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QTL Analysis for Resistance to Phytophthora Blight (*Phytophthora capsici* Leon.) Using an Intraspecific Doubled-Haploid Population of *Capsicum annuum*

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A doubled-haploid (DH) population (n = 176) obtained by anther culture of an F_1 hybrid between a line susceptible to *Phytophthora capsici* 'K9-11' (*Capsicum annuum* L.) and a line resistant to *P. capsici* 'AC2258' (*C. annuum* L.) was inoculated with *P. capsici*. QTL analysis of the resistance was performed using a linkage map consisting of 16 linkage groups (LGs), covering a total distance of 1100.5 cM. Three QTLs were detected on LG1, LG6 and LG7. The QTL with the highest LOD score, detected on LG7, explained 82.7% of the phenotypic variance with a LOD score of 67.02. This QTL was designated as *Phyt*-1. The nearest marker was an AFLP marker, M10E3-6. The second QTL, designated as *Phyt*-2, was found on LG1. It explained 6.4% of the phenotypic variance with a LOD score of 2.54. The nearest RAPD marker was RP13-1. The other QTL, designated as *Phyt*-3, which was found on LG6, explained 5.6% of the phenotypic variance with a LOD score of 2.20. The nearest AFLP marker was M9E3-11. It was confirmed that the lines with a high resistance could be efficiently selected by using two markers, M10E3-6 and RP13-1, simultaneously. The presence of both *Phyt*-1 and *Phyt*-2 under homozygous conditions may enable to breed resistant cultivars of sweet pepper. The molecular markers identified in the present study could be useful for marker-assisted selection (MAS) in order to breed sweet pepper cultivars with a high resistance to *P. capsici* using 'AC2258' as a source of resistance genes.

Key Words: sweet pepper, *Capsicum*, *Phytophthora capsici*, DNA marker, quantitative trait loci, inheritance.

Introduction

The genus *Capsicum* belongs to the *Solanaceae* family. This genus contains five species, i.e., *C. annuum*, *C. frutescens*, *C. baccatum*, *C. pubescens* and *C. chinense*. *C. annuum* is known as pepper, sweet pepper or paprika (*C. annuum* L. *var. angulosum* Miller or *var. grossum* Bailey), and is cultivated globally for use as spices, vegetables, colorants and medicinal products.

Phytophthora blight in sweet pepper is caused by *Phytophthora capsici* Leonian and is characterized by symptoms on the roots or the stems adjacent to the roots. The pathogen survives in a contaminated field for a long period of time, causing severe damage to *Capsicum* crops, and the disease occurs widely in sweet pepper cultivation areas in Japan. Since almost all the cultivars in sweet pepper are susceptible to this pathogen, the disease is being currently controlled mainly by the fungicide, methyl bromide. However, the use of this chemical will be strictly controlled in the near future because it disrupts the ozone layer. Thus, the use of resistant cultivars is an ideal strategy to overcome this disease.

Some pepper accessions have been reported to be resistant to *P. capsici* (Smith *et al*. 1967, Palloix *et al*. 1990, Bartual *et al*. 1991, Reifschneider *et al*. 1992). In particular, two wild accessions of *C. annuum*, 'AC2258' derived from PI 201234 from Central America and 'Criollo de Morelos 334' ('CM334') from Mexico display a high level of resistance to *P. capsici*. Regarding the mode of inheritance of the resistance to *P. capsici* in 'AC2258', Smith *et al*. (1967) reported that the resistance appears to be controlled by two distinct dominant genes without additive effect. In contrast, Yamakawa *et al*. (1979) reported that the resistance is considered to be controlled by a single gene with incomplete dominance. Since these two results are contradictory, it is important to determine the number of resistance genes, their location on the chromosomes, and their mode of inheritance. Although attempts have been made to breed resistant cultivars using these lines, resistant cultivars have not yet been

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developed using these accessions (Ogundiwin *et al*. 2005).

In the breeding process of disease-resistant cultivars, selection of a resistant genotype is based on the observation of the visual symptoms of the disease after inoculation of the pathogen. This procedure is rather simple and the results are clear in the case of some virus diseases. However, in the case of some soil-borne diseases, such as Phytophthora rot and Bacterial wilt, the procedure is labor-intensive and time-consuming. Moreover, various environmental factors (e.g. temperature and growth stage of the plants) may interfere with the attempts to clarify the relationship between genotype and resistance. In addition, the inoculation test may contaminate neighboring agricultural areas with the pathogen used. Therefore, the application of marker-assisted selection (MAS) (McCouch and Tanksley 1991), using appropriate molecular markers, is considered to be a suitable strategy for the breeding of sweet pepper.

