



Pathogenic diversity of *Phytophthora sojae* in the southeast of the Province of Buenos Aires

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Abstract

Phytophthora sojae causes root and stem rot of soybeans. In Argentina, this pathogen has shown dramatic shifts in pathotype variability since its first discovery during the 1970s. The aim of this study was to report the presence and distribution of different pathotypes of *P. sojae* isolates from 2013 to 2015 in the southeast of Buenos Aires Province in Argentina. Isolates were obtained from diseased soybean plants and soil in contact with the infected root. The pathotype evaluation of *P. sojae* was determined with a set of eight differential soybean lines. One hundred and twenty-four *P. sojae* isolates were obtained and forty pathotypes were determined, which is a considerable variability for a relatively new and recently infested soybean area.

Keywords Oomycetes · *Glycine max* · Pathotypes · Root and stem rot

Phytophthora sojae causes Phytophthora root rot (PRR) in soybean. Since its first discovery in the 1950s, the disease has been reported in all soybean-producing regions of the world (Hartman et al. 2015). New virulence gene combinations or pathotypes have been continuously emerging in the pathogen worldwide. Over 200 pathotypes have been reported so far (Dorrance et al. 2016), suggesting that the evolution of virulence in natural *P. sojae* populations is very dynamic. In Argentina, soybean has been produced as a commodity for more than thirty years and over 82% is produced in the northern Pampeana region (Ploper 2004). In this area, PPR physiological race 1 was originally identified in 1991 (Barreto et al. 1991) and continued being prevalent up to 1999 (Gally et al. 1999). After that, the disease showed a steady increase in prevalence, incidence and changes in pathogen virulence

(Grijalba and Gally 2015). Soybean cultivation in the southern Pampeana region, which includes the southeast of Buenos Aires Province (SEBA), where different agroecologic conditions prevail, is relatively new compared to the northern Pampeana region (NBA) (Pascale and Damario 2004). In the SEBA, PRR was not detected in different surveys carried out until 2004. The first isolation and pathotype determination was made in 2011 (Grijalba et al. 2011); no virulence studies have been done since. We herein communicate the presence and distribution of *P. sojae* pathotypes from diseased soybean plants and the soil of the root region isolated between 2013 and 2015 in the southeast of Buenos Aires Province in Argentina.

The areas sampled were intentionally selected based on reports of the occurrence of wilting and dead plants in collaboration with breeders, extension personnel and farmers. During the 2013/14 growing season, 15 commercial farms and experimental plots were sampled for PRR in three localities: Necochea, Balcarce and General Pirán. Similarly, in the 2014/15 growing season, 21 commercial farms and experimental plots were sampled in five localities: Necochea, Loberia, Balcarce, Bosch and El Moro (Fig. 1). From each field, 50 symptomatic plants and soil in contact with their roots were collected. Isolates from diseased plants were obtained from the advancing margin of stem lesions and the soybean seedling baiting technique was used to isolate *P. sojae* from soil samples (Schmitthenner et al. 1994; Dorrance et al. 2016).

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Cultures were characterized and identified as *P. sojae* by studying their morphological characters as being colorless, coenocytic mycelium with irregular hyphal swellings and abundant production of homothallic smooth-wall spherical oospores with both amphigynous and paragynous antheridia under the microscope (Erwin and Ribeiro 1996; Hartman et al. 2015; Abad et al. 2019). Pure cultures were transferred to slants of V8 Agar for storage at room temperature and kept at the Facultad de Agronomía, Universidad de Buenos Aires culture collection.

Virulence formulae (listing of genes defeated by the isolates) were used to describe pathotypes based on reactions to a known set of soybean genotypes containing eight different *Rps* genes. The hypocotyl technique was used to inoculate the differential genotypes (Dorrance et al. 2004; Schmitthener et al. 1994). The reactions were scored as resistant (R), intermediate (I), or susceptible (S) based on the number of seedlings killed: 30% or less seedlings; 31 to 69%; and 70% or more seedlings, respectively. In 2013–2014, soybean cultivars used for race determination were HARO 12 (*Rps* 1a), HARO 13 (*Rps* 1b), Corsoy 79 (*Rps* 1c), HARO 16 (*Rps* 1d), HARO 15 (*Rps* 1k), HARO 3272 (*Rps* 3a + *Rps* 7), HARO 6272 (*Rps* 6 + *Rps* 7) and Harosoy (*Rps* 7).

The susceptible controls were Haro (1–7)1. Due to the inconsistent reactions in 2015 (Grijalba et al. 2015), in some tests HARO (1–7)1 was substituted with Williams (*rps rps*); and HARO 16, HARO 15, HARO 3272 and Corsoy 79 were substituted with PI103091Hernan (*Rps*1d); Williams 82 (*Rps*1k); L83-570 (*Rps*3a) and Williams 79 (*Rps*1c), respectively. These substituted soybean cultivars (differentials) were obtained from S. Steward, INIA La Estanzuela Rep. Oriental del Uruguay. Inoculation tests were conducted twice for each isolate.

