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Pathogenic diversity of *Phytophthora sojae* pathotypes from Brazil

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Abstract Phytophthora root and stem rot has developed in commercial soybean fields since 2006 in Brazil, and cultivars with resistance to this disease have not been targeted for this region. Thus, the *Phytophthora sojae* pathotypes are expected to have virulence to few if any of the *Rps* genes. The objectives of this study were to characterize the pathotype diversity of *P. sojae* in Brazil, determine the distribution of the pathogen and predict which *Rps* genes will be effective and should be used in breeding programs. Isolates were collected in six states (Rio Grande do Sul, Santa Catarina, Paraná, Mato Grosso do Sul, Minas Gerais, and Goiás). The virulence formulae were based on the response of a differential set with 14 *Rps* genes (1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, and 8). None of the 17 pathotypes found was reported previously. The most common virulence formulas were: 1d, 2, 3c, 4, 5, 6, 7 (octal code 05471, representing 24 % of the occurrences); 1d, 2, 3b, 3c, 4, 5, 6, 7 (05671, 13 %); 1b, 1d, 2, 3a, 3c, 4, 5, 6, 7 (25571, 8 %); and 1d, 3a, 5, 7, 8 (01123, 8 %). Percentages of isolates with a susceptible interaction with each *Rps* gene was *Rps*1a (3 %), *Rps*1b (11 %), *Rps*1c (3 %), *Rps*1d (100 %), *Rps*1k (3 %), *Rps*2 (86 %), *Rps*3a (32 %), *Rps*3b (19 %), *Rps*3c (73 %), *Rps*4 (70 %), *Rps*5 (89 %), *Rps*6 (59 %), *Rps*7 (100 %), and *Rps*8 (22 %). There was apparently no relationship between pathotypes and origin. Stacking resistance genes *Rps*1a, *Rps*1b, *Rps*1c, and *Rps*1k with *Rps*3b or *Rps*8 would be highly effective for soybean cultivars targeted for Brazil.

Keywords *Glycine max* · Phytophthora root rot · Race · Soybean · Variability

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Introduction

Phytophthora root and stem rot (PRR) of soybean [*Glycine max* (L.) Merr.], caused by *Phytophthora sojae* Kaufm. & Gerd., affects susceptible soybean cultivars throughout the growing season. The most characteristic symptom is the discolouration of the lower stem from the soil line, which can affect lateral branches. Economic damage is related to extensive replanting early in the growing season and poor plant stand. Schmitthenner (1999) reported 100 % yield loss on highly susceptible soybean cultivars. Wrather and

Koenning (2010) considered PRR the second most damaging soybean disease in the USA, with an estimated mean loss of 46,631 million bushels/year from 2001 to 2010.

PRR incidence and severity are related to the presence of inoculum in the field, high soil moisture and susceptibility of the soybean cultivar. Oospores can survive for many years in crop residue and in soil. Zoospores are released when soil is flooded and are attracted to roots of germinating seeds, young roots, or exudates from older roots (Schmitthenner 1999). Soybean cultivars with very low levels of partial resistance or with *Rps* genes that have been already defeated by the predominant *P. sojae* pathotype are susceptible throughout the growing season (Dorrance et al. 2003; Jackson et al. 2004).

In South America, PRR was first described in Argentina in 1970 (Barreto et al. 1995) and in Brazil in 1995 (Costamilan et al. 2010). Occurrence of the disease remained insignificant and no practical control measures were utilized to manage PRR in Brazil until the 2005/2006 growing season, when great losses were first observed in Rio Grande do Sul and Paraná states (Costamilan et al. 2010). Until the 2011/2012 growing season, PRR was observed in the following Brazilian states: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), Mato Grosso do Sul (MS), Mato Grosso (MT), Minas Gerais (MG), Goiás (GO), and Tocantins (TO), sometimes only in isolated outbreaks. Since 2009, resistance to PRR is required as an additional description characteristic to seek patent protection on soybean cultivars in Brazil (Brasil 2009).

