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QTL molecular marker location of powdery mildew resistance in cucumber (*Cucumis sativus* L.)

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The cucumber lines, S94 (Northern China open-field type, powdery mildew (PM) susceptible) and S06 (European greenhouse type, PM resistant), and their $F_{6:7}$ populations were used to investigate PM resistance under seedling spray inoculation in 2005/Autumn and 2006/Spring. QTL analysis was undertaken based on a constructed molecular linkage map of the corresponding F_6 population using composite interval mapping. A total of four QTLs (*pm1.1*, *pm2.1*, *pm4.1* and *pm6.1*) for PM resistance were identified and located on LG 1, 2, 4 and 6, respectively, explaining 5.2%—21.0% of the phenotypic variation. Three consistent QTLs (*pm1.1*, *pm2.1* and *pm4.1*) were detected under the two test conditions. The QTL *pm6.1* was only identified in 2005/Autumn. The total phenotypic variation explained by the QTLs was 52.0% and 42.0% in 2005/Autumn and 2006/Spring, respectively. Anchor markers tightly linked to those loci (<5 cM) could lay a basis for both molecular marker-assisted breeding and map-based gene cloning of the PM-resistance gene in cucumber.

cucumber, powdery mildew, QTL, $F_{6:7}$families, seedling

Powdery mildew (PM), caused by the obligate biotrophic fungus, is one of the most serious foliage diseases in cucumber (*Cucumis sativus* L.) in both the greenhouse and the field. The two main pathogens of PM are *Podosphaera xanthii* and *Golovinomyces cichoracearum; P. xanthii* is predominant in China[1−2]. PM has a wide host range[3] and can easily develop under cooler conditions because the fungi conidia are readily detached and borne by air currents. Severe infection by powdery mildew before the flowering stage can reduce the yield of cucumber fruit by 20%—40%[4]. There are two ways to control the disease at present, by fungicide application or by the use of resistant varieties. The latter is an economical and environmentally safe strategy[5].

During traditional cucumber breeding, it is difficult to select PM high-resistance breeds as difficult resistance evaluation and long selection cycles. If the markers closely linked to resistance genes could be obtained, it would be a great advance for marker-assisted selection of resistant breeding.

The inheritance of PM resistance has been reported in many cucumber varieties and lines. Mao et al.[6] considered that this resistance was controlled by 3 genes in cucumber. Munger et al.[7] found a dominant gene for PM resistance in 'Spartan Salad 77-717' and 'PI197088'. However, Morishita et al.[8] reported that the PM resistance in PI197088-5 (a derivative line of PI197088) was inherited through 2 pairs of genes, a recessive and an incompletely dominant gene. Sakata et al.[9] reported that the PM resistance in PI197088-5 was a quantitative trait. It appears that the genetic mechanism of PM resistance in cucumber is moderately complex.

The molecular marker linked to cucumber PM resistance has rarely been reported. Zhang et al.[10] found an

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Amplified restriction fragment polymorphism (AFLP) marker linked to PM resistance (5.56 cM) in one population developed from a highly resistant parent Q9 and a highly susceptible parent Q10. With recombinant inbred lines (RILs) population ‘Santou× PI197088-1’, Sakata et al.[9] identified two and three loci for PM resistance under 26℃ and 20℃ conditions, respectively.

As the resistance to PM is affected greatly by different cucumber genotypes and environments, its inheritance has not been confirmed and needs to be investigated further. The objective of this study is to identify genetic resistance for powdery mildew, using an $F_{6:7}$ population (S94×S06) with 224 lines and parents, under the seedling spay inoculation. Based on a linkage map of the F_6 population and using WinQTLCart 2.5 software with a composite interval mapping method (LOD⩾2.5), quantitative trait loci (QTL) analysis for PM resistance in cucumber was first reported in China. The verification of the anchor markers tightly linked to the trait may be useful in marker-assisted breeding.

1 Materials and methods

1.1 Population and genetic map

The F_1 of S94 (susceptible to PM) crossed with S06 (resistant to PM) was self-pollinated to produce 252 F_2 individuals by means of single-seed descent (SSD), until 224 lines (F_6) were obtained for map construction.

