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Genetic mapping of gummy stem blight (*Didymella bryoniae*) resistance genes in *Cucumis sativus-hystrix* introgression lines

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Abstract Gummy stem blight (GSB, *Didymella bryoniae* (Auersw.) Rehm) is a devastating disease occurring worldwide in cucumber (*Cucumis sativus* L.) production and causing considerable yield loss. No commercially available cultivars are resistant to GSB. By screening 52 introgression lines (ILs) derived from the cross of *C. hystrix* × *C. sativus* and eight cucumber cultivar/lines through a whole plant assay, three ILs (HH1-8-1-2, HH1-8-5, HH1-8-1-16) were identified as GSB resistant lines. Six common introgression regions in these three ILs were on Chromosomes 1, 4, and 6. To further map the resistance in the ILs, three mapping populations ($2009F_2$, $2009F_2'$ and $2010F_2$) from a cross between resistant IL HH1-8-1-2 and susceptible 8419 were constructed and used for QTL mapping with SSR markers. Two quantitative trait loci (QTLs) were identified; one on Chromosome 4 and the other on Chromosome 6. The interval for Chromosome 4 QTL is 12 cM spanning 3.569 Mbp, and the interval for Chromosome 6 QTL is 11 cM covering 1.299 Mbp. The mapped QTLs provide a foundation for map-based cloning of the genes and establishing an understanding of the associated mechanisms underlying GSB resistance in cucumber.

Keywords *Cucumis hystrix* · *C. sativus* · Gummy stem blight · Mapping · Resistance gene

Introduction

Gummy stem blight (GSB) of cucumber (*Cucumis sativus* L.) is a devastating disease caused by the fungus *Didymella bryoniae* (Auersw.) Rehm, which can infect at least 12 genera and 23 species of cucurbits (Keinath 2011). Gummy stem blight has been found on six continents, occurring in open fields (Bala and Hosein 1986; McGrath et al. 1993; Sitterly and Keinath 1996) and greenhouses (Van Steekelenburg 1982) in cucumber-producing areas under warm and humid conditions. Although chemical application has been successful in controlling GSB, the repeated use of fungicides is not advisable because of their negative impact on the environment. Therefore, using cultivars resistant to GSB is considered an environmentally sustainable and economical strategy for disease management.

Though resistance to GSB has been reported in many cases, no dominant and effective resistance genes have been found in cucumber (Amand and

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Wehner 2001a, b). In the field, Homegreen #2 and PI 200818 were reported to be resistant in Wisconsin (Wyszogrodzka et al. 1986) as well as PI 164433, Slice, PI 390264, M17, and M12 in North Carolina (Wehner and Amand 1993). Under greenhouse screening in the Netherlands, several GSB resistant accessions were identified including Leningradsky, PI 200818, Wjarnikovsky, Pheinische Vorgebirge, and PI 339241 (Meer et al. 1978). Crosses between four resistant (PI 200818, Homegreen #2, NCSU M17, and Slice) and two susceptible (Wisconsin SMR18 and Marketmore76) lines were made to investigate the genetics of leaf and stem GSB resistance in cucumber at the Horticultural Crops Research Station, Clinton, NC (Amand and Wehner 2001a). The results showed that in these crosses, many genes with minor effects were responsible for the GSB resistance (Amand and Wehner 1995, 2001a).

Cucumber is a crop with a narrow genetic base (Horejsi and Staub 1999) that is susceptible to many diseases, including GSB (Amand and Wehner 1991). Introgressive hybridization, which is defined as the incorporation of genetic materials from one species into another through interspecific hybridization and repeated backcrossing, plays an important role in the evolution of plant species, genetic modification and gene pool enrichment for breeding (Anamthawat-Jónsson 2001). Interspecific hybridization in cucumber was first successfully performed between *C. hystrix* and the cultivated cucumber (Chen et al. 1997). *C. hystrix* is a wild *Cucumis* species possessing valuable traits, such as resistance to the root-knot nematode, downy mildew, GSB and fusarium wilts (Chen et al. 2004), as well as tolerance to low light and temperature (Qian et al. 2002; Zhuang et al. 2002). To introgress the value traits of *C. hystrix* into *C. sativus*, introgression lines (ILs) have been generated via the fertile interspecific hybridization between the synthesized allotetraploid species *C. hytivus* and *C. sativus* (Chen and Kirkbride 2000; Chen et al. 2003). It has been shown that ILs are suitable for studying the effect of individual quantitative trait loci (QTL) as well as interactions between QTLs (Gur and Zamir 2004; Muir and Moyle 2009). Thus, the *C. hytivus* ILs may be useful for elucidating the genetics and mechanisms underlying economically important traits in cucumber.

Using these ILs from interspecific hybridization, the objective of this research was to map the genes or QTLs conferring GSB resistance in *C. hytivus*. By exploring SSR markers derived from the whole genomes of three cucumber lines (Cavagnaro et al. 2010; Huang et al. 2009; Ren et al. 2009; http://csgenome.sggw.pl/data/), genetic mapping was conducted and a number of GSB resistant QTLs were identified that might be used in cucumber breeding and map-based cloning.

Materials and methods

Plant materials

C. hytivus was successfully synthesized via chromosome doubling of an interspecific hybrid between a Chinese cucumber, 'Beijing Jietou', and a wild cucumber, *C. hystrix* (Chen and Kirkbride 2000). The hybrids were subsequently self-pollinated for seven to eight generations during 2000–2008, producing ILs. The 52 ILs and eight cucumber cultivars that were used in this study are listed in Table S1. IL HH1-8-1-2 and cultivar 8419 with contrasting levels of GSB resistance were crossed to produce F_1 (HH1-8-1-2 × 8419) and F_2 generations. The reciprocal crosses were also made, producing F_1' and F_2' generations.