Commercial Brazilian soybean production started in the 1930s in Rio Grande do Sul, with great expansion during the 1970s, moving to Paraná and then to northern states (Hasse 1996). Soybean breeding programs in Brazil expanded during the 1960s, using US cultivars (e.g., Bienville, Bossier, Bragg, Cobb, Davis, Hale 7, Hardee, Hill, Hood, and Majos), which were well suited to the environmental conditions of south Brazil (Bonato and Bonato 2002). Currently, Brazil is the second largest producer and exporter of soybean in the world with total production of 75.3 million tons produced on 24.2 million hectares during 2010/2011 (USDA 2011; Conab 2011). In the Mercosur countries (Argentina, Brazil, Paraguay, and Uruguay), approximately 70 % of the total cultivated area uses the no-till production system (Derpsch et al. 2010). In southern Brazil, fields which have been cropped continuously to soybean for more than 30 years are common, and have been primarily managed under no-till production systems. *Phytophthora sojae* was recovered more frequently from the top (0 to 7.5 cm depth) level of soil collected in four out of five states in the USA under no-till compared to conventional-till cultivation (Workneh et al. 1998). According to Barreto et al. (1998), PRR increased in prevalence and incidence mainly under minimum and no-till systems and developed into major epidemics in the southern part of the soybean area in Argentina.

The most common way to manage PRR is the use of commercial soybean cultivars deployed with dominantly inherited resistance genes (*Rps*) (Dorrance et al. 2003; 2004; Grau et al. 2004). *Phytophthora sojae* has physiological specialization, called pathotypes, which impact *Rps* genes in a gene-for-gene manner. Fourteen *Rps* genes have been mapped to eight loci in the soybean genome, with an allelic series at two loci: *Rps*1 (1a, 1b, 1c, 1d, and 1k), *Rps*2, *Rps*3 (3a, 3b, and 3c), *Rps*4, *Rps*5, *Rps*6, *Rps*7 (Dorrance et al. 2004), and *Rps*8 (Burnham et al. 2003; Gordon et al. 2006). Two novel genes have been described recently, one of them temporarily designated as *RpsYu25* (Sun et al. 2011), and the other in the Japanese cultivar Waseshiroge, either allelic to *Rps*1, or at a tightly linked locus in a gene cluster (Sugimoto et al. 2011). Among these *Rps* genes, only five have been deployed to any great extent in soybean cultivars: *Rps*1a, *Rps*1c, *Rps*1k, *Rps*3a, and *Rps*6 (Abney et al. 1997; Dorrance et al. 2003). *Rps*1a was effective in the USA for approximately 8 years, and *Rps*1k for almost 20 years (Schmitthenner et al. 1994; Leitz et al. 2000; Dorrance et al. 2003; 2004; Malvick and Grunden 2004).

More than 55 races of *P. sojae* have been designated in the order that they were discovered based on inoculation of differentials. The most common series used eight *Rps* genes: *Rps*1a, *Rps*1b, *Rps*1c, *Rps*1d, *Rps*1k, *Rps*3a, *Rps*6, and *Rps*7. Each novel *Rps* gene incorporated in the differential series increases the number of possible races as well as the complexity the discussion about the population diversity. Characterization in terms of pathotypes or virulence phenotypes instead of races, and the use of octal codes to compare results, was proposed in order to standardize scientific discussions and to facilitate the reporting of results (Dorrance et al. 2003; Nelson et al. 2008).

Composition of the *P. sojae* population in terms of compatible reaction on *Rps* genes was observed to

have changed over the years, increasing the number of isolates with complex pathotypes (Dorrance et al. 2003). Numerous surveys for pathotype composition of *P. sojae* populations have been carried out primarily in the USA (Schmitthenner et al. 1994; Yang et al. 1996; Leitz et al. 2000; Kaitani et al. 2001; Dorrance et al. 2003; Jackson et al. 2004; Malvick and Grunden 2004; Nelson et al. 2008), as well as in Argentina (Barreto et al. 1995; Gally et al. 2007), and Australia (Ryley et al. 1998). Only the differential with *Rps*7 had a susceptible reaction (Costamilan et al. 2010) to one isolate from Passo Fundo, RS, in the first study about pathogenicity of *P. sojae* in Brazil. In Argentina, race 1 (virulence for *Rps* 7), race 4 (1a, 1c, 7) and a group that did not fit any known race virulence pattern were predominant (Barreto et al. 1998).

