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Journal of General Plant Pathology

ISSN 1345-2630
Volume 82
Number 3

J Gen Plant Pathol (2016) 82:132-141
DOI 10.1007/s10327-016-0654-y

Springer

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FUNGAL DISEASES

Pathotypes and metalaxyl sensitivity of *Phytophthora sojae* and their distribution in Heilongjiang, China 2011–2015

Miao Tian[1] · Liming Zhao[1] · Shuang Li[1] · Jing Huang[1] · Zhe Sui[1] · Jingzhi Wen[1] · Yonghao Li[2]

Received: 3 August 2015 / Accepted: 8 January 2016 / Published online: 25 March 2016

Abstract A total of 395 single-zoospore isolates of *Phytophthora sojae* that were obtained from 467 soybean fields in Heilongjiang, China from 2011 to 2015 were identified for pathotypes using differential soybean cultivars with *Rps*1a, *Rps*1b, *Rps*1c, *Rps*1d, *Rps*1k, *Rps*2, *Rps*3a, *Rps*3b, *Rps*3c, *Rps*4, *Rps*5, *Rps*6, *Rps*7, and *Rps*8 resistance genes. The results showed that *P. sojae* was widespread in Heilongjiang, but not evenly distributed. A heavy infestation of *P. sojae* in eastern Heilongjiang coincided with the serious diseases caused by this pathogen in that region. Among 135 pathotypes were identified, 20 isolates matched races of *P. sojae* based on published race definitions. To our knowledge, this is the first report of races 2, 8, 23, 25, 26, 29, 33, 39, 42, 43, and 48 in Heilongjiang. Race 1, previously considered as the dominant race in Heilongjiang, only comprised 1.52 % of the isolates in the present study. Virulence frequencies of 395 isolates to 14 *Rps* genes ranged from 17.85 to 82.41 %. Less than 30 % of the isolates were virulent on cultivars containing *Rps* genes 1k, 1c, or 3a, which indicated that these *Rps* genes were more effective than other genes in Heilongjiang. All isolates could defeat more than two *Rps* genes. Ninety-six percent of the isolates were virulent against more than four *Rps* genes, which indicated that multi-virulence isolates existed in Heilongjiang. All 223 tested isolates were sensitive to metalaxyl, but EC_{50} for this fungicide against the pathogen increased 18-fold in the last 20 years. The results of the present study suggest that incorporating *Rps* genes 1c, 1k, and 3a into soybean cultivars and the use of metalaxyl-coated seeds should provide integrated approach to manage Phytophthora root and stem rot of soybean in Heilongjiang, China.

Keywords *Phytophthora sojae* · Pathotype · Metalaxyl sensitivity · Distribution · Heilongjiang of China

Introduction

Phytophthora sojae Kaufm. & Gerd., the causal agent of soybean root and stem rot of soybean, has spread widely throughout the world (Doupnik 1993; Schmitthenner 1985; Wrather and Koenning 2006; Wrather et al. 1995) since it was first described in the United States in 1958 (Kaufmann and Gerdemann 1958). *P. sojae* is a soil-borne pathogen, and its oospores can survive in soil for several years. Wet or high moisture conditions in the soil facilitate oospore germination, sporangium production, and subsequent infection of soybean seeds and roots (Dorrance et al. 2007). Using resistant cultivars and treating seeds with metalaxyl are major strategies to control Phytophthora stem and root rot of soybean (Cui et al. 2010; Schmitthenner 1999).

Phytophthora sojae has a gene-for-gene relationship with its soybean host. To date, 14 *Rps* (resistance to *Phytophthora sojae*) genes have been described, including *Rps*1 (1a, 1b, 1c, 1d, and 1k), *Rps*2, *Rps*3 (3a, 3b, and 3c), *Rps*4, *Rps*5, *Rps*6, *Rps*7, and *Rps*8 (Burnham et al. 2003; Dorrance et al. 2004; Gordon et al. 2006; Grau et al. 2004). Many sets of soybean differentials that contain one *Rps* gene have been used to characterize the pathotypes (races)

✉ Jingzhi Wen
jzhwen2000@163.com

[1] Department of Plant Protection, College of Agriculture, Northeast Agricultural University, Harbin 150030, People's Republic of China

[2] Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT 06511, USA

of *P. sojae* (Dorrance et al. 2004), and 55 races have been identified using hypocotyl inoculations of these soybean differentials (Abney et al. 1997; Robertson and Yang 2004; Bernard et al. 1957; Förster et al. 1994; Haas and Buzzell 1976; Henry and Kirkpatrick 1995; Keeling 1980, 1982, 1984; Laviolette and Athow 1977, 1983; Layton et al. 1986; Leitz et al. 2000; Morgan and Hartwig 1965; Ryley et al. 1998; Schmitthenner 1972; Schmitthenner et al. 1994; Schwenk and Sim 1974; Wagner and Wilkinson 1992; White et al. 1983; Yang et al. 1996). Because of frequent virulence changes and pathotype shifts of the pathogen as well as incorporation of additional differentials, the number of races and diversity has become too great to record. So pathotypes rather than race codes have been used by more and more authors (Costamilan et al. 2013; Cui et al. 2010). A pathotype is denoted as a list of the *Rps* genes that were ineffective against the pathogen among the 14 soybean differential cultivars.