Disease assay

GSB resistance in seedlings was measured according to the method of Wehner and Shetty (2000). The A1 isolate of *D. bryoniae* was obtained from diseased melon plants growing in a plastic tunnel in Nanjing, China. To prepare the inoculum, the pathogen was multiplied on Potato dextrose agar (PDA) in Petri plates using mycelia plug inoculation, and mycelia colonies were cultured at 25 °C in the dark for 7 days followed by UV irradiation (40 w, 12 h/day) for 4 days to induce the spores. The mycelia were scraped from the agar surface and dissolved in 5–10 mL of distilled water. The solution was acidified to pH 3.5–4.0 using lactic acid and filtered through four layers of cheesecloth to remove mycelia, pycinidia and dislodged agar. The spore suspension was then adjusted to 1×10^6 spores mL^{-1} using a hemacytometer.

Seedlings in the four to six true-leaf stage were inoculated by spraying the fresh-made inoculum with a hand sprayer. Inoculation was done in the morning or late evening. Plants were overhead watered nearly to runoff at 1 day prior to inoculation and 3 days after

inoculation to ensure uniform disease development. After inoculation, plants were enclosed in a tight plastic tent with a relative humidity of 92 to 95 %, which was measured using a psychrometer. Two days post-inoculation, plants were fine-misted to provide free water on the leaves. Plants were re-inoculated 7 days after the first inoculation. Disease symptoms were scored 7–14 days after inoculation by rating leaf lesions, using a 0–9 rating scale defined as follows: 0 = no disease; 1 to 2 = a trace of infection; 3 to 4 = few small lesions on leaves; 5 to 6 = 20 to 50 % of the leaves covered with small lesions; 7 to 8 = wilting plant and more than 50 % of the leaves covered with lesions; and 9 = dead plant (Wehner and Shetty 2000).

Phenotypic data collection

Three F_2 populations with a total of 380 plants, named $2009F_2$, $2009F_2'$ and $2010F_2$, were used at different stages of this project. In total, 133 plants of the $2009F_2'$ population and 52 plants of the $2009F_2$ population were grown in greenhouses during autumn 2009 and evaluated for the GSB resistance. In spring 2010, 195 plants of the $2010F_2$ population were grown in greenhouses for disease testing. For adult plants, inoculations were conducted at the Nanjing Agriculture University, Jiangsu province, China. Plots with 15 plants each were arranged in two randomized blocks with unequal numbers of plots for each generation. Parents, F_1, and F_1' generations were each planted in one or two plots per block. F_2 and F_2' were each planted in two blocks with half of the population per block. Rows of susceptible control 8419 plants surrounding each test plot were used to enhance the uniformity of disease spread. One block of parents and F_1 generation plants in one plot each were used as uninoculated controls in this study.

SSR analysis, gene annotation and predication of gene function

Genomic DNA was isolated from young leaves using the cetyltrimethylammonium bromide (CTAB) procedure (Murray and Thompson 1980). DNA concentration was estimated on a 1 % agarose gel with 1 × TAE buffer stained with ethidium bromide.

SSR markers used in this study were described by Ren (2009). PCR amplification for SSRs was conducted following the procedure of Katzir et al. (1996). Gene annotation and function predictions were conducted according to the procedure provided by the cucumber database (CuGenDB) (http://www.icugi.org/cgi-bin/gbrowse/cucumber/).

Map construction and QTL analysis

Linkage analysis was performed using Join Map 4.0 software (Van Ooijen 2006). A minimum logarithm of odds (LOD) score of 4.0 was set as a threshold to assign marker loci into linkage groups, to order markers and to estimate interval distances (Kosambi function). The linkage analyses of the significance of the markers were executed by the *F* value and *t* test of SPSS (IBM SPSS Statistics 19). The additive-dominance genetic model from the classic analysis of QTL inheritance was used in calculating the additive and dominance effects of significant markers (Zhai and Wang 2007). By using the single locus method described by Lynch and Walsh (1998), the allele frequency, additive and dominant effects are incorporated into expressions for genetic variance at the locus.

Results

Identification of ILs with GSB resistance

Our previous study showed that *C. hystrix* possesses a high level of resistance to GSB (Chen et al. 2004). To identify *C. hystrix* ILs with GSB resistance, 52 ILs and eight cultivars were tested in 2 years (autumn 2008 and spring 2009). The cultivar 8419 was used as the susceptible control, which had a leaf disease rating above seven in the two tests, and was; therefore, considered as highly susceptible (HS) (Table 1). Three ILs (HH1-8-1-2, HH1-8-1-16, and HH1-8-5) had a disease rating between two and four, and they were regarded as resistant. The other 48 ILs and seven cultivars had inconsistent disease ratings between the two tests (data not shown) and were not analyzed further.

Inheritance of the GSB resistance in IL HH1-8-1-2

To study the inheritance of resistance present in these three ILs, crosses were made between the ILs and

Table 1 Estimation of disease rating in *Cucumis sativus-hystrix*, introgression lines (ILs) and cultivars in autumn 2008 and spring 2009

Code	[a]Leaf disease rating in autumn 2008	Leaf disease rating in spring 2009	[b]Resistance type(2008/2009)
8419	7.65	7.05	HS/HS
HH1-8-1-2	3.5	2.60	R/HR
HH1-8-1-16	4.01	3.74	R/R
HH1-8-5	3.84	2.45	R/HR
HH1-8-57	8.00	7.70	HS/HS

[a] Leaf disease rating. *0* no disease, *1 to 2* a trace of infection, *3* to *4* few small lesions on leaves, *5* to *6* = 20 to 50 % of the leaves covered with small lesions; *7* to *8* wilting plant and more than 50 % of the leaves covered with lesions; *9* dead plant

[b] Resistance type. 0–2 = HR, 2.1–4 = R, 4.1–6 = S, 6.1–9 = HS

Table 2 Estimates of disease ratings of *Cucumis sativus-hystrix* parents, gummy stem blight (*Didymella bryoniae)*-resistant IL HH1-8-1-2 and susceptible cultivar 8419, and F_1 generations in autumn 2009 and spring 2010

