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ORIGINAL PAPER

Fine genetic mapping localizes cucumber scab resistance gene *Ccu* into an *R* gene cluster

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Abstract Scab, caused by *Cladosporium cucumerinum*, is an important disease of cucumber, *Cucumis sativus*. In this study, we conducted fine genetic mapping of the single dominant scab resistance gene, *Ccu*, with 148 F_9 recombinant inbred lines (RILs) and 1,944 F_2 plants derived from the resistant cucumber inbred line 9110Gt and the susceptible line 9930, whose draft genome sequence is now available. A framework linkage map was first constructed with simple sequence repeat markers placing *Ccu* into the terminal 670 kb region of cucumber Chromosome 2. The 9110Gt genome was sequenced at 5× genome coverage with the Solexa next-generation sequencing technology. Sequence analysis of the assembled 9110Gt contigs and the *Ccu* region of the 9930 genome identified three insertion/deletion (Indel) markers, Indel01, Indel02, and Indel03 that were closely linked with the *Ccu* locus. On the high-resolution map developed with the F_2 population, the two closest flanking markers, Indel01 and Indel02, were 0.14 and 0.15 cM away from the target gene *Ccu*, respectively, and the physical distance between the two markers was approximately 140 kb. Detailed annotation of the 180 kb region harboring the *Ccu* locus identified a cluster of six resistance gene analogs (RGAs) that belong to the nucleotide binding site (NBS) type R genes. Four RGAs were in the region delimited by markers Indel01 and Indel02, and thus were possible candidates of *Ccu*. Comparative DNA analysis of this cucumber *Ccu* gene region with a melon (*C. melo*) bacterial artificial chromosome (BAC) clone revealed a high degree of micro-synteny and conservation of the RGA tandem repeats in this region.

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Introduction

Scab, caused by *Cladosporium cucumerinum*, is a very important disease of cucumber worldwide. The optimum temperature for disease development of scab is below 20°C (Walker 1952). Therefore, cucumber scab is especially a problem in those areas with cool growing seasons accompanied by intense fogs and dews. In China, scab is a serious problem in the ever increasingly utilized protected cultivation facilities such as high tunnels and greenhouses. Scab can cause death of seedlings and can considerably deteriorate fruit quality and commercial value of infected cucumbers. Because these pathogens can survive as resting spores for a long time in the soil, it is hard to control this disease

by traditional agricultural practices and agrochemicals. Thus, breeding of resistant cultivars is the best approach to minimize loss from infections. Bailey and Burgess (1934) first reported that scab resistance in cucumber was controlled by a single dominant gene, which was designated as *Ccu*. Unlike many other simply inherited plant resistance genes, the resistance conferred by *Ccu* has been very stable since its first deployment more than 70 years ago. Consistent with this, no physiological race differentiation has been reported in *C. cucumerinum* (Sitterly 1972).

Marker-assisted selection (MAS) is becoming a powerful tool in plant breeding. Development of molecular markers tightly linked with the target gene is one prerequisite for efficient MAS. Ideally markers should be designed from the target gene per se (perfect markers) if the gene has been cloned. Progress in genetic mapping in cucumber has been slow due to its narrow genetic base. The majority of molecular markers placed on low-resolution genetic maps are randomly amplified polymorphic DNAs (RAPDs) or amplified fragment length polymorphisms (AFLPs) (e.g., Bradeen et al. 2001; Serquen et al. 1997) that are not breeder friendly. However, recent advances in technology and instrumentation for sequencing plant genomes, such as the Roche/454 FLX and Illumina's Solexa platforms (Lister et al. 2009) provides exciting opportunities for cucumber and many other crops that lack adequate genomic resources to expedite molecular breeding. In cucumber, during the last 2 years, the draft genome of the Northern China type cucumber inbred line '9930' was released (Huang et al. 2009). Sequencing and assembly of two additional cucumber genotypes is near completion including a North American pickling cucumber inbred line 'Gy14' (available at http://cucumber.vcru.wisc.edu/ or http://www.phytozome.net/cucumber.php#A/) and a European line 'B' (Woycicki and Przybecki 2010). Hundreds of simple sequence repeat (SSR) or microsatellite markers have been developed from the whole genome sequence and a SSR-based high-resolution genetic map has been constructed (Ren et al. 2009). These genomic resources will greatly facilitate molecular mapping and gene cloning in cucumber.

Using SSR markers developed from the '9930' draft genome sequence, the co-authors conducted genetic mapping for the scab resistance gene *Ccu*, and identified two closely linked SSR markers flanking this locus, which were 0.7 and 1.6 cM away, respectively (Zhang et al. 2010). The objective of the present study was to continue our effort to develop a high-resolution genetic map for the *Ccu* gene toward eventual cloning of *Ccu* using a map-based cloning strategy. Cloning of *Ccu* resistance gene will not only enable design of perfect markers for MAS and pyramiding of multiple disease resistances, but also provide us a valuable tool to understand molecular mechanisms of host resistance against scab pathogen. Thus, in this study, we employed 148 F_9 RILs and 1,944 F_2 individuals as the mapping populations, and two strategies were used to develop molecular markers for fine genetic mapping of *Ccu*. We were able to delimit the *Ccu* locus into a 140 kb genomic DNA region that contained four nucleotide binding site (NBS)-type resistance gene analogs (RGAs). The details of the results are reported below.