Considering that PRR had not caused severe outbreaks until 2006 in Brazil and that the publicly and privately developed soybean cultivars have not been tested as a routine until 2009, it is very likely that the composition of the native *P. sojae* population has not had any selection pressure and therefore it is expected that there is limited if any variability for pathogenicity. Pathotypes which are highly complex (virulent to many *Rps* genes) are not expected to be present in Brazil. Thus, the objectives of this study were to characterize the pathotype diversity of *P. sojae* in soybean fields in Brazil, determine the distribution of the pathogen and to identify possible *Rps* genes which are effective towards this population to be used in soybean breeding programs.

Materials and methods

Origin of isolates *P. sojae* was isolated from symptomatic plants collected in fields from the 2006/07 to 2009/10 growing seasons. Stems with lesions were washed in tap water, disinfested with 70 % ethanol for 5–10 s, washed in sterile distilled water and placed on a sterile paper to dry. Small pieces of the stem on the edge between diseased and healthy tissue were excised aseptically and placed on the selective medium PBNIC (Schmitthenner and Bhat 1994) with some modifications: 40 ml tomato extract (Elefante®, Cargill) replacing V-8 juice in the same volume; $CaCO_3$ 0.6 g; bacto yeast extract 0.2 g; sucrose 1.0 g; bacto agar 20.0 g; distilled water 1000 ml; benomyl 0.0050 g; pentachloronitrobenzene 0.0405 g; iprodione 0.0200 g; neomycin sulphate 0.1000 g; and chloramphenicol 0.0100 g. Cholesterol was not added. The entire disc of agar medium was inverted in the petri plate, covering soybean stem pieces. Plates were incubated in a growth chamber for five days, at 25+−3 °C. Mycelial growths that appeared on the surface of the agar were transferred to petri plates with diluted tomato extract agar (tomato extract 40 ml; $CaCO_3$ 0.6 g; bacto yeast extract 0.2 g; sucrose 1.0 g; bacto agar 20.0 g; and distilled water 1000 ml). All colonies were examined with a microscope (at 40x magnification) for characteristic appearance of mycelium and for oospore formation. For each isolate, a single-zoospore isolate was recovered according to the technique described by Schmitthenner and Bhat (1994). Isolates were stored in liquid nitrogen (Tooley 1988) until their inoculation on to the differential series. The identification and origin of the isolates are presented in Table 1.

Inoculation technique The pathotypes were determined by the hypocotyl inoculation technique (Schmitthenner and Bhat 1994), using the slurry obtained by passing two times the 15-day-old mycelia and agar medium (diluted tomato extract agar with 10.0 g of bacto agar, poured into 9-cm-diameter petri plates) through a 20-ml syringe. With an 18-gauge needle, a slit was made in the hypocotyl approximately 1 cm below the cotyledons and 5 mm long in each seedling. With the syringe, 0.2 to 0.4 ml of the slurry was deposited and 15 seedlings for each differential were inoculated. For this study, the following differentials were used: PI 547677 (*Rps*1a), PI 547842 (*Rps*1b), PI 547834 (*Rps*1c), PI 103091 (*Rps*1d), Williams 82 (*Rps*1k), PI 547838 (*Rps*2), PI 547862 (*Rps*3a), PI 591509 (*Rps*3b), L92-7857 (*Rps*3c), L85-2352 (*Rps*4), PI 547876 (*Rps*5), PI 591511 (*Rps*6), Harosoy (*Rps*7), and PI 399073 (*Rps*8). The differentials were obtained from the USDA ARS Soybean Germplasm Collection (R. Nelson, University of Illinois, Urbana, IL) and were increased and maintained at Embrapa Trigo, Passo Fundo, RS. Cultivar BRS 244RR was used as universal suscept. All cultivars were initially tested in three replications, with five seeds of each differential being evaluated per replication for a total of 15 seedlings, planted in plastic cups (500 ml), in a substrate made of pine bark, vermiculite, turf soil, and vegetal charcoal (Tecnomax®, Ferticel Ltda.). Fifteen 11-day-old seedlings were inoculated per differential and the plants were incubated in a dew chamber for the next 48 h, at a temperature ranging from 18 to 20 °C, in the

