Breeding Science 61: 511–522 (2012)
doi:10.1270/jsbbs.61.511

Review

Pathogenic diversity of *Phytophthora sojae* and breeding strategies to develop Phytophthora-resistant soybeans

Takuma Sugimoto*[1)], Masayasu Kato[2)], Shinya Yoshida[1)], Isao Matsumoto[1)], Tamotsu Kobayashi[1)], Akito Kaga[3)], Makita Hajika[4)], Ryo Yamamoto[5)], Kazuhiko Watanabe[1,6)], Masataka Aino[1)], Toru Matoh[7)], David R. Walker[8)], Alan R. Biggs[9)] and Masao Ishimoto[3)]

1) *Hyogo Agricultural Institute for Agriculture, Forestry and Fisheries*, 1533 Minamino-oka, Kasai, Hyogo 679-0198, Japan
2) *National Agricultural Research Center*, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan
3) *National Institute of Agrobiological Sciences*, Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan
4) *NARO Institute of Crop Science, National Agriculture and Food Research Organization*, Kannondai 2-1-18, Tsukuba, Ibaraki 305-8518, Japan
5) *National Agricultural Research Center for Tohoku Region (NARCT)*, 4 Akahira, Shimokuriyagawa, Morioka, Iwate 020-0198, Japan
6) *Tokyo University of Agriculture, Department of Applied Biology and Chemistry*, 1-1-1 Sakuragaoka, Setagaya, Tokyo 156-8502, Japan
7) *Kyoto University, Laboratory of Plant Nutrition, Division of Applied Life Sciences, Graduate school of Agriculture*, Oiwake, Kitashirakawa, Sakyo, Kyoto 606-8502, Japan
8) *USDA-ARS, Soybean/Maize Germplasm, Pathology, and Genetics Research Unit, and University of Illinois, Department of Crop Sciences*, 232 National Soybean Research Center, 1101 W. Peabody Dr, Urbana, IL 61801, USA
9) *West Virginia University, Kearneysville Tree Fruit Research and Education Center*, P.O. Box 609, Kearneysville, WV 25430, USA

Phytophthora stem and root rot, caused by *Phytophthora sojae*, is one of the most destructive diseases of soybean [*Glycine max* (L.) Merr.], and the incidence of this disease has been increasing in several soybean-producing areas around the world. This presents serious limitations for soybean production, with yield losses from 4 to 100%. The most effective method to reduce damage would be to grow Phytophthora-resistant soybean cultivars, and two types of host resistance have been described. Race-specific resistance conditioned by single dominant *Rps* ("resistance to *Phytophthora sojae*") genes and quantitatively inherited partial resistance conferred by multiple genes could both provide protection from the pathogen. Molecular markers linked to *Rps* genes or quantitative trait loci (QTLs) underlying partial resistance have been identified on several molecular linkage groups corresponding to chromosomes. These markers can be used to screen for Phytophthora-resistant plants rapidly and efficiently, and to combine multiple resistance genes in the same background. This paper reviews what is currently known about pathogenic races of *P. sojae* in the USA and Japan, selection of sources of *Rps* genes or minor genes providing partial resistance, and the current state and future scope of breeding Phytophthora-resistant soybean cultivars.

Key Words: race-specific resistance, partial resistance, *Phytophthora sojae*, Phytophthora stem and root rot, *Rps* gene, soybean.

Introduction

Phytophthora stem and root rot (PSR), caused by the soil-borne Oomycete *Phytophthora sojae* (Kaufmann and Gerdemann 1958), is one of the most serious and widespread diseases of soybean [*Glycine max* (L.) Merr.] (Schmitthenner 1999). PSR is most often encountered when seeds are planted in poorly drained soils with a high clay content, and in fields subjected to temporary flooding and ponding. Disease can occur at any stage of soybean development from seedling to harvest, though it primarily affects seeds and seedlings (Schmitthenner 1985). When soybean plants are infected by *P. sojae*, the stem of the plant appears water-soaked and turns red-brown, and the infection results in wilting and the death of plants (Dorrance *et al.* 2003). *P. sojae* produces motile zoospores from infected tissues which can initiate further cycles of disease (Gijzen and Qutob 2009, Schmitthenner 1985). Large numbers of oospores can persist in the soil for many years without a host, and this may cause continuous crop losses (Kato 2010, Schmitthenner 1999). In

Communicated by Donghe Xu
Received July 22, 2011. Accepted December 6, 2011.
*Corresponding author (e-mail: Takuma_Sugimoto@pref.hyogo.lg.jp)

addition, plants infected with *P. sojae* may become more vulnerable to infection by other soilborne pathogens.

In the USA, PSR was first observed in the state of Indiana in 1948 and in Ohio in 1951 (Kaufmann and Gerdemann 1958). PSR now occurs in most of the soybean growing regions of the USA, but is most common in the northern half of the country, where environmental conditions are generally more favorable for the pathogen (Dorrance and Schmitthenner 2000). *P. sojae* has been reported in soybean-producing areas in Asia, Africa, Australia, Europe, and North and South America (Schmitthenner 1999). In Japan, PSR was first observed in 1977 on Hokkaido, the northernmost island (Tsuchiya *et al.* 1978), and has subsequently been observed in Shizuoka, Yamagata, Akita, Saga, Niigata, Fukuoka, Hyogo, Toyama, Fukui, and Miyagi Prefectures (Sugimoto *et al.* 2006). In recent years, the yields of soybean and the income of soybean producers have decreased dramatically in the USA, where Wrather and Koenning (2006) estimated that annual crop damage from PSR between 2003 and 2005 averaged about $251.6 million. It is therefore essential to construct effective disease management strategies as quickly as possible.

Methods for reducing economic losses due to PSR include fungicide applications (Anderson and Buzzell 1982), planting resistant cultivars (Dorrance *et al.* 2003, Schmitthenner 1999), improving soil drainage (Schmitthenner 1985), modifying tillage practices (Workneh *et al.* 1998), and applying calcium-containing compounds (Sugimoto *et al.* 2005, 2007, 2008b, 2009, 2010a). Increasing public concern about environmental and health consequences of the widespread use of conventional fungicides has encouraged research on alternative disease control strategies (Sugimoto *et al.* 2010a).

Schmitthenner (1999) reported that the most effective way to reduce damage from PSR would be to plant resistant cultivars, and numerous sources of resistance have been identified. Two distinct types of host resistance have been described: (i) race-specific resistance conditioned by a single dominant *Rps* ("resistance to *Phytophthora sojae*") gene and (ii) partial resistance conferred by multiple genes acting together. Partial resistance, sometimes referred to as tolerance or field resistance, is characterized by fewer rotted roots and disease progression at a much slower rate than what occurs in susceptible cultivars. Race-specific *Rps* genes have been widely used in commercial soybean cultivars (Dorrance *et al.* 2000, Slaminko *et al.* 2010). Agronomically competitive cultivars with resistance to PSR and other important diseases and pests would reduce both crop losses and production expenses, allowing producers to increase their incomes.

This review summarizes what is currently known about pathogenic races of *P. sojae* in Japan and the USA, methods used to identify and select soybean plants with *Rps* genes or partial resistance, and the current state and future scope of breeding soybean cultivars with race-specific or partial resistance to PSR.

Three characteristics of resistance to plant diseases

Van der Plank (1963) proposed the terms "vertical resistance" and "horizontal resistance" to describe two types of resistance found in plants. Vertical resistance confers absolute protection against some, but not all races of a pathogen, whereas horizontal resistance confers incomplete protection to all races of a pathogen. Fry (1982) proposed three characteristics of plant resistance to a pathogen: the magnitude of effect, the number of genes that govern the resistance, and the differential nature of the host's reaction to pathogen races. According to Fry's proposal, vertical resistance is conferred by a single gene with large and differential effects, and horizontal resistance is conditioned by multiple genes with small and non-differential effects. There are exceptions, however, such as the incomplete resistance that is conferred by a single gene in the rice/rice blast pathosystem (Zenbayashi *et al*. 2002). In this review, the terms race-specific resistance and partial resistance are used to refer to qualitatively and quantitatively inherited resistance, respectively. While this is convenient for the sake of simplifying discussion, the reader should bear in mind that some *Rps* genes may not confer complete immunity to certain races of the pathogen, resulting in a reaction that resembles quantitatively inherited partial resistance.