Materials and methods

Plant materials

Two cucumber inbred lines, '9110Gt' and '9930' were employed in the present study. 9110Gt is a European greenhouse type cucumber with scab resistance conferred by *Ccu*, and 9930 is a Northern China fresh market type that is susceptible to scab. The 9930 genome has recently been sequenced (Huang et al. 2009) and annotated (http://cucumber.genome.org.cn/).

The four populations derived from 9110Gt and 9930 for scab disease screening and fine genetic mapping are listed in Table 1. The 148 F_9 recombinant inbred lines (RILs)

Table 1 Segregation of responses to scab inoculation among different populations derived from resistant cucumber line 9110Gt and susceptible 9330

Materials	#Lines/plants tested	R:S:U observed	R:S ratio tested	χ^2 [a]	*P*
Recombinant inbred lines					
(9110Gt × 9930) F_9 RILs	148	73:75:0	1:1	0.03	0.87
F_2 plants					
(9110Gt × 9930)F_2	2,094	1,473:471:150	3:1	0.62	0.43
Backcross-1 (BC_1) plants					
(9110Gt × 9930) F_1 × 9930	112	60:52:0	1:1	0.57	0.45
(9110Gt × 9930) F_1 × 9110Gt	98	98:0:0	1:0	–	–

R resistant, *S* susceptible, *U* unassigned

[a] Unassigned (U) plants were excluded in calculation

represent the same population used by Zhang et al. (2010) to place the *Ccu* on cucumber Chromosome 2.

Disease screening

All experiments were conducted in a room with climate control at a mean temperature of 20°C. *C. cucumerinum* was isolated from infected tissue of cucumber using a single colony isolation method. The identity of the isolated *C. cucumerinum* was verified by morphological observation under a microscope. The pathogen was purified by single spore isolation and its virulence was further tested on the resistant cucumber variety Zhongnong No. 13 and susceptible line Jinza No. 4.

The design used for evaluation of responses to scab pathogen inoculation of the F_9 RILs was a randomized complete block design. There were three replications for each line and 10 plants in each replication. At the first true-leaf stage a water suspension with a concentration of 2×10^6 spores per ml was sprayed evenly on the cotyledons and first true leaf of the seedlings, which were then kept in dark at 17–20°C for 24 h with 100% relative humidity (RH). Afterward, the seedlings were maintained at the same temperature range with 60–85% RH and 16 h light/8 h dark of light. This experiment was repeated in a second year.

Scoring of disease responses was conducted at 7–10 days after inoculation. The disease rating used a six-step scale (0–5) following Zhang et al. (2010). Specifically, 0 = absence of disease symptoms; 1 = few disease spots on the leaf and no spots on stem; 2 = petiole shriveled, few disease spots on leaf, and small spots on stem; 3 = petiole shriveled, center of leaf wilted, sunken disease spots on stem; 4 = petiole shriveled, center of leaf dry and wilted, and sunken disease spots on stem; and 5 = petiole and leaf dry and wilted and shrunken disease spots on stem. Typical symptoms of each score are shown graphically in Fig. 1.

For the RIL population, disease resistance was evaluated using a disease index (DI) following Zhang et al. (2010) in which $DI = \sum[(s \times n)/(S \times N)] \times 100$, where n = number of plants with each disease rating; s = disease rating scale; N = total number of plants under investigation; and S = highest disease rating scale. Scab resistance for each RIL was determined based on DI value: $DI \leq 30$, resistant (R); $DI \geq 45$, susceptible (S); and $30 < DI < 45$, unassigned (U).

Phenotyping of disease resistance in the F_2 and backcross progeny was based on the rating of individual plants (Fig. 1): resistant (R) if disease rating = 0 or 1; susceptible (S) if disease rating = 3, 4 or 5; and unassigned (U) if rating = 2.