Table 1 Identification of *Phytophthora sojae* isolates collected in Brazil from the 2006/07 to 2009/10 growing seasons

Code	Place of origin	State[a]	Cultivar of origin
Ps 1/07	Passo Fundo	RS	CD FAPA 220
Ps 2/07	Passo Fundo	RS	BRS Charrua RR
Ps 3/07	Passo Fundo	RS	Genotype (breeding program)
Ps 4/07	Passo Fundo	RS	PI 398777
Ps 5/07	Passo Fundo	RS	PI 416764
Ps 6/07	Passo Fundo	RS	PI 423966
Ps 7/07	Coxilha	RS	BRS 245 RR
Ps 8/07	Coxilha	RS	BRS 256 RR
Ps 9/07	Ponta Grossa	PR	Not identified
Ps 10/08	Ronda Alta	RS	BRS 245 RR
Ps 11/08	Coxilha	RS	BRS 242 RR
Ps 12/08	Coxilha	RS	BRS 255 RR
Ps 13/08	Uberaba	MG	Not identified
Ps 14/08	Cachoeirinha	RS	Genotype (breeding program)
Ps 15/08	Carambeí	PR	NK 2555
Ps 16/08	Santo Ângelo	RS	Maradona
Ps 17/08	Passo Fundo	RS	Not identified
Ps 18/08	Pelotas	RS	Not identified
Ps 19/08	Arroio Grande	RS	MSOY 7979 RR
Ps 20/08	Camaquã	RS	BRS 244 RR
Ps 21/08	Maracaju	MS	BRS Charrua RR
Ps 22/09	Pato Branco	PR	NK 7054 RR
Ps 23/09	Colorado	RS	NK 7054 RR
Ps 24/09	Castro	PR	NK 3363
Ps 25/09	Ipiranga do Sul	RS	BRS 242 RR
Ps 26/09	Cachoeirinha	RS	BRS Charrua RR
Ps 27/09	Chapada	RS	Maradona
Ps 28/09	Sananduva	RS	Fundacep 53
Ps 29/09	Marau	RS	Not identified
Ps 30/09	Não-Me-Toque	RS	Fundacep 53
Ps 31/09	Ijuí	RS	BRS Charrua RR
Ps 32/10	Lagoa Vermelha	RS	Fundacep 53
Ps 33/10	Campos Novos	SC	CD 249 RR
Ps 34/10	Cachoeira do Sul	RS	Genotype (breeding program)
Ps 35/10	Cachoeira do Sul	RS	Genotype (breeding program)
Ps 36/10	Cachoeira do Sul	RS	Genotype (breeding program)
Ps 37/10	Montividiu	GO	Genotype (breeding program)

[a]Brazilian states: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), Mato Grosso do Sul (MS), Minas Gerais (MG), and Goiás (GO)

dark. Afterwards, the plants were placed in a greenhouse at temperatures ranging from 18 to 28 °C and under natural light. The number of dead plants was recorded within five to seven days after inoculation. Cultivars with results between 30 and 70 % were tested two or three times more. A differential was scored susceptible if at least 80 % of the plants in the replicate pots died after inoculations (Nelson et al. 2008).