Seasons	Generations	No. of plants	Mean disease rating ± standard deviation
Autumn 2009	HH1-8-1-2	25	3.98 ± 0.87
	8419	19	8.68 ± 0.63
	F1(HH1-8-1-2 × 8419)	24	4 ± 1.22
	F_1'(8419 × HH1-8-1-2)	5	5 ± 2.39
Spring 2010	HH1-8-1-2	37	2.36 ± 1.00
	8419	21	6.64 ± 1.44
	F_1(HH1-8-1-2 × 8419)	15	2.3 ± 1.01

susceptible cultivar 8419. Only the cross between HH1-8-1-2 and 8419 resulted in F_1 and F_2 generations including the reciprocal crosses ($F_1'$and F_2'). The F_2/F_2' populations, as well as the parent lines, were evaluated for GSB resistance after 2 years (Table 2). The average disease rating of each parental line was 3.98 ± 0.87 for HH1-8-1-2 and 8.68 ± 0.63 for 8419. The disease rating of F_1/F_1' was 4 or 5 in autumn 2009. Overall, the variation among the parental lines and F_1s was small, indicating a low environmental variation (Table 2). Segregation of resistance was observed in the F_2/F_2' populations (Fig. 1a, b, c). A continuous distribution pattern of disease ratings was observed for all three populations, although a nearly bimodal distribution with one peak toward HH1-8-1-2 was

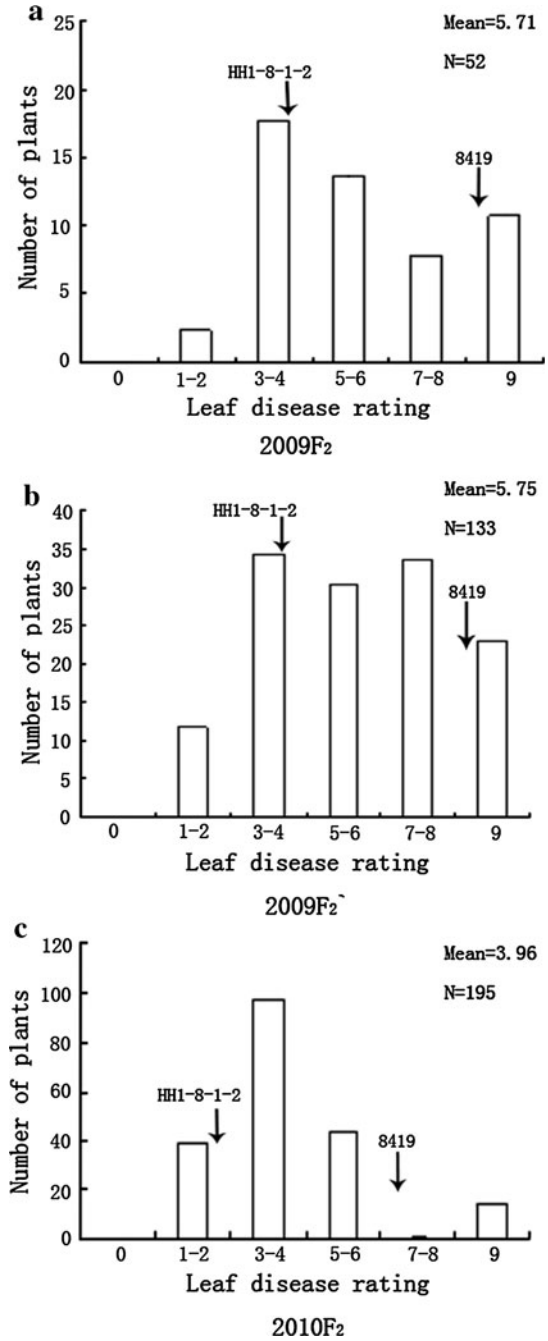

Fig. 1 Frequency distributions of leaf disease ratings for gummy stem blight (GSB, *Didymella bryoniae* (Auersw.) Rehm) on *Cucumis sativus-hystrix*: **a.** 2009 F_2 population, **b.** 2009 F_2' population; and **c.** 2010 F_2 population. *Mean* Average mean of disease ratings in the population. *N* Total number of plants in the population. *Arrows* average disease ratings of the parental IL, HH1-8-1-2 (resistance), and *inbred line*, 8419 (susceptible). Leaf disease ratings were based on a zero to nine scale with zero as resistance and nine as highly susceptible

shown in the 2009 F_2' population (Fig. 1b). It was thus expected that major and minor QTLs were involved in the GSB resistance in HH1-8-1-2.

Markers associated with the GSB resistance in IL HH1-8-1-2

To identify the introgression shared in the GSB resistant ILs, the genetic maps (Shi, 2011) describing the introgressed regions in these ILs were compared. Four introgressions, which were present in all the three resistant ILs, located on Chromosomes 1, 4, and 6 (Fig. 2), were identified. Potentially, these introgressions could carry genes that contribute to the GSB resistance in these ILs. To find markers linked to loci controlling the GSB resistance in the identified ILs, 375 primers from Ren's map (2009) were applied to the parental lines HH1-8-1-2 and 8419. Only 28 SSR primers generated polymorphic amplicons between parents, yielding a polymorphic rate of 7.47 %. These polymorphic primers were used to screen plants of the F_2/F_2' populations, and the significance of association between SSR markers with GSB resistance in F_2/F_2' populations was checked. Chromosome 4 markers, SSR 02697 at 32.3 cM and SSR 06347 at 35.1 cM in Ren's map (2009), showed significant linkage with the GSB resistance in the 2009 F_2' population (Table 3). SSR 02460 at 89.8 cM on Chromosome 6 in Ren's map was significantly associated with the GSB resistance in the 2009 F_2 population (Table 3).