Heilongjiang is a major soybean production province and grows more than 30 % of the soybeans in China. Phytophthora root and stem rot of soybean was first reported in Heilongjiang in 1991 (Shen and Su 1991) and has become widespread in this province. Over 150,000 ha of soybean fields have been estimated to be infested in Heilongjiang (Han et al. 1998; Lv et al. 2001; Xu et al. 2002). *P. sojae* has been isolated from soil in 31 counties (Cui et al. 2010), and 14 races (Ma and Li 1999; Wen and Chen 2002; Zhang et al. 2008, 2010) and 30 pathotypes (Cui et al. 2010) have been identified in the province. However, in the studies cited, the differential cultivar with the *Rps*8 gene was not used.

Although no metalaxyl-resistant isolates of *P. sojae* have been reported in China (Cui et al. 2010; Li et al. 1993), the continuous use of metalaxyl may result in metalaxyl-resistant isolates in *P. sojae* populations (Cui et al. 2013; Zuo et al. 2005). Considering that the distribution, pathotype, and metalaxyl sensitivity of *P. sojae* will change over time, the objectives of the present study were to determine the latest distribution of *P. sojae* in Heilongjiang, evaluate the metalaxyl sensitivity of the strains collected, and identify their pathotypes using 14 differential cultivars.

Materials and methods

Soil sampling

Soil samples were collected from 467 soybean fields in 65 counties in Heilongjiang, China from 2011 to 2015. Approximately 200 g of soil was collected 5–10 cm from a symptomatic plant or in an area with poor drainage. When symptomatic plants were not found in a field, soil samples were collected around the healthy soybean root area. Approximately 2 kg of soil that was collected from each field was bulked in an aseptic bag to represent a single composite sample. Then soil samples were air-dried in the shade. So, a total of 467 soil samples were analyzed in the present study.

Isolation

Phytophthora sojae was isolated from soil samples using a modified leaf disc baiting method on selective media (Canaday and Schmitthenner 1982; Meng et al. 1999; Schmitthenner et al. 1994). Each air-dried soil sample was ground with a sterile mortar and pestle to fine particles for easier mixing. Ten grams of ground soil was randomly taken from each sample and saturated with a water solution containing penicillin (500 ppm), rifampicin (200 ppm), pentachloronitrobenzene (PCNB 250 ppm), and carbendazim (250 ppm) in a Petri dish. The Petri dish was sealed with Parafilm (Bemis Co., Neenah, Wisconsin, USA) and incubated at 24 °C with 12 h light and 12 h dark. Five days after incubation, soil samples in Petri dishes were flooded with the same solution described above to 6 mm above the soil surface. Forty leaf discs (10 mm in diameter) freshly cut with a paper punch from the unifoliolates of 10-day-old susceptible soybean cv. Sloan seedlings were floated in each Petri dish and incubated under the same conditions described above. After 2 days of incubation, leaf discs were removed from the dish, washed twice in sterile water, and transferred to another sterile Petri dish containing 20 mL of sterile water. After another 2-day incubation in the same conditions, leaf discs with sporangia were transferred to an Eppendorf tube containing 1 mL of sterile water and incubated at 4 °C for 15 min and then at room temperature (22–26 °C) for 20–30 min for zoopores release. After the discs were removed from the Petri dish, 100 µL of the zoospore suspension was transferred onto selective medium CA (1000 mL of distilled water, 200 g of commercial carrot extract, and 20 g of agar), which was amended with the fungicide hymexazol (500 ppm) and the antibiotic ceftriaxone sodium (200 ppm) in a Petri dish. After 5–7 days incubation at 24 °C, Petri dishes were examined with a compound microscope for the presence of tenuous aseptate hyphae (approximately 3.5 µm width) with rectangular branching and abundant spherical catenulate hyphal swellings (23 µm diameter) and spherical homothallic oospores (30–35 µm diameter) (Wen and Zhang 1998). The target colonies were marked and transferred to selective CA for 6–7 rounds of purification. Pure cultures were obtained from hyphal tip sections if an isolate was contaminated by bacteria. All isolates from each sample were confirmed as *P. sojae* by their noncaducous, nonpapillate, inverted, pear-shaped sporangia (35–58 µm × 34–45 µm) (Wen and

Zhang 1998), and by PCR detection of a 288-bp band using primer pair 5′-CTGGATCATGAGCCCACT-3′/5′-TCTCC ATCCACCGACTACA-3′ and the method of Liu et al. (2007). The pure cultures were maintained on CA at 15 °C for single zoospore separation.

A single-zoospore culture was obtained by repeatedly pipetting 2 μL of zoospore suspension onto a sterile glass slide and examining the suspension with an inverted microscope for the presence of a single zoospore. The drop containing one zoospore was pipetted and sprayed on water agar and cultured at 25 °C for 2–3 days. The single zoospore colony was transferred to CA and stored at 15 °C for future use.

