Plant Breeding 127, 154—159 (2008)
© 2007 The Authors
Journal compilation © 2007 Blackwell Verlag, Berlin
doi:10.1111/j.1439-0523.2007.01440.x

Identification of SSR markers linked to the *Phytophthora* resistance gene *Rps1*-d in soybean

T. Sugimoto, S. Yoshida, K. Watanabe, M. Aino, T. Kanto, K. Maekawa and K. Irie

Hyogo Agricultural Institute for Agriculture, Forestry and Fisheries, 1533 Minamino-oka, Kasai City, Hyogo Prefecture 679-0198, Japan, E-mail: Takuma_Sugimoto@pref.hyogo.jp

With 2 figures and 4 tables

Received March 20, 2007/Accepted June 18, 2007
Communicated by W. E. Weber

Abstract

To identify markers for the *Phytophthora* resistance gene, *Rps1*-d, 123 $F_{2:3}$ families were produced from a cross between *Glycine max* (L.) Merr. 'Tanbakuro' (a Japanese traditional black soybean) and PI103091 (*Rps1*-d) as an experimental population. The results of virulence tests produced 33 homozygous resistant, 61 segregating and 29 homozygous susceptible $F_{2:3}$ families. The chi-squared test gave a goodness-of-fit for the expected ratio of 1 : 2 : 1 for resistant, segregating and susceptible traits, suggesting that the inheritance of *Rps1*-d is controlled by a monogenic dominant gene. Simple sequence repeat (SSR) analyses of this trait were carried out using the cultivars 'Tanbakuro' and PI103091. Sixteen SSR primers, which produced 19 polymorphic fragments between the two parents, were identified from 41 SSR primers in MLG N. Eight SSR markers were related to *Rps1*-d, based on 32 of the 123 $F_{2:3}$ families, consisting of 16 homozygous resistant and 16 homozygous susceptible lines. The remaining 91 families were analysed for these eight markers, and a linkage map was constructed using all 123 $F_{2:3}$ families. The length of this linkage group is 44.0 cM. The closest markers, Sat_186 and Satt152, are mapped at 5.7 cM and 11.5 cM, respectively, on either side of the *Rps1*-d gene. Three-way contingency table analysis indicates that dual-marker-assisted selection using these two flanking markers would be efficient.

Key words: *Glycine max* — *Phytophthora sojae* — marker-assisted selection — *Rps* gene — simple sequence repeat analysis

'Tanbakuro' [*Glycine max* (L.) Merr. cv.], one of the most famous commercial and traditional black soybean cultivars, is produced in Hyogo (Western Japan). Within this region, 'Tanbakuro' was grown on 1150 ha in 2003, 34% of the total production in Japan (Sugimoto et al. 2006). This soybean cultivar fetches a higher market price than other soybean lines in Japan, because its seeds are much larger, weighing about 80–85 g per 100 seeds. Black soybeans are reported to have many positive effects on the human body and on health (Kohama et al. 2005, Takahashi et al. 2005). Despite these desirable characteristics, 'Tanbakuro' is still susceptible to many pathogens (Sugimoto et al. 2006).

One of the most important diseases of soybeans, Phytophthora stem rot [*G. max* (L.) Merr.] is caused by *Phytophthora sojae*. It was first noted in Indiana in 1951 and in Ohio (Kaufmann and Gerdemann 1958). When soybeans are infected, the stem of the plant appears to be water-soaked and turns brown, and this may result in the wilting and the death of plants. In Japan, this disease was first observed in 1977 in Hokkaido, the northern island of Japan (Tsuchiya et al. 1978), and it then spread to other parts of Japan (Sugimoto et al. 2006). Furthermore, this disease remains a serious problem in other soybean-producing areas, such as Argentina, Australia, Brazil, Canada, China, Hungary, Italy, the former Soviet Union and the USA (Schmitthenner 1999). Disease management strategies require immediate attention and implementation.

Although this disease has been controlled with fungicides (Anderson and Buzzell 1982), calcium application (Sugimoto et al. 2005, 2007), partial resistance (Dorrance et al. 2003), soil drainage (Schmitthenner 1985) and tillage practices (Workneh et al. 1998) for over 40 years, the most effective method to reduce the damage would be to develop resistant and tolerant cultivars of soybeans (Schmitthenner 1999). Breeding cultivars to combine *Phytophthora* resistance with the desirable features of 'Tanbakuro' is important for minimizing losses from this disease and, simultaneously, to increase farmers' income. However, the conventional breeding method that is commonly used worldwide to produce new soybean cultivars involves screening for seedlings carrying the *Phytophthora* resistance gene and can result in time-consuming and costly phenotypic screening programmes (Young 1999). Therefore, molecular markers for marker-assisted selection (MAS) would obviously be of value.

