Genetic Analysis and QTL Mapping of Resistance to Gummy Stem Blight in *Cucumis sativus* Seedling Stage

Shulin Liu, Yanxia Shi, Han Miao, Min Wang, Baoju Li, Xingfang Gu, and Shengping Zhang, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

Abstract

Gummy stem blight (GSB), caused by *Didymella bryoniae* (syn. *Stagonosporopsis cucurbitacearum*), is an important disease of cucumber (*Cucumis sativus* L.) worldwide. To better understand the resistance to GSB in cucumber seedlings, a set of 160 F₉ recombinant inbred lines (RILs) and a total of 405 pairs of SSR primers were employed to detect quantitative trait loci (QTLs) conferring the resistance. Genetic analysis indicated that the resistance to GSB in PI 183967 seedlings was quantitative and mainly governed by two pairs of major QTLs and multiple minor QTLs. Six QTLs, *gsb3.1*, *gsb3.2*, *gsb3.3*, *gsb4.1*, *gsb5.1*, and *gsb6.1*,

for resistance to GSB in cucumber seedlings were detected. The stable locus *gsb5.1* on Chr.5 was repeatedly detected in three seasons. Locus *gsb5.1* accounted for the highest phenotypic variation, 17.9%, and was flanked by SSR15321 and SSR07711 within the genetic distance of 0.5 cM. There were 102 candidate genes predicted in the region harboring the stable QTL *gsb5.1*, of which seven genes were related to disease resistance. These results can provide a good base for further study and molecular markers for fine-mapping the major QTL conferring GSB resistance in cucumber.

Gummy stem blight (GSB) is a destructive disease of cucurbits, including cucumber (*Cucumis sativus* L.), that involves three genetically distinct species of the fungus, *Stagonosporopsis cucurbitacearum* (syn. *Didymella bryoniae*), *S. citrulli*, and *S. caricae* (Stewart et al. 2015). It affects root, stems, and leaves of cucumber, often causing losses in production of 15 to 30%, and up to 80% or more in severe infections.

Gummy stem blight was first described by Fautrey and Roumeguere in France as the disease caused on cucumber by *Ascochyta cucumis* in 1891 (Chiu and Walker 1949; Sherf and Mac Nab 1986). In 1917, gummy stem blight was reported for the first time in the U.S. affecting watermelon fruit (Sherbakoff 1917). So far, there are no reports on the inheritance of GSB in cucumber. However, resistance in watermelon, controlled by a single recessive gene (*db/db*), was detected using GSB-resistant PI 189225 and GSB-susceptible ‘Charleston Gray’ watermelons (*Citrullus lanatus* (Thunb.) Matsum. & Nakai (Norton 1979). Amand and Wehner (2001) reasoned that the major effect conferred by *db/db* is complemented by the expression of modifier genes, and that genetic factors are weaker than environmental factors.

In melons (*Cucumis melo* L.), five independent single genes conferring resistance to GSB were identified from different cultigens, four of these being dominant and one recessive (Frantz 2004; Wako et al. 2002; Zuniga et al. 1999).

As for molecular markers and genetic mapping of resistance to GSB in cucurbitaceae, Joseph (2009) identified four amplified fragment length polymorphism (AFLP) markers linked to GSB resistance in the melon cultivar PI 420145 with genetic distances of 2.0, 6.0, 5.4, and 6.0 cM. Molecular markers CMCT505, CMTC160a+b220, ISSR-57560, ISSR-100900, and CMTA170a have been closely linked with the melon GSB-resistance genes *Gsb-1*, *Gsb-2*, *Gsb-3*, and *Gsb-4*. Among these markers, CMTA170a and *Gsb-4* had the shortest genetic distance of 5.14 cM. The *Sb-x*

GSB-resistance gene in melon was identified and mapped to the LG4 linkage group using simple sequence repeats (SSR) markers and a double haploid (DH) melon population. A gene for resistance (*Sb-1*) was positioned to the melon LG1 linkage by Ha et al. (2010). These results provide valuable references for GSB-resistance molecular markers identification in cucumbers.