Using MAPMAKER/EXP3.0, with an LOD threshold ⩾3.0 [11] and the Kosambi function, a linkage map was constructed. The linkage map used in this study includes 257 molecular markers (206 sequence-related amplified polymorphisms (SRAPs), 22 simple sequence repeats (SSR), 25 sequence characterized amplification regions (SCARs), 1 sequence-tagged site (STS) and 3 morphologic traits[12–14]) resulting in a total map length of 1005.9 cM and a mean marker interval of 3.9 cM, which is suitable for QTL analysis[15].

1.2 Separation and purification of the pathogen

The inoculum of PM used in this test was isolated from the naturally infected PM leaves of cucumber plants in the greenhouse in the Qibao farm at Shanghai Jiaotong University. The spores of a single lesion were collected and inoculated directly to the seedlings in the tube culture, which were incubated under 22℃ and a 16-h photoperiod.

With the appearance of a new single lesion on a seedling, the spores of this lesion were transferred to another new seedling using a brush. The isolated pathogen, diagnosed as *P. xanthii* microscopically, was purified through 5 single lesion transfers. In the greenhouse, some susceptible potted cucumber seedlings of cultivar “Changchunmici” were separated for pathogen transference. The seedlings were placed in a culture room at temperature of 18℃－23℃ and relative humidity of 50%－75%.

1.3 Analysis of resistance to PM

In the autumn of 2005 and spring of 2006, the $F_{6:7}$ families, two parents and the F_1 generation were planted in the greenhouse of the Agriculture and Biology School of Shanghai Jiaotong University. Two independent trials, consisting of three randomized complete blocks composed of eight plants per genotype, were performed for resistance evaluation. Plants were grown in 12-cm plastic pots with a steamed compost–field soil–peat–sand (1 : 1 : 1 : 1 by volume) mixture. Twenty seeds each of the parental lines and their F_1 were sown as controls in all experiments. When the three true leaves of seedlings (three-weeks-old) were fully expanded, individuals were inoculated with the purified pathogen by spaying a spore suspension of 5×10^4 spores per mL and humidity was preserved at 75% ± 10% by moistener for 24 h[16]. The greenhouse temperature was 22℃－28℃ in the day and 15℃－18℃ at night, and relative humidity was over 70%.

After 12 d of inoculation, each leaf was investigated. Disease level scoring was performed in 10% steps according to the visual percentage of the first true leaf area covered with mildew (from 0% to 100%, corresponding to 0－10 grades). The average disease index (DI) of the three replications of each line was used for statistical and QTL analysis. For the inheritance study, lines with a DI⩽20 were considered resistant and lines with a DI>20 were considered susceptible.

$$\text{DI}=\frac{\sum(\text{disease grade}\times\text{number of leaves in that grade})}{\text{total number of leaves observed}\times\text{maximum disease grade}}\times100 .$$

1. 4 Statistical analysis and QTL location

The Microsoft ® Excel 2000 program was used for analysis of the variance of the grading data of disease per plant from each family in spring and autumn (α=0.05), calculating the DI means of families[17], and charting the column diagram of frequency distribution for DI grades in the permanent population.

QTL analysis was performed with WinQTLCart 2.5[18]

software, setting LOD≥2.5, composite interval mapping (CIM) and model 6, with a walking speed of 1 cM and background control parameters as follows: Control marker number, 15; Window size, 3 cM; and Regression method, positive.

The QTLs were named '*pm*' for powdery mildew, followed by two digits (separated by a dot), which represent the located linkage group (LG) number and a QTL number in the same group, respectively.

2 Results

2.1 Analysis of susceptibility to powdery mildew

The artificial inoculation results showed that S06 is a PM resistance line and exhibited average DI=11.5 and 7.5 in spring and autumn, respectively, compared with 79.0 and 87.0 for S94 which is a susceptible line. The DI of the F_1 was 57.5 in spring and 48.2 in autumn, which qualifies it as susceptible (Figure 1). The DI of the $F_{6:7}$ family showed a continuous distribution from resistant to susceptible phenotypes, without showing any typical segregation pattern according to the W statistics of Shapiro-Wilk (P>0.05) . Transgressive lines with more resistance and susceptibility than the parents were observed. These results indicated PM resistance as a typical quantitative trait fit for QTL analysis. An image of PM resistance and susceptibility in cucumber seedlings is shown (Figure 2).

