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FUNGAL DISEASES

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Race distribution of *Phytophthora sojae* on soybean in Hyogo, Japan

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Abstract Since 1987, Phytophthora root and stem rot of soybean [*Glycine max* (L.) Merr. cv. Tanbakuro], caused by *Phytophthora sojae* Kaufman and Gerdemann, has been increasing in the Sasayama, Nishiwaki, and Kasai regions in Hyogo, the most famous soybean (cv. Tanbakuro)-producing areas in Japan. In 2002 to 2004, 51 isolates (one from each field) of *P. sojae* were recovered from 51 fields in Hyogo. These isolates were tested for virulence on six Japanese differential soybean cultivars used for race determination in Japan, and three additional ones containing four *Rps* genes used in Indiana, USA. Race E was the most prevalent from 2002 to 2004, followed by races A, C, D, and four new races (proposed as races K, L, M, and N). Interestingly, none of the new races had high virulence on the Japanese differential cultivars, compared with other races in each area. One (race N) was avirulent on all six soybean differentials. There was a difference in race distribution on each of three individual areas; race E seemed to be a major component of the *P. sojae* population in Sasayama, whereas race A and the new race M were the most prevalent in Nishiwaki and Kasai, respectively. *Rps*6 (cv. Altona) and *Rps*1a + *Rps*7 (cv. Harosoy 63) were infected by 90.2% and 33.3% of all isolates, respectively. However, *Rps*1d (cv. PI103091) was not susceptible to any of the 51 isolates, nor was cv. Gedenshirazu-1. These two soybean cultivars were considered to be potential sources of resistance to breed new resistant cultivars with the desirable characteristics of cv. Tanbakuro for this region.

Key words *Glycine max* (L.) Merr. cv. Tanbakuro · Phytophthora stem and root rot · *Rps* gene

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Introduction

Tanbakuro [*Glycine max* (L.) Merr. cv.], which is one of the most famous commercial and traditional black soybean cultivars in Japan, is produced in the Sasayama, Nishiwaki, and Kasai regions of Hyogo (western Japan, Fig. 1). Within these regions, cv. Tanbakuro was grown on 1150 ha in 2003, making up 34% and 54% of the total production in Japan and Hyogo, respectively. This soybean cultivar is prized because its seeds are much larger than those of other cultivars, weighing about 80–85 g per 100 seeds; consequently, it fetches a higher market price than other soybeans in Japan. Furthermore, cv. Tanbakuro is reported to have many positive effects on the human body and health (Hirota et al. 2000). Despite these desirable characteristics, cv. Tanbakuro is susceptible to many pathogens (Irie et al. 1990).

One of the most important diseases on soybeans, Phytophthora root and stem rot caused by *Phytophthora sojae*, was first observed in Indiana and in Ohio in 1951 by Kaufman and Gerdemann (1958). When soybeans are infected, the stem of the plant appears water-soaked and turns brown, and the infection may result in wilting and the death of plants. In Japan this disease was first observed in 1977 on Hokkaido, a northern island of Japan (Tsuchiya et al. 1978), after which it has spread to other parts of Japan. The disease was also recorded in Shizuoka in 1978 (Suzuki et al. 1980), Yamagata in 1979 (Sato et al. 1981), Akita in 1980 (Tsuchiya and Furuya 1983), Saga in 1983 (Kan et al. 1984), and Hyogo in 1987 (Irie et al. 1990). Furthermore, this disease remains a serious problem in other soybean-producing areas of the rest of the world, such as Argentina, Australia, Brazil, Canada, The People's Republic of China, Hungary, Italy, the former Soviet Union, and the United States (Schmitthenner 1999). Because soybean producers' income has been rapidly decreasing, disease management strategies require immediate attention and implementation.