To date, there are no reports on molecular markers and genetic mapping of resistance to GSB in cucumber. In this study, a set of recombinant inbred lines (RILs) derived from the cross of wild-type and cultivated cucumbers with distinct resistance to GSB was used to conduct genetic analysis and quantitative trait locus (QTL) mapping. The aim of this study is to understand the inheritance of GSB resistance, and to map the genes controlling GSB-resistance, so as to facilitate fine mapping and molecular cloning of resistance genes leading to molecular marker-assisted selection (MAS) breeding.

Materials and Methods

Plant materials. The wild-type GSB-resistant cucumber accession PI 183967 (*Cucumis sativus* var. *hardwickii* (Royle) Alef.) was crossed with the cultivated GSB-susceptible cucumber accession designated 931 (*C. sativus* var. *sativus*). After single-seed descent (SSD) reproduction, a population was developed that consisted of 160 F₉ recombinant inbred lines (RILs).

Disease-resistance screen. The seedlings were arranged randomly with three replications of six plants each. Seeds of the two parental lines, PI 183967 and 931, the F₁, and 160 RILs were soaked in 5% NaOCl for 10 min, washed with tap water, and germinated in an incubator at 28°C. Germinations were carried out in spring 2013, autumn 2013, and spring 2014. When the average radicle length was 5 mm, the seeds were sown individually into plastic pots filled with a steam-sterilized propagation substrate composed of peat, vermiculite, and soil from a vegetable field (2:1:1, volume basis) that had been steam-sterilized at 134°C for 30 min.

The pathogen for inoculation was a specific strain *Ascochyta citrullina* of *Didymella bryoniae*. This was grown on cucumber fruits for 7 to 10 days to allow pycnidia to form. Spores were scraped from the infested fruits, suspended in 10 ml of sterile water, and the concentration adjusted to 1 × 10⁶ spores/ml in a hemocytometer. Seedlings were inoculated when the second true leaf was fully open by spraying all leaves uniformly on both sides using a hand pumped sprayer (YH-038, YUANHUA PENWU Factory, Taizhou, Zhejiang, China). They were then kept in dark for 24 h with 100% relative humidity (RH), and during the next 7 to 10 days, maintained at 80 to

Corresponding authors: Shengping Zhang, zhangshengping@caas.cn; Xingfang Gu, guxingfang@caas.cn

Shulin Liu and Yanxia Shi contributed equally to this work.

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100% RH and a constant temperature of 20 to 23°C with a 16 h light photoperiod.

Resistance assessment. Ten days after inoculation, all true leaves on each plant were assessed for the degree of infection using a disease index (DI) based on Zhang et al. (2013a):

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

The disease rating scale for each seedling was as follows: 0 = absence of GSB spots, 1 = $\leq 5\%$ GSB spots, 3 = $>5\%$ but $\leq 25\%$ GSB spots, 5 = $>25\%$ but $\leq 50\%$ GSB spots, 7 = $>50\%$ but $\leq 75\%$ GSB spots, 9 = $>75\%$ GSB spots or the whole leaf dead. A schematic diagram of the disease rating scale is shown in Figure 1, where the lines with the DI range of $0 < DI \leq 55$ was classified as GSB-resistant, and $55 < DI \leq 100$ was classified as GSB-susceptible.

Genetic analysis software and statistical analysis. The DI of each plant was recorded and summarized in Microsoft Excel 2003 and SAS 9.0 software. The mean DI of each line was calculated, and the genetic parameters of the parents, F_1 , and RILs were calculated. A joint analysis, assuming major genes plus the polygene model of RILs (Gai et al. 2003), was used for genetic analysis of the GSB resistance. The work was divided into four steps. The first step was to establish the genetic models. The second step was to estimate the iterated expectation and conditional maximization algorithm. The third step was to select the best genetic model through AIC (Akaike information criterion) value. The fourth step was to evaluate goodness-of-fit through the least squares method. The best genetic model was then used for estimation of other genetic parameters.