In recent years, DNA markers have been used for the breeding of and for molecular genetics research in many plants. In the *Capsicum* species, several linkage maps have been reported (Prince *et al*. 1993, Lefebvre *et al*. 1995, Livingstone *et al*. 1999, Kang *et al*. 2001). Thus, DNA markers linked to various agronomic characters were developed (Caranta *et al*. 1999, Moury *et al*. 2000, Blum *et al*. 2002, Matsunaga *et al*. 2003, Sugita *et al*. 2004). These DNA markers are now available for MAS for some traits. Similarly, QTLs for resistance to *P. capsici* have also been reported (Lefebvre and Palloix 1996, Thabuis *et al*. 2003, Thabuis *et al*. 2004, Quirin *et al*. 2005, Ogundiwin *et al*. 2005). Thabuis *et al.* (2003) performed a QTL analysis on three intraspecific populations generated from the crossing of the resistant accessions 'Vania', 'Perennial' and 'CM334'. They identified a major resistance QTL on pepper chromosome 5, which is common to the three populations. Ogundiwin *et al*. (2005) detected 16 and 5 QTLs in two intraspecific populations, 94 recombinant inbred lines and 94 F_2 lines generated by the crossing of the resistant accessions, PI 201234 and 'CM334', respectively.

Recently, a high-efficiency genome scanning (HEGS) system and a modified detection system for amplified fragment length polymorphism (AFLP) have been developed (HEGS/AFLP; Kawasaki and Murakami 2000, Kawasaki *et al*. 2003). For this combined system, non-labeled primers are used, and genetic maps can be easily constructed with efficient cost-performance. Furthermore, a genetic linkage map of sweet pepper mainly using HEGS/AFLP on an intraspecific doubled haploid (DH) population derived from a cross between 'K9-11'and 'AC2258', was constructed by Sugita *et al*. (2005) in a short period of time. The rapid construction of the linkage map may be associated with high performance for QTL analysis.

Accordingly, three QTLs for resistance to *P. capsici* were detected in the resistant accession 'AC2258' when we performed QTL analysis with the linkage map mainly obtained by the use of the HEGS/AFLP system and based on the inoculation results of *P. capsici* to an intraspecific DH population. Moreover, we evaluated the use of the linkage DNA markers as selection markers in MAS.

Materials and Methods

Plant materials and construction of the linkage map

A DH population (n=176) was developed by anther culture of an F_1 hybrid between two accessions of *C. annuum*, 'K9-11' and 'AC2258'. 'K9-11' harbors the L^3 gene, which is resistant to pepper mild mottle virus (PMMoV) derived from PI 159236. This non-pungent bell-shaped sweet pepper is susceptible to *P. capsici* (Yanokuchi *et al*. 1993, Sugita *et al*. 2004). 'AC2258' is highly resistant to *P. capsici* and is pungent (Smith *et al*. 1967, Yamakawa *et al*. 1979, Palloix *et al*. 1990). The immature fruit of this line is light yellow. Anther culture was performed using the method described by Dumas de Vaulx *et al*. (1981) with a minor modification. In brief, buds 4 to 6 mm in size were incubated in the dark at 4°C for four days. The buds were sterilized with 70% (v/v) ethanol for 20 seconds, followed by 20 minutes in a 0.5% (v/v) sodium hypochlorite solution with 2 drops of Tween 20 per 200 ml. Anthers in the sterilized buds were inoculated on CP medium (Dumas de Vaulx *et al*. 1981) containing 0.01 mg/l 2,4-dichlorophenoxyacetic acid and 0.01 mg/l kinetin, and cultured in the dark at 35°C for eight days. Thereafter, they were cultured in the dark at 25°C for three weeks and then under light at 25°C. Plantlets were regenerated after two to four months without transplanting to another medium.

Genomic DNA was extracted from young leaf tissues using the CTAB method (Murray and Thompson 1980) and the Nucleon Phyotopure™ DNA extraction kit (Amersham LIFE SCIENCE, Buckinghamshire).

The DNA markers applied to the linkage map for QTL analysis were obtained mainly based on the HEGS/AFLP system. Linkage analysis was performed using 518 molecular markers consisting of 382 AFLP markers, 122 random amplified polymorphic DNA (RAPD) markers, 3 restriction fragment length polymorphism (RFLP) markers, 7 sequence-characterized amplified region (SCAR) markers, 4 cleaved amplified polymorphic sequence (CAPS) markers and 2 phenotypic traits (Sugita *et al*. 2005), as well as the L^3 locus and the *C* locus for the expression of pungency. The isolate of PMMoV (P1.2 pathotype) used in the present study was collected in Miyazaki Prefecture. Young leaves were excised and mechanically inoculated with the purified virus (20 ng/ml) by rubbing with #500 carborundum powder. The inoculated leaves were kept in a growth chamber under moist conditions at 25°C for 24 hrs under light. Local lesions developed on the leaves harboring the L^3 gene after three to six days. Pungency was evaluated by the organoleptic method, in which the placenta tissue of the fruits was tasted at 30 to 40 days after plant blooming. Linkage analysis revealed that many molecular markers were located at the same loci: 224 markers with clear and reproducible banding patterns were selected as framework markers. Moreover, 83 pairs of

PCR primers described by Lee *et al*. (2004) and Ogundiwin *et al*. (2005) and 62 pairs of microsatellite primers obtained from the database, were used in association with already published linkage maps. The map, with a total of 16 linkage groups (LGs) and covering a total distance of 1100.5 cM was used for QTL analysis.