Although this disease has been controlled with fungicides, calcium application (Sugimoto et al. 2005), field resistance, soil drainage, and tillage practices for over 40 years,

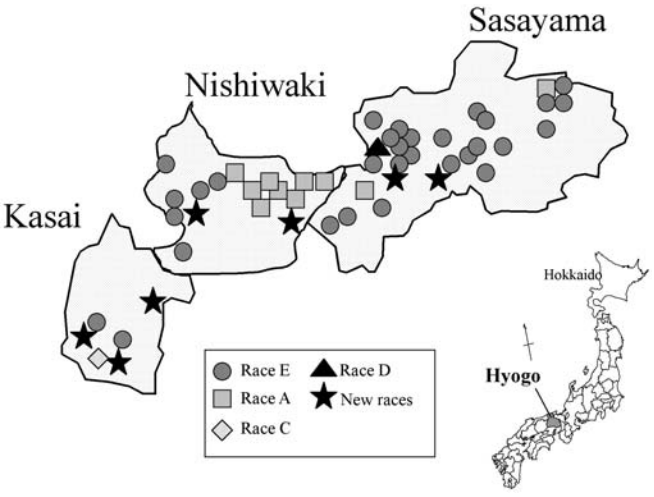

Fig. 1. Regions in Hyogo surveyed for races of *Phytophthora sojae* isolated from *Glycine max* (L.) Merr. cv. Tanbakuro from 2002 to 2004; Sasayama region (29 fields), Nishiwaki region (16 fields), Kasai region (6 fields)

the most effective method to reduce the damage is to develop resistant and tolerant cultivars of soybeans (Schmitthenner 1999). Breeding resistant cultivars having the desirable features of cv. Tanbakuro is important to minimize losses through the use of this cultivar and to increase farmers' incomes. Selecting parental lines with specific resistance is essential for breeding new cultivars.

Since 1955, physiologic races of *P. sojae* have been identified by many researchers. Fifty-five races of *P. sojae* have been reported in Ohio (Leitz et al. 2000). In Japan, ten races of *P. sojae* were identified by Tsuchiya et al. (1990) using six Japanese differential cultivars in 1990. However, there is no report about the race distribution of *P. sojae* and the races present in Japan, except for Hokkaido. Therefore, it is important to know which virulence phenotypes are present in fields in Hyogo.

The objectives of this study were to determine (1) the race distribution of *P. sojae* in cv. Tanbakuro-producing areas in Hyogo, (2) the effectiveness of *Rps* (resistance to *Phytophthora sojae*) genes and (3) the effectiveness of Phytophthora stem rot-resistant accessions to several races.

Materials and methods

Pathogens used in this study

A total of 51 diseased soybean plants (cv. Tanbakuro) were collected from 51 field areas in three regions of Hyogo from 2002 to 2004. All 51 fields surveyed were in the Sasayama, Nishiwaki, and Kasai regions of Hyogo (Fig. 1). Disease incidence was assessed in late August from 2002 to 2004, and measured on five spots in each field. It was calculated as the ratio of the number of infected plants to 100 soybean plants. The incidence of disease in each field was determined by an average of the ratio of the five spots in the field. Infected tissues were surface disinfected in 1.0% NaOCl for 5 min, and washed three times with distilled water. These tissue pieces were then placed on potato dextrose agar (PDA; 2.7 g of potato dextrose powder, 15 g of sugar, 10 g of agar per liter of distilled water) medium containing BNPRA-HMI (20 mg of Benomyl, 25 mg of nystatin, 25 mg of PCNB, 10 mg of rifampicin, 500 mg of anpicillin, and 25 mg of 3-hydroxy-5-methylisoxazole per liter of PDA medium) (Masago et al. 1977) for 5 days. The tips of the hyphae (3-mm-diameter plugs) were transferred to new PDA with BNPRA-HMI. Fifty-one isolates (one from each field) were isolated and examined for oospores, sporangia, antheridia size, shape, and optimum growth temperature on PDA using 7-day-old cultures (to identify that the isolates were *Phytophthora sojae*). MAFF isolates (MAFF 235802, MAFF 235803, and MAFF 235804 recovered from Hokkaido), which were obtained from the Ministry of Agriculture, Forestry, and Fisheries, Japan (MAFF) Genebank collection, were used as controls for measurement of the morphological features.