Race-specific resistance

Rps genes

Soybean cultivars and germplasm accessions differ in their reactions to different isolates of *P. sojae* (Kaufmann and Gerdemann 1958). Monogenic resistance conditioned by certain *Rps* genes has been providing reasonable protection against the majority of *P. sojae* populations in the USA for the last four decades (Bhattacharyya *et al.* 2005). *Rps* genes are thought to activate effector-triggered immune responses, similar to resistance (R) genes in other pathosystems (Dong *et al.* 2011). The first Phytophthora resistance gene was identified in the 1950s (Bernard *et al.* 1957). To date, 14 *Rps* genes at eight genomic loci have been reported (Sandhu *et al.* 2004) (Table 1). They are *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* (Gordon *et al*. 2006, Sandhu *et al.* 2005). The *Rps1* locus contains five functional alleles (*Rps1*a, *1*b, *1*c, *1*d and *1*k) (Bernard *et al.* 1957, Buzzell and Anderson 1992, Mueller *et al.* 1978), and the *Rps3* locus contains three (*Rps3*a, *3*b and *3*c) (Mueller *et al.* 1978, Ploper *et al.* 1985). With the exception of *Rps2*, which confers incomplete resistance and is root-specific (Mideros *et al.* 2007), *Rps* genes usually provide absolute protection (i.e., immunity) against incompatible *P. sojae* races. *Rps8* is the most recently identified *Rps* gene, and was discovered in PI 399073, a South Korean landrace (Sandhu *et al.* 2005). Near-isogenic lines (NILs), each carrying one of 14 *Rps*

Table 1. Phytophthora resistance genes in soybean

Rps gene	Source[a]	Molecular linkage group	Citation
*Rps1*a	L88-8470	N	Bernard *et al.* (1957)
*Rps1*b	L77-1863	N	Mueller *et al.* (1978)
*Rps1*c	L75-3735	N	Mueller *et al.* (1978)
*Rps1*d	L93-3312, PI 103091	N	Buzzell and Anderson (1992)
*Rps1*k	L77-1794	N	Bernard and Cremeens (1981)
Rps2	L76-1988	J	Kilen *et al.* (1974)
*Rps3*a	L83-570	F	Mueller *et al.* (1978)
*Rps3*b	L91-8347	F	Ploper *et al.* (1985)
*Rps3*c	L92-7857	F	R. Nelson, *personal communication*
Rps4	L85-2352	G	Athow *et al.* (1980)
Rps5	L85-3059	G	Buzzell and Anderson (1981)
Rps6	L89-1581	G	Athow and Laviolette (1982)
Rps7	L93-3258	N	Anderson and Buzzel (1992)
Rps8	PI 399073	F	Gordon *et al.* (2006), Sandhu *et al.* (2005)

[a] "L" lines are backcross lines developed by Bernard *et al*. (1991).

genes in the background of 'Williams' (*rps*; susceptible to *Phytophthora sojae*) were developed by Dr. Richard Bernard of the USDA-ARS in Urbana, IL, USA (Table 1; Bernard *et al.* 1991, Dorrance *et al.* 2004). This series of NILs included a line with the *Rps1*k gene from 'Kingwa' that was released as 'Williams 82' (Bernard and Cremeens 1988), the cultivar that was later used in the project to sequence the *G. max* genome (Schmutz *et al.* 2010).

Racial diversity of Phytophthora sojae, and germplasm with effective Rps Phytophthora resistance genes

Breeding Phytophthora-resistant cultivars requires selection of effective *Rps* genes or parental lines with Phytophthora resistance. Thus, it is essential to be familiar with the racial diversity of *P. sojae* in soybean-producing regions. Grau *et al.* (2004) summarized the virulence pathotypes of North American *P. sojae* races on a set of eight differentials with either *Rps1*a, *Rps1*b, *Rps1*c, *Rps1*d, *Rps1*k, *Rps3*a, *Rps6*, or *Rps7* (Table 2) using the hypocotyl inoculation method developed by Laviolette and Athow (1981). Single-zoospore isolates are generally used for the determination of race (Bhat *et al.* 1993). Since 1955, at least 55 physiologic races of *P. sojae* have been identified in the USA on the basis of the unique reaction patterns produced on the eight differential soybeans genotypes in Table 2 (Grau *et al.* 2004). The dominant races and racial diversity differ in each area (Grau *et al.* 2004). In the 1960s, *Rps1*a was the first resistance gene to be widely deployed in the USA, where it remained effective for approximately eight years (Grau *et al.* 2004, Schmitthenner 1985). *Rps1*a can still be found in approximately 5% of the commercial cultivars planted in the Midwestern USA (Slaminko *et al.* 2010). *Rps1*c, *Rps1*k, *Rps3*a and *Rps6* were subsequently deployed in the North Central/Midwest region of the USA (Dorrance *et al.* 2003, Gordon *et al.* 2007) (Table 3). *Rps1*k confers strong resistance against a large number of North American *P. sojae* races, and has been the most stable and widely used *Rps* gene in the last two decades (Gao *et al.* 2005, 2008, Schmitthenner 1994) (Table 3). Slaminko *et al*. (2010) tested 3,533 USA commercial cultivars for their resistance

Table 2. Races of *Phytophthora sojae* from the USA classified using eight differentials with *Rps1*a, *Rps1*b, *Rps1*c, *Rps1*d, *Rps1*k, *Rps3*a, *Rps6*, or *Rps7*[a]

Race	Virulence pathotype	Race	Virulence pathotype	Race	Virulence pathotype
0	—	19	1a, 1b, 1c, 1d, 1k, 3a	38	1a, 1b, 1c, 1d, 1k, 3a, 6, 7
1	7	20	1a, 1b, 1c, 1k, 3a, 7	39	1a, 1b, 1c, 1k, 3a, 6, 7
2	1b, 7	21	1a, 3a, 7	40	1a, 1c, 1d, 1k, 7
3	1a, 7	22	1a, 1c, 3a, 6, 7	41	1a, 1b, 1d, 1k, 7
4	1a, 1c, 7	23	1a, 1b, 6, 7	42	1a, 1d, 3a, 7
5	1a, 1c, 6, 7	24	1a, 3a, 6, 7	43	1a, 1c, 1d, 7
6	1a, 1d, 3a, 6, 7	25	1a, 1b, 1c, 1k, 7	44	1a, 1d, 7
7	1a, 3a, 6, 7	26	1b, 1d, 3a, 6, 7	45	1a, 1b, 1c, 1k, 6, 7
8	1a, 1d, 6, 7	27	1b, 1c, 1k, 6, 7	46	1a, 1c, 3a, 5, 7
9	1a, 6, 7	28	1a, 1b, 1k, 7	47	1a, 1b, 1c, 7
10	1a, 1b, 1c, 1d, 1k, 3a	29	1a, 1b, 1k, 6, 7	48 = 1[b]	(5), 7
11	6, 7	30	1a, 1b, 1k, 3a, 6, 7	49 = 5[b]	1a, 1c, (4), 6, 7
12	1a, 1b, 1c, 1d, 1k, 3a	31	1b ,1c, 1d, 1k, 6, 7	50 = 13[b]	(4), 6, 7
13	6, 7	32	1b, 1k, 6, 7	51	1c, 5, 6, 7
14	1c, 7	33	1a, 1b, 1c, 1d, 1k, 7	52 = 1[b]	(3b, 5), 7
15	3a, 7	34	1a, 1k, 7	53	1a, 1b,1c, 3a, 5, 7
16	1b, 1c, 1k	35	1a, 1b, 1c, 1d, 1k	54	1d, 7
17	1b, 1d, 3a, 6, 7	36	3a, 6	55	1d, 3a, 3c, 4, 5, 6, 7
18	1c	37	1a, 1c, 3a, 6, 7		

[a] This Table was adapted from Grau *et al*. (2004) with small changes.

[b] Additional differentials had been incorporated with *Rps2*, *Rps3*b, *Rps3*c, *Rps4*, or *Rps5*, but the original isolates were not reclassified.