Molecular marker analysis. Young leaf tissue of the parents, the F_1 , and each line in the RILs population was used to extract total DNA (Maniatis et al. 1982; Staub et al. 1996). The concentration of DNA was estimated after electrophoresis on 1% agarose gels with $1 \times$ TEA buffer and staining with ethidium bromide. The PCR components and amplified conditions were as described by Zhang et al. (2013b). Three microliters of the amplified products were separated on 6% polyacrylamide gels for 1 h and silver stained as described by Sambrook and Russell (2001).

Polymorphisms in the parental lines, PI 183967 and 931, were detected using 2,112 pairs of SSR primers (Ren et al. 2009). Polymorphic

markers were used to genotype individual lines of the RIL population for linkage map construction and QTL analysis.

Linkage construction and QTL mapping. Linkage groups (LGs) were developed with JoinMap 4.0 (Van Ooijen 2006) using a minimum logarithm of odds (LOD)-likelihood score of 2.5. The Kosambi map function (Kosambi 1944) was used to calculate the genetic distance between markers. Common markers between the present map and the high-resolution cucumber genetic map produced by Ren et al. (2009) were used to assign LGs to the seven chromosomes of cucumber. The averages of DI values for each year were used to estimate QTLs by multiple-QTL modeling (MQM) with MapQTL 4.0 (Van Ooijen et al. 2000) with composite interval mapping (CIM) analysis (Zeng 1994). Permutation tests were conducted 1,000 times to assess the LOD threshold at $\alpha = 0.05$ level. QTLs above the significance threshold determined by the permutation tests were considered significant. The abbreviation of the GSB followed by the chromosomal locations and the locus number is the same as the name of each detected QTL.

Bioinformatics analysis in the genomic region harboring the stable QTL. Using the whole genome sequence of inbred line 9930 (Huang et al. 2009), sequence annotation and gene prediction were performed on the genomic regions located as the stable QTL. An E-value cutoff of $1 \times e^{-10}$ to $1 \times e^{-20}$ with BLASTN was used when the DNA sequences were aligned to the reference genome. Gene prediction and annotation were performed only for retained sequences with an identity of $\geq 95\%$ as reported by Zhang et al. (2013a).

Results

GSB resistance in cucumber seedlings for the progeny of PI 183967 $\times$ 931. The disease indices for the GSB resistant parent PI 183967 in spring 2013, autumn 2013, and spring 2014 were 18.98 ± 1.39 (SE), 29.44 ± 2.42 (SE), and 17.10 ± 9.01 (SE), respectively, with a mean of 21.84 ± 3.84 (SE); the disease index for the GSB susceptible parent in the three seasons were 67.41 ± 2.67 (SE), 84.17 ± 4.63 (SE), and 64.20 ± 0.60 (SE), respectively, with a mean of 71.93 ± 6.19 (SE) (Table 1). The disease indices for the F_1 seedlings in the three assessments were 43.95 ± 3.49 (SE), 61.62 ± 3.26 (SE), and 51.00 ± 3.74 (SE), respectively, with a mean of 52.19 ± 5.14 (SE) (Table 1). The frequency distributions of the disease indices for GSB in the RIL population in the three trials showed normal

Fig. 1. Disease rating scale of each seedling was corresponding to the symptoms of all leaves. 0 = absence of GSB spots; 1 = $\leq 5\%$ GSB spots; 3 = $>5\%$ but $\leq 25\%$ GSB spots; 5 = $>25\%$ but $\leq 50\%$ GSB spots; 7 = $>50\%$ but $\leq 75\%$ GSB spots; 9 = $>75\%$ GSB spots or the whole leaf dead.

distributions ranging from resistant to susceptible phenotypes (Fig. 2). In addition, the disease indices for PI 183967, 931, and F_1 had significant positive correlation in the three seasons, and the correlation coefficient was up to 0.983.