ANOVA results showed that genotypic variation was highly significant for DI over a period of 2 years. The effects of environment, and genotype and environment interaction were also significant for DI over the same period (Table 1).

2.2 Resistance QTL analysis

(i) Autumn (2005): A total of four PM resistance QTLs (*pm1.1*, *pm2.1*, *pm4.1* and *pm6.1*) were identified in 2005 autumn (Table 2 and Figure 3), which were located on the LGs 1, 2, 4 and 6, respectively. The *pm1.1* on LG1 which was mapped between markers ME2EM4a (SRAP) and c162 (SSR) explained 7.0% of the observed phenotypic variation. Another QTL (*pm2.1*) on LG2 positioned between the markers CSEPGN11 (SSR) and F explained 15.3% of the observed phenotypic variation. The third QTL (*pm4.1*) was located on LG4 and bordered by SRAP markers e23m18f and ME11EM9c, with an R^2 value of 20.5%. The fourth QTL was located on LG6 and bordered by SCAR marker S_BC526_2 and SRAP marker ME11EM4f with an R^2 value of 9.2%. The total QTLs explained 52.0% of the phenotypic variation in the $F_{6:7}$ population.

(ii) Spring (2006): In the analysis of the $F_{6:7}$ families grown in spring, three QTLs were detected, and mapped to LG1, LG2 and LG4, respectively. In autumn (2005) and spring (2006), there were three QTLs (on LG1, LG2 and LG4) positioned in the same intervals, with different R^2 values. So, the three QTLs detected in the spring (2006) were given the same names: *pm1.1*, *pm2.1* and *pm4.1*. The QTL *pm4.1* had the largest effect on powdery mildew resistance among all QTLs, and explained 21.0% of the observed phenotypic variation with LOD=7.2. The *pm1.1 and pm2.1* had moderation associated R^2 values, explaining 5.2% and 15.8% of the

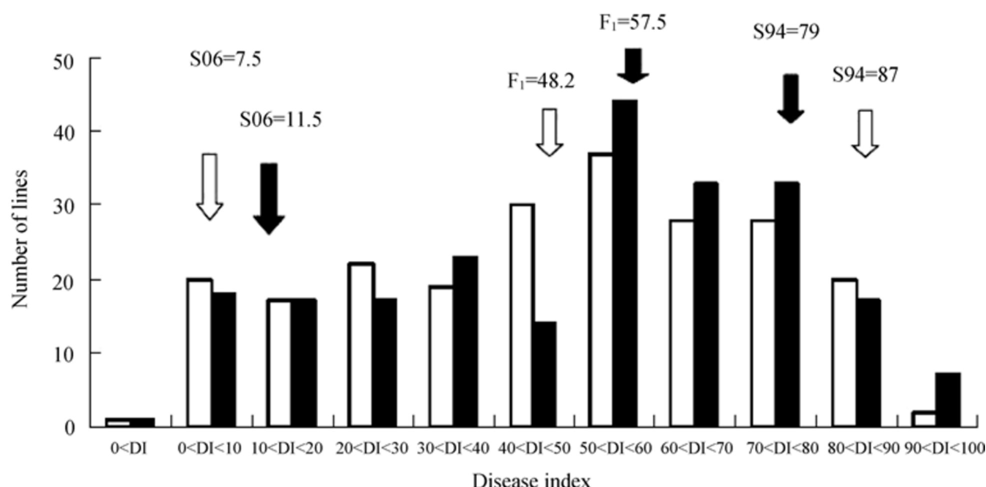

Figure 1 Frequency distribution of cucumber plant resistance to powdery mildew in $F_{6:7}$ families. The 224 $F_{6:7}$ families were obtained from the combination S94×S06. *X*-axis: disease index (DI); *Y*- axis: the number of $F_{6:7}$ lines.

Figure 2 An image of powdery mildew resistance and susceptibility in cucumber seedlings. Left: susceptible parent (S94, disease index=80); Right: resistant parent (S06, disease index=0); Middle: susceptible (F_1, disease index=15).

observed phenotypic variation, respectively. The total phenotypic variation explained by the three putative QTLs was 42.0% among the $F_{6:7}$ lines.