Pathotype evaluation

Pathotypes of *P. sojae* were determined using the universal hypocotyl inoculation method described by Meng et al. (1999). In the present study, 14 differentials including Harlon (*Rps*1a), Harosoy 13XX (*Rps*1b), Williams 79 (*Rps*1c), P.I.103 (*Rps*1d), Williams 82 (*Rps*1k), L76-1988 (*Rps*2), L83-570 (*Rps*3a), PI 172901 (*Rps*3b), PRX146-48 (*Rps*3c), L85-2352 (*Rps*4), L85-3059 (*Rps*5), Harosoy62XX (*Rps*6), Harosoy (*Rps*7), and PI 399073 (*Rps*8) were used. Sloan (*rps*) was used as the susceptible control. Twelve seeds of each differential were planted in a pot (10 cm diameter) and grown in a greenhouse at 19–27 °C for 10 days. For inoculation, a small disc (approximately 8 mm diameter) of 3–5-day-old pure culture of *P. sojae* on CA was inserted into a slit on a hypocotyl of a seedling by using a scalpel. Ten seedlings were inoculated for each differential–isolate combination. The inoculated seedlings were incubated in mist chambers at 19–25 °C for 24–48 h in a greenhouse. Reaction types of individual isolates on differentials were evaluated when all inoculated susceptible Sloan plants were dead. Reaction types were classified as resistant (R) if < 4 seedlings died, susceptible (S) if > 6 seedlings died, and intermediate (I) if 4–6 seedlings died. The test was repeated 2–4 times. A list of ineffective *Rps* genes in 14 soybean differentials was considered the name of a pathotype of *P. sojae*.

Metalaxyl sensitivity

Metalaxyl sensitivity was determined according to the method described by Taylor et al. (2006) with a minor modification. Technical grade metalaxyl (Shandong Zhanhua Chemical Co., Binzhou, China) was dissolved in dimethyl sulfoxide before adding it to autoclaved CA at 0, 0.01, 0.05, 0.1, 0.5, 1, and 5 μg/mL, respectively. Mycelial plugs, 5 mm in diameter, were cut from the margin of actively growing colonies that were grown on CA for 6 days in the dark and transferred to the metalaxyl-amended CA plates. After 7 days of incubation in the dark at 25 °C, growth of isolates at each concentration of metalaxyl was determined by subtracting 5 mm (the diameter of mycelial pug) from the mean diameter of a colony measured in two perpendicular directions. The relative growth reduction rate was calculated as follows: (100 – [growth in a treatment/growth in control plate] × 100). The EC_{50} value was estimated from the dose response curve that was plotted by using the percentage inhibition against the logarithmic scale of fungicide concentrations. The isolates were considered to be sensitive to metalaxyl if EC_{50} values were less than 1.0 μg/mL, resistant if EC_{50} values were higher than 100 μg/mL, and intermediate if EC_{50} values ranged between 1.0 and 100 μg/mL. The experiment was repeated twice for each isolate.

Results

Distribution of *P. sojae*

Among 467 soybean fields investigated throughout 65 counties in Heilongjiang Province, typical diseased plants caused by *P. sojae* or *P. sojae* strains were found or isolated in 43 counties, and the fungus was isolated from 281 fields in 28 counties. A heavy infestation of *P. sojae* was found in the eastern region including Qitaihe, Jixi, Shuangyashan, Mudanjiang, and Jiamusi where approximately 91 % of the isolates were obtained, and serious root and stem rot of soybeans were observed (Fig. 1; Table 1).

Pathotype evaluation

Based on the reactions on 13 differentials, 82 pathotypes were determined in 395 tested isolates. Each pathotype composed less than 2 % (0.25–1.79 %) of total isolates (Table 1). Twenty of the 82 pathotypes matched races 1, 2, 3, 5, 8, 9, 11, 13, 15, 17/26, 21, 23, 25, 29, 33, 39, 42, 43, 44, and 48 of *P. sojae* based on published race definitions (Grau et al. 2004). The isolation frequencies of the 20 identified races ranged from 0.25 to 5.82 %, in which races 8, 13, and 23 had higher isolation frequencies that comprised more than 5 % of the isolates; followed by races 11, 13, 2, 3, and 17/26, which made up more than 2 % of the isolates. Races 15, 21, 25, 33, 39, 42, 43, and 48 were detected infrequently, each with 1–3 isolates, representing 0.25–0.76 % of the population. Race 1, that has been considered the most dominant race in Heilongjiang, was only recovered from Jiamusi and Mudanjiang, for 1.52 % of the isolates in the present study (Table 1).

Virulence frequencies for the 14 *Rps* genes differed among the isolates and ranged from 17.85 to 82.41 %. Virulence frequencies for *Rps*3b, *Rps*1b, *Rps*6, *Rps*7, and