Inheritance of GSB resistance in the cucumber seedlings. A joint analysis, assuming major genes plus the polygene model of RILs, together with the DI measurement in the three trials, was used to generate 38 mixed genetic models. The maximum likelihood (ML) and defined AIC of these models were estimated. Based on the

principle that the smallest AIC value is the best-fitting genetic model, three models (F-3, E-2-0, and B-1-3) were prioritized based on the spring 2013 data and the lowest AIC was selected and further tested for goodness-of-fit (Tables 2 and 3). The number of statistically different parameters were 5, 3, and 8 for F-3, E-2-0, and B-1-3, respectively. Since model E-2-0 had the lowest number, it was considered the best fit for the spring 2013 GSB resistance inheritance analysis. The AIC values of E-1-9, E-2-9, and E-2-6 for the autumn 2013 data were relatively lower than other models, and therefore they were

Table 1. Gummy stem blight disease index of cucumber parental lines PI 183967 and 931, PI 183967 $\times$ 931 F_1 , and some genetic factors in the RIL population for three seasons

Season	Parental lines (mean $\pm$ SE)		F_1	RIL population			
	PI183967	931		Mean	SD ^a	Kurtosis	Skewness
Spring 2013	18.98 $\pm$ 1.39	67.41 $\pm$ 2.67	43.95 $\pm$ 3.49	55.49	15.38	0.13	-0.60
Autumn 2013	29.44 $\pm$ 2.42	84.17 $\pm$ 4.63	61.62 $\pm$ 3.26	62.86	13.79	-0.53	0.06
Spring 2014	17.10 $\pm$ 9.01	64.20 $\pm$ 0.60	51.00 $\pm$ 3.74	50.51	13.92	-0.16	-0.54

^a SD: standard deviation.

Fig. 2. Frequency distributions of gummy stem blight severity (index) of cucumber in the PI 183967 $\times$ 931 RIL population. The frequency distributions in spring 2013 (upper), autumn 2013 (middle), and spring 2014 (lower) trials each had a normal distribution ranging from resistant to susceptible phenotype. The mean index ratings for the parental lines and F_1 are indicated by arrows.

selected for goodness-of-fit testing (Table 3). The number of statistically different parameters was 2, 1, and 1 for the E-1-9, E-2-9, and E-2-6 models, respectively. Since model E-2-9 had the lowest number, it was considered the best fit model for analysis of the autumn 2013 GSB resistance inheritance. The same method was used to derive the best fit model E-2-6 for analysis of the spring 2014 GSB resistance inheritance (Tables 2 and 3).

Taken together, the best fit models for the inheritance of resistance to GSB in cucumber seedlings in spring 2013, autumn 2013, and spring 2014 were E-2-0, E-2-9, and E-2-6, respectively. The first order genetic parameters are shown in Table 4. The test of the best fit models indicated that two pairs of major genes and multiple minor genes were controlling GSB resistance in cucumber seedlings. The heritability of the two major genes was 61.79, 52.47, and 77.27%, respectively, for spring 2013, autumn 2013, and spring 2014.

QTL mapping of GSB resistance in cucumber seedlings. To identify polymorphisms between the two parental lines, 2,112 pairs of SSR primers were screened and of these, 1,125 (53.3%) were selected for further testing. We selected 405 markers that evenly distributed on the seven cucumber chromosomes (55 per chromosome except for 75 on Chr.6) to construct the genetic map in the RIL population. Seven linkage groups were corresponded to the seven cucumber chromosomes. The total genome length coverage was 993.3 cM with an average distance of 3.23 cM between markers (Wang et al. 2015) (Fig. 3).

QTL mapping was conducted using this linkage map. In spring 2013, one QTL for GSB resistance in cucumber seedlings, *gsb5.1*, was detected. The QTL was detected on Chr.5 and was placed in the marker interval between SSR15321 and SSR07711, with an LOD of 5.56, phenotypic variation of 15.1%, and additive effect of -6.91 (Table 5, Fig. 3).

