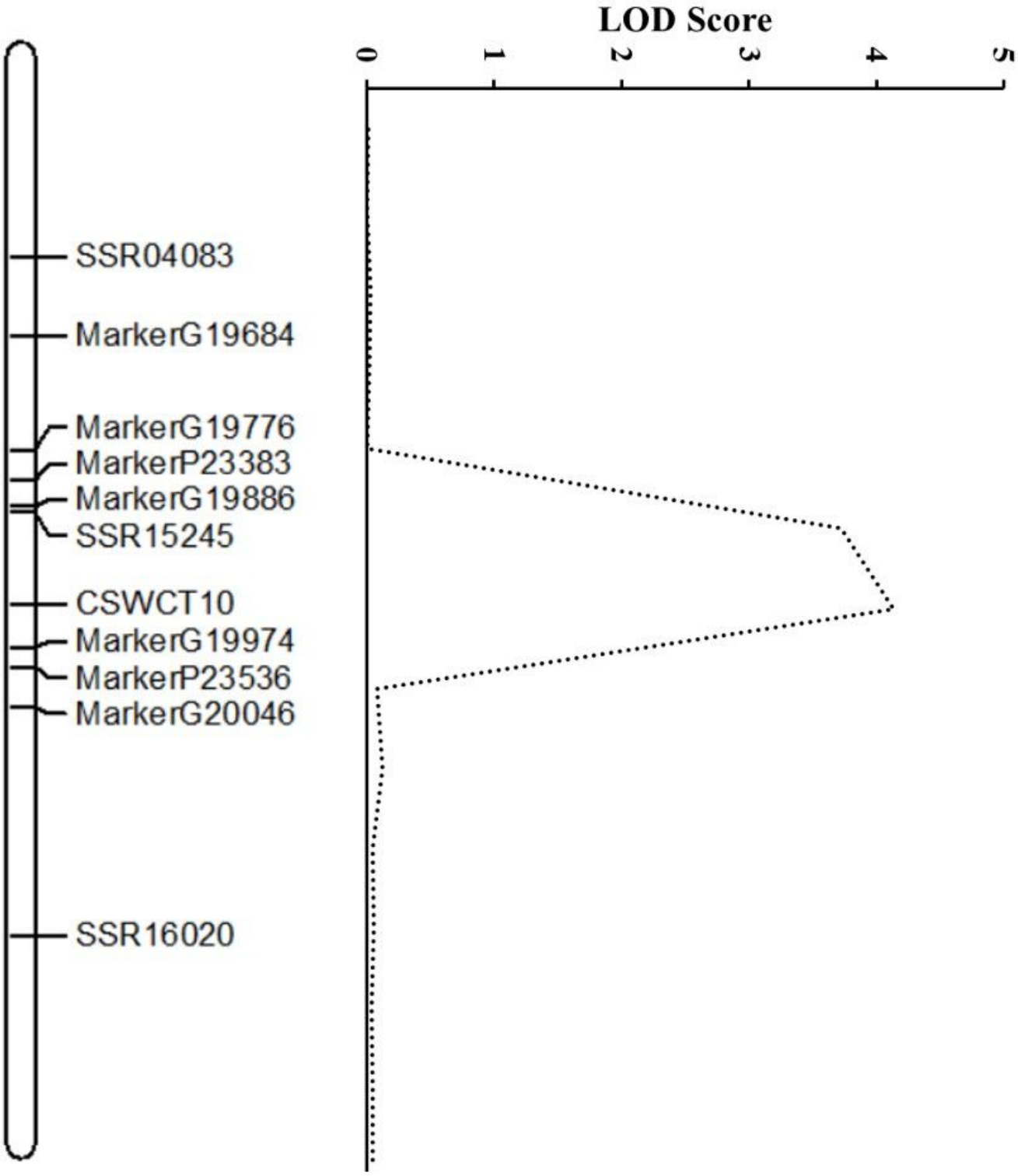

Figure 1

Genetic map of *gsb-s6.2* for GSB resistance at the adult stage on Chr.6. The curve indicates the physical position of markers against the LOD score. Markers SSR04083 and SSR16020 developed by (Zhang et al., 2017) are located at nucleotide 3,212,336 bp, and 5,421,428 bp respectively. In this work, we reduced the QTL interval which is now flanked by Markers G19776 and G20046, located at nucleotides 3,638,392 and 4,680,565, respectively.