Phytophthora resistance genes have been studied by many researchers and 14 *Rps* genes at eight genomic loci have been reported: *Rps1* (Bernard et al. 1957), *Rps2* (Kilen et al. 1974), *Rps3* (Mueller et al. 1978), *Rps4* (Athow et al. 1980), *Rps5* (Buzzell and Anderson 1981), *Rps6* (Athow and Laviolette 1982), *Rps7* (Anderson and Buzzell 1992) and *Rps8* (Sandhu et al. 2005, Gordon et al. 2006). *Rps1* and *Rps3* contain five (*Rps1*-a, *1*-b, *1*-c, *1*-d and *1*-k) and three (*Rps3*-a, *3*-b and *3*-c) functional alleles, and have been mapped to molecular linkage groups (MLG) N and F, respectively. *Rps2*, *Rps4*, *Rps5*, *Rps6*, *Rps7* and *Rps8* have been mapped to MLG J, G, G, G, N and F, respectively. *Rps4* and *Rps6* are most probably allelic or very tightly linked genes (Sandhu et al. 2004). A new integrated genetic linkage map of the soybean has been constructed using simple sequence repeat (SSR), restriction fragment length polymorphism, random amplified polymorphic DNA, amplified fragment length polymorphism (AFLP) markers, classical traits and isozymes (Diers et al. 1992, Cregan 2003, Song et al. 2004). This information allows researchers to detect molecular markers for MAS.

Sugimoto et al. (2006) determined the race distribution of *P. sojae* in Hyogo, Japan. A parental line (PI103091) carrying

the resistance gene *Rps1*-d (Buzzell and Anderson 1992) to *P. sojae* was selected for breeding new cultivars. There have been no published reports on DNA markers linked to the *Rps1*-d gene. Demirbas et al. (2001) failed to find molecular markers linked to *Rps1*-d, because monogenic segregation was not verifiable in *Rps1*-d populations derived from a cross between 'Williams' (*rps*) and 'L93-3312' (*Rps1*-d). It is therefore important to investigate DNA markers linked to *Rps1*-d for the efficient breeding of new resistant cultivars with the desirable characteristics of 'Tanbakuro' for the Hyogo region. The objectives of this study were to confirm single-gene segregation of *Rps1*-d, and to identify SSR markers linked to the *Rps1*-d gene.

Materials and Methods

Plant materials: One F_1 soybean plant was produced from a cross between *G. max* (L.) Merr. 'Tanbakuro' (Japanese black soybean) and PI103091 carrying *Rps1*-d (Buzzell and Anderson 1992). The F_1 plant was selfed to produce a population of 123 F_2 plants. The 123 F_2 plants were then selfed and threshed individually to produce $F_{2:3}$ seed progeny as the experimental population.

Pathogens used in this study: A total of 51 isolates of *P. sojae* were identified from 51 infected soybean plants obtained from several soybean-producing fields in Japan from 2002 to 2004. These isolates were tested using the virulence test on *G. max* 'Tanbakuro' and PI103091. Virulence evaluations of isolates were performed using the agar medium inoculation method, and conducted in a test bottle (width = 8 cm, height = 20 cm) using mycelia and not zoospores (Sugimoto et al. 2006, 2007). PI103091 was strongly resistant, but 'Tanbakuro' was susceptible to all 51 isolates. Two isolates, PJ-H30 (race E) and PJ-H42 (race A), showing strong virulence on 'Tanbakuro' (98.8%, PJ-H30 and 98.0%, PJ-H42), were chosen for further studies (Sugimoto et al. 2006). Soybean plants were therefore inoculated with PJ-H30 or PJ-H42.

***Phytophthora* virulence tests:** *Glycine max* 'Tanbakuro' and 'Williams' (*rps*) were used as susceptible control plants during virulence tests. The two cultivars are homozygous *rps1-d/rps1-d* at the *Rps1*-d allele. PI103091, containing the *Rps1-d* gene, was also tested using the virulence test as a resistant control. More than 20 individual F_3 seedlings per $F_{2:3}$ family were employed for the virulence test. The $F_{2:3}$ phenotypes were determined by the hypocotyl inoculation method (Laviolette and Athow 1981) modified by Sugimoto et al. (2003, 2006). Soybean seed surfaces were first sterilized with 0.7% NaOCl for seven min, rinsed three times with 100 ml of sterilized distilled water, placed on autoclaved 0.7% agar medium (130 ml total volume) containing 1.0% (w/v) sugar in a test bottle, and incubated at 23°C. After the first primary leaf appeared, approximately 10 days after sowing the seeds, the stem of the soybean near ground level was covered with two 3 mm in diameter plugs of 20-day-old mycelium of isolate PJ-H30 cultured on potato dextrose agar. Thereafter, the plants were incubated in a growth chamber at 23°C with a 16-h day length under fluorescent light (light intensity: 150 $\mu E/m^2/s$). About 10 days after inoculation, the number of dead or live plants in each bottle was recorded. Each $F_{2:3}$ family was scored as homozygous resistant (R), segregating (Rs), or homozygous susceptible (S) according to the method of Gordon et al. (2006). If all seedlings were considered homozygous resistant, then another 20 seedlings per family were used for inoculation to confirm the virulence results. Bioassays were repeated three times, and only reproducible data were used for phenotypic assay. The segregation ratios of several $F_{2:3}$ families were evaluated with the chi-squared test. Additionally, 60 of 123 $F_{2:3}$ families were inoculated with PJ-H42 (race A) to confirm single-gene segregation.