As stated above, three QTLs on LG1, LG2 and LG4 were consistent in the two seasons, in that they contributed similarly to phenotypic variation and additive effect direction between seasons.

Table 1 Analysis of the variance of the disease index of $F_{6:7}$ families for cucumber powdery mildew resistance in autumn (2005) and spring (2006)

Source of variation	DF	MS	*F*	*P* value
Family (genotype)	223	754.55	20.87	<0.01
Season (environment)	1	9877.64	273.24	<0.01
Genotype×environment	223	1677.05	43.63	<0.01
Error	447	36.15		

Table 2 QTL analysis of cucumber powdery mildew resistance in autumn (2005) and spring (2006)

Season	QTL [a)]	LG	LOD	Marker interval	R^2 (%)	Additive effect
Autumn(2005)	*pm1.1*	1	2.5	ME2EM4a-c162	7.0	−3.38
	pm2.1	2	3.7	F-CSEPGN11	15.3	3.82
	pm4.1	4	7.8	e23m18f-ME11EM9c	20.5	−3.30
	pm6.1	6	2.6	S_BC526_2-ME11EM4f	9.2	−1.59
Spring(2006)	*pm1.1*	1	2.5	ME2EM4a-c162	5.2	−2.74
	pm2.1	2	3.5	F-CSEPGN11	15.8	2.89
	pm4.1	4	7.2	e23m18f-ME11EM9c	21.0	−2.90

a) QTL name: Abbreviation of trait name + number of linkage group + number of locus.

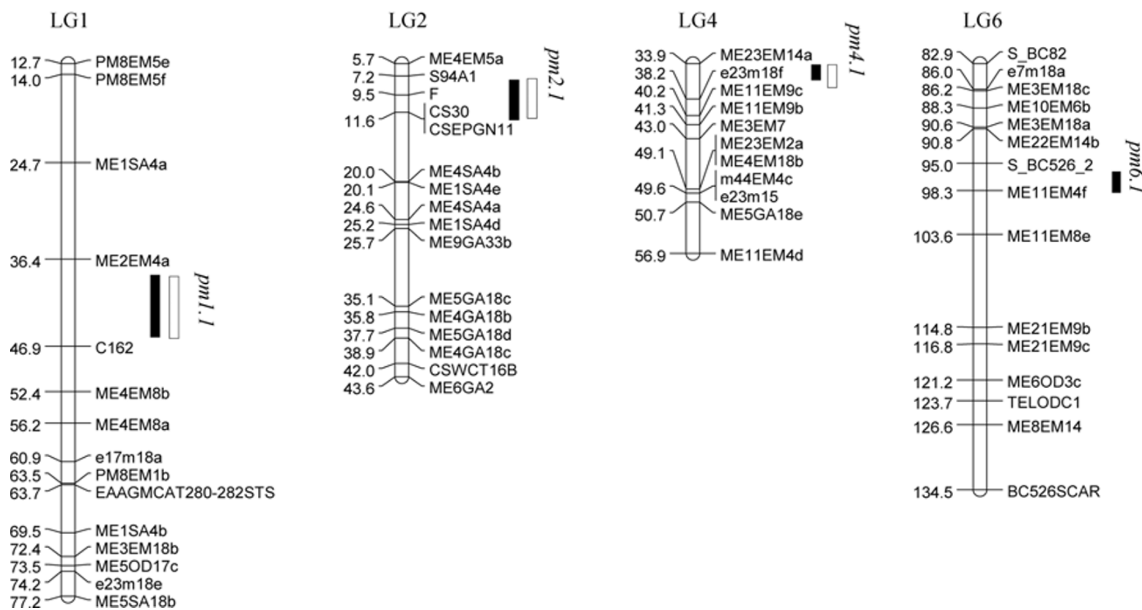

Figure 3 Genetic linkage map of cucumber based on 224 $F_{6:7}$ lines derived from crossing S94&S06. Only linkage groups (LG) holding the quantitative trait loci (QTL) are shown. ▮ and ▯ showed the QTLs detected in autumn (2005) and spring (2006), respectively. For QTL names, refer to Table 2.