Table 3. Sources of *Rps* genes for developing new cultivars with resistance to *P. sojae*

Region	Cultivar or line (*Rps* gene)	Citation
USA	L77-1794 (*Rps1*k), PI 103091 (*Rps1*d)	Buzzell and Anderson (1992), Schmitthenner (1999)
USA (northern area)	L75-3735 (*Rps1*c), L77-1794 (*Rps1*k), L83-570 (*Rps3*a), L89-1581 (*Rps6*)	Dorrance *et al.* (2003), Gordon *et al.* (2007)
Hokkaido, Japan	Hayagin-1, KLS733-1	Tsuchiya *et al.* (1990)
Hyogo, Japan	PI 103091 (*Rps1*d), Gedenshirazu-1 (ND[a]), Ohojyu (ND[a]), Waseshiroge (ND[a])	Sugimoto *et al.* (2006)
Hokkaido, Iwate, Miyagi, Yamagata, Fukushima, Ibaraki, Tochigi, Nagano, Shizuoka, Niigata, Toyama, Fukui, Hyogo, Tottori, Japan	PI 103091 (*Rps1*d), L77-1794 (*Rps1*k)	Moriwaki (2010)

[a] ND, not determined.

Table 4. Races of *Phytophthora sojae* reported in Japan using six Japanese cultivars as diferentials

Differentials	Races[a]														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Isuzu	S	S	R	S	S	S	S	S	R	S	S	S	R	R	R
Chusei Hikarikuro	R	S	S	R	S	S	S	S	S	S	R	R	R	R	S
Kitamusume	S	S	S	S	S	S	S	S	S	S	R	R	S	R	S
Toyosuzu	R	R	S	R	S	R	S	S	S	S	R	R	R	R	R
Gedenshirazu-1	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R
Ohojyu	R	R	R	S	R	S	S	R	S	S	S	R	R	R	R

[a] S, susceptible; R, resistant.

Races A to J of *P. sojae* were reported by Tsuchiya *et al.* (1990).

Races K, L, M and N were reported in Hyogo from 2002 to 2004 (Sugimoto *et al.* 2006).

Race O was first noted in this study in the Sasayama region in 2006 (Sugimoto *et al*. 2010b).

to *P. sojae*, and found that 51% carried at least one *Rps* gene. Half of them had *Rps1*c, while another 40% of them had *Rps1*k-mediated resistance to PSR. Gordon *et al.* (2007) stated that while single gene-mediated resistance has been an effective means for managing this soilborne disease, it is necessary to continue identifying novel *Rps* genes.

In Hokkaido, Japan, a total of 49 *P. sojae* isolates were recovered from soybean fields in 1985, and the reactions that each isolate induced on a set of eight differential cultivars (Table 1) from the USA were examined using the hypocotyl inoculation method (Tsuchiya *et al.* 1990). The reaction patterns that 45 of the 49 isolates produced on the eight differentials did not correspond to those of any of the 55 races previously identified in the USA. Six Japanese differential cultivars were subsequently selected to characterize the Hokkaido isolates (Tsuchiya *et al.* 1990) (Table 4). The 45 isolates represented 10 different virulence pathotypes (races A, B, C, D, E, F, G, H, I and J), and these were given letter designations to distinguish them from the numbers assigned to physiologic races from North America. Race D was the most prevalent, followed by races A, and J. Two cultivars, 'Hayagin-1' and 'KLS733-1', which were resistant to all 10 of the races from Hokkaido, were selected as resistance donors in soybean breeding programs (Tsuchiya *et al*. 1990) (Table 3).

In Hyogo Prefecture, which is famous in Japan for growing the black-seeded soybean cultivar 'Tanbakuro', PSR was first noted in 1987 (Sugimoto *et al*. 2006). *P. sojae* isolates were recovered from 164 fields between 2002 and 2008. The six Japanese differential soybean cultivars mentioned above were used for race determination of the 164 isolates with the agar medium inoculation method (Sugimoto *et al.* 2006). The results showed that race E was a major component of all the *P. sojae* populations, followed by races A, L, K, M, G, N and O (Sugimoto *et al.* 2010b). 'Gedenshirazu-1', PI 103091 (containing *Rps1*d) and 'Ohojyu' were resistant to most of the 164 isolates, and they were subsequently selected as sources of specific resistance from 10 Japanese cultivars and 14 differential cultivars with 14 *Rps* genes (Table 3). *Rps1*c, *Rps3*a and *Rps6*, which provided high levels of resistance to *P. sojae* races in the North Central USA, were ineffective in Hyogo (Sugimoto *et al.* 2011b).

Before 2009, Hokkaido and Hyogo were the only two regions of Japan for which there were published reports on the race distribution of *P. sojae* and the selection of cultivars that could be used as sources of resistance genes. Moriwaki (2010) collected 109 *P. sojae* isolates from 14 regions in Japan (Hokkaido, Iwate, Miyagi, Yamagata, Fukushima, Ibaraki, Tochigi, Nagano, Shizuoka, Niigata, Toyama, Fukui, Hyogo and Tottori), and examined the effectiveness of 14 *Rps* genes against them. *Rps1*d, *Rps1*k, *Rps8*, *Rps1*a,

*Rps1*c, *Rps7*, *Rps3*b and *Rps1*b were found to provide resistance to 47–81% of the 109 isolates. *Rps1*d and *Rps1*k were the most effective resistance genes (Moriwaki 2010). These results corresponded with those of previous studies (Buzzell and Anderson 1992, Sugimoto *et al*. 2006), and indicated that these two *Rps* genes are potential sources of resistance that can be used to breed new resistant cultivars in the USA as well as in Japan (Table 3).

DNA markers linked to Phytophthora resistance genes

From the 1960s to 1980s, conventional breeding methods used phenotypic assays to identify seedlings carrying a Phytophthora resistance (*Rps*) gene, but this process is both time-consuming and costly (Young 1999). Great progress in DNA technology was made in the 1990s, resulting in the development and use of restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers and analyses. These facilitated the development of genetic maps for mapping genes and quantitative trait loci (QTLs) and the use of marker-assisted selection (MAS) of plants carrying one or more genes of high importance. During the last decade, the development of single nucleotide polymorphism (SNP) markers has greatly improved marker coverage of the soybean genome, while providing a class of marker that is suited to semi-automated analysis of DNA samples. Molecular markers make it possible not only to reduce the amount of time and labor expended, but also to allow efficient and accurate selection of Phytophthora-resistant plants. Since 1999, integrated genetic linkage maps of the soybean have been constructed using SSR, RFLP, RAPD, AFLP and SNP markers, classical traits and isozymes (Cregan *et al.* 1999, Cregan 2003, Diers *et al.* 1992, Song *et al.* 2004, http://soybase.org/). Currently, a genetic linkage map consisting of 20 linkage groups with approximately 1,500 SNP, 1,000 SSR markers, 700 RFLP and 73 RAPD markers, in addition to 46 classical trait loci, is available (Cregan 2003, Hyten *et al*. 2010, Song *et al.* 2004). This information allows researchers to identify molecular markers linked to important genes to use for MAS. With this information, the *Rps1*, *Rps2*, *Rps3*, *Rps4*, *Rps5*, *Rps6*, *Rps7* and *Rps8* loci, have been mapped to molecular linkage groups (MLGs) N, J, F, G, G, G, N and F, respectively (Cregan *et al.* 1999, Cregan 2003, Demirbas *et al.* 2001, Gordon *et al.* 2006, Lohnes and Schmitthenner 1997, Sandhu *et al.* 2005, Weng *et al.* 2001) (Table 1 and Fig. 1). The *Rps4* and *Rps8* loci mapped close to the *Rps6* and *Rps3* regions, respectively (Gordon *et al.* 2006, Sandhu *et al.* 2005). Diers *et al.* (1992) reported that the RFLP marker pT-5 was linked to the *Rps5* locus (MLG G), but Demirbas *et al.* (2001) were unable to find SSR markers linked to it. Thus, SSR markers linked to each *Rps* gene except *Rps5* have been reported (Demirbas *et al.* 2001).

Many researchers have studied the *Rps1* locus, which carries five functional alleles, because of its effectiveness in controlling PSR. 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 (Bhattacharyya *et al*. 2005, Kasuga *et al*. 1997) were identified relatively early, but there were no published reports of molecular markers for the *Rps1*d gene until 2005 (Sugimoto *et al*. 2006), despite the fact that this gene was considered effective worldwide (Moriwaki 2010, Sugimoto *et al.* 2010b).