DNA samples: DNA was extracted using a modified CTAB method (Rowland and Nguyen 1993) to remove polysaccharides efficiently. The procedure was scaled down for the isolation of DNA from 200 mg fresh weight of plant tissue. Leaf tissue was sampled in the field from each parent and all 123 F_2 plants. Pre-expanded leaves were ground into fine powder using liquid nitrogen. The powder was transferred to a 1.5 ml Eppendorf tube and 400 µl of a buffer [75 mM Tris-HCl, pH 8.0, 15 mM EDTA, 1.05 mM NaCl, 0.75% polyvinyl pyrrolidone (Katayama Chemicals, Osaka, Japan), 1.5% CTAB and 1.5% mercaptoethanol] was added and mixed at 60°C for 25 min. Chloroform (700 µl) was added and mixed at room temperature for 15 min, and then centrifuged at 15 000 ***g*** for 20 min. The aqueous phase was used for DNA extraction. The DNA pellet was dissolved in 50 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNA samples were adjusted to 10 ng/µl for PCR analysis.

SSR analysis and linkage analysis: A total of 41 SSR primers in MLG N were used for PCRs. Primer sequences for developing SSR markers were obtained from the SoyBase internet site (http://soybase.agron.iastate.edu/resources/ssr.php). PCR amplifications were carried out in 20 µl reaction mixtures containing 20 ng genomic DNA, 1x PCR buffer (AmpliTaq Gold), 2.5 mM $MgCl_2$, 200 µM each of four dNTPs, 0.5 units AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA), and 1.5 pmol of each primer. The reaction was performed by predenaturing at 95°C for 9 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 47°C for 30 s, extension at 67°C for 1 min and with final extension at 72°C for 10 min. Amplification products were mixed with 10 µl of loading dye (95% formamide, 10 mM NaOH, bromophenol blue and xylene cyanol), heated for 5 min at 95°C and chilled on ice for 5 min. From this mixture, 12 µl were separated by electrophoresis on an 8.0% TBE (2.5 mM Tris-HCl, 2.5 mM boric acid, 0.05 mM EDTA, pH 8.0) polyacrylamide gel at 150 V/cm for 3 h, and stained with SYBR Green II gel stain (Cambrex Bio Science, Rockland, ME, USA) according to the instruction manual.

The number of polymorphic bands between 'Tanbakuro' and PI103091 were counted. Only primers that yielded reproducible polymorphic bands were used for SSR analysis. A DNA fragment was judged a scorable band if it was of sufficient intensity and it differed from neighbouring bands. SSR markers were scored as AA (homozygous for the PI103091 allele) or AB (heterozygous) or BB (homozygous for the 'Tanbakuro' allele) in 32 of the 123 $F_{2:3}$ families, consisting of 16 homozygous resistant and 16 homozygous susceptible lines, and were evaluated with respect to the *Phytophthora*-resistant trait. The segregation patterns of each selected SSR marker were then examined in the 123 $F_{2:3}$ families using the chi-squared test. Linkage analysis was performed using the MAPMAKER/EXP version 3.0 program (Lincoln et al. 1993). The error detection probability level was set at 5% in this study. The recombination rate was converted to map distance using the Kosambi mapping function (Kosambi 1944). Three-way contingency table analysis was performed to examine the relationship between the SSR marker genotype of two markers flanking the *Rps1*-d gene and the plant phenotype under the null hypothesis of no genotype association with the plant phenotype (Cheng et al. 2006).

Results

Segregation of resistant and susceptible lines in the F_2 soybean population

'Tanbakuro' and 'Williams' (*rps*) were susceptible to PJ-H30 (race E) and PJ-H42 (race A) isolates; the isolates were strongly virulent on those cultivars (Table 1). PI103091 was strongly resistant to PJ-H30 and PJ-H42. No inconsistent responses were found for any of the 123 $F_{2:3}$ families. The results of the virulence test of PJ-H30 on the 123 $F_{2:3}$ families identified 33 homozygous resistant, 61 segregating and 29 homozygous susceptible, which gives a 1 : 2 : 1 ratio (χ^2 = 0.27, P = 0.87). These results suggest that the *Phyto-*

Table 1: Genetic segregation of resistance to *Phytophthora sojae* isolate PJ-H30 (race E) in the F_2 soybean population ('Tanbakuro'/PI103091) consisting of 123 lines

Cultivars or parental type	Segregation of F_2 population[1] R	Rs	S	Total	Test and fit R : Rs : S	χ^2	P-value
'Tanbakuro'	0	–	98	98	0 : 0 : 1	–	–
'Williams'	0	–	87	87	0 : 0 : 1	–	–
PI103091	101	–	0	101	1 : 0 : 0	–	–
'Tanbakuro' × PI103091	33	61	29	123	1 : 2 : 1	0.27	0.87

[1]PJ-H42 (race A) phenotypic data were identical to those of PJ-H30.
R, homozygous resistant; Rs, segregating; S, homozygous susceptible.

phthora-resistant trait in PI103091 is controlled by a single dominant gene. The phenotypic data of 60 of 123 $F_{2:3}$ families to PJ-H42 were similar to those of PJ-H30.