Race determination of isolates

Glycine max (L.) Merr. cv. Tanbakuro and cv. Williams (*rps*) were used as susceptible control plants during virulence tests. Japanese soybean cultivars used for race determination in Hyogo were cvs. Isuzu, Chusei-Hikarikuro, Kitamusume, Toyosuzu, Gedenshirazu-1, and Ohojyu, which were obtained from the Hokkaido Prefectural Plant Genetic Resource Center. The race of each of the 51 isolates was identified according to their resistance reactions on the six Japanese differential cultivars (Table 1). Cv. PI103091 (*Rps*1d), cv. Altona (*Rps*6), and cv. Harosoy 63 (*Rps*1a + *Rps*7) were added to the other differentials (Laviolette and Athow 1981). An additional cultivar (in which the *Rps* gene was unknown), Waseshiroge, was resistant to most of the isolates of *P. sojae* obtained from Hokkaido (Tsuchiya et al. 1990). The virulence of each isolate was evaluated after hypocotyl inoculation (Laviolette and Athow 1981), as modified by Sugimoto et al. (2003, 2005). After the first primary leaf appeared, the stem of the soybean near ground level was covered with two 3-mm-diameter plugs of 20-day-old mycelium cultured on PDA. Thereafter, the plants were incubated in a growth chamber at 23°C with a 16-h day length under fluorescent light (light intensity: 150 $\mu Em^{-2}s^{-1}$). About 10 days after inoculation, the number of dead or survived plants in each bottle was recorded, and cultivars were rated as resistant or susceptible. Each cultivar was considered resistant if less than 20% of the plants died and susceptible if more than 20% of the plants were infected. Bioassays were repeated three times, and only reproducible data was used for race determination. The race determination data was applied to the region where the isolate was obtained to investigate race distribution. If the isolate was classified as a new race, the virulence test was repeated more than three times to confirm the results.

Table 1. Races of *Phytophthora sojae* reported in Japan using Japanese differential cultivars

Differential cultivars	Races of *P. sojae*[a]													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Isuzu	S	S	R	S	S	S	S	S	R	S	S	S	R	R
Chusei-Hikarikuro	R	S	S	R	S	S	S	S	S	S	R	R	R	R
Kitamusume	S	S	S	S	S	S	S	S	S	S	R	R	S	R
Toyosuzu	R	R	S	R	S	R	S	S	S	S	R	R	R	R
Gedenshirazu-1	R	R	R	R	R	R	R	S	S	S	R	R	R	R
Ohojyu	R	R	R	S	R	S	S	R	S	S	S	R	R	R

S, susceptible; R, resistant

[a] Races A to J of *P. sojae* were reported by Tsuchiya et al. in 1990 using six Japanese differential cultivars. Races K, L, M, and N were first reported in Hyogo from 2002 to 2004

Table 2. Disease incidence of Phytophthora root and stem rot on soybean at 51 fields in three regions of Hyogo from 2002 to 2004

Region surveyed	Disease incidence (%)[a]	Year	Region surveyed	Disease incidence (%)[a]	Year
Mananjyo, Sasayama	14	2002	Higashihonjyo, Sasayama	20	2004
Hatai, Sasayama	24	2002	Shimoharayama, Sasayama	30	2004
Hatai, Sasayama	16	2002	Yagami, Sasayama	6	2004
Ichinono, Sasayama	16	2002	Ishihara, Nishiwaki	20	2002
Honjyo, Sasayama	18	2002	Kurodasyo, Nishiwaki	24	2002
Kamionohara, Sasayama	10	2003	Nakayasuda, Nishiwaki	42	2002
Yagami, Sasayama	4	2003	Sakamoto, Nishiwaki	2	2002
Ichinono, Sasayama	22	2003	Kurodasyo, Nishiwaki	8	2002
Ohyama, Sasayama	26	2003	Ishihara, Nishiwaki	10	2002
Shinjyo, Sasayama	8	2003	Ishihara, Nishiwaki	8	2003
Ichinono, Sasayama	10	2003	Sakamoto, Nishiwaki	8	2003
Wada, Sasayama	16	2003	Nakayasuda, Nishiwaki	46	2003
Hatai, Sasayama	24	2003	Kurodasyo, Nishiwaki	4	2004
Hatamiya, Sasayama	20	2003	Ishihara, Nishiwaki	6	2004
Kan, Sasayama	8	2003	Nakayasuda, Nishiwaki	40	2004
Ogura, Sasayama	16	2003	Sakamoto, Nishiwaki	2	2004
Kawakita, Sasayama	10	2004	Arata, Nishiwaki	8	2004
Kawakita, Sasayama	8	2004	Shimomihara, Nishiwaki	2	2004
Kawakita, Sasayama	10	2004	Kurodasyo, Nishiwaki	16	2004
Kawakita, Sasayama	16	2004	Kenzaka, Kasai	6	2002
Kawakita, Sasayama	18	2004	Nishiosa, Kasai	10	2002
Ajima, Sasayama	4	2004	Yamashita, Kasai	8	2002
Mananjyo, Sasayama	8	2004	Befu, Kasai	4	2004
Ichinono, Sasayama	6	2004	Yamashita, Kasai	6	2004
Hatai, Sasayama	10	2004	Higashikenzaka, Kasai	4	2004
Ogami, Sasayama	12	2004			