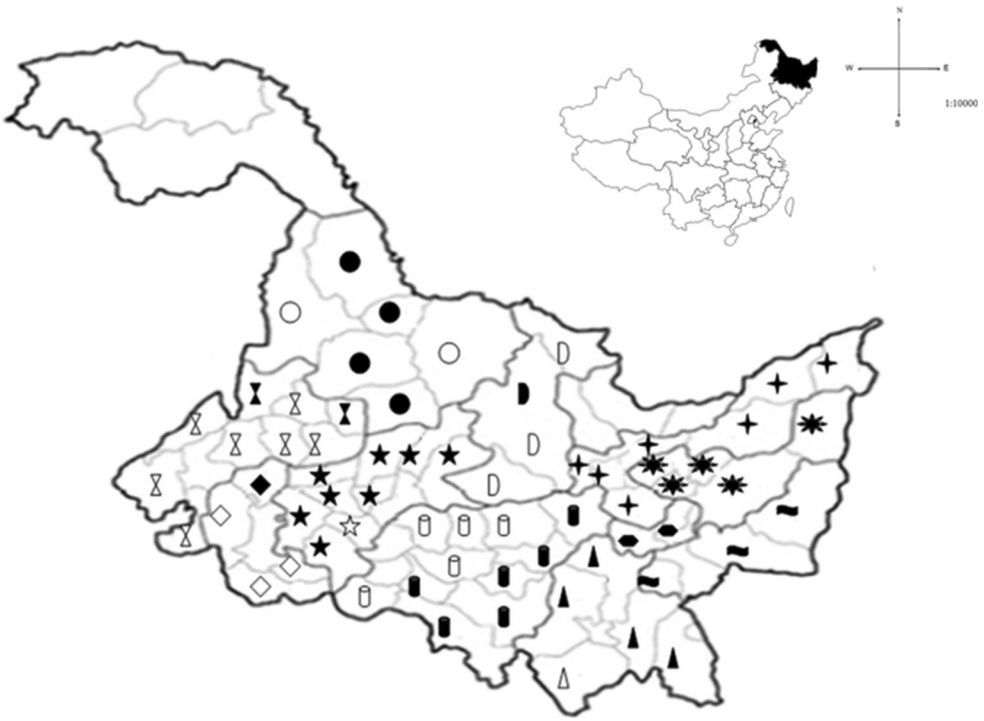

Fig. 1 Distribution of *Phytophthora sojae* in Heilongjiang Province from 2011 to 2015. Figures represent 65 counties sampled. *Black symbols* represent 43 counties where *Phytophthora sojae* was detected or typical Phytophthora root and stem rot of soybean was found. *Symbols* represent the 11 cities in Table 1. ✦, Jiamusi; ✷, Shuangyashan; ◇, Daqing; ☆, Suihua; △, Mudanjiang; ⌭, Harbin; ⬡, Qitaihe; ⏢, Jixi; ○, Heihe; ⏣, Yichun; ⧖, Qiqihaer. Heilongjiang Province is located in northeastern China (*small map*)

*Rps*8 genes were 54.07, 58, 69.03, 78.74, and 82.41 % of the isolates, respectively, whereas 17.85, 18.9, and 26.77 % of the isolates were virulent to cultivars with single *Rps* genes 1k, 1c, and 3a, respectively (Fig. 2). Various virulence combinations were detected in the tested isolates that could defeat soybean cultivars with 2–11 multiple *Rps* genes. Ninety-six percent and 29 % of the isolates in the present study defeated more than 4 and 7 combined *Rps* genes, respectively. Approximately 54 % of the highly virulent pathotypes, that could defeat 7–11 multiple *Rps* genes, were found in eastern Heilongjiang (Qitaihe, Jixi, Shuangyashan, Mudanjiang and Jiamusi) where a high incidence (18 %) of soybean stem and root rot was recorded and a high number of *P. sojae* isolates were obtained. Eighty-eight percent of the isolates defeated *Rps* 8 gene although they were virulent to only 2 or 3 different *Rps* genes (Table 1).

Metalaxyl sensitivity

Two hundred twenty-three isolates were randomly chosen and tested for their sensitivity to metalaxyl. The results showed that all tested isolates were sensitive to metalaxyl. EC_{50} values of these isolates ranged from 0.081 to 0.56 μg/mL (mean EC_{50} = 0.18 μg/mL). More than 90 % of the isolates had EC_{50} values less than 0.28 μg/mL. Isolates with the highest mean EC_{50} value were found in Jiamusi (Fig. 3).

Discussion

Phytophthora sojae is a soil-borne pathogen that has a gene-for-gene relationship with its soybean host. Although a single soybean plant could be infected by more than one pathotype, isolates from infected soybean plants only reflect a small portion of *P. sojae* populations in soil (our unpublished data). To avoid the deviation derived from genotype of the soybean cultivar, in the present study, *P. sojae* was isolated from soil samples instead of the diseased plants.

Phytophthora sojae is an oomycete with diploid genetic characteristics that indicate a high degree of genetic variability and capabilities of producing a variety of genotypes. Many races and pathotypes of *P. sojae* have been reported in various regions (Costamilan et al. 2013; Cui et al. 2010; Dorrance et al. 2003; Jackson et al. 2004; Kaitany et al. 2001; Nelson et al. 2008), and differences in pathotype diversity of *P. sojae* were found among different years (Jackson et al. 2004; Robertson et al. 2009; Schmitthenner et al. 1994; Yang et al. 1996), which were mainly related to resistant soybean genotypes. Fourteen races of *P. sojae* including races 1, 3, 4, 5, 9, 11, 13, 15, 17, 21, 24, 38, 44, and 54 have been reported previously in Heilongjiang (Ma et al. 2005; Wen and Chen 2002; Xu et al. 2003; Zhang et al. 2008, 2010; Zhu et al. 2000). Among 20 races that were identified in the present study, 11 races (races 2, 8, 23, 25, 26, 29, 33, 39, 42, 43, and 48) are newly reported in Heilongjiang. Four races including races 4, 24, 38, and 54