Identification of SSR markers linked to the *Rps1*-d gene

Simple sequence repeat banding patterns were reproducible for DNA samples. Sixteen of the 41 primer pairs selected from the *Rps1* region (Song et al. 2004) produced 19 scorable polymorphic fragments, ranging in size from 100 to 600 bp between 'Tanbakuro' and PI103091 (Table 2). Each of these primer pairs generated one or more polymorphisms.

The results of SSR analysis of the 123 $F_{2:3}$ families using seven of the 19 selected SSR markers revealed a marker segregation pattern that almost fits a 1 : 2 : 1 ratio (Table 3). One marker (Sat_186) did not produce a heterozygous DNA banding pattern. The segregation pattern of plant SSR genotype and phenotype (resistant : susceptible) of this marker fits the expected 3 : 1 ratio (χ^2 = 0.13, P = 0.72). Chi-squared tests of the phenotypic segregation pattern (R + Rs/genotype AA + AB, R + Rs/genotype BB, S/genotype AA + AB or S/genotype BB) in Table 3 for 123 $F_{2:3}$ families indicated the presence of linkage between each of the eight SSR markers and the *Rps1*-d gene ($P < 0.001$). Banding patterns of Sat_186, Satt631 and Satt009, which displayed a higher level of fitness to the traits (91.9–92.7%), are illustrated in Fig. 1. The data were used to construct a linkage map with a length of 44.0 cM, as illustrated in Fig. 2. Seven of the eight SSR markers (Sat_186, Satt631, Satt009, Satt675, Satt683, Satt624 and Satt080) are located on one side of the gene, while the remaining marker (Satt152) is mapped on the other side. The closest marker, Sat_186, was mapped at 5.7 cM, and Satt152 was mapped at 11.5 cM distance from the gene. Furthermore, *Rps1*-d was located at the same position when PJ-H42 phenotypic data were used. Chi-squared contingency table analysis indicated that the SSR marker genotype of Satt152 and Sat_186 were significantly associated with the plant phenotype, suggesting that the two markers were closely linked to *Rps1*-d.

Table 2: Simple sequence repeat (SSR) markers for soybeans in MLG N, which produced polymorphic bands between 'Tanbakuro' and PI103091

SSR markers[1]	cM position on composite MLG N
Satt152	22.67
Satt631	26.14
Satt009	28.52
Sat_186	30.11
Satt683	34.52
Satt675	34.65
Satt624	35.32
Sat_280	43.45
Satt080	45.14
Sat_033	58.38
GMABAB	73.10
Satt237	74.99
Satt255	76.49
Sat_091	79.51
Satt239	87.35
Satt022	102.06

[1]SSR markers were obtained from the SoyBase internet website (http://soybase.agron.iastate.edu/resources/ssr.php).

Discussion

Totally five functional alleles, *Rps1*-a, *1*-b, *1*-c, *1*-d and *1*-k, were mapped to the *Rps1* locus. To date, SSR markers linked to *Rps1* (Cregan et al. 1999), *Rps1*-a (Weng et al. 2001), *Rps1*-b (Demirbas et al. 2001), *Rps1*-c (Demirbas et al. 2001) and *Rps1*-k (Kasuga et al. 1997, Bhattacharyya et al. 2005) have been developed. On the other hand, there has been no published report of molecular markers for the *Rps1*-d gene.

In the present study, 123 $F_{2:3}$ families produced from a cross between 'Tanbakuro' (*rps1-d*/*rps1-d* at *Rps1*-d allele) and PI103091 were used to identify SSR markers linked to the Phytophthora stem rot disease resistance gene of PI103091 (*Rps1*-d). During the disease resistance tests, two isolates of PJ-H30 (race E) and PJ-H42 (race A) were selected, because race E was the most prevalent from 2002 to 2004, followed by race A in the Hyogo region (Sugimoto et al. 2006). According to virulence tests (Table 1) and SSR analysis of 123 $F_{2:3}$ families (Table 3), the phenotypic traits and the pattern of seven of the eight markers segregated into a 1 : 2 : 1 ratio with respect to resistant vs. segregating vs. susceptible or AA vs. AB vs. BB of SSR marker genotype. The segregation pattern of another marker (Sat_186) fits a 3 : 1 ratio; this marker was considered dominant. These results confirmed the validity of the single dominant inheritance model in describing the Phytophthora stem rot resistant gene (*Rps1*-d). This result was identical to a previous report on PI103091 (Buzzell and Anderson 1992).