Molecular marker development and linkage analysis

Zhang et al. (2010) confined that *Ccu* was located in cucumber Chromosome 2. Thus, we started developing a skeleton map for *Ccu* using the RIL population. SSR markers

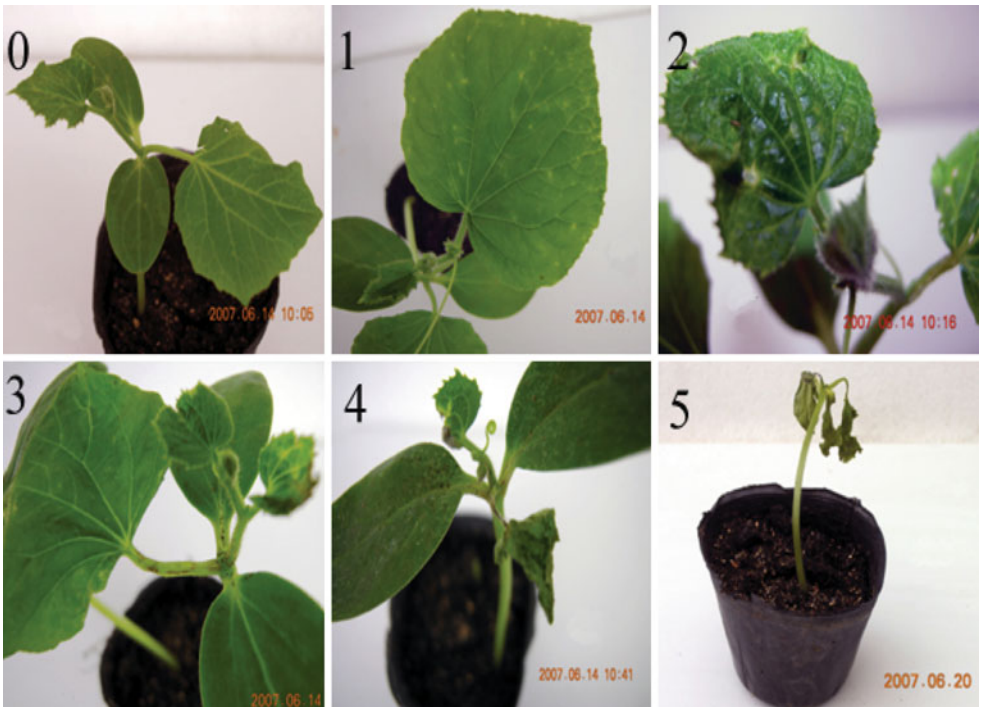

Fig. 1 Typical symptoms for each score of resistance phenotype after inoculation of the cucumber seedling with the fungus *Cladosporium cucumerinum*. *Number* in each image corresponds the rating index (0–5)

in Chromosome 2 reported by (Ren et al. 2009) were used to screen the two parental lines 9110Gt and 9930, and all polymorphic markers were applied to the RIL population.

From genetic mapping with the RIL population, two high quality SSR markers flanking the *Ccu* locus, SSR06576 and SSR16226 were identified, which were approximately 670 kb apart in the 9930 reference genome (Huang et al. 2009; http://cucumber.genomics.org.cn). Two strategies were then used for fine mapping of *Ccu* with the F_2 population. The first strategy used next-generation sequencing for marker development. Genomic DNA of 9110Gt was digested with restriction enzyme *Bam* HI and sequenced with the Solexa platform (http://www.genomics.cn/en/index.php). The 9110Gt genome was sequenced at 5× genome coverage and contigs were assembled with the computer program SOAP (Li et al. 2008; available at http://soap.genomics.org.cn/). The assembled contig sequences were analyzed with BLASTn (Altschul et al. 1997) and BLAT (Kent 2002) using the 670 kb *Ccu* gene region as the subject sequence. Single nucleotide polymorphism (SNP) and insertion/deletion DNA sequence variations (Indels) between 9110Gt and 9930 were identified from sequence alignment. However, due to technical difficulties in SNP detection, only Indels were explored in the present study for new marker development. In the second strategy, based on the early annotation of the 9930 genome (Huang et al. 2009), the 670 kb *Ccu*-resident region of the 9930 draft genome was re-annotated manually. Several NBS-type RGAs were identified. PCR primer pairs were designed from these RGAs to amplify 9330 and 9110Gt genomic DNAs. The PCR products from both parents were sequenced with an ABI 3730 DNA sequencer. The quality of the sequences was evaluated by the software Sequence Scanner v1.0 (available from Applied Biosystems at http://www.appliedbiosysytems.com/). Only high quality sequences were used for sequence alignment to identify Indels between 9110Gt and 9930 for new marker development. Because some small Indels (less than three base pairs) were difficult to resolve with polyacrylamide gel electrophoresis (PAGE), these PCR products were sequenced directly to confirm the genotypes.

DNA was extracted from cucumber leaves by the CTAB method (Murray and Thompson 1980). PCR and PAGE procedures followed Zhang et al. (2010).

Data analysis

For disease screening data in RIL and F_2 plants, χ^2-tests for goodness-of-fit were used to test for deviations of the observed data from the theoretically expected segregation.

Linkage analysis of the *Ccu* resistance locus with molecular markers in RIL and F_2 mapping populations was performed with JOINMAP 3.0 (Stam 1993; Van Ooijen and Voorrips 2001) using a minimum LOD threshold of 4.0 and the Kosambi mapping function (Kosambi 1944). RILs or F_2 individual plants labeled with 'U' (unassigned) in disease screening were excluded in linkage analysis. The linkage map was drawn using Perl and SVG (scalable vector graphics, http://search.cpan.org/~ronan/SVG-2.28/SVG/Manual.pm).