Table 1 Pathotypes identified with 13 and 14 differential cultivars of soybean for *Phytophthora sojae* isolates baited from soil samples collected from soybean fields in Heilongjiang Province, China from 2011 to 2015

No. of *Rps* genes defeated	Virulence pathotype[a]	Race[b]	No. of isolates[c]	Source[d]
11	1a, 1b, 1c, 1d, 1k, 2, 3b, 3c, 5, 7, 8	33	3	Jiamusi
11	1b, 1c, 1d, 1k, 2, 3a, 3b, 4, 5, 6, 8	UD	2	Jiamusi
11	1a, 1b, 1c, 1k, 2, 3a, 3c, 4, 6, 7, 8	39	3	Jiamusi
10	1a, 1b, 1c, 1d, 2, 3a, 3c, 6, 7, 8	UD	4	Jiamusi
10	1a, 1b, 1d, 2, 3a, 3b, 3c, 6, 7, 8	UD	4	Jiamusi
9	1b, 1c, 1k, 2, 3a, 3b, 3c, 7, 8	UD	4	Jiamusi
9	1a, 1b, 1d, 2, 3a, 3b, 3c, 6, 7	UD	3	Jiamusi
9	1a, 1b, 1k, 2, 3b, 4, 6, 7, 8	29	4	Jiamusi
9	1a, 1b, 1d, 3b, 3c, 4, 5, 6, 7	UD	4	Jiamusi
9	1a, 1b, 2, 3a, 3b, 3c, 6, 7, 8	UD	5	Jiamusi
9	1a, 1b, 1d, 3a, 3b, 3c, 6, 7, 8	UD	3	Jiamusi
9	1b, 1d, 2, 3b, 3c, 4, 6, 7, 8	UD	5	Jiamusi
9	1a, 1b, 1d, 2, 3c, 5, 6, 7, 8	UD	4	Suihua
9	1a, 1b, 1d, 2, 3a, 4, 6, 7, 8	UD	2	Jiamusi
9	1d, 1k, 2, 3a, 3c, 4, 6, 7, 8	UD	1	Shuangyashan
9	1a, 1b, 1c, 1d, 2, 5, 6, 7, 8	UD	1	Jiamusi
8	1c, 1d, 2, 3b, 3c, 5, 6, 8	UD	2	Jiamusi
8	1a, 1b, 2, 3b, 5, 6, 7, 8	23	4	Daqing (2), Suihua (2)
8	1b, 1d, 2, 3a, 4, 6, 7, 8	17/26	2	Daqing
8	1a, 1b, 1d, 2, 5, 6, 7, 8	UD	4	Shuangyashan
8	1a, 1b, 3c, 4, 5, 6, 7, 8	23	7	Jiamusi
8	1c, 2, 3a, 3b, 3c, 5, 7, 8	UD	3	Shuangyashan
8	1a, 1k, 3b, 3c, 4, 6, 7, 8	UD	3	Mudanjiang
8	1a, 1b, 1d, 4, 5, 6, 7, 8	UD	5	Jiamusi
8	1a, 1b, 1k, 3a, 3b, 5, 7, 8	UD	3	Jiamusi
8	1a, 1b, 1k, 2, 3a, 4, 7, 8	UD	2	Jiamusi
8	1a, 1d, 2, 3b, 5, 6, 7, 8	8	2	Harbin
8	1a, 1d, 2, 3a, 3c, 5, 7, 8	42	2	Shuangyashan
8	1c, 1d, 1k, 2, 3b, 6, 7, 8	UD	1	Qitaihe
8	1k, 2, 3a, 3b, 3c, 4, 6, 8	UD	1	Mudanjiang
8	1a, 2, 3c, 4, 5, 6, 7, 8	9	3	Jiamusi
8	1b, 3b, 3c, 4, 5, 6, 7, 8	11	1	Jiamusi
8	1a, 2, 3b, 3c, 4, 5, 7, 8	3	2	Jiamusi
8	1a, 1b, 3b, 3c, 4, 6, 7, 8	23	2	Jiamusi
8	1a, 1d, 1k, 2, 3b, 5, 6, 8	UD	4	Jiamusi
8	1a, 1b, 1c, 1d, 3c, 6, 7, 8	UD	5	Jiamusi
8	1a, 1b, 1d, 3a, 3c, 6, 7, 8	UD	5	Jiamusi
7	1a, 1b, 1c, 1k, 3c, 4, 7	25	1	Jiamusi
7	1a, 1b, 1c, 1d, 2, 5, 6	UD	4	Jiamusi
7	1b, 1d, 1k, 3b, 5, 6, 7	UD	4	Jiamusi
7	1b, 1d, 3b, 3c, 2, 5, 7	UD	5	Jiamusi
7	1a, 1b, 1d, 3b, 6, 7, 8	UD	4	Jiamusi
7	1b, 2, 3a, 3b, 6, 7, 8	UD	5	Jiamusi
7	1b, 1d, 2, 3b, 3c, 4, 7	UD	5	Jiamusi
7	2, 3b, 3c, 4, 5, 6, 7	13	5	Jiamusi
7	1b, 1d, 2, 3a, 3b, 6, 7	17/26	6	Jiamusi
7	1a, 1d, 4, 5, 6, 7, 8	8	7	Mudanjiang
7	1b, 3b, 3c, 4, 6, 7, 8	11	5	Daqing (1), Mudanjiang (4)
7	1b, 1d, 4, 5, 6, 7, 8	UD	4	Jiamusi
7	1b, 1d, 2, 3b, 5, 6, 8	UD	4	Jixi
7	1a, 1d, 2, 5, 6, 7, 8	8	4	Shuangyashan
7	1a, 3b, 3c, 4, 6, 7, 8	9	3	Jiamusi (1), Mudanjiang (2)