Rps1-d was flanked by Sat_186 (5.7 cM) on one side and Satt152 (11.5 cM) on the other side (Fig. 2a). This is the first report of DNA markers for *Rps1*-d in soybeans. Cregan (2003) reported that *Rps1* was flanked by Sat_186 (1.4 cM) and Satt530 (1.4 cM) (Fig. 2b). *Rps1*-a was located north of Satt009, and flanked by Satt159 (0.7 cM) and Satt009 (3.2 cM) (Weng et al. 2001), as illustrated in Fig. 2c. Satt530 and Satt159 might be closely linked to *Rps1*-d; however, these did not show polymorphism in the present study. The length of the linkage group between Satt152 and Satt080 is almost similar to that of Weng et al. (2001) (Fig. 2a,c); the map distance of this study, and that calculated by Weng et al.

Table 3: Segregation of eight simple sequence repeat (SSR) markers selected among 123 $F_{2:3}$ families

Markers[1]	Fragment size (bp)	Plant SSR genotype[2]			Expected ratio (1 : 2 : 1) or (3 : 1)		Markers[3]						Phenotype[4]		
							AA + AB		BB		Expected ratio (3 : 1)		Expected ratio (9 : 3 : 3 : 1)		Recombination frequencies
		AA	AB	BB	χ^2	P-value	R + Rs	S	R + Rs	S	χ^2	P-value	χ^2	P-value	
Satt152	230	37	49	37	5.08	0.08	82	4	12	25	1.69	0.19	62.4	< 0.001	0.130
Sat_186	310	94	–	29	0.13	0.72	89	5	5	24	0.13	0.72	68.6	< 0.001	0.081
Satt631	70, 100	35	59	29	0.78	0.67	89	5	5	24	0.13	0.72	68.6	< 0.001	0.081
Satt009	240	36	59	28	1.24	0.54	90	5	4	24	0.33	0.57	70.8	< 0.001	0.073
Satt675	110	36	57	30	1.24	0.54	86	7	8	22	0.02	0.88	51.8	< 0.001	0.122
Satt683	210	36	58	29	1.20	0.55	86	8	8	21	0.13	0.72	46.8	< 0.001	0.130
Satt624	90	37	55	31	1.96	0.38	83	9	11	20	0.02	0.96	37.3	< 0.001	0.155
Satt080	160	36	59	28	1.24	0.54	81	14	13	15	0.33	0.57	16.9	< 0.001	0.220

[1]Markers Satt152, Sat_186, Satt631, Satt009, Satt675, Satt683, Satt624 and Satt080 were named according to the SoyBase internet site (http://soybase.agron.iastate.edu/resources/ssr.php).
[2]AA, homozygous for PI103091 allele; AB, heterozygous; BB, homozygous for 'Tanbakuro' allele.
[3]R, resistant; Rs, segregating; S, susceptible.
[4]Phenotype, R + Rs/genotype AA + BB, R + Rs/genotype BB, S/genotype AA + AB, S/genotype BB.

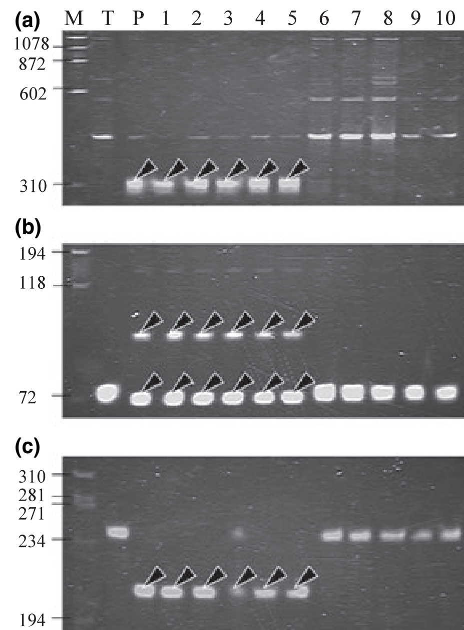

Fig. 1: Simple sequence repeat (SSR) analysis of the cultivar 'Tanbakuro', PI103091 and 10 $F_{2:3}$ families using SSR primers Sat_186 (a), Satt631 (b) and Satt009 (c). M, molecular weight marker (ΦX174/*Hae*III digested); T, 'Tanbakuro'; P, PI103091; lanes 1–5, homozygous resistant $F_{2:3}$ families; lanes 6–10, homozygous susceptible $F_{2:3}$ families. Diamonds indicate PI103091-specific SSR fragment of Sat_186 (a), Satt631 (b) and Satt009 (c)