Inoculation of Phytophthora capsici and evaluation of resistance

The *P. capsici* isolate 'Keihoku' used in the present study was provided by Kyoto Prefectural University. The *P. capsici* inoculum was prepared according to the method described by Bosland and Lindsey (1991) with a minor modification and inoculation was performed on a V8 medium in a 90 mm Petri dish. The dish was sealed with Parafilm and incubated in the dark at 25°C for ten days. Thereafter, the Parafilm was removed and the isolate was further incubated under light at 25°C for three days to promote zoosporangium formation. After incubation, 10 ml of distilled water was poured into the Petri dish and the zoosporangia were gently collected with a writing brush. The concentration of the zoosporangia was adjusted to 2.0×10^4/ml. Furthermore, the collected zoosporangia were incubated at 4°C for 0.5 hours and then at 25°C for 3 hours to release the zoospores. Ten milliliter of inoculum was poured onto the stumps of five plants three to four weeks after seeding. The plants were misted every three hours to promote infection. The number of surviving plants was counted after two weeks, and the percentage of surviving plants was calculated. These inoculation tests were performed five times in a glass house, and the mean survival rate was used as an index of resistance.

Data analysis

Linkage analysis was performed using the program MAPMAKER Ver. 3.0 (Lander *et al*. 1987) with a maximum recombination fraction of 25 cM and a minimum LOD score above 3.0. All the co-dominant markers were confirmed to correspond to a homozygous genotype at each locus in all the DH lines. QTL analysis was performed using the program MAPMAKER/QTL Ver. 1.1 (Lincoln *et al*. 1993) with a minimum LOD score above 2.0.

Results and Discussion

Evaluation of the phenotypic data

After inoculation with *P. capsici*, the resistant lines continued to grow, while the susceptible lines exhibited wilting symptoms on the leaves. Later, the stems began to rot, and then the plants died (Fig. 1). In five inoculation tests using the DH population, the rate of survival showed the same tendency as that of each DH line (Table 1). The frequency distribution of the mean survival rates in the five inoculation tests is presented in Figure 2. The survival rates for the parents in the five inoculation tests were 85% for 'AC2258' and 0% for 'K9-11'. A continuous distribution was observed for the survival rate, indicating that the resistance to *P. capsici* was polygenic. However, the survival rate tended to be distributed toward both ends, and 24% of all the lines were concentrated in the range of 10%. A small peak was identified in the range of 30%. Although in various reports the number of resistance genes to *P. capsici* in 'AC2258' had been determined (Smith *et al*. 1967, Yamakawa *et al*. 1979, Ogundiwin *et al*. 2005), the results suggested that the resistance was controlled by a single major gene with a large effect, and some minor genes.

QTL analysis

For the association with previously published linkage

Fig. 1. Symptoms of Phytophthora rot. The resistant lines (right) continued to grow, while the susceptible lines (left) developed wilting symptoms on the leaves. Later, the stems began to rot, and then the plants died.

Table 1. Frequency distribution of the survival rate in the five inoculation tests using a DH population of sweet pepper

Tests	Duration of seedling period (days)	Survival rate (%)		No. of DH lines						No. of DH lines examined	Mean survival rate (%)
		K9-11	AC2258	Survival rate (%)	0–20	21–40	41–60	61–80	81–100		
1st	27 days	0.0	100.0		49	18	13	37	58	175	58.5
2nd	27 days	0.0	100.0		61	11	16	16	68	172	55.6
3rd	24 days	0.0	75.0		61	9	18	24	64	176	55.6
4th	23 days	0.0	100.0		71	16	12	22	51	172	48.5
5th	20 days	0.0	50.0		81	14	16	24	37	172	40.9

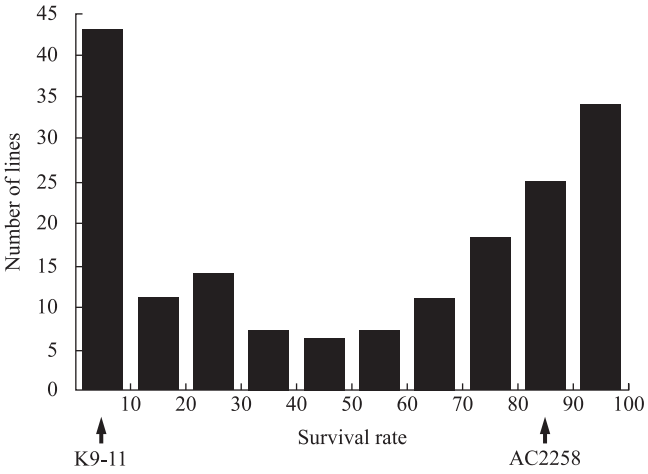

Fig. 2. Frequency distribution of the mean survival rate in the five inoculation tests using a DH population of sweet pepper. The vertical axis indicates the number of lines, and the horizontal axis indicates the survival rate.