To further map the resistance genes, we selected 10 and 20 more SSR primers in or near the region carrying the significant markers on Chromosomes 4 and 6, respectively (Table 3). Using all these SSR primers to screen the 2009 F_2' population, we constructed two linkage groups for parts of Chromosomes 4 and 6. Group 1 contained 12 markers spanning 12 cM, which corresponds to a region of 2.8 cM (from 32.3 to 35.1 cM) on Chromosome 4 in Ren's map (2009). Group 2 had 21 markers spanning 44 cM, which matches a region of 13.9 cM (from 81.1 to 105 cM) on Chromosome 6 in Ren's map (2009). Except for SSR 07747 in Group 1, the other SSR markers were significantly associated with GSB resistance in the 2009 F_2' population (Table 3). The most significant markers on Chromosome 4 were SSR 26165 and SSR 06347, as well as the clustered markers SSR 15203, SSR 28327 and SSR 30478. Based on the genetic map, the significant clustered markers were located between SSR 26165 and SSR 06347. The results indicated that the chromosomal region flanked by SSR 26165 and SSR 06347 may harbor a QTL for GSB resistance. As for Chromosome 6, only one marker, SSR 02460, was significantly associated with the GSB resistance in this 2009 F_2 population.

To verify the results obtained in the 2009 F_2' population, all of the SSR markers used in this population were applied to the 57 F_2 plants of the 2010 F_2 population. Surprisingly, only two markers, SSR 02697 on Chromosome 4 and SSR 06632 on Chromosome 6, showed a significant association with the GSB resistance in the 2010 F_2 population (Table 3). The results obtained on the 2010 F_2 population also indicated that at least two QTLs contribute to the GSB resistance in IL1-8-1-2, one on Chromosome 4 and the other on Chromosome 6. Further QTL analysis showed that the additive effect of these markers was larger than their dominance effect. The percentage of variance explained by these markers varied from 6.29 to 13.58 % (Table 4).

Fig. 2 The script map showing the *C. hystrix* introgressions in different introgression lines (ILs). *Rows* IL and *columns* indicate chromosomes. For each chromosome, the *top* and *bottom* are from the *left* to *right*. *Solid* segments show introgressions in the IL based on Shi (2011). The *arrows* the introgressed regions overlapping in the ILs, which contains the most likely QTLs for resistance to gummy stem blight (GSB, *Didymella bryoniae* (Auersw.) Rehm)

Table 3 The significant level of gummy stem blight (*Didymella bryoniae)* resistance markers in the genetic linkage groups of the *Cucumis sativus-hystrix* 2009 F_2, 2009 F_2' and 2010 F_2 populations

Chr	Genetic position (cM)	Locus	Population	*P* value	Chr	Genetic position (cM)	Locus	Population	*P* value	Chr	Genetic position (cM)	Locus	Population	*P* value
4	0	SSR26165	$2009F_2'$	0.002**	6	0	SSR21936	–	–	6	27	SSR06632	$2010F_2$	0.049*
4	2	SSR04534	$2009F_2'$	0.028*	6	13	SSR23856	–	–	6	28	SSR19755	–	–
4	3	SSR17406	$2009F_2'$	0.018*	6	17	SSR12833	–	–	6	28	SSR16683	–	–
4	4	SSR04649	$2009F_2'$	0.012*	6	18	SSR15316	–	–	6	32	SSR02460	$2009F_2$	0.007**
4	5	SSR15203	$2009F_2'$	0.005**	6	19	SSR02906	–	–	6	37	SSR12510	–	–
4	5	SSR28327	$2009F_2'$	0.006**	6	20	SSR13251	–	–	6	38	SSR05946	–	–
4	5	SSR30478	$2009F_2'$	0.007**	6	20	SSR03147	–	–	6	40	SSR07477	–	–
4	6	SSR02697	$2009F_2'$/ $2010F_2$	0.021/ 0.019*	6	22	SSR12944	–	–	6	42	SSR20704	–	–
4	7	SSR17459	$2009F_2'$	0.012*	6	23	SSR15606	–	–	6	44	SSR03150	–	–
4	9	SSR06347	$2009F_2'$	0.001**	6	24	SSR20599	–	–					
4	12	SSR04454	$2009F_2'$	0.05*	6	25	SSR20218	–	–					
4	–	SSR07747	–	–	6	26	SSR21115	–	–					

* Significant at 0.05

** Significant at 0.01

– No significance

Table 4 Allelic effect of markers closely linked to QTLs on chr4 and chr6 on gummy stem blight (GSB, *Didymella bryoniae)* resistance in the *Cucumis sativus-hystrix* 2009 F_2' and 2009 F_2 populations based on data from autumn 2009

Populations	Markers	$\bar{P}2$/no. of plants/f_0	$\bar{P}1$/no. of plants/f_2	$\bar{F}1$/no. of plants/f_1	Total plants	Mid-parent (m)	Additive effect(a)	Dominance effect(d)	Genetic variance V_G	Percentage of variance explained PVE (%)
2009F_2'	SSR26165	4.38/30/0.24	6.45/29/0.23	5.90/67/0.53	126	5.415	1.035	0.485	0.56	10.17
	SSR15203	4.77/31/0.26	6.63/34/0.24	5.70/66/0.50	131	5.7	0.93	0	0.43	7.72
	SSR28327	4.77/31/0.24	6.64/32/0.25	5.70/66/0.51	129	5.705	0.935	−0.005	0.43	7.69
	SSR30478	4.74/29/0.24	6.58/32/0.26	5.75/61/0.50	122	5.66	0.92	0.09	0.42	7.62
	SSR02697	4.91/28/0.23	6.57/34/0.28	5.73/60/0.49	122	5.74	0.83	−0.01	0.35	6.29
	SSR06347	4.74/35/0.27	6.69/34/0.27	5.69/59/0.46	128	5.715	0.975	−0.025	0.51	9.23
2009F_2	SSR02460	4.59/14/0.27	6.88/12/0.23	5.30/26/0.50	52	5.735	1.145	−0.435	0.68	13.58