Table 1 continued

No. of *Rps* genes defeated	Virulence pathotype[a]	Race[b]	No. of isolates[c]	Source[d]
7	1a, 1b, 1d, 2, 5, 6, 8	UD	4	Jiamusi
7	1d, 1k, 2, 3a, 5, 6, 8	UD	3	Harbin
7	1c, 1k, 2, 3c, 6, 7, 8	UD	3	Qitaihe
7	1b, 3c, 4, 5, 6, 7, 8	11	3	Jiamusi
7	1a, 1b, 3a, 4, 6, 7, 8	UD	3	Jiamusi
7	1a, 1d, 3c, 4, 5, 7, 8	44	3	Jiamusi
7	1a, 1d, 3c, 2, 6, 7, 8	8	2	Suihua
7	1b, 1d, 3b, 5, 6, 7, 8	UD	2	Qitaihe
7	1a, 1b, 1d, 2, 6, 7, 8	UD	2	Mudanjiang
7	1a, 1c, 1k, 2, 3b, 4, 7	UD	1	Mudanjiang
7	1d, 1k, 3a, 3c, 4, 5, 7	UD	1	Mudanjiang
7	1a, 1b, 1d, 2, 3b, 5, 8	UD	2	Jiamusi
7	1a, 1d, 2, 4, 6, 7, 8	8	2	Jiamusi
7	1a, 1d, 1k, 3a, 6, 7, 8	UD	1	Qitaihe
7	1a, 2, 3a, 3b, 3c, 5, 8	UD	1	Shuangyashan
7	1a, 1b, 1d, 4, 5, 7, 8	UD	2	Mudanjiang (1), Jiamusi (1)
7	1c, 2, 3a, 3b, 3c, 7, 8	UD	1	Mudanjiang
7	1a, 1b, 3c, 4, 5, 6, 7	23	5	Jiamusi (1), Shuangyashan (4)
7	1a, 1b, 1d, 4, 5, 6, 7	UD	2	Jiamusi
7	1a, 1b, 1k, 3a, 3b, 5, 7	UD	1	Heihe
6	3b, 3c, 4, 6, 7, 8	13	7	Jiamusi (5), Mudanjiang (2)
6	1b, 3b, 4, 6, 7, 8	11	5	Jiamusi
6	1a, 1d, 3c, 6, 7, 8	8	4	Harbin (2), Jiamusi (2)
6	1b, 1k, 3b, 4, 5, 8	UD	3	Harbin
6	1b, 3b, 4, 5, 7, 8	2	3	Daqing
6	1b, 1c, 1d, 6, 7, 8	UD	3	Jixi
6	1k, 3b, 3c, 6, 7, 8	UD	3	Jiamusi
6	1b, 2, 3b, 6, 7, 8	11	3	Jiamusi
6	1b, 2, 3b, 4, 6, 8	UD	3	Jiamusi
6	1c, 1d, 2, 3b, 7, 8	UD	3	Jiamusi
6	1c, 1d, 1k, 3a, 6, 8	UD	3	Jiamusi
6	1c, 1d, 2, 6, 7, 8	UD	2	Jixi
6	1a, 1b, 1d, 2, 6, 8	UD	2	Jiamusi
6	1d, 3b, 5, 6, 7, 8	UD	1	Qitaihe
6	1d, 1k, 2, 6, 7, 8	UD	1	Shuangyashan
6	2, 3a, 3b, 3c, 7, 8	15	1	Shuangyashan
6	1a, 3a, 3b, 3c, 4, 7	21	1	Shuangyashan
6	1a, 1d, 4, 5, 7, 8	44	1	Mudanjiang
6	1d, 3a, 3b, 3c, 5, 8	UD	1	Mudanjiang
6	1a, 1c, 1d, 4, 7, 8	43	1	Jiamusi
6	1b, 1c, 1d, 3c, 6, 8	UD	3	Jiamusi
6	1a, 1d, 1k, 5, 7, 8	UD	5	Jiamusi
6	1a, 1c, 3c, 2, 6, 7	5	4	Jiamusi
6	1a, 1b, 1c, 3b, 7, 8	UD	5	Jiamusi
6	1a, 1b, 3b, 6, 7, 8	23	3	Jiamusi
6	1a, 1d, 3c, 4, 6, 7	8	1	Shuangyashan
6	1a, 1b, 1k, 3a, 4, 7	UD	2	Heihe
5	1a, 3b, 3c, 7, 8	3	3	Jiamusi
5	1b, 1c, 1d, 1k, 6	UD	2	Jiamusi
5	1c, 1d, 3b, 5, 7	UD	4	Jiamusi (3), Qitaihe (1)
5	1b, 3a, 4, 6, 8	UD	3	Jiamusi
5	1a, 1b, 2, 5, 8	UD	3	Jiamusi
5	1a, 3a, 3b, 6, 8	UD	4	Jiamusi