[a] Disease incidence was measured in late August from 2002 to 2004

Results

Isolation from diseased plants at three regions in Hyogo

From 2002 to 2004, 51 isolates of *Phytophthora sojae* were recovered in Hyogo from 51 field areas with a disease incidence ranging from 2% to 46% (Table 2). Disease incidence in 2002, 2003, and 2004 was 15.6%, 16.1%, and 10.9%, respectively. Disease incidences in Sasayama, Nishiwaki, and Kasai region from 2002 to 2004 were 15.2%, 13.5%, and 5.3%, respectively. Oogonia were spherical, and the diameter of oospores varied from 32 to 41 μm, with an average of 36 μm; 29 to 40, average 34 μm (MAFF 235802, MAFF 235803, and MAFF 235804; MAFF isolates). Sporangia were typically nonpapillate pyriform and ranged from 35.0 to 48.0 μm in length (average 41 μm), and 21.0 to 27.5 μm in width (average 24 μm) (Hyogo isolates); 28.0 to 43.0 μm in length (average 35 μm), and 23.0 to 25.0 μm in width (average 22 μm) (MAFF isolates). Antheridia ranged from 12.5 to 20.0 μm in length (average 15.4 μm) and from 9.4 to 20.0 μm in width (average 11.1 μm) (Hyogo isolates); 11.0 to 21.0 μm in length (average 13 μm), and 9.0 to 15.0 μm in width (average 12 μm) (MAFF isolates). Antheridia were paragynous. The optimum temperature for growth on PDA ranged from 22° to 25°C (Hyogo isolates); 23° to 24°C (MAFF isolates). As a result, there was no significant difference in the morphological features between the MAFF isolates and the Hyogo isolates. The morphological characteristics of sporangia, oogonia, antheridia, oospores, and the optimum growth temperature of isolates were identical to those of *P. sojae* (Hildebrand 1959).

Table 3. Race components of *Phytophthora sojae* in 2002 to 2004 and race distribution in each of three regions in Hyogo

Year	Number of isolates[a]	Regions surveyed	Races of *P. sojae* (no. of isolates)												
			A	B	C	D	E	F	G	H	I	J	K	L	M	N
2002	14	Sasayama	0	0	0	0	5	0	0	0	0	0	0	0	0	0
		Nishiwaki	4	0	0	0	1	0	0	0	0	0	0	0	0	1
		Kasai	0	0	1	0	2	0	0	0	0	0	0	0	0	0
2003	14	Sasayama	2	0	0	1	7	0	0	0	0	0	1	0	0	0
		Nishiwaki	1	0	0	0	2	0	0	0	0	0	0	0	0	0
2004	23	Sasayama	0	0	0	0	12	0	0	0	0	0	0	1	0	0
		Nishiwaki	3	0	0	0	3	0	0	0	0	0	0	1	0	0
		Kasai	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Total	51		10	0	1	1	32	0	0	0	0	0	1	2	3	1