$\bar{P}_2$ average disease rating of F_2 plants with the HH1-8-1-2 genotype, $\bar{P}_1$ average disease rating of F_2 plants with the 8419 genotype, $\bar{F}_1$ average disease rating of F_2 plants with the F_1 genotype; f_0, f_1 and f_2 are the genotypic frequencies of HH1-8-1-2, F_1 and 8419 in the F_2 population, respectively, $m = (P_1 + P_2)/2$, $a = (P_1 - P_2)/2$; $d = F_1 - m$; $V_G = [f_2 + f_0 - (f_2 - f_0)^2]\, a^2 - 2\, f_1\, (f_2 - f_0)\, ad + (f_1 - f_1^2)\, d^2$, $PVE = V_G/V_{P(F2)} \times 100$ %

Physical map and predicted genes in the chromosomal regions harboring QTLs for GSB resistance

Based on the published cucumber genome sequence (http://www.icugi.org/cgi-bin/gbrowse/cucumber/), we located the markers linked to GSB QTLs on the physical map (Figs. 3, 4). All of the markers except one, SSR 26165 on Chromosome 4, which had no homologs in Genbank, were mapped physically on the genome (Fig. 3). The SSR markers in Group 1 were found to be in two adjacent physical regions, one from 13,760,190 to 15,935,774 and the other from 17,329,145 to 20,864,936. Rearrangements between markers at the 4 and 5 cM loci were observed in linkage Group 1 (Fig. 3).

On Chromosome 6, only two SSR markers were found that were significantly associated with GSB resistance. Their physical and genetic locations are collinear. The marker SSR 06632, identified in the 2009 F_2' population, was flanked by markers SSR 21115 and SSR 19755 covering a physical distance of 528.7 Kbp. The marker SSR 02460, identified in the 2010 F_2 population, was flanked by markers SSR 19755 and SSR 12510. However, the physical location of SSR 12510 was not in agreement with its genetic location, which made it very difficult to predict the physical area of GSB resistance (Fig. 4).

Discussion

GSB, which started as a disease of a native plant in central Europe and grew to a worldwide threat to cucurbits cultivated in humid environments (Keinath 2011), causes severe defoliation and stem necrosis in cucumber production. While several cultivars resistant to GSB have been reported (Amand and Wehner 1995; Meer et al. 1978; Wehner and Amand 1993; Wyszogrodzka et al. 1986), it has been shown that no single gene of major effect controls GSB resistance and that the heritability of GSB resistance is low to moderate in detected populations (Amand and Wehner 2001a, b). No resistance genes or QTLs have been mapped in cucumber yet.

In our previous studies, we generated and tested ILs derived from the interspecific hybridization between *C. hystrix* and a Chinese cucumber 'Beijing Jietou' (*C. sativus* L.) for their resistance to different pathogens