Sequence annotation and gene prediction in genomic region harboring *Ccu* locus

Gene prediction was performed with the computer program BGF (http://bgf.genomics.org.cn) and verified by FGENESH (http://sunl.softberry.com/) (Salamov and Solovyev 2000), GENESCAN (http://genes.mit.edu/GENSCAN.html) (Burge and Karlin 1997), TwinScan (http://mblab.wustl.edu/software/twinscan) (Korf et al. 2001) and lastly checked manually. InterProScan (http://www.ebi.ac.uk/InterProScan) (Zdobnov and Apweiler 2001) was used for gene annotation. For comparison with syntenic regions in melon, homologous melon bacterial artificial chromosome (BAC) clone sequences were downloaded from GenBank (accession number AF499727). Sequences alignment between 9110Gt and 9930 was performed using the software ClustalW (Thompson et al. 1994).

Results

Inheritance of scab resistance gene *Ccu* in cucumber

Responses of plants to scab inoculation in different populations are summarized in Table 1. Results for the RIL population in 2 years were highly consistent. Of the 148 RILs, 75 were homozygous susceptible (DI ≥ 45), which was very close to that observed by Zhang et al. (2010). Of the 2,094 F_2 plants screened, 1,473 were resistant and 471 susceptible (total 1,944). However, we were unable to assign the remaining 150 F_2 plants (disease rating = 2) into either category. Of the 112 BC_1 plants derived from backcross with the susceptible parent 9930, 60 and 52 were resistant and susceptible, respectively; whereas all 98 BC_1 plants from backcross with 9110Gt were resistant (Table 1). Goodness-of-fit Chi-square tests among all four populations indicated that the scab resistance in 9110Gt is controlled by a single dominant gene, which was consistent with previous studies (Bailey and Burgess 1934; Walker 1952; Zhang et al. 2010).

Genetic mapping of *Ccu* gene with the RIL population

Ren et al. (2009) mapped 126 SSRs in cucumber Chromosome 2. In the present study, SSRs were genotyped using PAGE method as reported in Zhang et al. (2010), and 15