Frequency distributions of virulence to specific *Rps* genes, isolate complexities (the number of differentials with which an isolate has a susceptible interaction), and diversity indices were calculated using the “hagis” R package (McCoy et al. 2019).

One hundred and twenty-four *P. sojae* isolates were recovered, 74 from diseased plants and 50 from the soil. As fields sampled were intentionally selected based on the symptoms of diseased plants, all of them were positively identified as *P. sojae*. Forty virulence patterns were determined, which represented 9 known races (Table 1). All isolates resulted pathogenic on the susceptible differential cultivars, Haro (1–7)1 or Williams, as well as on Harosoy (*Rps*7).

Fig. 1 Distribution of *Phytophthora sojae* in the southeast of Buenos Aires Province (Argentina). On top left corner: Argentinian agricultural regions. Numbers represent the 6 localities where isolates were collected. 1: Necochea, 2: Lobería, 3: El Moro, 4: Balcarce, 5: General Pirán, and 6: Bosch. Adapted from Pascale and Damario 2004



Table 1 Distribution of virulence of *Phytophthora sojae* isolated in the southeast Buenos Aires Province (Argentina) from 2013 to 2016, tested on 8 *Rps*

Pathotype ¹	Race	N° of isolates ²	Area	Years
7	1	4 pl	N	14
1a,7	3	2 pl	L-N	13–14
1a,6,7	9	1 s	B	14
1a,1c,7	4	4 pl	B-L-N	13-14.15
1a,1b,1c,1d,1 k,6,7		4 pl 2 s	L-N-B	14–15
1a,1b,1c,1d(I),1 k(I),7		1 pl 1 s	Bo-B	14–15
1a,1b,1c,1d,1 k,7		5 pl 1 s	Bo-L	15
1a,1b,1c,1d, 7		1 pl	L	14
1a,1b,1c,1d,1 k, 3a, 7		1 s	L	15
1a,1b,1c,1d,1 k, 3a,6 (I), 7	38	1 pl 1 s	B-L	15
1a,1b,1c,1 k,7		1 pl 3 s	B-L	14–15
1a,1b,1c,1 k,6,7		1 pl	N	15
1a,1b,1d,1 k,3a,7		1 s	L	15
1a,1b,1d,1 k,6,7		3pl	N	14
1a,1c,1d,7	43	2 pl	B-L	14–15
1a,1c,1d,1 k (I), 7		1 pl	L	15
1a,1c,1d,1 k, 7		17 pl	B-L-N-M	14–15
1a,1c,1d,1 k, 3a,7		8 s 3 pl	B-N-L-T	13-14.15
1a,1c,1d,1 k, 6(I),7		1 pl	Bo	15
1a,1c,1d,1 k, 6, 7		1 pl	L	15
1a,1c,1d, 6(I),7		1 pl	M	15
1a,1c,1d,1 k,6,7		2 pl 2 s	L	15
1a,1c,1d,1 k,3a,7		3Pl	L	14
1a,1c,1d,1 k, 3a,6,7		2 s	L	15
1a,1c,1 k, 3a,7		1 PL	B	15
1a,1c,1 k,6,7		1 pl	M	15
1a,1c,1 k,7	25	8 pl	M-N-L-B	14–15
1a,1c,1 k, 3a,6, 7		1 s	B	15
1a,1c,1d,3a,7		1 s 2 pl	L	14–15
1a,1d,1 k,7		3 s	B	14–15
1a,1d,1 k,3a, 7		1 pl 3 s	L-B-N	14–15
1a,1d,3a, 7		1 s	L	15
1a,1 k,3a, 7		5 pl 3 s	L-B	13–14
1a,1 k,3a,6, 7		2 pl	Bo-N-L	14–15
1a,1 k,7	34	2 s 2 pl	N-P-B	14–15
1b,1d, 1 k, 3a,7		1 s	N	14
1c,7	14	1 pl	L	13
1c,1 k, 7		1 pl	N	13
1 k,3a, 7		2 pl	L-B	14–15
1 k,7		2 pl	N	13

¹ Listing of defeated *Rps* alleles (1a, 1b, 1c, 1d, 1 k, 3a, 6, 7) ²pl= Plant s = Soil. N = Necochea, B = Balcarce, P = General Pirán, L = Lobería, Bo = Bosch and M = El Moro.

Most isolates were virulent on *Rps1a* (90.32%), *Rps1c* (67.74%) and *Rps1k* (79.03%), the resistance genes more frequently deployed in the Argentinian soybean fields (Rossi and

Nari 1995). *Rps 6* and *Rps 1d* resulted more effective resistance genes against the isolates obtained in this study (19.35 and 20.97, respectively) (Table 2). Race 1 (pathogenic on *Rps 7*) was only determined in four isolates from symptomatic plants from Necochea, which represented 3.2% of the recovered isolates, a very low percentage compared to the prevalence of 25% during surveys carried out between 1998 and 2004 in the northern Pampeana region (Grijalba and Gally 2015). The present report, conversely, depicts a very complex scenario regarding isolate virulence (Fig. 2), with every diversity index (Simple = 0.2823 Gleason = 7.054; Shannon = 3.178; Simpson = 0.9413 and Evenness = 0.894) yielding considerably high results.