Table 1 continued

No. of *Rps* genes defeated	Virulence pathotype[a]	Race[b]	No. of isolates[c]	Source[d]
5	1b, 3b, 4, 7, 8	2	6	Jiamusi
5	1a, 1d, 3b, 6, 8	UD	3	Suihua
5	1b, 3a, 3b, 5, 8	UD	3	Mudanjiang
5	1a, 2, 3a, 3c, 8	UD	2	Shuangyashan
5	1a, 3b, 3c, 6, 8	UD	2	Mudanjiang
5	3a, 3b, 5, 7, 8	15	2	Mudanjiang
5	3b, 3c, 4, 7, 8	1	4	Mudanjiang
5	1a, 3c, 6, 7, 8	9	2	Jiamusi
5	3a, 3b, 6, 7, 8	UD	2	Jiamusi
5	1k, 4, 5, 7, 8	UD	1	Jiamusi
5	1b, 1d, 3a, 7, 8	UD	1	Jiamusi
5	1k, 2, 6, 7, 8	UD	4	Jiamusi
5	1b, 2, 3b, 6, 8	UD	1	Jiamusi
5	1a, 1d, 3c, 6, 7	8	1	Jiamusi
4	1b, 3a, 6, 7	UD	4	Jiamusi
4	3a, 3c, 6, 7	UD	4	Jiamusi
4	1a, 3b, 3c, 7	3	5	Jiamusi
4	1b, 2, 3b, 8	UD	5	Jiamusi
4	1c, 2, 6, 7	UD	3	Jiamusi
4	1b, 5, 7, 8	2	2	Jiamusi
4	1c, 1k, 3a, 3c	UD	1	Shuangyashan
4	2, 6, 7, 8	13	1	Jiamusi
4	1b, 1d, 2, 8	UD	1	Jiamusi
3	6, 7, 8	13	5	Jiamusi
3	5, 7, 8	48	1	Jiamusi
3	1b, 3b, 8	UD	1	Jixi
3	2, 6, 7	13	2	Shuangyashan
2	1d, 8	UD	3	Shuangyashan
2	7, 8	1	2	Jiamusi
2	3b, 8	UD	3	Yichun

[a] Virulence pathotype, which is a list of ineffective *Rps* genes among 14 soybean differential cultivars (1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8); virulence pathotypes in yellow are ineffective *Rps* genes among 13 soybean differential cultivars (1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7)

[b] Race identified with 13 differentials (Grau et al. 2004). UD = undefined races of *P. sojae*

[c] In total, 395 isolates were evaluated

[d] Place where *P. sojae* was isolated. Values in the parentheses are number of isolates. Each place name is a city that encompasses several counties. In total, *P. sojae* was found in 28 counties. Jiamusi city includes 7 counties, and all had *P. sojae*; Suihua city includes 9 counties, 2 had *P. sojae*, 6 had diseased plants; Shuangyashan city includes 5 counties, all had *P. sojae*; Daqing city includes 4 counties, 1 had *P. sojae*; Mudanjiang city includes 5 counties, 4 had *P. sojae*; Harbin city includes 11 counties, 3 had *P. sojae*, another 3 had diseased plants; Qitaihe city includes 2 counties, and all had *P. sojae*; Jixi city includes 3 counties, 2 had *P. sojae* and another 1 had no *P. sojae* isolate but had diseased plants; Heihe city includes 6 counties, 1 had *P. sojae*, 3 others had diseased plants; Yichun city includes 4 counties, and 1 had *P. sojae*. Among all 11 cities in Heilongjiang, only Qiqihaer (including 9 counties) did not have *P. sojae*; however, diseased plants were found in 2 counties in this city

that were reported in previous reports were not found in the present study. Race 1, previously considered as the dominant race with the highest isolation frequency of 60 % in Heilongjiang (Zhang et al. 2010), was detected infrequently (1.52 %) in the present study. Since race 1 is only virulent to *Rps*7 gene, new races can develop by integrating newly defeated *Rps* genes into the virulence formula. This finding suggests that races with integrative virulence have formed in Heilongjiang and was supported by the pathotypes identified using the 14 differential cultivars in the present study. That means that a single isolate can defeat more *Rps* genes, thus challenging the resistance of soybean cultivars in Heilongjiang. However, a majority of soybean cultivars commonly grown in Heilongjiang have multi-resistant genes that can defeat several races or several pathotypes; 73 soybean cultivars grown in Heilongjiang can defeat 1–8 races (Xu et al. unpublished data), which is good news for Heilongjiang soybean producers.