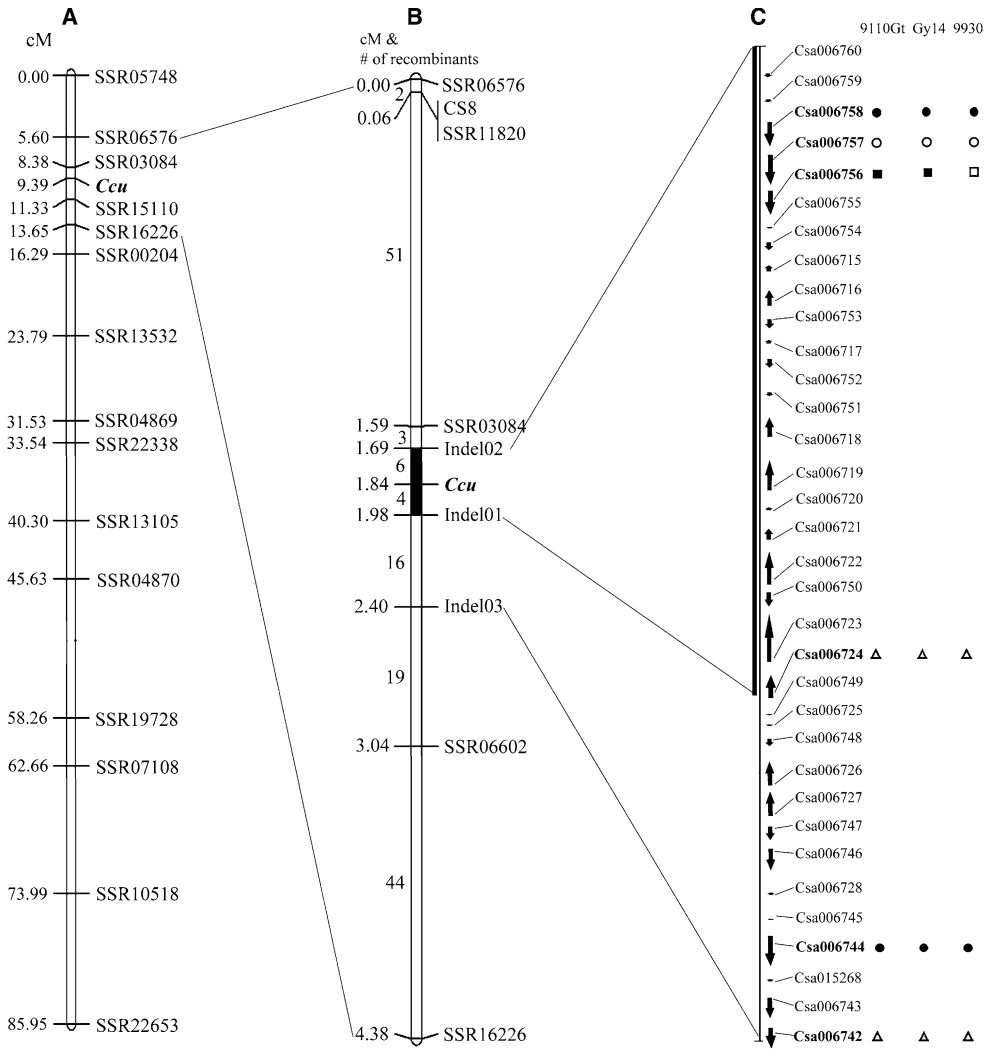

Fig. 2 Fine mapping of *Ccu* locus in cucumber. **a** Linkage analysis with the RIL population placed the *Ccu* gene in the short terminal of cucumber Chromosome 2. **b** Fine mapping with 1,944 F2 individuals delimited *Ccu* into a 140 kb genomic DNA region flanked by two Indel markers Indel01 and Indel02. In **a** and **b**, *vertical bars* represent the chromosome. Genetic distances in centimorgan (cM) and marker or gene locus names that are connected by *horizontal lines* (loci) are shown to the *left* and *right* of the *vertical bar*, respectively. In **b**, *number* in between two marker loci are recombination events occurred in this region. **c**. Annotation of the 140 kb region harboring *Ccu*. Six NBS-type RGAs were identified which are *boldface* typed. The predicted R gene domain structures of the 6 RGAs in 9110Gt, Gy14 and 9930 cucumber lines are shown to the far *right*. The domains represented by different symbols are: *filled square* TIR-NBS-LRR, *open square* NBS-LRR, *open circle* TIR-CC-NBS-AAA+ATPase, *triangle* TIR-NBS, *filled circle* TIR-NBS-LRRAAA + ATPase

SSRs were found polymorphic between 9110Gt and 9930. The genetic map from linkage analysis of these 15 SSRs and *Ccu* in 148 RILs is shown in Fig. 2a. SSR03084 and SSR15110 were the two closest flanking markers of *Ccu*. However, two other markers next to them, SSR06576 sand SSR16626 were more robust and easy to use, and thus were used as the starting point for high-resolution genetic mapping with the F_2 population. SSR06576 and SSR16626 were located in the 9930 whole genome scaffolds, scaffold000020 and scaffold000056, respectively (Huang et al. 2009; http://cucumber.genome.org.cn). Because the 9930 scaffolds overlapped a scaffold from another sequenced cucumber line, Gy14 (http://cucumber.vcru.wisc.edu/), we were able to estimate that the physical distance between the two markers in 9930 was approximately 670 kb.

Fine mapping of *Ccu* locus

Two flanking markers, SSR06576 and SSR16226 identified from linkage analysis in the RIL population were used to screen the F_2 population with 1,944 individuals (those with 'unassigned' disease screening ratings were excluded), and 145 recombinants between the two markers were identified which were used as the high-resolution mapping population. Nearly, simultaneously, (Zhang et al. 2010) had identified closely linked SSR markers for *Ccu*. Three markers, SSR11820, CS8, and SSR06602 were thus included in linkage mapping of the F_2 population. On the resulting map shown in Fig. 2b, SSR06602 was closer to *Ccu* than to SSR16226, but the cosegregating CS8 and SSR11820 were only 0.06 cM away from SSR06576. There were still 48 recombination events between SSR03084 and SSR06602 flanking *Ccu* (Fig. 2B). Therefore, development of new markers was initiated for fine mapping of *Ccu*.

Two strategies were employed for new marker development. First, the whole genome of 9110Gt was sequenced with a Solexa next-generation sequencing machine at 5× genome coverage. Alignment of the 9110Gt sequences with the 670 kb region of 9930 draft genome assembly delimited by SSR06576 and SSR16626 one indel sequence variations.

We used the indel polymorphism between 9110Gt and 9930 identified in this region. This marker, Indel02, was located between SSR03084 and *Ccu*, and was 0.10 cM away from SSR03084 (Fig. 2b). No additional indel markers were found in this region through alignment of the 9110Gt Solexa contigs with the 9330 draft genome.