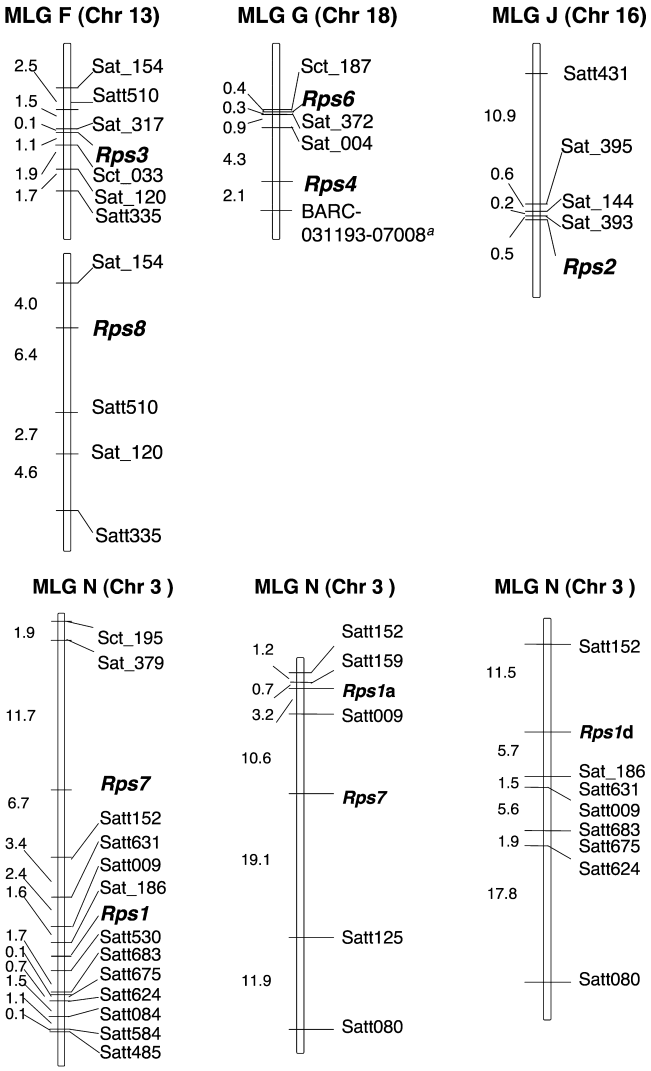

Fig. 1. SSR-based genetic linkage map of *Rps* genes on soybean molecular linkage groups (MLG), with corresponding Chromosomes (Chr) in parentheses. Genetic linkage map of the *Rps3* and *Rps8* region on MLG F (Chr 13) with the map distances reported by Cregan (2003) and Sandhu *et al*. (2004). Genetic linkage map of the *Rps4* and *Rps6* region on MLG G (Chr 18) with the map distances reported by Cregan (2003). Genetic linkage map of the *Rps2* on MLG J (Chr 16) with the map distances reported by Cregan (2003). Genetic linkage map of the *Rps1* and *Rps7* region on MLG N (Chr 3) with the map distances reported by Cregan (2003). Genetic linkage map of the *Rps1*a and *Rps7* region on MLG N (Chr 3) with the map distances reported by Weng *et al.* (2001). Genetic linkage map of the *Rps1*d region on MLG N (Chr 3) with the map distances reported by Sugimoto *et al.* (2008). [a] Molecular marker for single nucleotide polymorphism (SNP), because no SSR marker was found distal to *Rps4*.

Demirbas *et al*. (2001) were unable to find molecular markers linked to *Rps1*d because none of the markers closely linked to *Rps1* on MLG N were polymorphic in a population derived from a cross between 'Williams' (*rps*) and L93-3312 (*Rps1*d). To identify markers for *Rps1*d, Sugimoto *et al.* (2008a) developed 123 $F_{2:3}$ lines from a cross between the traditional black soybean cultivar 'Tanbakuro' and PI 103091 (*Rps1*d). The results of virulence tests showed that the inheritance of *Rps1*d is controlled by a single dominant gene. Seven SSR markers (Sat_186, Satt631, Satt009, Satt675, Satt683, Satt624 and Satt080) on MLG N were linked to *Rps1*d, and a linkage map 44.0 cM in length was constructed. The closest markers, Sat_186 and Satt152, were mapped to positions 5.7 cM and 11.5 cM of this linkage map, respectively, on each side of the *Rps1*d gene. The accuracy of MAS was estimated to be 92.7% and 87.0% for MAS using Sat_186 and Satt152, respectively, by using progeny tests to confirm the presence of *Rps1*d. Selection efficiency was theoretically estimated to be 99.05% for MAS using both markers. We subsequently identified novel molecular markers even more closely linked to the *Rps1*d gene (Sugimoto *et al.* 2011b).

In Japan, Tazawa and Tezuka (2003) developed 143 $F_{2:3}$ lines from a cross between 'Hayagin-1' (resistant) and 'Toyokomachi' (susceptible) in order to identify markers for the *Rps* allele from Hayagin-1, which is resistant to all of the 10 *P. sojae* races in Hokkaido. Seven SSR markers on MLG N were associated with the *Rps* gene in Hayagin-1, and a linkage map was constructed. The closest marker, Satt152, was mapped 6.7 cM from the *Rps* gene, but the investigators were unable to identify a closer marker on the other side of the gene. These results suggest that the *Rps* gene in 'Hayagin-1' may be *Rps1* or *Rps7* according to information available at the SoyBase/Soybean Breeders Toolbox website (www.soybeanbreederstoolbox.org).

Isolation, characterization and evolution of Rps genes

*Rps1*k is the most frequently examined of the 14 *Rps* genes because *Rps1*k has provided stable and broad-spectrum Phytophthora resistance in the major soybean-producing regions of the USA for 40 years (Bhattacharyya *et al.* 2005, Schmitthenner *et al.* 1994). Kasuga *et al.* (1997) constructed a high-density linkage map of the *Rps1*k region, and the locus was mapped to a 0.13-cM interval between two AFLP markers. These markers were used to screen bacterial artificial chromosome (BAC) libraries to identify a BAC clone containing the *Rps1*k gene (Bhattacharyya *et al.* 2005). *Rps1*k was then isolated through positional cloning and transformation experiments (Gao *et al.* 2005, Gao and Bhattacharyya 2008). Sequence analysis of the cDNA clone showed that the sequence is a member of the coiled coil-nucleotide binding site-leucine rich repeat (CC-NBS-LRR) class of disease resistance genes. Bhattacharyya *et al.* (2005) reported that the soybean genome contains about 38 copies of a similar sequence, and that most of the copies are clustered in approximately 600 kb of contiguous DNA from the *Rps1*k region. Analysis of *G. max* sequence data released by the Soybean Genome Project, Department of Energy, Joint Genome Institute (http://www.phytozome.net/soybean; Schmutz *et al.* 2010) indicates the presence of 30 loci with genes on MLG N (Chr 3) that share sequence homology with *Rps1*k (Sugimoto *et al.* 2011b). The clustering of related genes at the *Rps1*k locus might have facilitated the expansion of *Rps1* gene numbers and the generation of new recognition specificities. Graham *et al.* (2002) reported that resistance genes in soybean tend to be clustered in groups of genes that confer resistance to more than one type of pathogen. Plant disease resistance (R) genes often occur in clusters (Richly *et al.* 2002), which may facilitate the expansion of R gene loci and the generation of new R gene specificities through recombination and positive selection (Michelmore and Meyers 1998).

Although *Rps1*k was previously considered to be a single gene, two functional *Rps* genes (*Rps1*k-1 and *Rps1*k-2) were cloned from the *Rps1*k locus by Bhattacharyya *et al.* (2005), who sequenced the *Rps1*k region to try to gain a better understanding of the possible evolutionary steps that shaped the generation of Phytophthora resistance genes in soybean. As a result, *Rps1*k-3 from this *Rps1*k genomic region was evolved through intramolecular recombination between *Rps1*k-1 and *Rps1*k-2. They hypothesize that crossing over was one of the mechanisms involved in tandem duplication of CC-NBS–LRR sequences in the *Rps1*k region (Gao and Bhattacharyya 2008). Analyses of recombinants strongly indicated that at least one additional functional *Rps* gene maps next to the *Rps1* locus (Bhattacharyya *et al.* 2005). Gao and Bhattacharyya (2008) also proposed from the sequencing analysis of the *Rps1*k region that the *Rps1* locus is located in a gene-poor region where only a few full-length genes were predicted. These include two coiled coil-nucleotide binding-leucine rich repeat (CC-NB-LRR)-type *Rps1*k genes and retrotransposons (Gao and Bhattacharyya 2008). The abundance of repetitive sequences in the *Rps1*k region suggested that the location of this locus is in or near a heterochromatic region. Low recombination frequencies along with the presence of two functional *Rps* genes at this locus may explain why *Rps1*k has provided stable Phytophthora resistance in soybean for several decades.