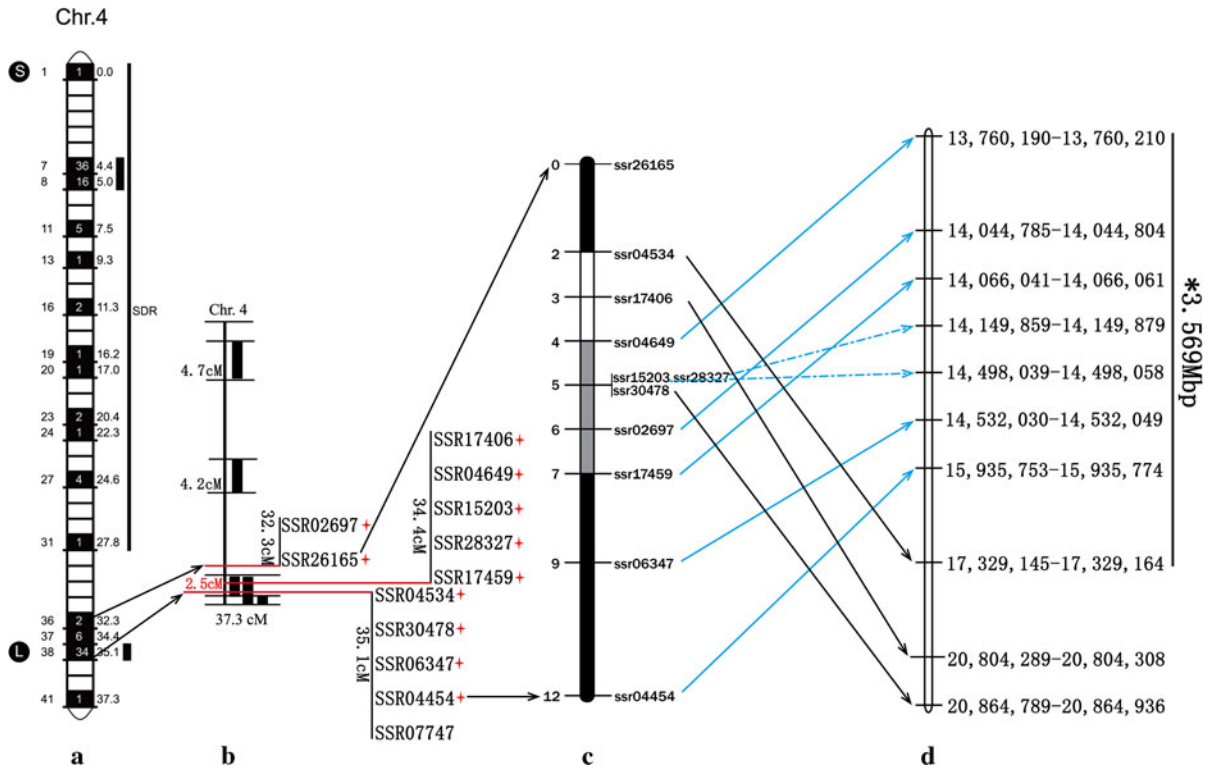

Fig. 3 SSR markers linked to the QTL for gummy stem blight (GSB, *Didymella bryoniae* (Auersw.) Rehm) resistance on chr4 of HH1-8-1-2. **a.** Genetic map of Ren (2009). The Bin names and genetic distance in cM are listed, respectively, on the *left* and *right* of the chromosome. The number of SSR markers in each filled Bin is indicated in the *boxes*. **b.** Genetic map of introgression lines (Shi et al. 2011). **c.** Genetic map from this study. **d.** Physical map of SSR markers. Numbers to the *right* of the physical map indicate the physical length (bp) of the chromosome. *Asterisk* the possible site of the GSB resistance QTL. ✦ shows significant markers linked to the GSB resistance QTL. (Color figure online)

(Chen et al. 2004). In total, 12 *C. hystrix* ILs have been discovered harboring resistances to various pathogens (Shi et al. 2011). Considering the low genetic diversity in cucumber (Dijkhuizen et al. 1996; Knerr et al. 1989), these ILs from wild *Cucumis* species (*C. hystrix* Chakr.) are very valuable for broadening the gene pool of cucumber by an introgression strategy.

In this study, a nearly bimodal distribution was displayed with one peak toward HH1-8-1-2 in the 2009 F_2' population (Fig. 1b), which indicated that a few genes with potentially large effects may be present in the IL. However, no clear differences between resistant and susceptible plants could be identified in the 2009 F_2 (Fig. 1a) and 2010 F_2 (Fig. 1c) populations, suggesting that environmental effects play a role in the effect of GSB resistance. Thus, the effect of the two identified QTLs needs to be further confirmed using different generations, such as the F_3 families.

We tested the *C. hystrix* ILs and identified three with GSB resistance. In the three GSB resistant ILs, four *C. hystrix* introgressions are commonly present, two regions in Chromosome 1, one in Chromosome 4 and one in Chromosome 6. Potentially, these shared introgressed regions harbor resistant QTLs (Fig. 2). To further map the GSB resistance, it would have been ideal to cross these three ILs with the susceptible cucumber cultivar. However, because of a failure in the possible crosses producing F_1 generations, only the crosses between HH1-8-1-2 and 8419 with reciprocals were successful, and these were used in this study for genetic mapping of the GSB resistance.

SSR markers located in the introgressions shared by all the resistant ILs were applied to the three F_2 populations derived from the crosses between HH1-8-1-2 and 8419 for further mapping of the GSB QTLs. Since no markers on Chromosome 1 were found to be