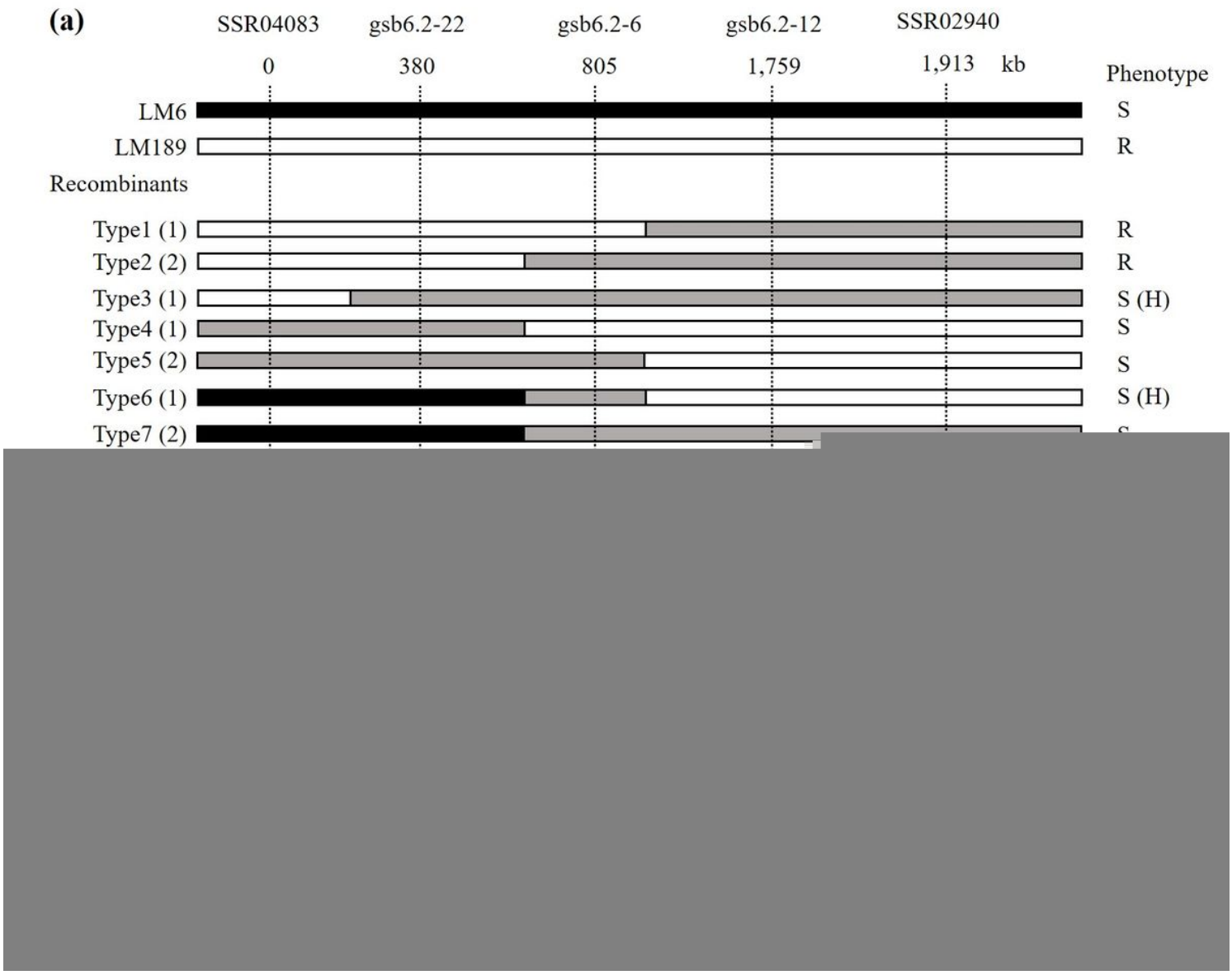

Figure 2

Genetic and physical mapping of *gsb-s6.2*. (a) Genetic mapping of *gsb-s6.2*. Key recombinants from an F_2 segregated population that were used for fine mapping *gsb-s6.2*. The interval containing this locus was narrowed from a 425 kb to a 34 kb region. The numbers below the markers indicate their relative physical position. Markers gsb6.2-22 and gsb6.2-6 were located at nucleotide position 3,671,399 and 4,017,385 bp respectively. (b) The six annotated genes found located in the 34 kb region using the cultivar '9930'_v2 reference genome are shown. R: resistant, S: susceptible, S (H): susceptible (dominant heterozygous).