The region containing *Rps2* has been cloned and sequenced (Sandhu *et al.* 2005). It is comprised of three functional genes: (1) the powdery mildew resistance gene *Rmd-c*, (2) an ineffective nodulation gene named *Rj2* and (3) *Rps2* (Graham *et al.* 2000, 2002). This region contains at least nine resistance gene analogues (RGAs) similar to the Toll/Interleukin-1 receptor (TIR)-NBS-LRR class of resistance genes (Graham *et al.* 2002, Polzin *et al.* 1994). *Rps2* was therefore proposed to be in the TIR-NBS-LRR class of resistance genes (Graham *et al.* 2002).

The *Rps4* region also has been recently cloned and characterized, and genes similar to the CC-NBS-LRR resistance genes in the *Rps1* locus were identified (Sandhu *et al.* 2004). Deletion of a disease resistance gene-like sequence leads to

a loss of *Rps4* function (Sandhu *et al.* 2004). Although Athow and Laviolette (1982) reported no linkages between *Rps4* and *Rps6*, recent studies indicated that the two genes are either allelic or clustered (Demirbas *et al.* 2001, Sandhu *et al.* 2004) (Fig. 1).

The *Rps3* locus has been mapped to a gene–rich region containing three additional disease resistance loci with one bacteria resistance gene (*Rpg1*) and two virus resistance genes (*Rsv1* and *Rpv1*) (Sandhu *et al.* 2005). Three functional Phytophthora resistance genes were mapped to the *Rps3* locus (Sandhu *et al.* 2005, http://soybeanbreederstoolbox.org/). Sandhu *et al.* (2005) reported that a novel Phytophthora resistance gene has been mapped to the *Rps3* region.

The *Rps8* gene mapped closely to the disease resistance gene-rich *Rps3* region and this gene was located "below" *Rps3* (Sandhu *et al.* 2005). Sandhu *et al.* (2005) reported that at least 11 disease resistance genes, including *Rps8*, have been mapped to this small genomic region. Other *Rps* genes could be cloned and characterized using information from SoyBase (http://soybeanbreederstoolbox.org/) and the sequence data from the soybean genome (http://www.phytozome.net/soybean). In addition, resequencing of regions where resistance genes reside could identify SNPs that may be directly responsible for phenotypic differences.

Partial resistance

Effectiveness of partial resistance in soybean

Continuous utilization of stable *Rps* genes in soybean cultivars grown in North America has resulted in selection pressures that promote the evolution of more pathogenic races of *P. sojae* (Grau *et al.* 2004). Numerous physiological races of *P. sojae* that can overcome the resistance conferred by the known *Rps* genes have been identified (Dorrance *et al.* 2003). Single *Rps* genes have been effective for 8 to 15 years, depending on inoculum density and environmental conditions (Schmitthenner 1985). The time cycle for loss of disease control with *Rps1*a and *Rps1*c was 8 to 10 years for each gene from the time of introduction of resistant cultivars with these genes (Schmitthenner *et al*. 1994). Cultivars having partial resistance are usually not severely damaged by *P. sojae* in the field, but are killed when hypocotyls or roots are inoculated with a compatible race (Schmitthenner 1985). It is reported that partial resistance is effective against all races of *P. sojae* (Dorrance *et al.* 2003, Schmitthenner 1985). Partial resistance can be evaluated by planting cultivars in the field and rating the incidence of diseased plants or assessing yield loss, or by using an assay in which agar cultures of a compatible race are placed 5 cm below the seeds of cultivars in long pots (Walker and Schmitthenner 1984a). The latter method gives more reliable results due to the better control over environmental conditions compared with the former method, which is influenced by the amount of rainfall and the population pathotypes. There is little *P. sojae* colonization of the roots of soybean cultivars with high levels of partial resistance (McBlain *et al.* 1991). Tooley and Grau (1984) proposed that this type of resistance would limit the lesion growth rate of the pathogen in host tissues and reduce the severity of root rot. Generally, partial resistance has been described as the relative ability of susceptible plants to survive infection without showing severe symptoms like death, stunting, or yield loss (Glover and Scott 1998). Dorrance *et al.* (2003) examined the effect of partial resistance on PSR incidence and seed yield of soybean in Ohio, and demonstrated that yields of soybean cultivars with partial resistance were not significantly different from those of cultivars with single *Rps* genes or *Rps* gene combinations in an environment with low disease pressure, indicating that genetic traits associated with high levels of partial resistance do not have a negative effect on yield. Walker and Schmitthenner (1983b) examined the heritability of tolerance to PSR in soybean. Heritability estimates were not affected by race-specific resistance, although soybean lines having incompatible *Rps* genes had a higher mean tolerance rating than lines which did not have *Rps* genes, indicating that race-specific resistance and tolerance were not completely independent.

'Conrad', a cultivar with high levels of partial resistance, developed more disease when inoculated 0–4 days after planting compared to infection 5 or more days afterwards, while 'Resnik', a cultivar with the *Rps1*k gene and moderate levels of partial resistance, showed no PSR symptoms (Dorrance and McClure 2001). Even if a cultivar with partial resistance is planted, additional control measures such as a combination of race-specific resistance with partial resistance, improved soil drainage, hilled row planting, or seed treatment with a fungicide might be necessary.

Evaluation of soybean cultivars with partial resistance to P. sojae

Dorrance and Schmitthenner (2000) examined 887 soybean plant introductions (from PI 273483 to PI 427107) from the USDA Soybean Germplasm Collection for partial resistance to *P. sojae* races 7, 17, 25, 30 and 31. A total of 438 (55.5%) accessions had high levels of partial resistance or tolerance to *P. sojae* (Table 5). Interestingly, 67.6% of the lines with high levels of partial resistance had originally been collected from South Korea, indicating that *P. sojae* may be endemic to East Asia. Dorrance *et al.* (2003) evaluated the partial resistance of 12 soybean cultivars in seven growing environments (i.e., site × year combinations). They found that the cultivar 'Conrad' (Fehr *et al*. 1989) possessed a high level of partial resistance or tolerance to PSR, and that partial resistance may provide protection when plants were subjected to diverse *P. sojae* populations (Table 5). Several other studies have also demonstrated that 'Conrad' was somewhat susceptible to all of the *P. sojae* races, but has strong partial resistance to the pathogen (Han *et al.* 2008, Jia and Kurle 2008, Weng *et al.* 2007). Mideros *et al.* (2007) reported that 'General' (with *Rps1*k) and 'Jack' had higher levels of partial resistance than other germplasm tested (Table 5) with the slant board assay. Jia and Kurle (2008) examined 113 early maturity group (MG) soybean PIs from the

Table 5. Potential sources with partial resistance to *Phytophthora sojae* to breed new resistant cultivars

Cultivar or line with partial resistance trait	Citation
Conrad	Dorrance *et al.* (2003), Han *et al.* (2008), Jia and Kurle (2008), Sugimoto *et al.* (2010a), Weng *et al.* (2007)
General (with *Rps1*k)	Mideros *et al.* (2007)
Jack	Mideros *et al.* (2007)
438 accessions (PI 273483 to PI 427107) from the USDA Soybean Germplasm Collection	Dorrance *et al.* (2000)
MN0902, MN0302, 91B53, PI 437161, PI 437700, PI 438148, PI 445831, PI 449459, PI 468377, PI 504484, PI 549051, PI 561308, PI 561389B, PI 592919 and PI 593975	Jia and Kurle (2008)
Syoutou-1, Kitamijiro, Yuuhime, Horokanai-zairai, Wabash and Tim144	Yamashita (2008)

USDA germplasm collection for partial resistance to PSR, and 'MN0902' was found to have even higher partial resistance than 'Conrad'. Two American cultivars ('MN0302' and '91B53') and twelve accessions (PI 437161, PI 437700, PI 438148, PI 445831, PI 449459, PI 468377, PI 504484, PI 549051, PI 561308, PI 561389B, PI 592919 and PI 593975) had levels of partial resistance equivalent to 'Conrad' (Table 5).