[a] Fifty-one isolates (one from each field) of *Phytophthora sojae* were recovered from 51 fields in Hyogo from 2002 to 2004

Race determination and distribution of isolates in Hyogo

The race determination of the 51 isolates recovered from 2002 to 2004 is summarized in Table 3 and Fig. 1. Cvs. Tanbakuro and Williams (*rps*) were susceptible to all 51 isolates during the test; the isolates were strongly virulent on those cultivars (90% to 100%). No intermediate or inconsistent responses occurred on any of the differential cultivars. Race E was the most prevalent, representing 62.7% of the 51 isolates, followed by races A (19.6%), C (2%), D (2%), and new races (K, L, M, and N) (13.7%). Races B, F, G, H, I, and J were not recovered at all from 2002 to 2004 (Table 3). Results of the races of *P. sojae* isolated for 2002 to 2004 are also shown in Table 3, and no shifts in the frequency of the prevalent races was seen. Although race E tended to be dominant in Sasayama, race A was the most prevalent in Nishiwaki (50%), followed by race E (37.5%), and the new races (12.5%), as shown in Fig. 1. In Kasai, new race M was considered prevalent (50%), followed by race E.

Four new races (K, L, M, and N) were discovered from Hyogo (Table 1). Race K (obtained from Sasayama) was virulent on cvs. Isuzu and Ohojyu, but was nonpathogenic on cvs. Chusei-Hikarikuro, Kitamusume, Toyosuzu, and Gedenshirazu-1. Race L (isolated from Nishiwaki and Sasayama) was only virulent on cv. Isuzu. These two races, which were avirulent on cv. Kitamusume, were first noted in Japan. Race M (obtained from Kasai) was only pathogenic on cv. Kitamusume. These three new races were not highly virulent on the Japanese differential cultivars, compared with the other races in each of three areas; race K, L, or M versus race A, C, D, or E. Moreover, one isolate (PJ-H12) that was obtained from Nishiwaki was not virulent on any of the differentials, although the isolate was pathogenic to cvs. Williams and Tanbakuro. Therefore, the PJ-H12 is *P. sojae*, which was also identified for the first time in Japan and classified as new race N on six differentials used in Japan (Table 1).

Reaction of Japanese differential cultivars to isolates and the effectiveness of *Rps* genes in Hyogo

The percentage of *P. sojae* isolates that could defeat each of the six Japanese cultivars and the effectiveness of genes *Rps*1d, *Rps*6, and *Rps*1a + *Rps*7 in Hyogo are summarized in Table 4. Cv. Gedenshirazu-1 was strongly resistant to all 51 isolates, and cv. Ohojyu was slightly resistant to 49 of 51 isolates. Cv. PI103091 containing *Rps*1d was resistant to all isolates; cv. Altona containing *Rps*6 and cv. Harosoy 63 containing *Rps*1a + *Rps*7 were defeated by 90.2% and 33.3% of all isolates, respectively. Cv. Waseshiroge, considered to be strongly resistant in Hokkaido, was susceptible to 64.7% of the isolates. This cultivar was ineffective. Cvs. Isuzu and Altona (*Rps*6) had the same reaction pattern to all 51 isolates.

Table 4. Percentage of *Phytophthora sojae* isolates that defeated each of six Japanese differential cultivars and additional cultivars with four *Rps* genes in 2002 to 2004

Japanese differential cultivars	Virulent isolates (%)			
	2002	2003	2004	Total
Isuzu	86	100	87	90
Chusei-Hikarikuro	64	64	65	65
Kitamusume	93	93	91	92
Toyosuzu	64	64	65	65
Gedenshirazu-1	0	0	0	0
Ohojyu	0	14	0	4
PI103091 (*Rps*1d)	0	0	0	0
Altona (*Rps*6)	86	100	87	90
Harosoy 63 (*Rps*1a + *Rps*7)	29	29	39	33
Unknown[a]	64	64	65	65

[a] Genotype of resistant gene in cv. Waseshiroge has not been determined

Interaction between several *Rps* genes and races in Hyogo

When *Rps*1d, *Rps*6, and *Rps*1a + *Rps*7 genes were applied in the virulence phenotype of seven races in Hyogo, race E was classified into two types of races (Table 5); the virulence phenotype of race E-1 [*Rps*1d (resistant), *Rps*6 (susceptible), and *Rps*1a + *Rps*7 (susceptible)] and E-2 [*Rps*1d (resistant), *Rps*6 (susceptible) and *Rps*1a + *Rps*7 (resistant)], comprised 62.5% and 37.5% of race E, respectively. Races A, C, D, K, L, M, and N each had only one reaction to four *Rps* genes.