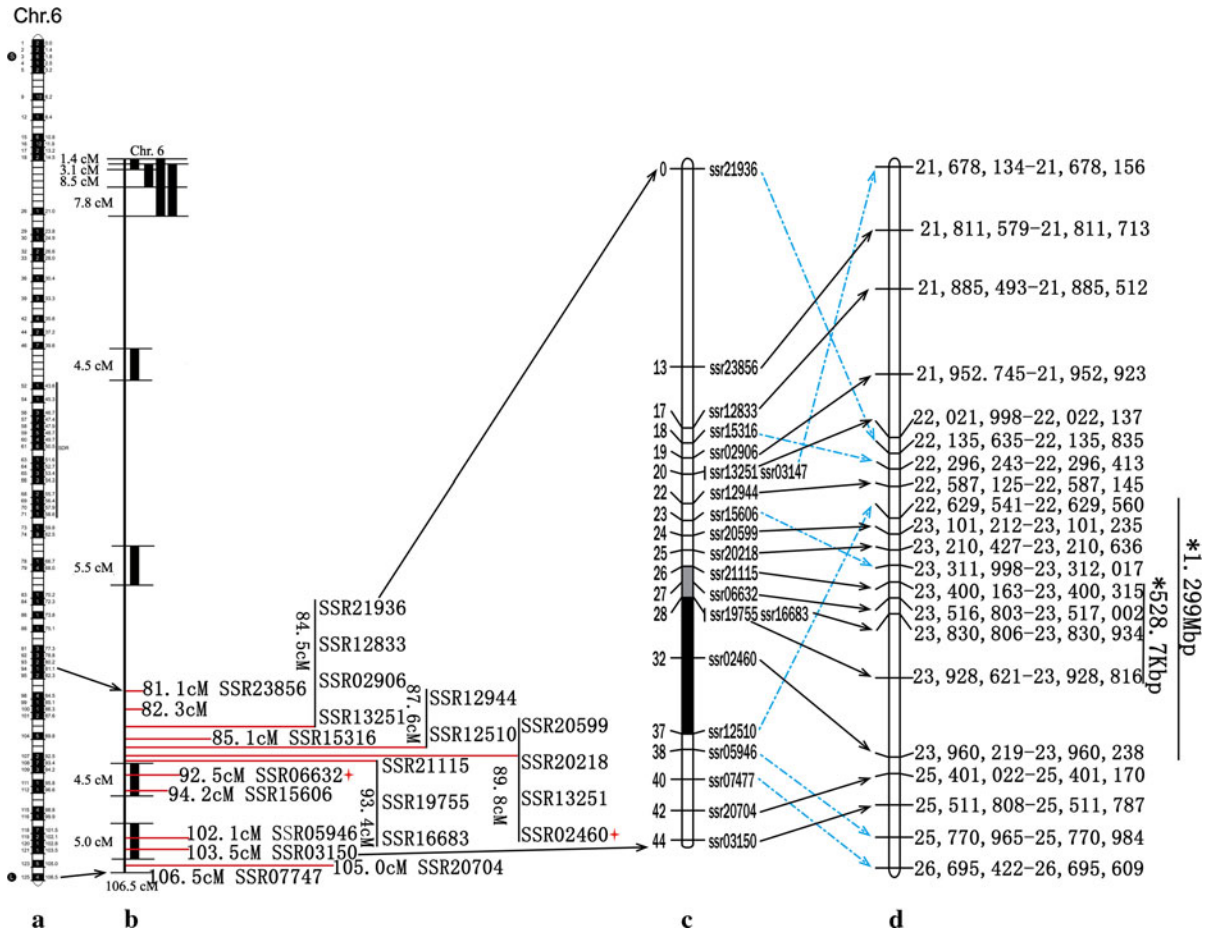

Fig. 4 SSR markers linked to the QTL for gummy stem blight (GSB, *Didymella bryoniae* (Auersw.) Rehm) resistance on chr6 of HH1-8-1-2. **a.** Genetic map of Ren (2009). The Bin names and genetic distance in cM are listed, respectively, on the *left* and *right* of the chromosome. The number of SSR markers in each filled Bin is indicated in the *boxes*. **b.** Genetic map of introgression lines (Shi et al. 2011). **c.** Genetic map from this study. **d.** Physical map of SSR markers. Numbers to the *right* of the physical map indicate the physical length (bp) of the chromosome. *Asterisk* the possible site of the GSB resistance gene. The *solid lines* shows the adjacent physical distance on the map and the *dotted arrow* shows non-adjacent physical markers. ✦ shows significant markers linked to the GSB resistance gene. (Color figure online)

significantly associated with GSB resistance, no QTLs could be detected for the introgressions (GSB1a, GSB1b) on this chromosome. The same situation was true for the introgression GSB6a on Chromosome 6. Markers located in/near the introgression on Chromosome 4 were found to be significantly associated with GSB resistance in both 2009 F_2 and 2009 F_2' populations, suggesting the presence of a QTL in the introgression (GSB4a) contributing to the GSB resistance identified in these three ILs. For the introgression GSB6b on Chromosome 6, two markers were identified that were significantly linked with the resistance, one in the 2009 F_2 population and the other in the 2010 F_2 population. Although these two markers were not identified in the same population, two ILs, HH1-8-5 and HH1-8-1-2, carrying the introgression covering these two markers showed a higher level of resistance than ILHH1-8-1-16 in which this introgression is lacking. Thus, these results suggest that this region harbors a potential QTL for GSB resistance, GSB6b.

Because of the low polymorphism rate, SSR markers used in this study could not cover all the introgressions in the three GSB resistant ILs. Thus, we could not exclude the possibility that the introgressed regions on Chromosomes 1 and 6 carry QTLs for GSB resistance. Nevertheless, the two potential QTLs (GSB4 and

GSB6b) provide a promising application for breeding GSB resistance in cucumber. In agreement with other studies, our data show that ILs are suitable for identifying the regions that potentially carry QTLs (Gur and Zamir 2004; Muir and Moyle 2009).

Using the cucurbit genomics database (CuGenDB), we identified some predicted genes related to resistance responses in the target physical region on both Chromosomes 4 and 6. The direct association of the predicted candidate gene with GSB resistance has not been well documented. Since too many candidate genes were predicted in the target regions, additional research is required to narrow the QTL regions before functional analysis.

Inverted marker orders were shown when the genetic and physical locations of these markers were compared (Fig. 3). These could be due to possible inversions or other minor chromosomal structural changes of the parental lines, although mapping errors could not be excluded in the early study (Li et al. 2011; Ren et al. 2009). This region was part of a paracentric inversion identified by Yang et al. (2012) in the long arm of Chromosome 4. There are six inversions found in the study of Yang et al. (2012), and all six inversions were probably common between cultivated (*C. sativus* L.) and wild (*C. sativus var. hardwickii*) cucumbers (Yang et al. 2012). In our study, IL HH1-8-1-2 was the progeny of wild cucumber (*C. hystrix*). Whether this inversion was common in wild cucumber requires further studies, for example by fluorescence in situ hybridization (FISH) (Szinay et al. 2010). Inversions have been shown to be present in many wild relatives of crops, which may hamper the introgression of valuable traits from wild relatives into cultivated crops, as demonstrated in tomato (Verlaan et al. 2011).

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References

Amand PCS, Wehner TC (1991) Crop loss to 14 disease of cucumber in North Carolina from 1983 to 1988. Cucurbit Genet Coop Rpt 14:15–17

Amand PCS, Wehner TC (1995) Greenhouse, detached-leaf, and field testing methods to determine cucumber resistance to gummy stem blight. J Am Soc Hortic Sci 120:673–680

Amand PCS, Wehner TC (2001a) Generation means analysis of leaf and stem resistance to gummy stem blight in cucumber. J Am Soc Hortic Sci 126:95–99

Amand PCS, Wehner TC (2001b) Heritability and genetic variance estimates for leaf and stem resistance to gummy stem blight in two cucumber populations. J Am Soc Hortic Sci 126:90–94

Anamthawat-Jónsson K (2001) Molecular cytogenetics of introgressive hybridization in plants. Methods Cell Sci 23:141–150. doi:10.1023/A:1013182724179

Bala G, Hosein F (1986) Studies on gummy stem blight disease of cucurbits in Trinidad. Trop Agric 63:195–197

Cavagnaro PF, Senalik DA, Yang L, Simon PW, Harkins TT, Kodira CD, Huang S, Weng Y (2010) Genome-wide characterization of simple sequence repeats in cucumber (*Cucumis sativus* L.). BMC Genomics 11:569. doi:10.1186/1471-2164-11-569

Chen J, Kirkbride JH (2000) A new synthetic species of *Cucumis* (Cucurbitaceae) from interspecific hybridization and chromosome doubling. Brittonia 52:315–319. doi:10.2307/2666583