In Japan, 16 cultivars that are susceptible to ten *P. sojae* races from Hokkaido were examined for partial resistance in the field from 2005 to 2007 (Yamashita 2008). Six cultivars, including 'Syoutou-1', 'Kitamijiro', 'Yuuhime', 'Horokanai-zairai', 'Wabash', and 'Tim144', had higher partial resistance to PSR than the other ten cultivars (Table 5). The partial resistance of 'Conrad' was evaluated in field experiments conducted at five sites in Hyogo between 2006 and 2008. Disease incidence for 'Conrad' ranged from 0 to 3.4%, which was much lower than the 11.7 to 52.0% incidence observed on the susceptible cultivar 'Tanbakuro' (Sugimoto *et al.* 2010a). One Japanese cultivar with partial resistance significantly higher than 'Conrad' was discovered in the field experiments and the resistance was confirmed in laboratory examinations (Sugimoto *et al.* 2011a). These cultivars may be useful as sources of resistance for breeding new cultivars adapted to other parts of Japan or other countries where PSR is a problem.

DNA markers linked to partial resistance in soybean

The heritability of quantitatively inherited partial resistance is relatively high (Burnham *et al.* 2003, Han *et al.* 2008, Weng *et al.* 2007). To date, six QTLs associated with the partial resistance of 'Conrad' have been mapped to four different MLGs in soybean [two QTLs on MLG D1b+W (Chr 2), three QTLs on MLG F (Chr 13) and one on MLG J (Chr 16)] (Burnham *et al.* 2003, Han *et al.* 2008, Weng *et al.* 2007) (Table 6). Burnham et al. (2003) identified two QTLs in three F_4 populations (66 lines from 'Conrad' × 'Sloan', 79 lines from 'Conrad' × 'Williams' and 64 lines from 'Conrad' × 'Harosoy') using lesion length as an indicator of the level of partial resistance. The QTLs were mapped to MLG F (Chr 13) and D1b+W (Chr 2) using phenotypic data from growth chamber assays conducted at the seedling stage (Burnham *et al.* 2003). SSR markers Satt252 and Satt149 on MLG F and Satt579, Satt266 and Satt600 on MLG D1b+W were significantly associated with variation in stem lesion lengths. The QTLs on MLG F and D1b+W explained 21.4–35.0% and 10.6–20.7% of the genotypic variation for the three different populations, respectively. Han *et al.* (2008) detected three QTLs (named QGP1, QGP2 and QGP3) underlying tolerance to PSR in an F_7 population ('Conrad' × 'OX 760-6-1') consisting of 112 lines using growth chamber tests with three *P. sojae* isolates from China. Two markers, Satt509 (2.3–8.6 cM) and Satt343 (2.4–5.1 cM) on MLG F (Chr 13), were located near QGP1 and QGP2, respectively and marker $OPL18_{800}$ (2.35–10.63 cM from the QTL) on MLG D1b+W (Chr 2) was located near QGP3. Weng *et al.* (2007) recently identified a putative QTL on MLG J (Chr 16) in a 'Conrad' × 'OX 760-6-1' F_6 population consisting of 62 lines evaluated in the field at two locations. Satt414 and Satt596 were significantly ($P < 0.005$) associated with variation in reactions to PSR. The putative QTL was flanked by Satt414 (2.0 cM from the QTL) and Satt596 (4.5 cM from the QTL), which explained 13.7% and 21.5% of the total phenotypic variance, respectively.

Tucker *et al.* (2010) first identified three QTLs for partial resistance to a Midwestern USA isolate on MLG J (Chr 16), I (Chr 20) and G (Chr 18) using an interspecific recombinant inbred line (RIL) population consisting of 298 F_{11} individuals derived from a cross between 'V71-370' (a *G. max* cultivar with moderate partial resistance) and PI 407162 (a *G. soja* accession with lower partial resistance) (Table 6). One PI 407126 gene at a major QTL on MLG J accounted for 22–42% of the phenotypic variation in three experiments. This QTL corresponds to the previously identified QTL on MLG J in 'Conrad' (Weng *et al.* 2007). It is unclear why the resistance gene at the QTL with the largest effect was inherited from the more susceptible parental line. A minor QTL gene from 'V71-320' that mapped to MLG I (Chr 18) explained 7–12% of the phenotypic variation, and the location of the QTL coincided with a previously mapped RGA designated as RGA018. Another QTL on MLG G, which explained 9–11% of the variation in three experiments, was

Table 6. Soybeans with partial resistance and the QTLs associated with Phytophthora stem and root rot tolerance

Cultivar or line with partial resistance	Population	Generation	*P. sojae* isolate	Number of QTL	Molecular linkage group (Chromosome)	Marker	Citation
Conrad	66 lines from Conrad × Sloan, 79 lines from Conrad × Williams, and 64 lines from Conrad × Harosoy	F4	OH25 (vir 1a, 1b, 1c, 1k, 7)	2	MLG F (Chr 13), MLG D1b+W(Chr 2)	Satt252 and Satt149 on MLG F, Satt579, Satt266, and Satt600 on MLG D1b+W	Burnham *et al.* (2003)
Conrad	62 lines from Conrad × OX 760-6-1	F6	Woodslee and Weaver	1	MLG J (Chr 16)	Satt414 and Satt596 on MLG J	Weng *et al.* (2007)
Conrad	112 lines from Conrad × OX 760-6-1	F7	JiXi, ShuangYaShan, JianSanJiang, Woodslee	3	MLG F (Chr 13), MLG D1b+W (Chr 2)	Satt509 and Satt343 on MLG F, OPL18800 on MLG D1b+W	Han *et al.* (2008)
PI 407162	298 lines from V71-370 × PI 407162	F11	C2S1(vir 1a, 1b, 1c, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7)	3	MLG J (Chr 16), MLG I (Chr 20), MLG G (Chr 18)	Satt529 and Satt414 on MLG J, Sat_105 and Satt239 on MLG I, Satt235 and Satt163 on MLG G	Tucker *et al.* (2010)

derived from PI 407162 (susceptible), and the marker SLP142, a disease-related EST-SSR, was linked to this QTL. Tucker *et al.* (2010) did not have a good explanation for why resistance genes at the QTLs on MLG-J and G had been contributed by the more susceptible parent, PI 407162. The partial resistance QTLs reported here map to different genomic regions from those in which the known *Rps* genes have been mapped.

Mechanisms involved in partial resistance to Phytophthora stem rot

The molecular mechanisms and the defense responses associated with partial resistance to *P. sojae* in soybean have been examined in 'Conrad' and 'OX 20-8' (which carries *Rps1*a, but has a low level of partial resistance) (Vega-Sanchez *et al.* 2005). Correlations between the expression of defense-related genes and partial resistance between two cultivars were examined, and effective lesion-limiting mechanisms in 'Conrad' were found to occur primarily in the upper root section. At 72 hours after inoculation, transcript levels for PR1a, matrix metalloproteinase (MMP) and basic peroxidase (IPER) at the inoculation site and for IPER above the inoculation site were significantly higher in 'Conrad' compared with 'OX 20-8'. They concluded that defense responses associated with the accumulation of PR1a, MMP, IPER and β-1,3-endoglucanase (EGL) mRNAs may contribute to the partial resistance response of soybean to *P. sojae*.

Thomas *et al*. (2007) indicated that the content of soybean root suberin (a complex biopolymer with a poly component associated with the cell wall and plasma membrane) was involved in partial resistance to *P. sojae*. 'Conrad' was found to contain significantly higher amounts of suberin than 'OX 760-6', a susceptible line. This correlation was supported by data from an analysis of nine cultivars and 32 recombinant inbred lines derived from a 'Conrad' × 'OX 760-6' cross.

Current status and future scope of developing Phytophthora-resistant soybean cultivars

Since *P. sojae* is now firmly established in many soybean production regions around the world, resistance to PSR will continue to be an important objective in many breeding programs. Slaminko *et al.* (2010) reported that a large percentage of soybean cultivars currently or recently marketed in the USA carry at least one *Rps* gene. Although race-specific resistance from certain *Rps* genes has been effective for many years, pathologists and breeders nevertheless recognize the value of partial resistance, which should be more durable and broader than resistance dependent on a single major gene. Similar benefits could also be achieved by "stacking" two or more *Rps* genes or an *Rps* gene and partial resistance genes in the same background. Molecular markers linked to *Rps* genes have been identified, and could be used for MAS to efficiently identify seedlings carrying the resistance gene. With this technique, backcrossing can be performed two or three times in a year to transfer the *Rps* gene into a different elite genetic background. In Hyogo Prefecture in Japan, the black-seeded Phytophthora-resistant line 262-1 (BC4) with *Rps1*d was developed as an alternative to the high-value, but PSR-susceptible cultivar 'Tanbakuro'. This line was evaluated in the field from 2006 to 2008. The incidence of disease in this line was 0%, whereas control plants of 'Tanbakuro' had a disease incidence of 11.7–52.0% (Sugimoto *et al.* 2010a), showing that resistance conferred by some *Rps* genes provides adequate protection against PSR in Japan, where few Phytophthora-resistant cultivars have been developed.