maps, 76 pairs of microsatellite primers (Lee *et al*. 2004) and seven pairs of PCR primers (Ogundiwin *et al*. 2005) were used. Six SSR markers and two SCAR markers could be mapped (Table 2). Furthermore, 62 pairs of microsatellite primers designed from the sequencing data obtained from the database were used. Consequently, four markers could be mapped (Table 2). Some LGs could be assigned to the pepper chromosomes using these common markers and two morphological markers, L^3 for resistance to PMMoV and *C* for the pungency trait. LG1 was assigned to the first chromosome of pepper based on the presence of the markers, Hpms1-43 and CCS, while LG2 was assigned to the 12th chromosome based on the marker TG523. LG3 was assigned to the second chromosome based on the morphological marker *C* and LG6 was assigned to the 11th chromosome based on the morphological marker L^3. LG7 was assigned to the fifth chromosome based on the marker LCYB. LG9 was assigned to the ninth chromosome based on the marker $D11Scar_{650}$. LG11 was assigned to the eighth chromosome based on the marker HpmshpMADS. LG14 was also assigned to the eighth chromosome based on the markers TG510 and Hpms1-41. LG14 may merge to LG11 in the future, with the use of additional DNA markers. Thus far, the remaining LGs could not be assigned to the pepper chromosomes. They should be associated with those on the already published linkage maps by mapping additional common markers on our map.

As a result of the QTL analysis, three QTLs were detected on three LGs (Fig. 3 and Table 3). A QTL on LG7, designated as *Phyt*-1, explained 82.7% of the phenotypic variance. The peak position corresponded to a LOD score of 67.02. The nearest marker was M10E3-6, a dominant AFLP marker present in 'AC2258'. The second QTL, designated as *Phyt*-2, was located on LG1, which explained 6.4% of the phenotypic variance with a LOD score of 2.54. The nearest RAPD marker, RP13-1, was a dominant marker for the 'AC2258' allele. Another QTL, designated as *Phyt*-3, was located on LG6, which explained 5.6% of the phenotypic variance with a LOD score of 2.20. The nearest AFLP marker, M9E3-11, was a dominant marker present in 'AC2258' (Table 3). Ogundiwin *et al*. (2005) detected 16 QTLs from *Phyto.A* to *P* on the RILs derived from the same resistant parent as that we used. *Phyt*-1 detected on LG7 (pepper chromosome 5) appeared to correspond to *Phyto.P* detected by Ogundiwin *et al*. (2005), because it was located on the same chromosome. Moreover, it was assumed that *Phyt*-1 was the same QTL as *Phyto.U* (Ogundiwin *et al*. 2005) on the F_2 map derived from the resistant parent, 'CM334', and *Phyt.5* (*Phyt.5.1* and *Phyt.5.2*) (Thabuis *et al*. 2003, Thabuis *et al*. 2004, Quirin *et al*. 2005). *Phyt.5* was detected across three intraspecific populations derived from unrelated resistant parents. This QTL seemed to be stable across the *P. capsici* isolates, susceptible pepper genotypes, and different locations (Ogundiwin *et al*. 2005). Accordingly, this QTL is likely to be very useful in pepper breeding because it conferred a significant effective resistance to *P. capsici* isolates. Recently, Quirin *et al*. (2005) have developed a SCAR marker, OpD04, linked to *Phyt.5.2*. Although we attempted to apply the SCAR marker to our map, we could not locate it because neither parent was amplified. The difference

Table 2. Nucleotide sequences and information about the primers used in the present study

Locus	Forward primer	Reverse primer	Repeat	Reference
Hpms 1-41	5′-GGGTATCATCCGTTGAAAGTTAGG-3′	5′-CAAGAGGTATCACAACATGAGAGG-3′	(AT)6(GT)32	Lee *et al.* 2004
Hpms 1-43	5′-AACCAGCAATCCCATGAAAACC-3′	5′-GGGCTTTGGGGAGAATAGTGTG-3′	(GT)9T(TG)7	Lee *et al.* 2004
Hpms 2-24	5′-TCGTATTGGCTTGTGATTTACCG-3′	5′-TTGAATCGAATACCCGCAGGAG-3′	(CT)17(CA)5A21	Lee *et al.* 2004
Hpmshp MADS	5′-TGCTTTCAAAACAATTTGCATGG-4′	5′-GCGTCTAATGCAAAACACACATTAC-3′	(AT)17	Lee *et al.* 2004
AF242731	5′-GGGCTGACGGCCATTAAGAAC-3′	5′-CAGACAGCTAGAAAGAGAGGAATTCTG-3′	T18	Lee *et al.* 2004
SSR6	5′-TGGGAAGAGAAATTGTGAAAGC-3′	5′-AGACCCAATGTGGTCCAATC-3′	(CAT)n	Ogundiwin *et al.* 2005
D11Scar650	5′-AATCACACTGGGTTGTTGAC-3′	5′-CTGGATAAGATGGAAGAGGA-3′		Ogundiwin *et al.* 2005
Bs2	5′-TGCCTGGGCTACCATATCTC-3′	5′-ACAGATCCACTTGGGCAATC-3′		Ogundiwin *et al.* 2005
PM12	5′-GCAGAAGCCATAATTGGCTG-3′	5′-GGAGTTAACTCAAAGGTTGC-3′	(ATT)12	BM067867
PM18	5′-CGACAGTCTTTCAAGAACTAGA-3′	5′-AGTGGAGCAAACACAGCAGA-3′	(AG)11	CA516439
PM37	5′-CGGAAACTAAACACACTTTCTC-3′	5′-CGGTTCCGGCAACGGCTATT-3′	(CA)9	CA525390
PM53	5′-CGCGCCAGTTCAACTTCCGA-3′	5′-GCAGCAAAGTCTACAACCTCAG-3′	(AGA)7	CA847557