Pathotype evaluation Virulence data were converted into an octal format using the program Habgood-Gilmour Spreadsheet (HaGiS), developed by Herrman

et al. (1999). The HaGiS program quantifies the number of phenotypes, calculates frequency distribution of virulence to specific *Rps* genes, isolate complexities (the number of differentials in which an isolate has a susceptible interaction), and quantifies the pathogenic diversity with the Shannon index. Octal codes, using a reverse octal system, were established based on incompatible reaction to *Rps* genes or resistance (0), and on compatible reaction or susceptibility (1) in the following triplets of *Rps* genes: 1a, 1b, 1c; 1d, 1k, 2; 3a, 3b, 3c; 4, 5, 6; and 7, 8. According to the octal nomenclature, a single number was given for each triplet, based on the reaction of each differential, as follows: 000 (= 0), 100 (= 1), 010 (= 2), 110 (= 3), 001 (= 4), 101 (= 5), 011 (= 6), and 111 (= 7). For example, an isolate with the virulence pathotype 1d, 2, 3a, 7 will be described by the octal code as 05101 (Herrmann et al. 1999; Dorrance et al. 2003; Malvick and Grunden 2004).

Results

Phytophthora sojae isolates were recovered from 2007 to 2010 from samples collected in 25 counties in six Brazilian states (Table 1, Fig. 1). Thirty-seven isolates were obtained and 17 pathotypes (identified based on the same octal code) were identified with 14

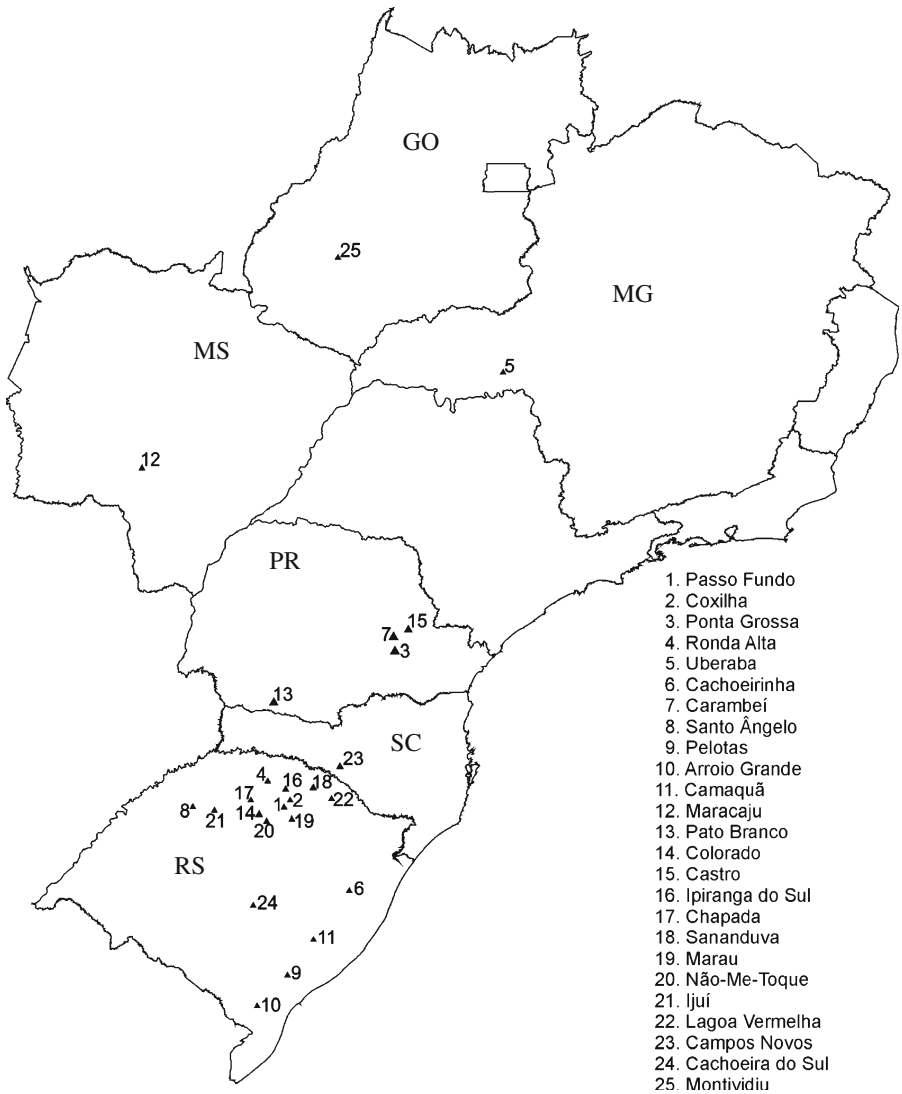

Fig. 1 Distribution of *Phytophthora sojae* in Brazil. Numbers represent the 25 counties where isolates were collected. Letters represent Brazilian states: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), Mato Grosso do Sul (MS), Minas Gerais (MG), and Goiás (GO)

differentials (Table 2). The great majority of the isolates was from Rio Grande do Sul state.