In autumn 2013, three QTLs for GSB resistance were detected, which were *gsb3.1*, *gsb3.2*, and *gsb5.1*. QTLs *gsb3.1* and *gsb3.2* were located on Chr.3 at 5.0 cM and 43.0 cM, between SSR02451-SSR07456 and

SSR18541-SSR20863, respectively. QTLs *gsb3.1* and *gsb3.2* provided additive effects of 3.91 and -4.31, and contributed to the GSB resistance phenotype by 6.0% (LOD = 2.50) and 7.4% (LOD = 3.52), respectively. The QTL *gsb5.1* was located on Chr.5 between

Table 3. Akaike information criterion values of different genetic models for the resistance to gummy stem blight in cucumber seedlings for three seasons

Season	Model code	AIC value
Spring 2013	F-3	1,411.293
	E-2-0	1,412.484
	B-1-3	1,415.827
Autumn 2013	E-1-9	1,281.233
	E-2-9	1,282.735
	E-2-6	1,284.477
Spring 2014	F-3	1,285.416
	E-1-6	1,289.324
	E-2-6	1,291.244

Table 4. Parameter estimation of the first order is shown for the optimal model for resistance to gummy stem blight in cucumber seedlings for three seasons

Season	Model code	Content	Mean	Weight	Content ANAVA
Spring 2013	E-2-0	1	22.21	0.17	89.31
		2	53.25	0.32	
		3	54.36	0.32	
		4	80.67	0.18	
Autumn 2013	E-2-9	1	74.53	0.69	108.72
		2	51.01	0.31	
Spring 2014	E-2-6	1	29.72	0.24	56.69
		2	62.23	0.52	
		3	45.89	0.24	

Table 2. Goodness-of-fit of alternative genetic models for resistance to gummy stem blight in cucumber accession PI 183967 seedlings for three seasons^a

Season	Model	Populations ^b	$U1^2$	$U2^2$	$U3^2$	nW^{2c}	D_n^d
Spring 2013	F-3	P1	0.081 (0.77639)*	0.547 (0.45949)*	3.455 (0.06307)*	0.206	0.507
		P2	2.890 (0.08915)*	2.545 (0.11067)*	0.041 (0.83931)	0.373	0.707
		RILs	0.017 (0.89612)	0.116 (0.73391)	0.729 (0.39310)*	0.034	0.042
	E-2-0	P1	0.003 (0.95625)	0.140 (0.70859)	2.916 (0.08773)*	0.118	0.388
		P2	0.180 (0.67121)	0.669 (0.41330)*	2.652 (0.10344)*	0.165	0.51
		RILs	0.071 (0.78964)	0.083 (0.77313)	0.014 (0.90455)	0.022	0.031
	B-1-3	P1	0.906 (0.34129)*	1.576 (0.20931)*	1.786 (0.18146)*	0.285	0.627
		P2	1.164 (0.28063)*	1.564 (0.21105)*	0.679 (0.40985)*	0.217	0.592
		RILs	0.552 (0.45752)*	0.073 (0.78695)	3.227 (0.07245)*	0.235	0.083
Autumn 2013	E-1-9	P1	0.037 (0.84693)	0.012 (0.91355)	0.098 (0.75400)	0.07	0.328
		P2	0.216 (0.64239)	0.547 (0.45937)*	1.348 (0.24561)*	0.113	0.44
		RILs	0.086 (0.76932)	0.016 (0.89916)	0.396 (0.52939)	0.06	0.058
	E-2-9	P1	0.000 (0.99313)	0.003 (0.95495)	0.037 (0.84724)	0.076	0.343
		P2	0.107 (0.74332)	0.332 (0.56470)	1.072 (0.30055)*	0.099	0.409
		RILs	0.002 (0.96413)	0.006 (0.93830)	0.018 (0.89225)	0.04	0.057
	E-2-6	P1	0.038 (0.84579)	0.044 (0.83397)	0.007 (0.93211)	0.081	0.38
		P2	0.034 (0.85304)	0.155 (0.69365)	0.737 (0.39077)*	0.085	0.373
		RILs	0.004 (0.95213)	0.006 (0.93820)	0.006 (0.93812)	0.043	0.059
Spring 2014	F-3	P1	1.064 (0.30241)*	0.446 (0.50432)*	1.751 (0.18572)*	0.163	0.507
		P2	0.597 (0.43987)*	1.280 (0.25795)*	2.351 (0.12516)*	0.263	0.609
		RILs	0.994 (0.31874)*	1.320 (0.25054)*	0.540 (0.46259)*	0.199	0.081
	B-1-1	P1	1.927 (0.16503)*	1.088 (0.29694)*	1.452 (0.22821)*	0.225	0.553
		P2	0.049 (0.82469)	0.066 (0.79677)	3.565 (0.05901)*	0.218	0.499
		RILs	0.297 (0.58567)	0.160 (0.68915)	0.261 (0.60914)	0.075	0.047
	E-2-6	P1	0.026 (0.87172)	0.153 (0.69606)	0.878 (0.34865)*	0.052	0.316
		P2	0.502 (0.47873)*	0.084 (0.77211)	2.512 (0.11298)*	0.247	0.569
		RILs	0.000 (0.98525)	0.001 (0.97370)	0.004 (0.95190)	0.04	0.048