between the resistance genes in 'CM334' and 'AC2258' is interesting. *Phyt*-2 detected on LG1 (pepper chromosome 1) seemed to correspond to the QTLs *Phyto.D*, *Phyto.E* and *Phyto.F* on the RILs map. However, no QTL corresponded to it on the F_2 map derived from the resistant parent 'CM334' constructed by Ogundiwin *et al*. (2005) and on the maps reported by Thabuis *et al*. (2003). This QTL might be specific to 'AC2258'. *Phyt*-3 detected on LG6 (pepper chromosome 11) seemed to correspond to the QTLs *Phyto.N*, *Phyto.O* or *Phyto.T*, on the RILs and F_2 map reported by Ogundiwin *et al*. (2005). This QTL was found to be linked to the *L* locus. On the maps reported by Thabuis *et al*. (2003), it was estimated to correspond to *Phyt.11.1*, originating from the susceptible parent.

The effectiveness of the present linkage markers for MAS was further analyzed. The DH lines harboring 'AC2258' alleles at all the three DNA marker loci were highly resistant to the pathogen. Similarly, the lines harboring the 'K9-11' allele only at the M9E3-11 marker locus exhibited a high resistance (Fig. 4-A and 4-B). In contrast, the lines harboring the 'K9-11' allele only at the RP13-1 marker locus were resistant but showed a peak at 80% (Fig. 4-C). The lines with the other combinations of alleles at the three loci showed a susceptibility or intermediate resistance (Fig. 4-D to 4-H). Clearly, the lines harboring three resistance genes with 'AC2258' alleles exhibited a very high resistance, and those with the two markers, M10E3-6 and RP13-1, displayed a similar level of resistance. Although the QTL *Phyt*-3 was found around the marker M9E3-11, the effect of this QTL may be rather small. Thus, we limited our analysis to the two linkage markers, M10E3-6 and RP13-1. The lines harboring the 'AC2258' allele at the M10E3-6 marker locus showed a resistance above 50% (Fig. 5-A). The lines harboring the 'AC2258' allele at the RP13-1 marker locus showed a continuous and bimodal distribution, with peaks at 30% and 100% (Fig. 5-B). The DH lines with the

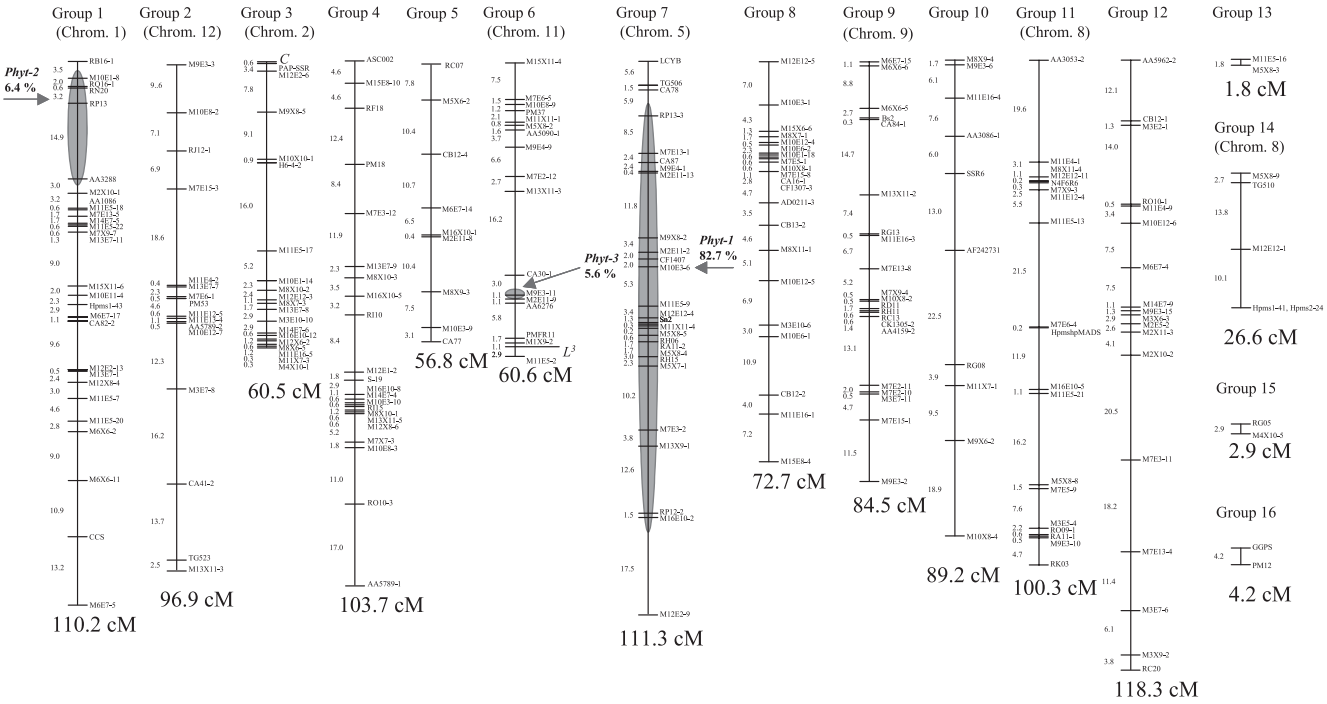

Fig. 3. QTLs for resistance to *P. capsici* using an intraspecific DH population of sweet pepper. Map distances in centiMorgans (cM) calculated by the Kosambi function are denoted on the left side of each linkage group. The portion enclosed with a circle on the linkage groups indicates the putative region of the QTLs with a LOD score of 2.0.