Pathotypes with octal codes 05471 (1d, 2, 3c, 4, 5, 6, 7), 05671 (1d, 2, 3b, 3c, 4, 5, 6, 7), 25571 (1b, 1d, 2, 3a, 3c, 4, 5, 6, 7), and 01123 (1d, 3a, 5, 7, 8) were the most common, representing 53 % of the total pathotype frequency distribution. Pathotypes 05471 and 05671, collected in Passo Fundo, Ipiranga do Sul, Ijuí, Coxilha, Ronda Alta (RS), Uberaba (MG), Montividiu (GO), and Ponta Grossa (PR), were very similar to each other (93 %) with the only difference in virulence to *Rps*3b.

All isolates had a compatible reaction on the differentials containing *Rps*1d and *Rps*7, and at least 59 % had a susceptible interaction on the differentials *Rps*2 (86 %), *Rps*3c (73 %), *Rps*4 (70 %), *Rps*5 (89 %), and *Rps*6 (59 %). Less than 40 % of the isolates had a susceptible interaction on *Rps*1b (11 %), *Rps*3a (32 %), *Rps*3b (19 %), and *Rps*8 (22 %). Differentials containing genes *Rps*1a, *Rps*1c, and *Rps*1k were resistant to all but one isolate (Fig. 2).

The pathotype complexity ranged from 3 (isolates from Castro and Pato Branco, PR, and Maracaju, MS) to 10 (isolate from Pelotas, RS). The average complexity value was 6.7.

Discussion

This is the first characterization of the virulence pathotypes of *P. sojae* collected in six Brazilian states, using a differential set representing 14 *Rps* genes. These data are essential for breeding programs, leading to a selection of effective *Rps* genes for cultivar development. Pathotypes most commonly found had a compatible reaction to eight *Rps* genes: *Rps*1d, *Rps*2, *Rps*3a, *Rps*3c, *Rps*4, *Rps*5, *Rps*6 and *Rps*7, which are not useful to control PRR in Brazil.

Table 2 Virulence formulae of the pathotypes of *Phytophthora sojae* isolated from soybean plants in Brazil

Pathotype[a]	Virulence formulae (14 genes)[b]	No. of isolates (%)	Origin
05471	1d, 2, 3c, 4, 5, 6, 7	9 (24)	Passo Fundo (2), Ipiranga do Sul, Ijuí, Uberaba, Coxilha (3), Ronda Alta
05671	1d, 2, 3b, 3c, 4, 5, 6, 7	5 (13)	Montividiu, Coxilha, Passo Fundo (2), Ponta Grossa
25571	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7	3 (8)	Cachoeira do Sul (2), Arroio Grande
01123	1d, 3a, 5, 7, 8	3 (8)	Passo Fundo, Chapada, Não-Me-Toque
01021	1d, 5, 7	2 (5)	Castro, Maracaju
05123	1d, 2, 3a, 5, 7, 8	2 (5)	Carambeí, Sananduva,
05431	1d, 2, 3c, 4, 5, 7	2 (5)	Santo Ângelo, Campos Novos
05573	1d, 2, 3a, 3c, 4, 5, 6, 7, 8	2 (5)	Cachoeirinha (2)
57411	1a, 1c, 1d, 1k, 2, 3c, 4, 7	1 (3)	Cachoeira do Sul
25771	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	1 (3)	Pelotas
05001	1d, 2, 7	1 (3)	Pato Branco
05523	1d, 2, 3a, 3c, 5, 7, 8	1 (3)	Marau
05651	1d, 2, 3b, 3c, 4, 6, 7	1 (3)	Colorado
05401	1d, 2, 3c, 7	1 (3)	Passo Fundo
05421	1d, 2, 3c, 5, 7	1 (3)	Passo Fundo
05031	1d, 2, 4, 5, 7	1 (3)	Camaquã
05071	1d, 2, 4, 5, 6, 7	1 (3)	Lagoa Vermelha
Total		37	