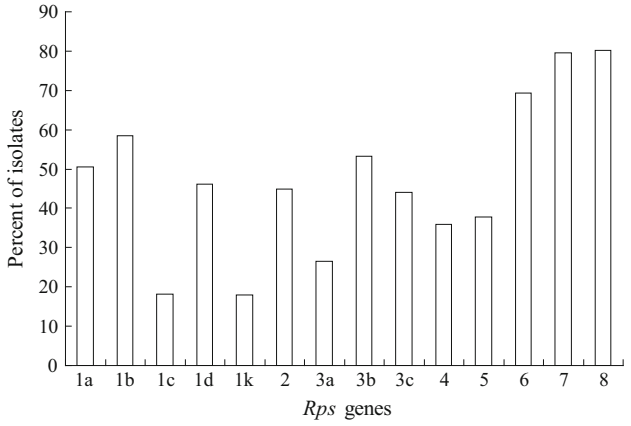

Fig. 2 Virulence frequency of *Phytophthora sojae* isolates from Heilongjiang Province for individual *Rps* gene in 14 differential cultivars

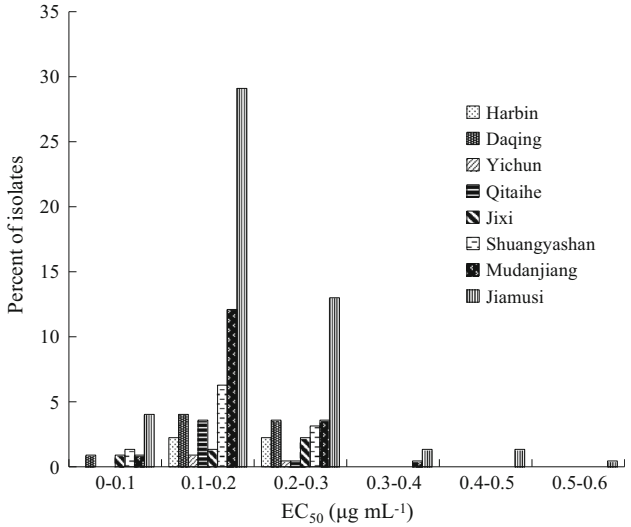

Fig. 3 Frequency of 223 *Phytophthora sojae* isolates at different ranges of EC_{50} values according to county in Heilongjiang Province. Any isolate with an EC_{50} equal to the minimum end of a range (i.e., 0.1, 0.2, 0.3, 0.4, or 0.5), was assigned to the next lower range of EC_{50} values

In the present study, all virulent genes that defeated 14 *Rps* genes were detected in the population of *P. sojae* in Heilongjiang. Virulence frequencies for these 14 *Rps* genes ranged from 17.85 to 78.74 %, a narrower range than the 2–93 % previously reported (Cui et al. 2010), which suggests a reduction in the predominance of some dominant races in Heilongjiang, indicating that some effective *Rps* genes reported previously like *Rps*1a, 1c, and 1k are losing their advantage in resistance to *P. sojae* in Heilongjiang. Compared with a previous report (Cui et al. 2010), virulence frequencies for *Rps* genes 1a, 1b, 1c, 1d, 1k, 3b, and 7 increased, but decreased for *Rps* genes 2, 3c, 4, and 5, and were unchanged for *Rps* genes 3a and 6 in the present study. Virulence frequencies for *Rps* genes 1a, and 1b were approximately 25 and 15 times higher, respectively, than reported in the previous study (Cui et al. 2010), which indicates that *Rps* genes 1a and 1b could become ineffective in Heilongjiang.

Stem and root rot is a common and serious disease of soybean in Heilongjiang, China, and *P. sojae* was found previously in 31 of 45 counties in this province (Cui et al. 2010). In the present study, the pathogen was isolated from 28 of 65 counties. Moreover, typical Phytophthora root and stem rot of soybean were found in 15 counties, with 21 counties having their first record of *P. sojae* or Phytophthora root and stem rot of soybean. However, in the present study, neither *P. sojae* nor diseased plants were found in 11 counties where the pathogen had been isolated previously (Cui et al. 2010). Differences in the distribution of *P. sojae* in Heilongjiang between these studies could be due to the sampling of different fields in the individual counties. Nevertheless, *P. sojae* was distributed widely in Heilongjiang, especially in the eastern part. Previous investigations including our unpublished data showed that the incidence of this disease was heavy in eastern Heilongjiang. In the present study, approximately 91 % of the *P. sojae* isolates were from the eastern region (Qitaihe, Jixi, Shuangyashan, Mudanjiang and Jiamusi), indicating an uneven distribution of the pathogen and supports previous reports of a high incidence of soybean stem and root rot in the east (Li and Wen 2012; Wang et al. 1998). In addition, both mollisol and albic luvisol soils, which are favorable for the development of *P. sojae* (unpublished result), are widely distributed in eastern Heilongjiang.

In the present study, all isolates of *P. sojae* in Heilongjiang were sensitive to metalaxyl, and the mean EC_{50} value was 0.18 μg/mL, 18 times greater than reported previously (Li et al. 1993), indicating that the sensitivity of *P. sojae* to metalaxyl has decreased over the years. This finding is related to the increasing use of metalaxyl to control this disease. There was no relationship between the distribution of the pathotypes and metalaxyl sensitivity. However, the population of *P. sojae* in Heilongjiang is still sensitive to metalaxyl, which suggests that this fungicide can be continue to be used in Heilongjiang, China.

Acknowledgments This research was supported by the Special Fund for Agro-scientific Research in the Public Interest (201303018) and the National Natural Science Foundation of China (Grant No. 31370449).

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