Table 3. Effect of QTLs for resistance to *Phytophthora capsici* detected in the DH population derived from a cross between 'AC2258' and 'K9-11'

LG	Flanking marker[1)]	Survival rate of the lines		LOD	Var. Exp. (%)[2)]	Direction[3)]
		'K9-11' alleles (n)	'AC2258'alleles (n)			
1	RP13-1	60.6 (91)	41.2 (83)	2.54	6.4	AC2258
6	M9E3-11	61.7 (76)	44.2 (100)	2.20	5.6	AC2258
7	M10E3-6	83.9 (89)	17.0 (85)	67.02	82.7	AC2258

1) The closest marker to the QTL.

2) Percentage of phenotypic variance explained.

3) Indicates the parent that contributes to increased resistance.

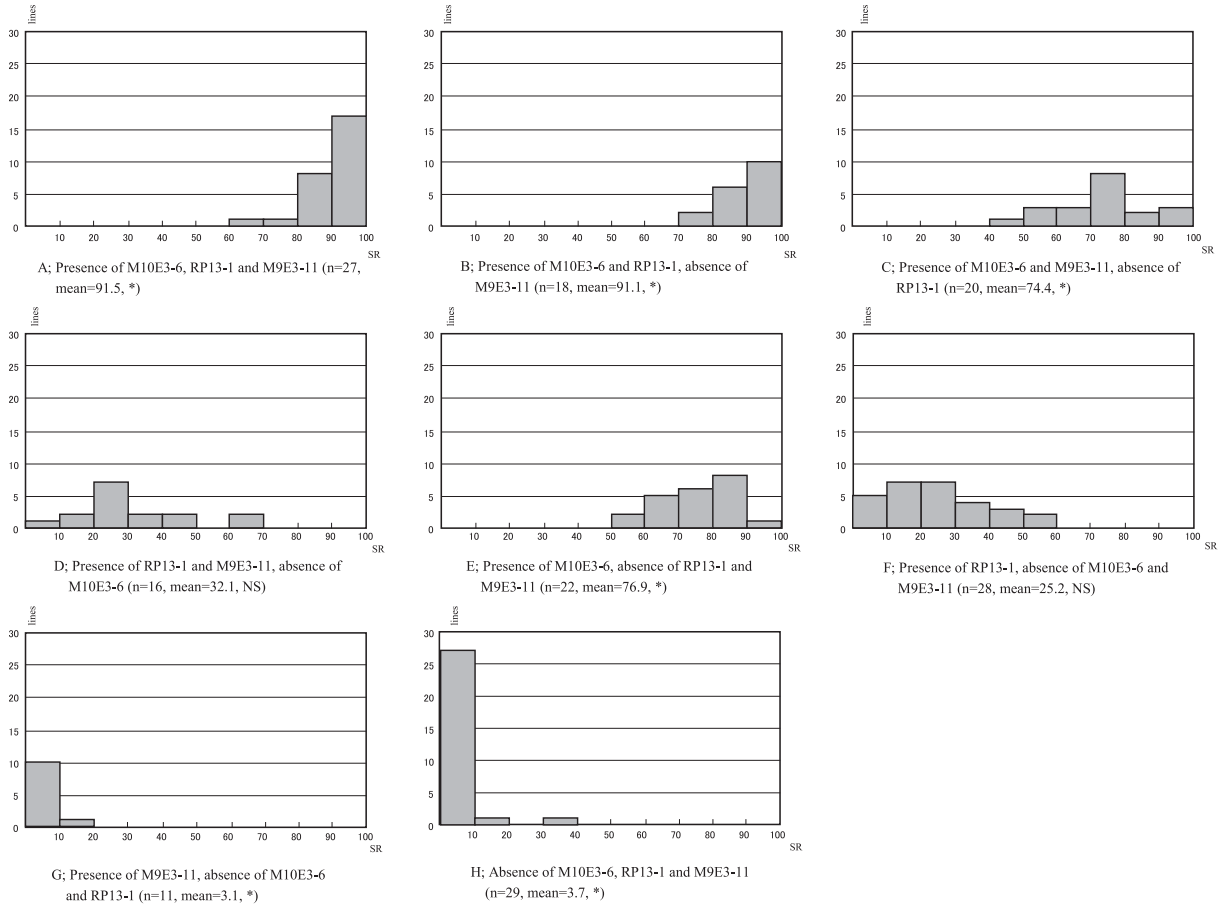

Fig. 4. Relationship between the genotype of the marker locus and the survival rate of the plants for three markers. The vertical axis indicates the number of lines, and the horizontal axis indicates the percentage of surviving plants. M10E3-6 was linked to *Phyt*-1, RP-13-1 was linked to *Phyt*-2, and M9E3-11 was linked to *Phyt*-3. They were the dominant markers present in the resistant parent 'AC2258'. NS, *: Non-significant or significant at $P < 0.05$ by Steel's test.