^a $U1^2$, $U2^2$, and $U3^2$ are the statistic of uniformity test; nW^2 is the statistic of Smirnov test; D_n is the statistic of Kolmogorov test.

^b P₁: PI 183967; P₂: 931; RILs: recombinant inbred lines.

^c $nW_{0.05}^2 = 0.4610$.

^d $D_{n0.05} = 1.3600$.

^e The relevant probability of $U1^2$, $U2^2$, and $U3^2$ is shown in parentheses. * indicates that the value is significant at $P \leq 0.05$.

SSR15321 and SSR07711, with a LOD value of 8.01 and phenotypic variation of 17.9%.

Four QTLs for GSB resistance in cucumber seedling were detected in spring 2014. The QTL *gsb3.3* on Chr.3 was flanked by SSR12383 and SSR18541 at genetic distances of 33.0 and 40.4 cM, respectively. This QTL explained 5.7% of the total phenotypic variance with a LOD of 3.01. On chromosome 4, the QTL (*gsb4.1*) was detected and accompanied by SSR21051 and SSR21501 with an LOD of 3.03 and an R^2 of 6.4%. The QTL *gsb5.1* was also detected in this season with an LOD value of 4.17 and phenotypic variation of 8.1%. Locus *gsb6.1*, accounting for 6.5% of the phenotypic variance with an LOD of 3.31, was mapped to Chr.6 at the position of 166.6 cM.

In summary, a total of six QTLs were identified for GSB resistance in the PI 183967 × 931 RIL population tested over three trials in this

study. The QTL *gsb5.1* was detected in different seasons and accounted for the highest phenotypic variation to GSB resistance (Fig. 4).

Gene prediction and annotation in the stable QTL genome region. In the cucumber 9930 draft genome, 61 NBS and LRR type resistance gene analogs (RGAs) were identified and were distributed mostly in 11 clusters (Huang et al. 2009). In this study, gene prediction and annotation was performed in the genomic region harboring the stable QTL (*gsb5.1*) associated with GSB resistance. Of the 102 genes predicted in this region, there were seven disease resistance-related genes and three (Csa5M467390, Csa5M467900, and Csa5M485190) belonged to the NBS-LRR gene. The three NBS-type RGAs will be candidate genes for further research associated with GSB resistance.

Fig. 3. Construction and chromosomal quantitative trait locus (QTL) mapping for gummy stem blight resistance in a cucumber RIL seedling population using SSR markers. Six QTLs for gummy stem blight resistance were detected in three different seasons of screening for resistance in the F_3 RIL population: *gsb5.1* was detected in spring 2013; *gsb3.1*, *gsb3.2*, and *gsb5.1* in autumn 2013; and *gsb3.3*, *gsb4.1*, *gsb5.1*, and *gsb6.1* in spring 2014. The loci of *gsb3.1*, *gsb3.2*, and *gsb3.3* were on chromosome 3 (Chr.3) and *gsb4.1*, *gsb5.1*, and *gsb6.1* were on Chr.4, Chr.5, and Chr.6, respectively.