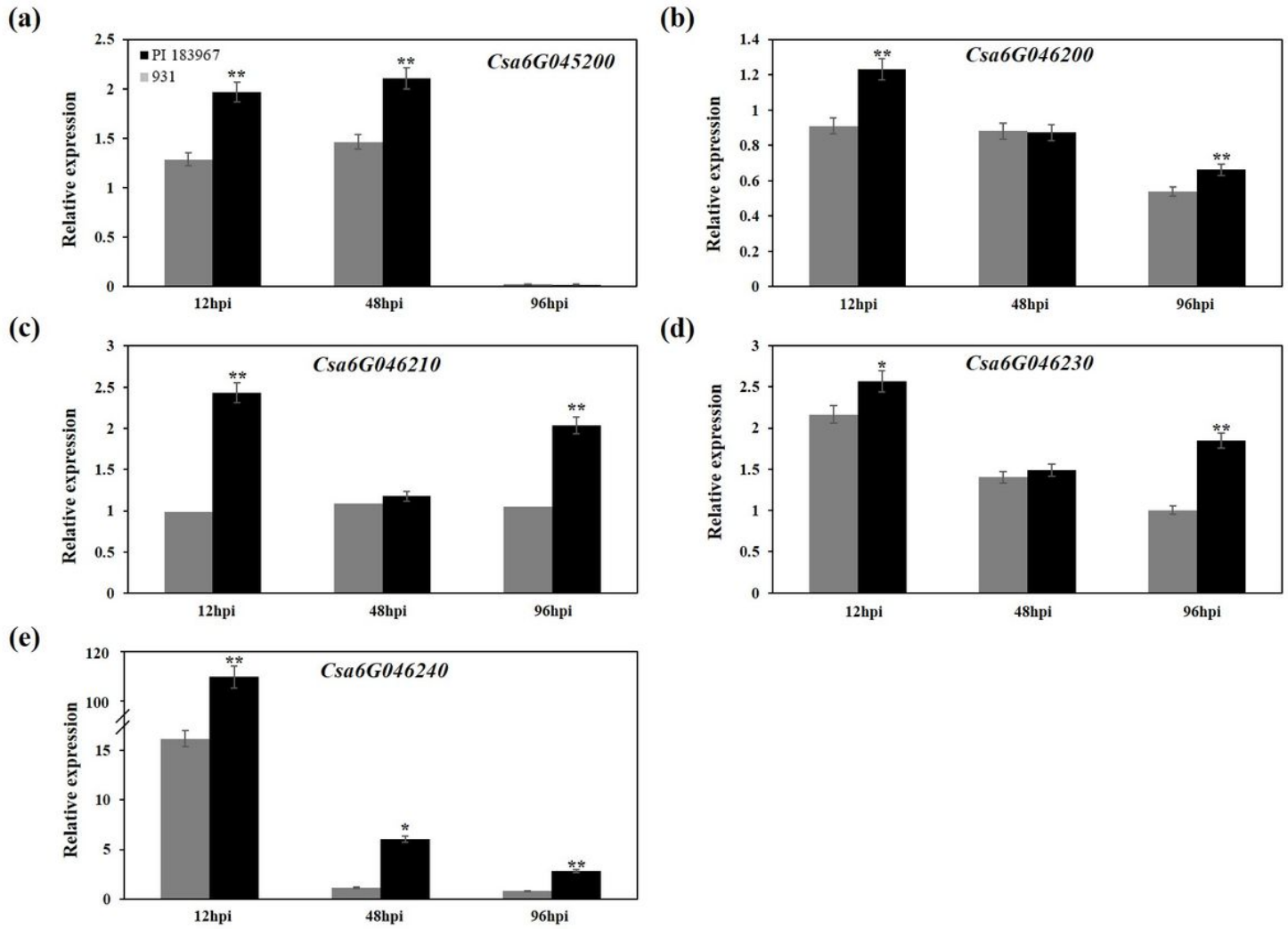

Figure 3

Relative expression levels of five candidate genes in 'PI 183967' and '931' after inoculation with *Didymella bryoniae*. The Y-axis represents relative expression levels of the candidate genes at 12 hpi, 48 hpi and 96 hpi, compared with 0 hpi. Asterisks indicate significant differences as determined by ANOVA (**$P < 0.01$).