'AC2258' alleles at two DNA marker loci displayed a high resistance (Fig. 5-C). The lines with the 'AC2258' allele at the M10E3-6 marker locus and the 'K9-11' allele at the RP13-1 marker locus exhibited a distribution with a peak at 80% (Fig. 5-D). The lines with the 'K9-11' allele at the M10E3-6 marker locus and the 'AC2258' allele at the RP13-1 marker locus showed a distribution with a peak at around 30% (Fig. 5-E). The DH lines with the 'K9-11' alleles at two DNA marker loci were susceptible (Fig. 5-F). These results suggest that the resistance of the lines with both markers was higher than that of the lines with two independent QTLs, indicating that the presence of both *Phyt*-1 and *Phyt*-2 under homozygous conditions may enable to breed resistant cultivars of sweet pepper.

To determine the precise location of *Phyt*-1, we further analyzed the genotype at the marker locus and the resistance of the DH lines that exhibited recombination around *Phyt*-1. Marker CF1407 was located at a distance of 2.0 cM from M10E3-6, and M11E5-9 was located at a distance of 5.3 cM from M10E3-6 (Fig. 6). Figure 5 clearly indicates that the DH lines with the 'AC2258' allele at the M10E3-6 locus were resistant. However, in the DH lines with the 'AC2258' allele at the CF1407 or M11E5-9 locus, clear resistance could not be detected. It was assumed that the resistance gene might be located at a distance in the range of 7.3 cM between CF1407 and M11E5-9.

Although the resistant materials for Phytophthora rot, such as 'CM334' and 'AC2258', have been well documented (Smith *et al*. 1967, Yamakawa *et al*. 1979, Palloix *et al*. 1990), apparently no resistant cultivars have been developed using these lines. It may be difficult to introduce resistance QTLs by a conventional breeding method. Recently, however, QTL analysis using statistical analysis and the linkage map constructed using numerous molecular markers have provided a great deal of useful genetic information. DNA markers linked to various desirable agronomic characters have now been identified to promote MAS. Thus, MAS is a most efficient system for the implementation of plant breeding programs aimed at introducing QTLs that could not have been selected by conventional methods. In previous classic studies related to the mode of inheritance of *P. capsici* resistance in 'AC2258', it was reported that the resistance appears to be controlled by two distinct dominant genes (Smith *et al*. 1967) or by a single gene with incomplete dominance

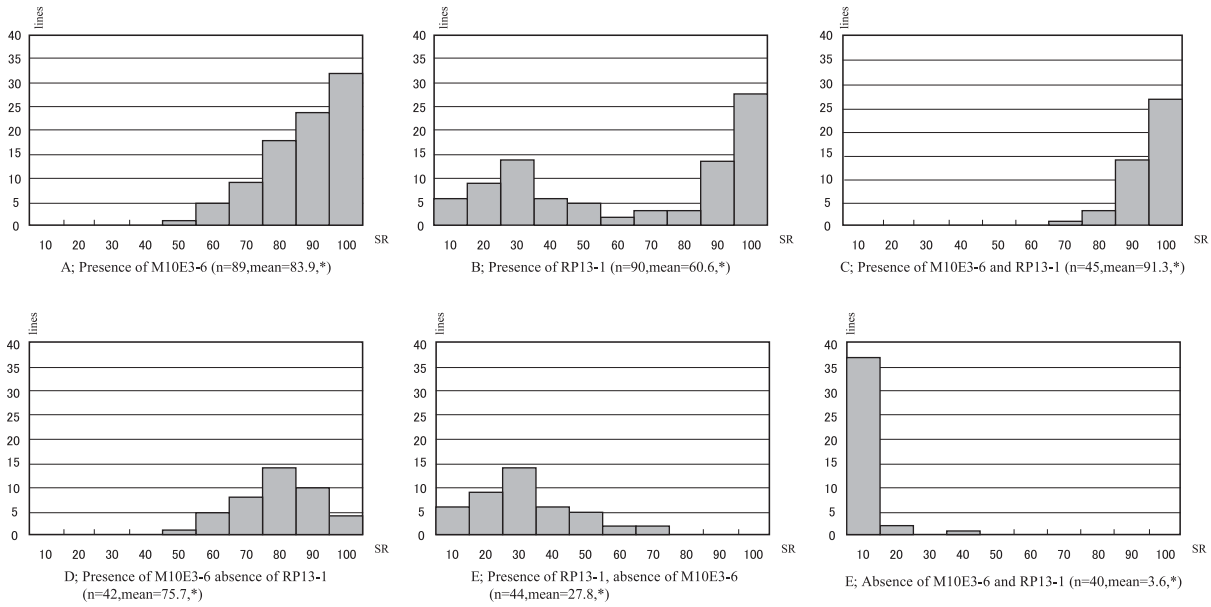

Fig. 5. Relationship between the genotype of the marker locus and the survival rate of the plants for two markers. The vertical axis indicates the number of lines, and the horizontal axis indicates the percentage of surviving plants. M10E3-6 was linked to *Phyt*-1, and RP-13-1 was linked to *Phyt*-2. They were the dominant markers present in the resistant parent 'AC2258'. *: significant at $P < 0.05$ by Steel's test.