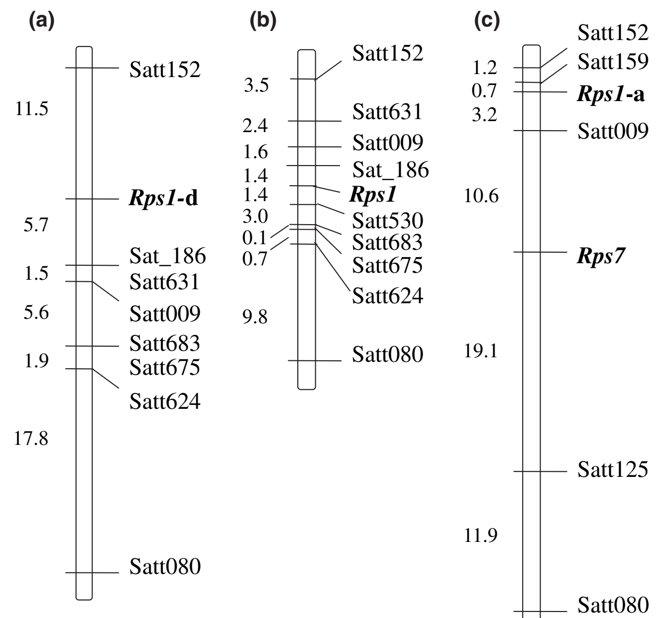

Fig. 2: Simple sequence repeat-based genetic linkage map of the *Rps1* region. (a) Genetic linkage map developed using 123 $F_{2:3}$ families produced from a cross between *Glycine max* (L.) Merr. 'Tanbakuro' and PI103091. (b) Genetic linkage map developed by Cregan (2003). (c) Genetic linkage map reported by Weng et al. (2001). Marker names and distances are written on the right and left of the linkage map, respectively. Map distance is reported in Kosambi units

(2001) being 44.0 cM and 46.7 cM, respectively. However, Cregan (2003) reported an estimate of 22.5 cM (Fig. 2b) with the order of the SSR markers being different. The present

marker order of Sat_186 as being north of Satt009 and Satt631 contrasts with Cregan (2003) who stated that Sat_186 was mapped south of these two markers. These differences might be caused by different mapping populations; Cregan (2003) used the mapping population of USDA/Iowa State University or the University of Nebraska. These differences can also be seen in previous studies (Cregan et al. 1999, Weng et al. 2001, Sandhu et al. 2005).

The accuracy of genotyping was estimated to be 92.7% and 87.0% for MAS using Sat_186 and Satt152, respectively, to genotype progeny on *Rps1*-d. It was theoretically estimated to be 99.05% for dual-MAS using both markers. Three-way contingency table analysis indicates that the selection of *Phytophthora*-resistant lines with mainly homozygous types [25 homozygous (R) and 11 segregating (Rs) in Table 4] could be possible by detecting only AA + AB = A./AA for Sat_186/Satt152 SSR genotypes. This selection would be much more efficient and reliable than one marker alone, because the ratio of contamination of susceptible lines was zero.

Identification of further markers flanking the *Rps1*-d gene is required, because Satt186 and Satt152 were not tightly linked to the gene. Map-based cloning of *Rps1*-d using either of the markers alone will be inefficient. Kasuga et al. (1997) developed a high-density linkage map of molecular markers for the *Rps1*-k gene, and mapped the gene between two AFLP markers with a 0.13 cM interval. These markers were recently used to screen bacterial artificial chromosome (BAC) libraries to identify the BAC clone containing the *Rps1*-k gene (Bhattacharyya et al. 2005). Consequently, the gene was isolated through positional cloning and transformation experiments (Gao et al. 2005). It was reported that *Rps1*-k and *Rps1*-d are either allelic with *Rps1* or very tightly linked clustered genes (Demirbas et al. 2001, Bhattacharyya et al. 2005). Sequence information about *Rps1*-k and BAC clones (BAC18, BAC43 or BAC99) containing the DNA fragment will be useful for identifying additional markers flanking the *Rps1*-d gene.

Table 4: Contingency table for association of simple sequence repeat (SSR) plant genotype with plant phenotype in the F_2 soybean population ('Tanbakuro'/PI103091) consisting of 123 lines

SSR genotype[1]		Phenotype[3]			
Sat_186[2]	Satt152[2]	R	Rs	S	Total
AA + AB = A	AA	25	11	0	37
AA + AB = A	AB	4	38	2	44
AA + AB = A	BB	2	9	3	14
AA + AB = A	Total	31	58	5	94
χ^2	(P)	42.42	P < 0.001		
BB	AA	1	0	0	1
BB	AB	1	2	2	5
BB	BB	0	1	22	23
BB	Total	2	3	24	29
χ^2	(P)	22.95	P < 0.001		
Sum	AA	26	11	0	37
Sum	AB	5	40	4	49
Sum	BB	2	10	25	37
Sum	Total	33	61	29	123
χ^2	(P)	98.32	P < 0.001		

[1]AA, homozygous for PI103091 allele; AB, heterozygous; BB, homozygous for 'Tanbakuro' allele.
[2]Markers Sat_186 and Satt152 were named according to the SoyBase internet site.
[3]Phenotype: R, resistant; Rs, segregating; S, susceptible.

Acknowledgements

The authors thank Professor (Dr) Anne E. Dorrance for valuable discussions, Professor (Dr) Katsura Izui (Kyoto University) and Dr Ken Tatsuda for reviewing this manuscript, Eiji Hinomoto for collecting diseased plants, Toshiharu Ohnishi and Sanae Shikata for supporting this study and Dr R. L. Nelson (USDA-ARS) and Akiko Tazawa for providing seeds of the soybean differential cultivars.