Partial resistance controlled by QTLs would be more durable than resistance conditioned by single *Rps* genes, especially in the USA, where *P. sojae* races that can defeat even the most effective *Rps genes* have been detected (Dorrance *et al.* 2003). Several QTLs for partial resistance to *P. sojae* have been reported, and the molecular markers linked to the QTLs could be used for developing cultivars with partial resistance (Burnham *et al.* 2003, Han *et al.* 2008, Tucker *et al.* 2010, Weng *et al.* 2007). However, it is still difficult to integrate multiple QTLs at independent loci into a cultivar without some adverse effects. Dorrance *et al.* (2003) reported that plants with high partial resistance are still infected by *P. sojae*, which can impact yield under conditions of high inoculum and weather favorable to the pathogen. One of the most promising future strategies for controlling PSR would therefore be to combine partial resistance and race-specific resistance in Japan, as well as in the USA. This could maintain the effectiveness of resistance genes.

In response to the emergence of novel pathotypes, and to provide complete control of *P. sojae*, the identification of additional unique *Rps* genes is required. Sugimoto *et al.* (2011b) found a novel *Rps* gene from the Japanese soybean cultivar Waseshiroge. This *Rps* gene is either allelic to *Rps1*, or resides at a tightly linked locus in a gene cluster. Dorrance and Schmitthenner (2000) reported that the Republic of Korea is an area with many sources of resistance to *P. sojae* for both specific *Rps* genes and partial resistance.

Resistance to PSR could also be enhanced and prolonged if it is used in combination with other available tools and tactics in an integrated approach to disease management. While treatment of seeds with fungicides has proven effective for reducing seedling losses to *P. sojae*, more environmentally benign alternatives are needed. It was recently demonstrated that calcium compounds could reduce the severity of PSR in laboratory and field experiments (Sugimoto *et al*. 2008a, 2010a). $Ca(HCOO)_2$-A [Suicaru—a commercially available calcium formate formulation (Koei Chemical, Nagoya, Japan)] was the most effective in suppressing disease incidence among seven calcium compounds tested. By combining methods such as this with the planting of cultivars having both appropriate *Rps* genes and genes conditioning partial resistance, strategies for the integrated management of PSR of soybean can be both more sustainable and less threatening to the environment than some of the management practices currently used.

Acknowledgements

The authors thank Professor Dr. Anne E. Dorrance of The Ohio State University for valuable discussions and for providing *P. sojae* isolates from Ohio; Eiji Hinomoto for collecting diseased plants; Tomoko Okudaira, Kozue Akamatsu, Toshiharu Ohnishi, Sanae Shikata, Natsuko Ichieda, Masanobu Kawai, Atsuko Mineyama and Seiko Mori for supporting this study; and Akiko Tazawa and Yoko Yamashita (Hokkaido Prefectural Plant Genetic Resource Center), as well as Randall L. Nelson (USDA-ARS) for providing seeds of the soybean differential cultivars. This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan [Genomics for Agricultural Innovation (DD-3113) and Development of mitigation and adaptation techniques to global warming in the sectors of agriculture, forestry, and fisheries (1005)], and by a grant from the Hyogo Prefectural Government (Overseas training programs for young staff, 2007).

Literature Cited

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., 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.

Athow, K.L. and F.A. Laviolette (1982) *Rps6*, a major gene for resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. Phytopathology 72: 1564–1567.

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

Bernard, R.L. and C.R. Cremeens (1981) An allele at the rps_1 locus from the variety 'Kingwa'. Soybean Genet. Newsl. 8: 40–42.

Bernard, R.L. and C.R. Cremeens (1988) Registration of 'Williams 82' soybean. Crop Sci. 28: 1027–1028.

Bernard, R.L., R.L. Nelson and C.R. Cremeens (1991) USDA Soybean Genetic Collection: Isoline collection. Soybean Genet. Newsl. 18: 27–57.

Bhat, R.G., B.A. McBlain and A.F. Schmitthenner (1993) Development of pure lines of *Phytophthora sojae* races. Phytopathology 83: 473–477.

Bhattacharyya, M.K., N.N. Narayanan, H. Gao, D.K. Santra, S.S. Salimath, T. Kasuga, Y. Liu, B. Espinosa, L. Ellison, L. Marek *et al*. (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.

Burnham, K.D., A.E. Dorrance, T.T. VanToai and S.K. St. Martin (2003) Quantitative trait loci for partial resistance to *Phytophthora sojae* in soybean. Crop Sci. 43: 1610–1617.

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.

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 *et al*. (1999) An integrated genetic linkage map of the soybean genome. Crop Sci. 39: 1464–1490.

Cregan, P.B. (2003) Soybean_Composite_Genetic_Map_2003 http://soybeanbreederstoolbox.org/

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.

Dong, S., D. Yu, L. Cui, D. Qutob, J. Tedman-Jones, S.D. Kale, B.M. Tyler, Y. Wang and M. Gijzen (2011) Sequence variants of the *Phytophthora sojae* RXLR effector Avr3a/5 are differentially recognized by *Rps*3a and *Rps*5 in soybean. PLoS ONE 6: e20172. doi:10.1371/journal.pone.0020172.

Dorrance, A.E. and A.F. Schmitthenner (2000) New sources of resistance to *Phytophthora sojae* in the soybean plant introductions. Plant Dis. 84: 1303–1308.

Dorrance, A.E. and S.A. McClure (2001) Beneficial effect of fungicide seed treatments for soybean cultivars with partial resistance to *Phytophthora sojae*. Plant Dis. 85: 1063–1068.

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.

Dorrance, A.E., H. Jia and T.S. Abeny (2004) Evaluation of soybean differentials for their interaction with *Phytophthora sojae*. Plant Health Prog. doi:10.1094/PHP-2004-0309-01-RS.

Fehr, W.R., S.R. Cianzio, B.K. Voss and S.P. Schultz (1989) Registration of 'Conrad' soybean. Crop. Sci. 29: 830.

Fry, W.E. (1982) Plant resistance: effects and mechanisms. *In*: Principles of Plant Disease Management, Academic Press, San Diego, California, USA, pp. 195–217.

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.

Gao, H. and M.K. Bhattacharyya (2008) The soybean-Phytophthora resistance locus *Rps1*-k encompasses coiled coil-nucleotide binding-leucine rich repeat-like genes and repetitive sequences. BMC Plant Biol. 8: 29. doi: 10.1186/1471-2229-8-29.

Gijzen, M. and D. Qutob (2009) *Phytophthora sojae* and soybean. *In*: Lamour, K. and S. Kamoun (eds.) Oomycete Genetics and Genomics, John Wiley & Sons, Hoboken, New Jersey, USA, pp. 303–329.

Glover, K.D. and R.A. Scott (1998) Heritability and phenotypic variation of tolerance to Phytophthora root rot of soybean. Crop Sci. 38: 1495–1500.

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.

Gordon, S.G., K. Kowitwanich, W. Pipatpongpinyo, S.K. St. Martin and A.E. Dorrance (2007) Molecular marker analysis of soybean plant introductions with resistance to *Phytophthora sojae*. Phytopathology 97: 113–118.

Graham, M.A., L. F. Marek, D. Lohnes, P. Cregan and R.C. Shoemaker (2000) Expression and genome organization of resistance gene analogs in soybean. Genome 43: 86–93.

Graham, M.A., L.F. Marek and R.C. Shoemaker (2002) Organization, expression and evolution of a disease resistance gene cluster in soybean. Genetics 162: 1961–1977.

Grau, C.R., A.E. Dorrance, J. Bond and J. Russin (2004) Fungal diseases. *In*: Boerma, H.R. and J.E. Specht (eds.) Soybeans: Improvement, Production and Uses. 3rd ed., Agronomy Monogr. American Soc. Agron. Madison, WI, pp. 679–763.

Han, Y.P., W.L. Teng, K.F. Yu, V. Poysa, T. Anderson, L.J. Qiu, D.A. Lightfoot and W.B. Li (2008) Mapping QTL tolerance to Phytophthora root rot in soybean using microsatellite and RAPD/ SCAR derived markers. Euphytica 162: 231–239.