Table 5. Quantitative trait locus (QTL) analysis of gummy stem blight resistance genes in cucumber seedlings for three seasons

Season	QTL ^a	Chromosome	Position(cM)	Marker interval	LOD	R ² (%)	AE
Spring 2013	<i>gsb5.1</i>	5	141.3	SSR15321-SSR07711	5.56	15.1	-6.91
Autumn 2013	<i>gsb3.1</i>	3	5	SSR02451-SSR07456	2.5	6	3.91
	<i>gsb3.2</i>	3	43	SSR18541-SSR20863	3.52	7.4	-4.31
	<i>gsb5.1</i>	5	141.3	SSR15321-SSR07711	8.01	17.9	-7.35
Spring 2014	<i>gsb3.3</i>	3	34.4	SSR12383-SSR18541	3.01	5.7	-3.55
	<i>gsb4.1</i>	4	52.2	SSR21051-SSR21501	3.33	6.4	-3.92
	<i>gsb5.1</i>	5	141.3	SSR15321-SSR07711	4.17	8.1	-4.64
	<i>gsb6.1</i>	6	166.6	SSR18079-SSR18956	3.31	6.5	-3.72

^a One (*gsb5.1*), three (*gsb3.1*, *gsb3.2*, and *gsb5.1*) and four (*gsb3.3*, *gsb4.1*, *gsb5.1*, and *gsb6.1*) QTLs were detected in spring 2013, autumn 2013, and spring 2014, respectively. For all seven QTLs except for *gsb3.1*, the additive effect were negative value showing the QTL favorable allele came from the parent PI 183927. For QTL *gsb3.1*, the QTL favorable allele came from the parent 931.

Fig. 4. The stable QTL (*gsb5.1*) for resistance to gummy stem blight in PI 183967 × 931 F₉ RIL population seedlings was detected on chromosome 5. The QTL was detected in spring 2013 (upper), autumn 2013 (middle), and spring 2014 (lower) trials with a highest LOD value between SSR15321 and SSR07711.

Table 6. Annotation and gene prediction for stable quantitative trait locus (QTL) region for resistance to gummy stem blight in seedlings of cucumber accession PI 183967

Candidate R genes	Function of R gene	Type of R gene
Csa5M467390	IPR003590 Leucine-rich repeat ribonuclease inhibitor subtype	leucine rich repeat (LRR)
Csa5M467900	IPR003590 Leucine-rich repeat ribonuclease inhibitor subtype	leucine rich repeat (LRR)
Csa5M485190	IPR001611 Leucine-rich repeat;	leucine rich repeat (LRR)
	IPR003591 Leucine-rich repeat typical subtype;	
	IPR013210 Leucine-rich repeat-containing N-terminal	
Csa5M471090	IPR001878 Zinc finger CCHC-type;	Zinc finger
	IPR004859 Putative 5-3 exonuclease;	
	IPR013084 Zinc finger CCHC retroviral-type;	
Csa5M477610	IPR007087 Zinc finger C2H2-type;	Zinc finger
	IPR013087 Zinc finger C2H2-type/integrase DNA-binding	
Csa5M484625	IPR000767 Disease resistance protein;	Disease resistance protein
	IPR002182 NB-ARC ^a	
Csa5M494390	IPR000157 Toll-Interleukin receptor;	Toll-Interleukin receptor
	IPR000767 Disease resistance protein;	
	IPR001611 Leucine-rich repeat;	
	IPR002182 NB-ARC;	
	IPR003593 ATPase AAA+ type core	

^aNB-ARC: nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4 (www.plantphysiol.org/content/140/4/1233.abstract).