In the second approach, the 670 kb genomic DNA region of the 9930 draft genome between markers of SSR06576 and SSR16226 was annotated and gene functions were predicted. Sequence analysis revealed that there were approximately 100 genes in this region, and 6 of them were putative RGAs carrying a NBS domain (Fig. 2c). PCR primers were designed from these RGAs (Table 2), which were then used to screen 9110Gt and 9930 genomic DNAs for polymorphisms. Consequently, two polymorphic markers, Indel01 and Indel03 derived from CDS (coding sequences) Csa006724 and Csa006742, respectively, were mapped (Fig. 2c). Both Indel01 and Indel03 were mapped on the same side of *Ccu* and were 0.42 cM apart. Now, the two closest flanking markers of *Ccu* were Indel01 and Indel02, which were 0.14 and 0.15 cM from *Ccu*, respectively. The number of recombinant events (RE's) in the *Ccu* region is also indicated in Fig. 2c. There were in total 10 RE's between Indel01 and Indel02. The genetic distances between *Ccu* and Indel01 as well as between *Ccu* and Indel02 were calculated based on the assumption that 4 and 6 TEs occurred in the two regions, respectively. However, the genotypes of the two markers suggested six RE's must have occurred between Indel02 and *Ccu*, but it was not known if the remaining four RE's occurred between Indel01 and *Ccu*, or Indel02 and *Ccu* because we were not able to recover the four recombinant F_2 plants, which were not kept for generating F_3 after disease screening. Thus, we were unable to infer the genotypes at the *Ccu* locus in the four critical F_2 recombinants (homozygous or heterozygous resistant).

Physically, both Indel01 and Indel02 were located in scaffold000020 of the 9930 draft genome that were 140 kb apart suggesting that the *Ccu* gene is also in this region.

In addition, 90 SNPs between 9110Gt and 9930 were confirmed in the 180 kb DNA region between Indel01 and Indel03. These SNPs are shown in supplement Table S1. 9110Gt re-sequences data in this 180 kb region are shown in supplement file S3.

Annotation and gene prediction in 180-kb *Ccu*- gene region

The ~180 kb genomic DNA region in scaffold000020 of the 9930 draft genome delimited by Indel02 and Indel03 were manually annotated. There were 34 predicted genes in this region, and the details of these genes are provided in supplemental Table S2. Interestingly, it seems that there is a cluster of NBS-type RGAs in the 180 kb DNA sequences (Fig. 2c; supplement Table S2) which included six members, Csa006724, Csa006742, Csa006744, Csa006756, Csa006757, and Csa006758. Of these, Csa006724 and Csa006742 encode TIR-NBS domains, Csa006756 encodes NBS-LRR domain, Csa006757 encodes TIR-CC-NBS-AAA+ATPase domain, and Csa006744 and Csa006758 encode TIR-NBS-LRR-AAA+ATPase domains.

Comparative analysis of six NBS-type RGAs among 9930, 9110Gt, and Gy14

Based on the 9930 draft genome sequence, PCR primers were designed to amplify the alleles of above six RGAs from the resistant parent 9110Gt. By this time, the whole genome of a pickling cucumber inbred line Gy14 was also sequenced with the 454/Titanium next-generation sequencing platform (with 36× genome coverage) and assembled (Weng et al., unpublished data, Gy14 genome assembly is available at http://cucumber.vcru.wisc.edu/, and annotation

Table 2 Information of markers linked with cucumber scab resistance gene *Ccu*

Marker	Scaffold	Left primer (5′–3′)	Right primer (5′–3′)	Amplicon size (bp)	Amplicon size (bp)	
					9930	9110Gt
SSR06576	scaffold000056	TGATCATGGGAAGAGAGAGACA	TCAAGAAATGTGATGAATGGAAA	~200	–	
SSR11820	scaffold000020	ACGACGCCGTATTTGCTTAG	AAGCTCGTTCATTATTACCCAA	~200	–	
SSR03084	scaffold000020	GACAAGGGATTCATCCGAGA	CAGACCCTGAAGCGGATAAA	~200	–	
Indel02	scaffold000020	TTTGGTGCATTCTAGGATTT	TCTGGAGGCAAGATAAATAA	–	271	263
Indel01	scaffold000020	ATTCATCGGCTGTAGACTCA	ATGGATGTAGTATTGAGAATGA	–	295	439
Indel03	scaffold000020	GTGAGGGTTAGGGTTCTATT	TCGACATACTTTCGTCTTTT	–	273	275
SSR06602	scaffold000020	CCCTGCCTTCCTTTCCTATC	AGAAAGGATCGGATCGAACA	~200	–	
SSR16226	scaffold000020	TGATGGGAGAAAGGTAAACAAGA	TTAAAATTCCCAACGGAAACC	~200	–	