Chen J, Staub JE, Tashiro Y, Isshiki S, Miyazaki S (1997) Successful interspecific hybridization between *Cucumis sativus* L. and *C. hystrix* Chakr. Euphytica 96:413–419. doi:10.1023/A:1003017702385

Chen J, Staub J, Qian Ch, Jiang J, Luo X, Zhuang F (2003) Reproduction and cytogenetic characterization of interspecific hybrids derived from *Cucumis hystrix* Chakr. × *Cucumis sativus* L. Theor Appl Genet 106:688–695. doi:10.1007/s00122-002-1118-7

Chen J, Moriarty G, Jahn M (2004) Some disease resistance tests in *Cucumis hystrix* and its progenies from interspecific hybridization with cucumber. In: Lebeda A, Paris HS (eds) Progress in cucurbit genetics and breeding research. Proceeding of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on cucurbit genetics and breeding. Czech Republic, Olomouc, p 189–196

Dijkhuizen A, Kennard WC, Havey MJ, Staub JE (1996) RFLP variation and genetic relationships in cultivated cucumber. Euphytica 90:79–87

Gur A, Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. PLoS Biol 2:e245. doi:10.1371/journal.pbio.0020245

Horejsi T, Staub JE (1999) Genetic variation in cucumber (*Cucumis sativus* L.) as assessed by random amplified polymorphic DNA. Genet Res Crop Evol 46:337–350

Huang S, Li R, Zhang Z et al (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281. doi:10.1038/ng.475

Katzir N, Danin-Poleg Y, Tzuri G, Karchi Z, Lavi U, Cregan P (1996) Length polymorphism and homologies of microsatellites in several Cucurbitaceae species. Theor Appl Genet 93:1282–1290. doi:10.1007/BF00223461

Keinath AP (2011) From native plants in central Europe to cultivated crops worldwide: the emergence os *Didymella bryoniae* as a cucurbit pathogen. HortScience 46:532–535

Knerr LD, Staub JE, Holder DJ, May BP (1989) Genetic diversity in *Cucumis sativus* L. assessed by variation at 18

allozyme coding loci. Theor Appl Genet 78:119–128. doi:10.1007/BF00299764

Li Y, Yang L, Pathak M, Li D, He X, Weng Y (2011) Fine genetic mapping of *cp*: a recessive gene for compact (dwarf) plant architecture in cucumber, *Cucumis sativus* L. Theor Appl Genet 123:973–983. doi:10.1007/s00122-011-1640-6

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Inc., Sunderland

McGrath DJ, Vawdrey L, Walker IO (1993) Resistance to gummy stem blight in muskmelon. HortScience 28:930–931

Meer QP, Bennekom JL, Giessen AC (1978) Gummy stem blight resistance of cucumbers (*Cucumis sativus* L.). Euphytica 27:861–864. doi:10.1007/BF00023726

Muir CD, Moyle LC (2009) Antagonistic epistasis for ecophysiological trait differences between Solanum species. New Phytol 183:789–802. doi:10.1111/j.1469-8137.2009.02949.x

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucl Acids Res 8:4321–4326. doi:10.1093/nar/8.19.4321

Qian CT, Chen JF, Zhuang FY, Xu YB, Li SJ (2002) Several photosynthetic characters of the synthetic species *Cucumis hytivus* Chen and Kirkbride under weak light condition. Plant Physiol Commun 38:336–338

Ren Y, Zhang Z, Liu J et al (2009) An integrated genetic and cytogenetic map of the cucumber genome. PLoS ONE 4:e5795. doi:10.1371/journal.pone.0005795

Shi JL, Lou QF, Qian CT, Wan HJ, Zhou XH, Chen JF (2011) Construction and genetic evaluation of chromosome segment introgression lines in cucumber. J Nanjing Agric Univ 34:20–24

Sitterly WR, Keinath AP (1996) Gummy stem blight. In: Zitter TA, Hopkins DL, Thomas CE (eds) Compendium of Cucurbit Diseases. APS Press, St. Paul, MN., pp 27–28

Szinay D, Bai Y, Visser R, de Jong H (2010) FISH applications for genomics and plant breeding strategies in tomato and other solanaceous crops. Cytogenet Genome Res 129:199–210

Van Ooijen JW (2006) JoinMap® 4.0. Software for the calculation of genetic linkage maps in experimental populations. Plant Research International, Wageningen

Van Steekelenburg NAM (1982) Factors influencing external fruit rot of cucumber caused by *Didymella bryoniae*. Eur J Plant Pathol 88:47–56. doi:10.1007/BF01977338

Verlaan MG, Szinay D, Hutton SF, de Jong H, Kormelink R, Visser RG, Scott JW, Bai Y (2011) Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the TYLCV resistance gene *Ty-1*. Plant J 68:1093–1103

Wehner TC, Amand PCS (1993) Field tests for cucumber resistance to gummy stem blight in North Carolina. HortScience 28:327–329

Wehner TC, Shetty NV (2000) Screening the cucumber germplasm collection for resistance to gummy stem blight in North Carolina field tests. HortScience 35:1132–1140

Wyszogrodzka AJ, Williams PH, Peterson CE (1986) Search for resistance to gummy stem blight (*Didymella bryoniae*) in cucumber (*Cucumis sativus* L.). Euphytica 35:603–613. doi:10.1007/BF00021869

Yang L, Koo D-H, Li Y, Zhang X, Luan F, Havey MJ, Jiang J, Weng Y (2012) Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. Plant J 71:895–906. doi:10.1111/j.1365-313X.2012.05017.x

Zhai HQ, Wang JK (2007) Applied quantitative genetics. China Agricultural Science and Technology Press, Beijing

Zhuang FY, Chen JF, Qian ChT, Li ShJ, Ren G, Wang ZhJ (2002) Responses of seedings of *Cucumis* × *hytivus* and progenies to low temperature. J Nanjing Agric Univ 25:27–30