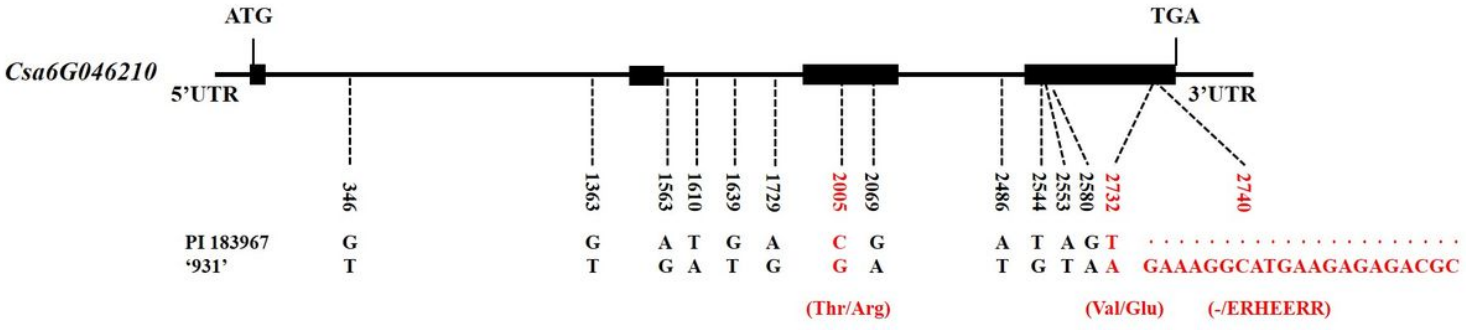

Figure 4

Polymorphisms between parental genotypes ‘PI183967’ and ‘931’ in the coding sequence of candidate gene *Csa6G046210*. Characters in red font indicate nonsynonymous variants, their nucleotide position and the corresponding amino acids.

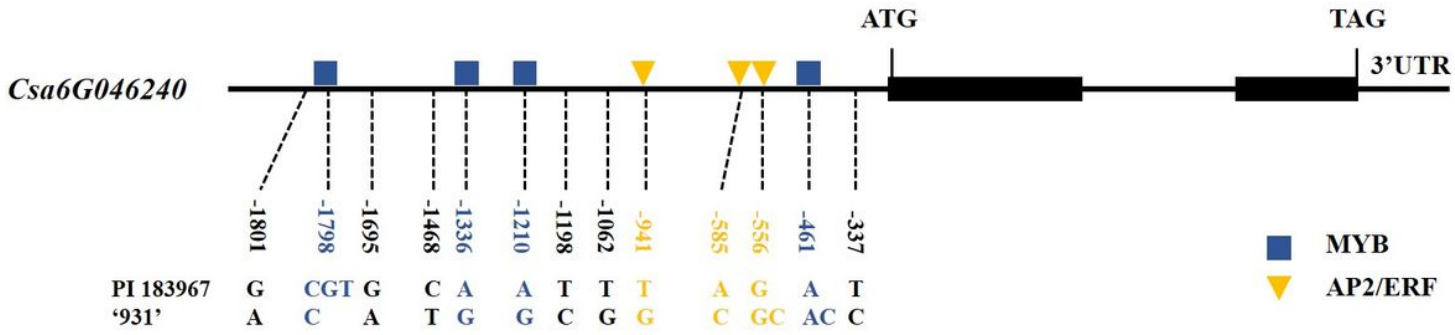

Figure 5

Sequence variations in the 5’ regulatory regions of candidate gene *Csa6G046240* in the parental genotypes ‘931’ and ‘PI 183967’. Blue rectangles and letters represent the MYB motif, and the yellow triangles and letters represent the AP2/ERF motif.

Supplementary Files

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