[a] Octal digits were assigned as follows: 000 (= 0), 100 (= 1), 010 (= 2), 110 (= 3), 001 (= 4), 101 (= 5), 011 (= 6), and 111 (= 7), according to the results in triplets of *Rps* genes: 1a, 1b, 1c; 1d, 1k, 2; 3a, 3b, 3c; 4, 5, 6; and 7, 8. 0 indicates an incompatible reaction, and 1 indicates a compatible reaction on the differentials

[b] Differential series tested was composed of the following soybean cultivars: PI 547677 (*Rps*1a), PI 547842 (*Rps*1b), PI 547834 (*Rps*1c), PI 103091 (*Rps*1d), Williams 82 (*Rps*1k), PI 547838 (*Rps*2), PI 547862 (*Rps*3a), PI 591509 (*Rps*3b), L92-7857 (*Rps*3c), L85-2352 (*Rps*4), PI 547876 (*Rps*5), PI 591511 (*Rps*6), Harosoy (*Rps*7), and PI 399073 (*Rps*8)

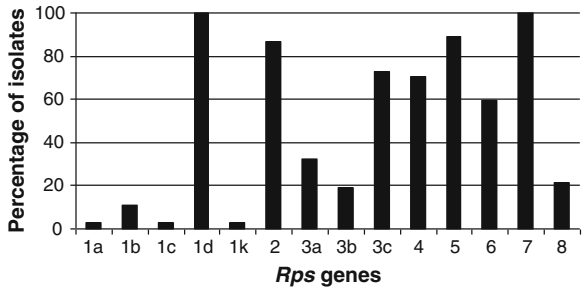

Fig. 2 Percentage of the *Phytophthora sojae* isolates collected in Brazil with a susceptible interaction with *Rps* genes

None of the 17 Brazilian pathotypes described in this study was previously reported. The most common pathotypes reported in the USA prior to 1990 included virulence to *Rps*1a, *Rps*1c, *Rps*1d, *Rps*6, and *Rps*7; currently, most areas are reporting virulence either to *Rps*1b and *Rps*1k (Grau et al. 2004). This contrasts to the development of virulence within the *P. sojae* populations in Brazil, in which *Rps* 1a, 1c and 1k are still highly effective. This fact could indicate that Brazilian and American *P. sojae* populations do not share the same origin, or that the Brazilian population has had different selection pressure induced by cultivars with *Rps* gene that were unknowingly deployed.

With this study, there was an increase in virulence to a greater number of *Rps* genes within the Brazilian isolates ranging from race 1 (vir. 7) to 1d, 3a, 6, and 7, which were present in 100, 39, 60 %, and 100 % of the *P. sojae* isolates evaluated, respectively. Barreto et al. (1998) also related an increase in virulence from 1992 to 1997 in Argentina. In the USA, *P. sojae* populations reportedly have a low incidence of virulence for *Rps*1d collected in Arkansas (10 %), Illinois (38 %), Ohio (3 %), North Dakota (19 %), Iowa (2 %), and Indiana (14 %) (Yang et al. 1996; Abney et al. 1997; Dorrance et al. 2003; Jackson et al. 2004; Malvick and Grunden 2004; Nelson et al. 2008). In contrast to USA populations which almost all have virulence to *Rps*1a, the great majority of Brazilian populations have no virulence response to *Rps*1a, *Rps*1c, or *Rps*1k, which are the most commonly deployed resistance genes in American soybean cultivars (Slaminko et al 2010).