Table 5. Reaction of races of *Phytophthora sojae* to four *Rps* genes in Hyogo from 2002 to 2004

Races[b]	*Rps* genes[a]		
	*Rps*1d	*Rps*6	*Rps*1a + *Rps*7
A	R	S	R
C	R	R	R
D	R	S	R
E-1	R	S	S
E-2	R	S	R
K	R	S	R
L	R	S	R
M	R	R	R
N	R	R	R

[a] *Rps*1d in cv. PI103091, *Rps*6 in cv. Altona, *Rps*1a + *Rps*7 in cv. Harosoy 63

[b] Race E has two virulence phenotypes; cv. Harosoy 63 containing *Rps*1a + *Rps*7 was susceptible (E-1) or resistant (E-2) to 62.5% or 37.5% of race E, respectively

Discussion

In this study, to determine the race distribution of *Phytophthora sojae* in Hyogo, Japan, 51 isolates represented eight different virulence phenotypes. Race E was the most prevalent in Hyogo in 2002–2004, accounting for 62.7% of the 51 isolates (Table 3). Based on the race distribution and reaction of differentials to isolates in Hyogo, race diversity in Hokkaido differed from that in Hyogo: in Hokkaido, race D was the most prevalent, followed by races A and J (Tsuchiya et al. 1990).

Shifts in the frequency of prevalent races were not seen during the 3-year survey (Table 3). In worldwide research, temporal changes in the racial composition of *P. sojae* were caused by the resistance genes used in commercial cultivars, that is, by shifts in the compatible and incompatible genotypes in cultivars (Anderson and Bussell 1992; Ryley et al. 1998). Such changes in racial dynamics did not occur in Hyogo from 2002 to 2004 because few soybean cultivars were grown in the regions surveyed. Long-term research is needed in the future.

In the 2002 to 2004 survey, race A and new race M were the major races in the Nishiwaki and Kasai areas, respectively, although race E was the most prevalent throughout the rest of Hyogo. This result indicates each of the isolates evolves in particular areas. It has also been suggested that several races can exist randomly in one region, even in the presence of a predominant race. This tendency also can be seen in Ohio (Schmitthenner et al. 1994), Indiana (Laviolette and Athow 1981), Iowa (Yang et al. 1996), and Australia (Ryley et al. 1998). As a consequence, several new resistant cultivars must be bred to the dominant races in each region. Such a breeding strategy would be suitable for the traditional cultivation of Tanbakuro.

Ten soybean cultivars were tested as parental lines for breeding new resistant cultivars having the desirable traits of cv. Tanbakuro. Gedenshirazu-1 and PI103091 (*Rps*1d) were strongly resistant to all 51 isolates. Similarly, *Rps*1d could be used to control a majority of races in the world and is an effective gene (Abney et al. 1997). It is believed from this study that *Rps*1d and one other *Rps* gene (contained in cv. Gedenshirazu-1) should be incorporated into cv. Tanbakuro in combination or singly, according to the race distribution in each field.

There are two virulence phenotypes for race E (Table 5); race E was divided into E-1 and E-2 when cv. Harosoy-63 is added to six Japanese differential cultivars. During the virulence test, seven isolates were designated as new races, proposed as races K, L, M, and N (Table 3). These results indicate that at least 15 races of *P. sojae* exist in Japan.

Recently, *P. sojae* has evolved into new races that rapidly overcome the commonly used *Rps* genes (Schmitthenner 1999). Even more races of *P. sojae* may develop, rendering the *Rps* genes currently used ineffective. To prevent this, we must search for additional cultivars that are resistant to Phytophthora root and stem rot before new races arise. *Rps*1k in cv. Kingwa is used as the predominant resistant gene in soybean-producing areas around the world (Abney et al. 1997). Cultivars containing the *Rps*1k allele should be tested in Japan because this gene may be also effective against Japanese races.