References

Anderson, T. R., and R. I. Buzzell, 1982: Efficacy of metalaxyl in controlling *Phytophthora* root and stalk rot of soybean cultivars differing in field resistance. Plant Dis. **66,** 1144—1145.

Anderson, T. R., and R. I. Buzzell, 1992: Inheritance and linkage of the *Rps7* gene for resistance to *Phytophthora* rot of soybean. Plant Dis. **76,** 958—959.

Athow, K. L., and F. A. Laviolette, 1982: *Rps*$_6$, a major gene for resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. Phytopathology **72,** 1564—1567.

Athow, K. L., F. A. Laviolette, E. H. Mueller, and J. R. Wilcox, 1980: A new major gene for resistance to *Phytophthora megasperma* var. *sojae* in soybean. Phytopathology **70,** 977—980.

Bernard, R. L., P. E. Smith, M. J. Kaufmann, and A. F. Schmitthenner, 1957: Inheritance of resistance to *Phytophthora* root and stem rot in the soybean. Agron. J. **49,** 391.

Bhattacharyya, M. K., N. N. Narayanan, H. Gao, D. K. Santra, S. S. Salimath, T. Kasuga, Y. Liu, B. Espinosa, L. Ellison, L. Marek, R. Shoemaker, M. Gijzen, and R. I. Buzzell, 2005: Identification of a large cluster of coiled coil-nucleotide binding site-leucine rich repeat-type genes from the *Rps1* region containing *Phytophthora* resistance genes in soybean. Theor. Appl. Genet. **111,** 75—86.

Buzzell, R. I., and T. R. Anderson, 1981: Another major gene for resistance to *Phytophthora megasperma* var. *sojae* in soybean. Soybean Genet. Newsl. **18,** 30—33.

Buzzell, R. I., and T. R. Anderson, 1992: Inheritance and race reaction of a new soybean *Rps1* allele. Plant Dis. **76,** 600—601.

Cheng, P. E., J. W. Liou, M. Liou, and J. A. D. Aston, 2006: Data information in contingency tables: a fallacy of hierarchical loglinear models. J. Data. Sci. **4,** 387—398.

Cregan, P. B., 2003: An Integrated Genetic Linkage Map of the Soybean-March 2003: (Soybean_Composite_Genetic_Map_2003-N). Personal Communication to Soybean Staff. Available at: (http://bateson.agron.iastate.edu/cgi-bin/cmap/viewer?ref_map_set-aid_=GmComposite2003;ref_map_aids=GmComposite2003_N;comparative_maps=-1%3Dmap_aid%3DGmWmPhys2005_N;data_Source=CMAP).

Cregan, P. B., T. Jarvik, A. L. Bush, R. C. Shoemaker, K. G. Lark, A. L. Kahler, N. Kaya, T. T. VanToai, D. G. Lohnes, J. Chung, and J. E. Specht, 1999: An integrated genetic linkage map of the soybean genome. Crop Sci. **39,** 1464—1490.

Demirbas, A., B. G. Rector, D. G. Lohnes, R. J. Fioritto, G. L. Graef, P. B. Cregan, R. C. Shoemaker, and J. E. Specht, 2001: Simple sequence repeat markers linked to the soybean *Rps* genes for *Phytophthora* resistance. Crop Sci. **41,** 1220—1227.

Diers, B. W., L. Mansur, J. Imsande, and R. C. Shoemaker, 1992: Mapping of *Phytophthora* resistance loci in soybean with restriction fragment length polymorphism markers. Crop Sci. **32,** 377—383.

Dorrance, A. E., S. A. McClure, and S. K. St. Martin, 2003: Effect of partial resistance on Phytophthora stem rot incidence and yield of soybean in Ohio. Plant Dis. **87,** 308—312.

Gao, H., N. N. Narayanan, L. Ellison, and M. K. Bhattacharyya, 2005: Two classes of highly similar coiled coil-nucleotide binding-leucine rich repeat genes isolated from the *Rps1*-k locus encode *Phytophthora* resistance in soybean. Mol. Plant Microbe Interact. **18,** 1035—1045.

Gordon, S. G., S. K. St. Martin, and A. E. Dorrance, 2006: *Rps*8 maps to a resistance gene rich region on soybean molecular linkage group F. Crop Sci. **46,** 168—173.

Kasuga, T., S. S. Salimath, J. Shi, M. Gijzen, R. I. Buzzell, and M. K. Bhattacharyya, 1997: High resolution genetic and physical mapping of molecular markers linked to the *Phytophthora* resistance gene *Rps1*-k in soybean. Mol. Plant Microbe Interact. **10,** 1035—1044.

Kaufmann, M. J., and J. W. Gerdemann, 1958: Root and stem rot of soybean caused by *Phytophthora sojae* n. sp. Phytopathology **48,** 201—208.