Pathotype surveys are essential for development of breeding strategies to ensure that effective genes will be deployed in cultivars targeted for specific regions. Based on the results of this study, stacking resistance genes *Rps*1a, *Rps*1b, *Rps*1c, and *Rps*1k with *Rps*3b or *Rps*8 would be highly effective for soybean cultivars targeted for Brazil. However, in this process, care should be taken to combine these *Rps* genes in cultivars with high levels of partial resistance, in order to avoid selection pressure that could lead to a change in the virulence composition of the pathogen (Dorrance and St. Martin 2000).

The *P. sojae* isolates within the Brazilian population are surprisingly complex with virulence to seven different *Rps* genes. The variability of *P. sojae* observed in this study is representative of commercial grower fields, especially those located in RS state. In this study, it was observed that large proportions of the *P. sojae* populations had individuals that could cause disease on soybean with a great number of the known *Rps* genes. This is expected with a long period of continuous soybean production, as occurred in RS, but only if the cultivars have *Rps* genes. Even the pathotype from Montividiu, GO, a state more recently engaged in soybean commercial production, also had isolates of *P. sojae* with virulence to eight genes (Table 2). The American soybean cultivars used in the development of Brazilian cultivars initially may have been unintentional sources of *Rps* genes that remained effective for a long time. What types of PRR resistance were present in the cultivars used since 2006 is difficult to identify. In the USA and Australia, the increased selection pressure exerted by *Rps* genes included in commercial soybean cultivars may have driven the development of a diverse *P. sojae* population with greater genetic variability, and the production of more pathotypes (Schmitthenner et al. 1994; Ryley et al. 1998; Jackson et al. 2004). In Ohio, after a systematic soil survey, it was observed that between 51 and 96 % of the locations had at least one isolate with virulence to commonly deployed *Rps* genes 1a, 1b, 1c, 1k, 3a, and 6 (Dorrance et al. 2003). In a 10-year interval, Nelson et al. (2008) also found an increasing numbers of pathotypes (from 4 to 16) infecting plants with the most common resistance genes deployed in the soybean cultivars adapted to North Dakota.

In Argentina, considerable diversity within *P. sojae* populations from Buenos Aires and Santa Fe provinces suggested rapid evolution, and the higher variability found in Buenos Aires was probably related to a longer evolution compared to the other sites (Gally et al. 2007). The pathogenic diversity identified in the Brazilian *P. sojae* population (Shannon index=2.53) was smaller than those found among isolates of *P. sojae* recovered from Ohio, which ranged from 2.71 in 1990/1991 to 4.82 in 1997/1999 (Dorrance et al. 2003). The difference in pathogenic diversity could be related to the non-intentional

deployment of soybean cultivars with resistance to PRR in Brazil compared to this state in the USA, which gradually led to a selection of more complex pathotypes.

Based on this limited survey, no relationship was observed between Brazilian isolates and their geographic origins. For a soilborne pathogen that is homothallic, it is expected that isolates from the same region would be similar. However, great variability in pathotype was observed, with different pathotypes collected from the region as well as the same pathotypes recovered from states as far away as RS and MG or GO (Table 2). High intraspecific variability among *P. sojae* isolates from the same geographic origin was also observed in Argentina (Gally et al. 2007) and in the USA, by Dorrance et al. (2003) in Ohio, by Jackson et al (2004) in Arkansas, by Malvick and Grunden (2004) in Illinois, and by Nelson et al. (2008) in North Dakota.

The large adoption of the conservation soil tillage system in Brazilian soybean areas could be part of the cause of the widespread distribution of PRR in the country. No-till has been shown to have impacts on the major concentration of *P. sojae* propagules in the first 7.5 cm of soil depth (Workneh et al. 1998) and on the development of complex populations of *P. sojae* (Dorrance et al. 2003). In Brazil, some practices used in no-till production could favour the development of PRR: (1) intense traffic of heavy machinery on fields with excessive moisture; (2) double cropping in the same growing season; and (3) cultivation in the rainy season, generating a compacted soil layer between 10 and 20 cm depth (Franchini et al. 2009). Further studies are necessary to quantify the impact of no-till agriculture on the occurrence of PRR and the development of new *P. sojae* pathotypes in Brazilian soybean fields.

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