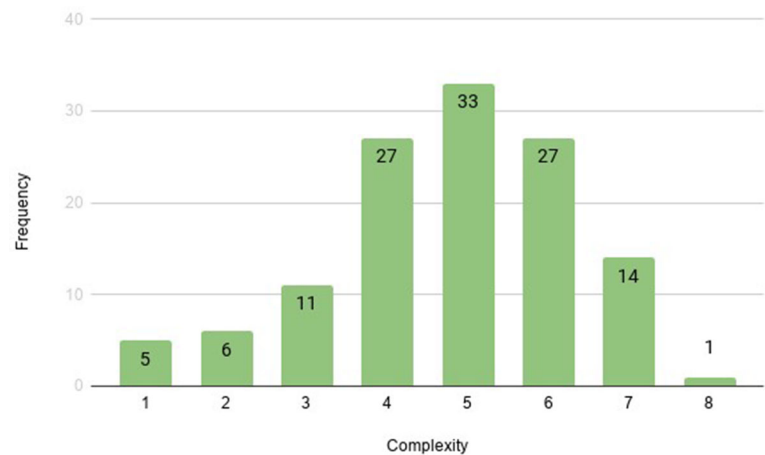
These results strongly suggest a profound pathogenicity shift in the study area. Similarly, virulence in *P. sojae* has continued increasing in North America. Reports from 1990s indicated that *P. sojae* pathotype composition was growing in complexity (Anderson and Buzzell 1992; Dorrance et al. 2004; Nelson et al. 2008; Schmitthenner et al. 1994; Xue et al. 2015; Zhang et al. 2010, Stewart et al. 2016; Dorrance et al. 2016).

These findings suggest the occurrence of complex mechanisms, other than directional selection, in SEBA soybean fields. It is likely that epigenetic phenomena as well as disruptive selection and/or other (yet unidentified) forces are modulating the evolution of *P. sojae* pathogenicity under a strong environmental stress, imposed by different cultural and chemical management practices. A very similar situation has been reported in Quebec (Canada), where soybean production has only recently started, but pathotype diversity reported is much greater than in any other area (Dorrance and Grunwald 2009) where soybean had been produced for long.

Some Argentinian seed companies commercialize allegedly resistant or tolerant cultivars to *P. sojae* without specifying where or how these were tested or which virulence pathotypes were used. Other companies indicate resistant cultivars, particularly due to the incorporation of the *Rps 1 k* gene. We can conclude from this study that nowadays most fields in Buenos Aires Province have detectable populations of *P. sojae* that may defeat *Rps1k* resistance, a situation that was not foreseen a few years ago. The complexity in individual isolates pathogenicity, along with the admixture in pathotype composition within single production fields show that cultivars carrying single gene resistance to *P. sojae* are not effective in controlling PRR anymore.

Development of new resistance mechanism is required, as well as pyramidization and new management practices. Some *Rps* genes are not being used at present. Examination of Tables 1 and 2 may indicate some gene combinations that would be effective against most pathotypic formulae (i.e. *Rps 6* and *Rps1b*). In addition,

Fig. 2 Frequency of the number of *Rps* defeated by each individual isolate (complexity) in a sample of 124 isolates and 35 pathotypes



isolates should be tested with the expanded set of 14 differentials in order to broaden the scope and precision of analysis and downstream cultivar development (Stewart et al. 2016; Dorrance et al. 2016; Schmitthenner et al. 1994). The use of soybean cultivars with partial resistance or tolerance has also been advocated (Hartman et al. 2015; Dorrance et al. 2003; Ferro et al. 2006; Thomison et al. 1991), but the response of Argentinian cultivars must be corroborated with local isolates of *P. sojae*.

To shed more light on the reasons for the outbreak of this soybean root rot in a new area, an analysis of the populations in the whole Pampeana region is currently underway to determine the level of molecular and pathotypic diversity in Argentina. Similarity, pathotype complexity might suggest a significant flow among the *P. sojae* populations. In addition, selective media are being used to identify seed-transmitted *P. sojae* isolates. The results of *P. sojae* pathotype surveys are essential for breeding programs. Thus, this study demonstrates the need for additional research on soybean root rot disease caused by *P. sojae* in the Pampeana region, including identification of effective sources of resistance, determination

Table 2 The number and frequency of virulent *Phytophthora sojae* isolates and different *Rps* genes defeated

Gene	N° of virulent isolates	Percent Pathogenic
Susceptible	124	100
<i>Rps</i> 1a	112	90.32
<i>Rps</i> 1b	26	20.97
<i>Rps</i> 1c	84	67.74
<i>Rps</i> 1d	77	62.1
<i>Rps</i> 1 k	98	79.03
<i>Rps</i> 3a	46	37.1
<i>Rps</i> 6	24	19.35
<i>Rps</i> 7	124	100

of the race structure of pathogen populations, and implementation of disease management strategies suitable for local environmental conditions.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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