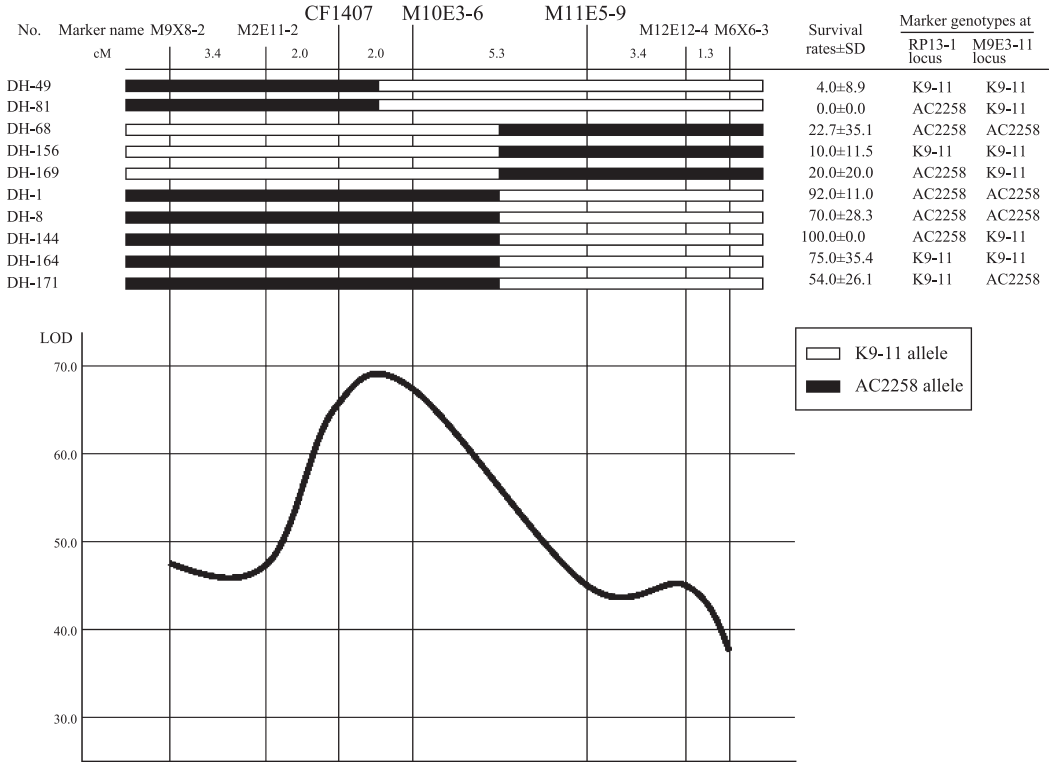

Fig. 6. Graphical genotype of the DH lines around *Phyt*-1. The upper figure indicates the graphical genotypes and the survival rates of ten DH lines that exhibited recombination between CF1407 and M11E5-9. The lower figure indicates the LOD score around *Phyt*-1.

(Yamakawa *et al*. 1979). In a recent study, Ogundiwin *et al*. (2005) detected 16 QTLs from *Phyto.A* to *P* on 97 RILs derived from PI 201234, the same resistant parent as that we used. Thabuis *et al.* (2003) detected the major resistance QTL common to the three intraspecific populations on pepper chromosome 5. In the present study, we detected at least

three QTLs: one which displayed a large effect was located on pepper chromosome 5, and two which displayed minor effects were located on pepper chromosomes 1 and 11. However, the effect of the QTL *Phyt*-3 located on pepper chromosome 11 may be rather small, suggesting that the presence of only two of the three QTLs (*Phyt*-1 and *Phyt*-2) in a single cultivar was associated with acceptable resistance.

The present markers linked to the resistance QTLs consisted of dominant AFLP markers and of one RAPD marker. In order to use these markers in MAS, they should be converted to PCR-based specific markers by cloning and sequencing. Moreover, we should locate more common markers such as RFLP or SSR on our map, for comparison to other published maps.

Agronomic traits were not mapped in the present population. 'AC2258' is a pungent wild accession that produces small immature fruits weighting around 10 grams. In contrast, 'K9-11' as the other parent, produces large immature fruits weighting around 40 grams and exhibits some useful traits for agriculture as sweet pepper cultivar. The DH population used in the present study is suitable for mapping these agronomic traits. In future studies, we plan to identify loci or QTLs for agronomic traits using this population. Furthermore, some resistant DH lines that showed a recombination between the markers located near the *Phyt*-1 locus were obtained in the present study. Hereafter, we plan to investigate the relationship between a resistance QTL and undesirable traits, and further promote the MAS programs using these resistant lines.

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