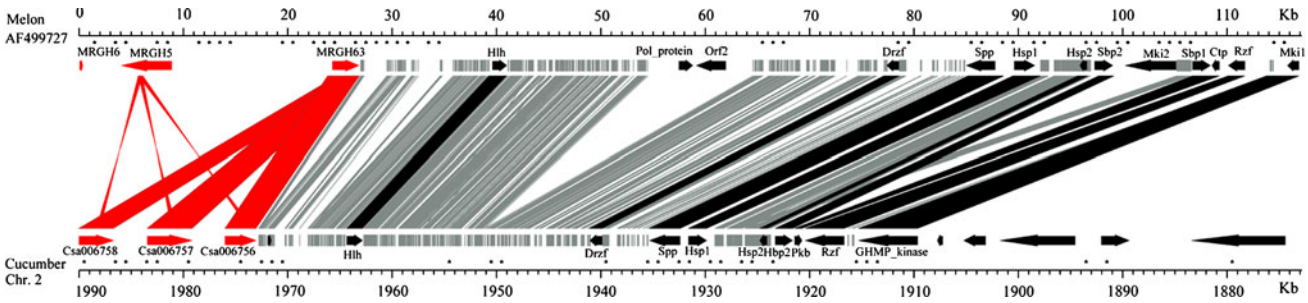

Fig. 3 Microsynteny of the Ccu region between melon and cucumber. The 120 kb melon BAC clone sequence AF499727 (*top*) was aligned with 80 kb Ccu region of cucumber (*down*). The *arrow* shows the gene with orientation. The *gray* region linking the melon and cucumber sequences are intergenic regions. DNA repeat region of miniature inverted-repeat transposable elements (MITE) (*asterisk*); MRGH6, MRGH5, MRGH63: melon NBS encoding genes; Csa006758, Csa006757, Csa006756: cucumber NBS encoding genes. Hlh: helix-loop-helix-like protein encoding gene; Orf1, Orf2: gagprotease polyprotein; Drzf: RING/C3HC4/PHD zinc finger-like protein; Spp: Signal peptidase 22 kDa subunit; Hsp1, Hsp2: Heat shock protein Hsp20; Sbp1, Sbp2: selenium-binding protein; Mki1, Mki2: mevalonate kinase-like protein;Ctp, Rzf: conserved site of ATP binding Protein kinase; GHMP: C-terminal GHMP kinase, ATP-binding conserved site

available at http://www.phytozome.net/). Gy14 is scab resistant. Thus, the genomic DNAs of the six RGAs among the three cucumber genotypes were compared. The details of the DNA and deduced protein sequence alignment are included in Supplement Files 1 and 2 and diagrammatically presented in Fig. 2c. The sequences of the six RGAs of 9930 and 9110Gt have also been submitted to GenBank (accession numbers HM064415 to HM064420). For Csa006724, Csa006742, Csa006744, Csa006757, and Csa006758, all three genotypes seem to share the same predicted domains. Csa006724 and Csa006742 seem to be pseudogenes. Interestingly, Csa006756 encodes a NBS-LRR RGA, but no TIR domain in 9930, but its alleles in 9110Gt and Gy14 both encode a TIR-NBS-LRR domains (Supplement Files 2).

Micro-synteny of *Ccu* region between cucumber and melon genomes

Garcia-Mas et al. (2001) and van Leeuwen et al. (2005) reported resistance gene homologs (MRGHs) in the melon genome. One BAC clone (GenBank accession # AF499272) contains a RGA cluster with three MRGH members. We used the melon BAC clone AF499272 DNA sequence as the query sequence and the cucumber inbred line 9930 whole genome sequence as the subject database, and aligned the sequences by the BLAST program. We found that they shared high homology, and there was one copy of AF499272 in the cucumber genome. Significant syntenic blocks were found between the aligned cucumber and melon sequences. The microsyntenic relationship within the *Ccu* region is graphically shown in Fig. 3. In the syntenic regions, there were 16 and 13 predicted genes in the melon and cucumber genome region, respectively. They included one helix-loop-helix protein encoding gene (Hlh), one signal peptidase 22 kDa subunit protein encoding gene (spp), one zinc finger-like protein encoding gene (Drzf), two heat shock (Hsp 20 family) protein encoding genes (Hsp1 and Hsp2), one ATP binding protein kinase encoding protein gene (Rzf). The other genes in the region shared no homology. Three RGAs were present in the overlapping region. The three RGAs in both cucumber and melon are arranged in tandem arrays (Fig. 3).

Discussion

Fine genetic mapping of *Ccu* locus in cucumber facilitated by draft genome sequence, next-generation genome sequencing and bioinformatic analysis

In this study, we conducted fine genetic mapping of the scab resistance locus *Ccu* with two segregating populations (F_9 RIL and F_2) derived from the resistant cucumber inbred line 9110Gt and the susceptible 9930. Based on the high-resolution cucumber genetic map produced by Ren et al. (2009) and the 9930 draft genome (Huang et al. 2009), we were able to quickly locate *Ccu* into a sub-telomeric 670 kb region of cucumber Chromosome 2 (Fig 2a) delimited by two flanking SSRs markers. This result was consistent with our previous study (Zhang et al. 2010). We took two approaches to identify more closely linked markers for *Ccu*. In the first approach, we sequenced the 9110Gt genome with Solexa next-generation sequencing technology with low genome coverage (5×). Alignment of the Solexa sequences with the 9930 draft genome enabled us to identify an Indel marker, Indel02 in the *Ccu* target region (Fig. 2b). Since the two populations derived from 9110Gt and 9910 are also segregating for a number of other horticulturally important genes such as uniform immature fruit color (*u*), fruit ridging (*Fr*), and bitterfree foliage (*bi*) (Xingfang Gu, manuscript in preparation), these Solexa sequences should be an invaluable resource for fine mapping of these genes.