Hyten, D.L., I.-Y. Young Choi, Q. Song, J.E. Specht, T.E. Carter, R.C. Shoemaker, E.Y. Hwang, L.K. Matukumalli and P.B. Cregan (2010) A high density integrated genetic linkage map of soybean and the development of a 1536 universal soy linkage panel for quantitative trait locus mapping. Crop Sci. 50: 960–968.

Jia, H. and J.E. Kurle (2008) Resistance and partial resistance to *Phytophthora sojae* in early maturity group soybean plant introductions. Euphytica 159: 27–34.

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.

Kato, M. (2010) Recent reseach on Phytophthora root and stem rot of soybean in Japan. Plant Protection 64: 497–500.

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.

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.

Lohnes, D.G. and A.F. Schmitthenner (1997) Position of the Phytophthora resistance gene *Rps7* on the soybean molecular map. Crop Sci. 37: 555–556.

McBlain, B.A., J.J. Hacker, M.M. Zimmerly and A.F. Schmitthenner (1991) Tolerance to Phytophthora rot in soybean: II. Evaluation of three tolerance screening methods. Crop Sci. 31: 1412–1417.

Michelmore, R.W. and B.C. Meyers (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. 8: 1113–1130.

Mideros, S., M. Nita and A.E. Dorrance (2007) Characterization of components of partial resistance, *Rps*2, and root resistance to *Phytophthora sojae* in soybean. Phytopathology 97: 655–662.

Moriwaki, J. (2010) Aiming of the construction of the race distinction system of *Phytophthora sojae*. Plant Prot. 64: 508–510.

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.

Ploper, L.D., K.L. Athow and F.A. Laviolette (1985) A new allele at *Rps3* locus for resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. Phytopathology 75: 690–694.

Polzin, K.M., D.G. Lohnes, C.D. Nickell and R.C. Shoemaker (1994) Integration of *Rps2*, *Rmd*, and *Rj2* into linkage group J of the soybean molecular map. J. Hered. 85: 300–303.

Richly, E., J. Kurth and D. Leister (2002) Mode of amplification and reorganization of resistance genes during recent *Arabidopsis thaliana* evolution. Mol. Biol. Evol. 19: 76–84.

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 *Rps4* 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*: Hartman,

G.L., J.B.Sinclair and J.C.Rupe (eds.) Compendium of Soybean Diseases. 4th edn., The American Phytopathological Society Press, St. Paul, Minnesota, pp. 39–42.

Schmitthenner, A.F., M.Hobe and R.G.Bhat (1994) *Phytophthora sojae* races in Ohio over a 10-year interval. Plant Dis. 78: 269–276.

Schmutz, J., S.B.Cannon, J.Schlueter, J.Ma, T.Mitros, W.Nelson, D.L. Hyten, Q.Song, J.J.Thelen, J.Cheng *et al.* (2010) Genome sequence of the palaeopolyploid soybean. Nature 463: 178–183.

Slaminko, T.L., C.R.Bowen and G.L.Hartman (2010) Multi-year evaluation of commercial soybean cultivars for resistance to *Phytophthora sojae*. Plant Dis. 94: 368–371.

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., 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.

Sugimoto, T., S.Yoshida, K.Watanabe, M.Aino, T.Kanto, K.Maekawa and K.Irie (2008a) Identification of SSR markers linked to the Phytophthora resistance gene *Rps1*-d in soybean. Plant Breed. 127: 154–159.

Sugimoto, T., K.Watanabe, S.Yoshida, M.Aino, K.Irie, T.Matoh and A.R.Biggs (2008b) Select calcium compounds reduce the severity of Phytophthora stem rot of soybean. Plant Dis. 92: 1559–1565.

Sugimoto, T., K.Watanabe, M.Furiki, D.R.Walker, S.Yoshida, M. Aino, T.Kanto and K.Irie (2009) The effect of potassium nitrate on the reduction of Phytophthora stem rot disease of soybeans, the growth rate and zoospore release of *Phytophthora sojae*. J. Phytopathol. 157: 379–389.

Sugimoto, T., K.Watanabe, S.Yoshida, M.Aino, M.Furiki, M.Shiono, T.Matoh and A.R.Biggs (2010a) Field application of calcium to reduce Phytophthora stem rot of soybean, and calcium distribution in plants. Plant Dis. 94: 812–819.

Sugimoto, T., S.Yoshida, M.Aino, K.Maekawa, T.Kanto, T.Matoh, Y.Koroda, K.Irie and K.Watanabe (2010b) Pathogenic races of *Phytophthora sojae* in *Glycine max* cv. Tanbakuro producing areas in Hyogo and selection of parental soybeans for breeding new resistant cultivars. J. Crop Res. 55: 19–25.

Sugimoto, T., S.Yoshida, M.Aino, R.Yamamoto, T.Kuroda, M.Maeda and K.Irie (2011a) Evaluation of several soybeans for field resistance to *Phytophthora sojae* and selection of parental soybeans for breeding new resistant cultivars. Jpn. J. Phytopathol. 71:157.

Sugimoto, T., S.Yoshida, A.Kaga, M.Hajika, K.Watanabe, M.Aino, K.Tatsuda, R.Yamamoto, T.Matoh, D.R.Walker *et al*. (2011b) Genetic analysis and identification of DNA markers linked to a novel *Phytophthora sojae* resistance gene in the Japanese soybean cultivar Waseshiroge. Euphytica 182: 133–145.

Tazawa, A. and M.Tezuka (2003) DNA markers related with resistance to *Phytophthora sojae* and its effectiveness. Breed. Res. 5: 192.

Thomas, R., X.Fang, K.Ranathunge, T.R.Anderson, C.A.Peterson and M.A.Bernards (2007) Soybean root suberin: Anatomical distribution, chemical composition, and relationship to partial resistance to Phytophthora sojae. Plant Physiol. 144: 299–311.

Tooley, P.W. and C.R.Grau (1984) The relationship between rate-reducing resistance to *Phytophthora megasperma* f. sp. *glycinea* and yield of soybean. Phytopathology 74: 1209–1216.

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.

Tsuchiya, S., F.Tanaka and T.Adachi (1990) Classification of race of *Phytophthora sojae* and screening of resistant soybean cultivars to Phytophthora stem rot. Ann. Phytopathol. Soc. Jpn. 56: 144.

Tucker, D.M., M.A. Saghai Maroof, S. Mideros, J.A. Skoneczka, D.A.Nabati, G.R.Buss, I.Hoeschele, B.M.Tyler, S.K.St. Martin and A.E.Dorrance (2010) Mapping quantitative trait loci for partial resistance to *Phytophthora sojae* in a soybean interspecific cross. Crop Sci. 50: 628–635.

Van der Plank, J.E. (1963) Vertical and horizontal resistance against potato late blight. *In*: Plant Diseases: Epicemics and Contol, Academic Press, New York, New York, USA, pp. 171–205.

Vega-Sanchez, M.E., M.G.Redinbaugh, S.Costanzo and A.E.Dorrance (2005) Spatial and temporal expression analysis of defense-related genes in soybean cultivars with different levels of partial resistance to *Phytophthora sojae*. Physiol. Mol. Plant Pathol. 66: 175–182.

Walker, A.K. and A.F.Schmitthenner (1984a) Comparison of field and greenhouse evaluations for tolerance to Phytophthora rot in soybean. Crop Sci. 24: 487–490.

Walker, A.K. and A.F.Schmitthenner (1984b) Heritability of tolerance to Phytophthora rot in soybean. Crop Sci. 24: 490–491.

Weng, C., K.Yu, T.R.Anderson and V.Poysa (2001) Mapping genes conferring resistance to *Phytophthora* root rot of soybean, *Rps1*a and *Rps7*. J. Hered. 92: 442–446.

Weng, C., K.Yu, T.R.Anderson and V.Poysa (2007) A quantitative trait locus influencing tolerance to Phytophthora root rot in the soybean cultivar 'Conrad'. Euphytica 158: 81–86.

Wilcox, J.R. and S.K.St. Martin (1998) Soybean genotypes resistant to *Phytophthora sojae* and compensation for yield losses of susceptible isolines. Plant Dis. 82: 303–306.

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.

Wrather, J.A. and S.R.Koenning (2006) Estimates of disease effects on soybean yields in the United States 2003 to 2005. J. Nematol. 38: 173–180.

Yamashita, Y. (2008) Studies on resistance to *Phytophthora sojae* in soybean in Hokkaido. Plant Protec. 62: 457–460.

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

Zenbayashi, K., T.Ashizawa, T.Tani and S.Koizumi (2002) Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32. Theor. Appl. Genet. 104: 547–552.