3 Discussion

The spread and occurrence of PM can be affected by pathogenic races, the development stages of the plant, the genotype of plant, and complicated environmental factors[19,20]. The cucumber PM pathogen spores may spread with wind and airstream, which increases the complexity of the PM pathogen in a region. The PM pathogen of cucumber cannot be cultivated on the culture medium because of its characteristic of strong autoeciousness. Due to the multiple factors above, the pace of detailed PM resistance studies in cucumber is slow. In this study, the isolated single spore progenitive pathogen was used in seedling spray inoculation and in addition, plenty of colonies and many repetitions were included in the design in order to reduce sampling errors, to allow easy checking of the exchange between the tight loci and to improve locate veracity. We detected 4 QTLs, of which three QTLs were consistent between two seasons; the *pm2.1* explained more than 10% of the phenotypic variation, and the effect of *pm4.1* was large and stable, i.e., more than 15% of the phenotypic variation. Therefore, it is plausible that cucumber resistance to PM is a quantitative trait regulated by at least 4 loci; and the major gene for PM resistance can be determined. According to the total phenotypic variation explained in autumn/2005 and spring/2006, the minor-gene effect for PM resistance cannot be removed from the equation. The positive and negative signs of the additive gene effect at the loci indicate the contribution of both two parental lines (Table 3), which is consistent with many reports[21–23].

Until now, only Sakata et al.[9] reported five QTLs for the resistance of cucumber seedlings to PM on linkage groups 1, 2, 3 and 4. However, the lack of SSR markers and common linkage group nomenclature in their map makes it difficult to compare the location of QTLs detected in our study with those detected by Sakata et al.[9]. Only two markers (c162，EAAGMCAT280- 282STS) linked to the resistance were mapped to the LG1 of the cucumber map of this study. The reason for this may be that there is an essential difference in the resistant materials of cucumber and pathogen races of PM. However, the QTL (*pm1.1*) was positioned in the vicinity of SSR marker c162 (space=0.1 cM). Thus, we speculated that *pm1.1* may be allelic to the QTL (peak marker: EAACMCAC391-395STS) reported by Sakata et al.[9]. If possible, an exchange of the pathogen or genetic population with Sakata's lab would provide the advantage of comparing the QTLs of PM resistance reported and thereby strengthen the analysis of cucumber PM resistance.

Many studies of disease resistance have showed that resistance genes on LGs have a tendency toward cluster distribution[24–26]. Grumet et al.[27] reported that the resistance gene to the watermelon strain of the papaya ringspot virus in cucumber was co-segregated with other potyvirus resistance genes. Perchepied et al. discovered that among the QTLs of melon powdery mildew and downy mildew resistances on RIL molecular marker mapping ("PI 124112/Védrantais"), some of the former conferred resistance to several races and some were overlap positioned with the latter on LG XII[28]. According to the results of this study, the QTLs of cucumber downy mildew resistance on the RIL ("S94/S06") molecular marker map were identified in LG1 and LG6, respectively[15]. A QTL (*dm6.1*) on LG6 was positioned between ME3EM18a (SRAP) and ME22EM14b (SRAP). The *dm6.1* is 7.0 cM from the nearest PM resistance QTL (*pm6.1*). However, we could not find an overlap position between PM resistance QTLs and downy mildew resistance QTLs. In general, the studies of identifying and classifying PM pathogen races progress relatively slowly, which hinders the in-depth study of PM resistance in cucumber. This study may contribute to interrelated disease research in cucumber.

In this study, four QTLs of cucumber powdery mildew resistance were identified by $F_{6:7}$ families ("S94/S06"). Three QTLs were consistent between two seasons and each of them had contiguous markers. The c162 was 0.1 cM away from *pm1.1.* The *pm2.1* is 0.2 cM and 0.9 cM from the nearest two markers, F and CSEPGN11 (SCAR), respectively; *pm4.1* is 0.2 cM and 2.0 cM from the nearest two markers, e24m18f (SRAP) and ME11EM9c (SRAP). For the next step, the SRAP markers closely linked to the three QTLs should be transformed into sequence-specific markers in order to facilitate cucumber breeding for PM resistance by molecular marker-assisted selection[9,29-30].

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