Discussion

In the present study, we used an RIL population derived from PI 183967 × 931 and 405 pairs of SSR primers to identify candidate genes for GSB resistance in the cucumber seedlings. The frequency distributions of the disease indices suggested that the GSB resistant phenotype of parental line PI 183967 has quantitative-trait heritability characteristics rather than conferred by a single gene. So far, research progress on the genetic analysis of GSB resistance is very slow and limited in cucumber. The reasons may lie in the following four aspects. Firstly, the inheritance of GSB resistance appears to be complex in cucumber. Secondly, different cultivars or breeding lines have been used as resistance sources in different studies. Thirdly, it is difficult to compare research results because of different inoculation methods and rating scales in previous studies. Fourthly, variable environmental conditions can strongly affect the initiation and development of cucumber GSB, so it is crucial to maintain stable symptom assessment conditions during testing. In this study, we used the GSB resistant parent PI 183967 and kept the seedlings in a growth chamber at stable humidity and constant temperature after inoculation. This ensures the accuracy and repeatability of the experimental results.

Molecular marker-assisted selection (MAS) for resistance may be a more effective and efficient way compared with phenotypic selection in a conventional cucumber resistance-breeding program because a great deal of time and labor is needed and multiple tests with several replications and years are required. Therefore, MAS for GSB resistance breeding as one of the major objectives in cucumber should be enforced (Van der Meer et al. 1978; Wehner et al. 1996). In our study, we detected six QTLs for GSB resistance in cucumber seedlings. Based on its stable performance, contribution level, and LOD, *gsb5.1* was postulated to be one of the stable QTLs for GSB resistance and was mapped in the region between SSR15321 and SSR07711 with genetic distance of 0.5 cM on Chr.5. On Chr.3, three QTLs were detected associated with GSB resistance: *gsb3.1* was flanked by SSR02451 and SSR07456 within 12.6 cM; *gsb3.2* was linked to the makers SSR18541 and SSR20863 with genetic distance of 3.0 cM; *gsb3.3* was flanked by SSR12383 and SSR18541 within 7.4 cM; *gsb4.1* was located in the region between SSR21051 and SSR21501 with genetic distance of 3.5 cM; *gsb6.1* was detected between SSR18079 and SSR18956 within 6.9 cM. These results will lay a good foundation for future studies of GBS resistance in cucumber.

In recent years, with the release of cucumber genome and melon genome, comparative genomics research saw rapid development. Previous studies had shown that cucumber Chr.3 was syntenic to melon chromosome IV and VI, cucumber Chr.5 was syntenic to

melon chromosome IX and X, and cucumber Chr.6 was syntenic to melon chromosome III, VIII, and IX (Huang et al. 2009; Fukino et al. 2010; Li et al. 2011). For gummy stem blight resistance genes in melon, only *Gsb-1* was located to melon chromosome I, which was syntenic to cucumber Chr.7. In the present study, no QTL was detected on cucumber Chr.7, so we did not find QTL located in the syntenic regions of cucumber and melon.

Biological analysis was performed to predict GSB resistance related genes in the SSR15321-SSR07711 region. BLAST analysis showed that this region had a physical distance of 1,075.5 kb and contained 102 annotated genes based on the gene annotation. These genes were mainly classified as DNA binding site, transcriptional factors, protein kinase, cell signal transduction factor, transmembrane protein, leucine rich repeat, Zinc finger, et al. Many genes conferring resistance (*R* genes) to a diverse array of pathogens, including bacteria, fungi, oomycetes, viruses, and nematodes, have been isolated in plants. Nucleotide binding site (NBS) type and leucine-rich repeat (LRR) domains are encoded in the major largest *R* gene family (Dangl and Jones 2001; Hulbert et al. 2001; Meyers et al. 2003). Among predicted genes, there are seven annotated genes related to resistance to cucumber disease, including three leucine rich repeat (LRR) resistance genes (Csa5M467390, Csa5M467900, and Csa5M485190), two Zn-finger motifs genes (Csa5M471090 and Csa5M477610), one disease resistance protein gene (Csa5M484625), and one Toll-Interleukin receptor gene (Csa5M494390) (Table 6). The large number of genes in this region makes it difficult to determine the specific cucumber gene(s) that have large effects on GSB resistance. However, these predicted GSB resistance-related genes will be key candidates for further study.

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