In conclusion, race E is a major component of the *P. sojae* population in Hyogo, and cvs. Gedenshirazu-1 and PI103091 are effective as parental lines for breeding. Continuous monitoring of race diversity and distribution is the most important factor for the management of Phytophthora root and stem rot of soybean in conjunction with the discovery and development of new cultivars containing resistant genes, in addition to Gedenshirazu-1 or PI103091.

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References

Abney TS, Melgar JC, Richards TL, Scott DH, Grogan J, Young J (1997) New races of *Phytophthora sojae* with *Rps*1-d virulence. Plant Dis 81:653–655

Anderson TR, Buzzell RI (1992) Diversity and frequency of races of *Phytophthora megasperm*a f. sp. *glycinea* in soybean fields in Essex County, Ontario, 1980–1989. Plant Dis 76:587–589

Hildebrand AA (1959) A root rot and stalk rot of soybeans caused by *Phytophthora megasperma* Drechsler var. *sojae* var. nov. Can J Bot 37:927–957

Hirota T, Tabata K, Fukushima A, Inoue Y (2000) Evaluation of proper harvest period and effects of holding conditions before freezing on quality in immature black soybean "Tanbakuro" served as a vegetable (in Japanese). Kinki Chugoku Agr Res 100:33–37

Irie K, Aino M, Kinugasa T (1990) Occurrence of damping-off on black soybean in Hyogo Prefecture (in Japanese). Proc Kansai Plant Protect 32:52

Kan M, Yokho H, Matsuzaki M (1984) Damping-off of soybean seedling caused by *Phytophthora megasperma* var. *sojae* Hildebrand (in Japanese). Proc Assoc Pl Prot Kyushu 30:36–38

Kaufmann MJ, Gerdemann JW (1958) Root and stem rot of soybean caused by *Phytophthora sojae* n. sp. Phytopathology 48:201–208

Laviolette FA, Athow KL (1981) Physiologic races of *Phytophthora megasperm*a f. sp. *glycinea* in Indiana, 1973–1979. Plant Dis 65:884–885

Leitz RA, Hartmann GL, Pedersen WL, Nickell CD (2000) Races of *Phytophthora sojae* on soybean in Illinois. Plant Dis 84:487

Masago H, Yoshikawa M, Fukuda M, Nakanishi N (1977) Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soils and plants. Phytopathology 67:425–428

Ryley MJ, Obst NR, Irwin JAG, Drenth A (1998) Changes in the racial composition of *Phytophthora sojae* in Australia between 1979 and 1996. Plant Dis 82:1048–1054

Sato T, Kobayashi N, Komada H (1981) Comparison of resistance of some soybean cultivars to several isolates of stem rot Phytophthora (in Japanese). Ann Rept Plant Protect North Jpn 32:115–116

Schmitthenner AF (1999) Phytophthora rot of soybean. In: Hartman GL, Sinclair JB, Rupe JC (eds) Compendium of soybean diseases, 4th edn. American Phytopathological Society, St Paul, MN, pp. 39–42

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

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

Sugimoto T, Aino M, Sugimoto M, Watanabe K (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

Suzuki T, Nakamura H, Morita H (1980) Disease occurrence of Phytophthora stem rot and physiologic race of *Phytophthora megasperma* var. *sojae* Hildebrand in Shizuoka Prefecture (in Japanese). Ann Phytopathol Soc Jpn 46:108

Tsuchiya S, Furuya H (1983) Physiologic race of *Phytophthora megasperma* f. sp. *glycinea* from soybean root and stem rot occurred in Hachirogata reclaimed land, Akita Prefecture (in Japanese). Ann Rept Plant Protect North Japan 34:121–123

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

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

Yang XB, Ruff RL, Meng XQ, Workneh F (1996) Races of *Phytophthora sojae* in Iowa soybean fields. Plant Dis 80:1418–1420