Kilen, T. C., E. E. Hartwig, and B. L. Keeling, 1974: Inheritance of a second major gene for resistance to Phytophthora root rot in soybeans. Crop Sci. **14,** 260—262.

Kohama, T., H. Kobayashi, and M. Inoue, 2005: The effect of soybeans on the anovulatory cycle. J. Med. Food **8,** 550—551.

Kosambi, D. D., 1944: The estimation of map distances from recombination values. Ann. Eugen. **12,** 172—175.

Laviolette, F. A., and K. L. Athow, 1981: Physiologic races of *Phytophthora megasperma* f. sp. *glycinea* in Indiana, 1973–1979. Plant Dis. **65,** 884—885.

Lincoln, S. E., M. J. Daly, and E. S. Lander, 1993: Construction of a Genetic Linkage Map with MAPMAKER/EXP v3.0: a Tutorial and Reference Manual. An Whitehead Institute Technical Report, Cambridge, Massachusetts.

Mueller, E. H., K. L. Athow, and F. A. Laviolette, 1978: Inheritance of resistance to four physiologic races of *Phytophthora megasperma* var *sojae*. Phytopathology **68,** 1318—1322.

Rowland, L. J., and B. Nguyen, 1993: Use of PEG for purification of DNA from leaf tissue of woodly plants. BioTechniques **14,** 735—736.

Sandhu, D., H. Gao, S. Cianzio, and M. K. Bhattacharyya, 2004: Deletion of a disease resistance nucleotide-binding-site leucine-rich repeat-like sequence is associated with the loss of the *Phytophthora* resistance gene *Rps*4 in soybean. Genetics **168,** 2157—2167.

Sandhu, D., K. G. Schallock, N. Rivera-Velez, P. Lundeen, S. Cianzio, and M. K. Bhattacharyya, 2005: Soybean *Phytophthora* resistance gene *Rps8* maps closely to the *Rps3* region. J. Hered. **96,** 536—541.

Schmitthenner, A. F., 1985: Problems and progress in control of *Phytophthora* root rot of soybean. Plant Dis. **69,** 362—368.

Schmitthenner, A. F., 1999: *Phytophthora* rot of soybean. In: G. L. Hartman, J. B. Sinclair, and J. C. Rupe (eds), Compendium of Soybean Diseases, 4th edn, 39—42. The American Phytopathological Society Press, St Paul, Minnesota.

Song, Q. J., L. F. Marek, R. C. Shoemaker, K. G. Lark, V. C. Concibido, X. Delannay, J. E. Specht, and P. B. Cregan, 2004: A new integrated genetic linkage map of the soybean. Theor. Appl. Genet. **109,** 122—128.

Sugimoto, T., S. Yoshida, M. Aino, T. Ohnishi, and K. Shiwaku, 2003: Development of the agar medium inoculation method for the pathogenic test of *Phytophthora sojae*. Proc. Kansai Plant Prot. **45,** 93—96 (in Japanese with English summary).

Sugimoto, T., M. Aino, M. Sugimoto, and K. Watanabe, 2005: Reduction of Phytophthora stem rot disease on soybeans by the application of $CaCl_2$ and $Ca(NO_3)_2$. J. Phytopathol. **153,** 536—543.

Sugimoto, T., S. Yoshida, M. Aino, K. Watanabe, K. Shiwaku, and M. Sugimoto, 2006: Race distribution of *Phytophthora sojae* on soybean in Hyogo, Japan. J. Gen. Plant Pathol. **72,** 92—97.

Sugimoto, T., K. Watanabe, S. Yoshida, M. Aino, M. Matsuyama, K. Maekawa, and K. Irie, 2007: The effects of inorganic elements on the reduction of Phytophthora stem rot disease of soybean, the growth rate and zoospore release of *Phytophthora sojae*. J. Phytopathol. **155,** 97—107.

Takahashi, R., R. Ohmori, C. Kiyose, Y. Momiyama, F. Ohsuzu, and K. Kondo, 2005: Antioxidant activities of black and yellow soybeans against low-density lipoprotein oxidation. J. Agric. Food. Chem. **53,** 4578—4583.

Tsuchiya, S., F. Kodama, J. Akai, and Y. Matsumura, 1978: Phytophthora stem rot caused by *Phytophthora megasperma* var. *sojae* Hildebrand (new disease). Ann. Phytopathol. Soc. Jpn **44,** 351 (abstract, in Japanese).

Weng, C., K. Yu, T. R. Anderson, and V. Poysa, 2001: Mapping genes conferring resistance to *Phytophthora* root rot of soybean, *Rps*1a and *Rps7*. J. Hered. **92,** 442—446.

Workneh, F., X. B. Yang, and G. L. Tylka, 1998: Effect of tillage practices on vertical distribution of *Phytophthora sojae*. Plant Dis. **82,** 1258—1263.

Young, N. D., 1999: A cautiously optimistic vision for marker-assisted breeding. Mol. Breed. **5,** 505—510.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.