Our mapping effort enabled us to locate *Ccu* in a 180 kb region thus allowing identification of NBS-type RGAs and design of new indel markers from these RGAs (Indel01 and Indel03, Fig. 2b). The Indel markers delimited the *Ccu* gene in a 140 kb region. Our study suggested that combined use of the draft genome sequence, and next generation sequencing could accelerate development of molecular markers for target genes. With the continuing decreasing cost for next-generation sequencing, we believe that this methodology is a method of choice for fine genetic mapping and gene cloning in cucumber and other crops.

R gene cluster in cucumber may provide broad-spectrum fungus disease resistance

Many disease resistance genes (*R*-genes) conferring resistance to a diverse array of pathogens, including bacteria, fungi, oomycetes, viruses, and nematodes, have been isolated in plants. The largest *R* gene family encodes NBS and leucine-rich repeats (LRR) domains (Dangl and Jones 2001; Hulbert et al. 2001; McHale et al. 2006; Meyers et al. 2003). These R proteins have been shown to function as intracellular immune receptors that recognize, directly or indirectly, specific pathogen effectors encoded by avirulence (*Avr*) genes (Bent and Mackey 2007). In the 9930 draft genome, 61 NBS-type RGAs were identified and mostly were distributed in 11 clusters in the cucumber chromosomes (Huang et al. 2009). In the present study, we annotated the 180 kb region bearing the *Ccu* gene (Fig. 2C, supplemental Table S2). Of the 34 genes predicted, six belonged to NBS-type RGAs, and three of which were in tandem repeats. Two Indel markers, Indel01 and Indel03 were actually designed from part of two RGAs (Csa006742, and Csa006724, respectively). Of the 1,944 F_2 plants, ten recombination events were identified between the two markers Indel01 and Indel02 flanking *Ccu* (Fig. 2C), six of which were between *Ccu* and Indel02. Because we only had F_2 phenotypic data for the four recombinant plants, their genotypes at the *Ccu* locus (homozygous or heterozygous resistant) were not known. Therefore, at present we were unable to deduce precisely the genetic distances of Indel01 or Indel02 to *Ccu*. Thus, which of the four RGAs is the candidate gene is also not known.

Comparison of the RGA sequences among the scab resistant 9110Gt, Gy14 and the susceptible 9930 cucumber genotype revealed that the DNA and deduced protein sequences of the RGAs Csa006724 and Csa006756 in 9110Gt and Gy14 were almost identical, but they were obviously different from those in 9930 (Fig. 2c, Supplemental Files 1 and 2). In particular, 9930 seemed to have lost the TIR domain in Csa006756. Thus, both Csa006724 and Csa006756 could be potential candidate genes of *Ccu*. It is also possible that multiple RGA members in the cluster cooperate to provide the durable scab resistance in cucumber. However, further evidence is needed to verify the possible functions of each RGA member. One approach is to identify cosegregating markers of *Ccu* to pin down a smaller region thus identifying target candidate gene of *Ccu*. For more precise fine mapping of *Ccu*, we are using the two flanking markers Indel01 and Indel02 to screen a large F_2 population (>2,000 plants) from 9110Gt × 9930 to identify recombinants. F_3 families from these recombinant F_2 plants will be produced for disease screening to infer F_2 genotypes at the *Ccu* locus. We have identified a number of SNPs in this region (Supplement Table S1). These SNPs will be used in *Ccu* fine mapping in the high-resolution mapping population. We are also developing a transformation system in cucumber. Transgenic cucumber plants will be produced from the four candidate RGAs to verify their functions in complementation tests.

Of the 61 NBS-type RGAs detected in the 9930 draft genome, 20 were distributed in Chromosome 2 (Huang et al. 2009) including six identified in the present study (Fig. 2c). This may suggest that multiple NBS-LRR type disease resistance genes may be located in this chromosome. Indeed, previous studies suggested that in cucumber resistances to scab and *Fusarium* wilt caused by *Fusarium oxysporum* were linked (Vakalounakis 1993; Mao et al. 2008). The two parental lines 9110Gt and 9930 used in this study have different reactions upon *Fusarium* wilt pathogen inoculation: 9110Gt is resistant and 9930 is susceptible. Our molecular mapping study using the 9110Gt × 9930 RIL population revealed that scab resistance and a major QTL for *Fusarium* wilt resistance were co-localized in the *Ccu* region (Kang et al., unpublished data). If *Fusarium* resistance in cucumber is controlled by NBS-type R gene, this may implicate that the NBS cluster members may condition multiple disease resistances in this region.

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