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CHANGES IN RACIAL COMPOSITION OF *PHYTOPHTHORA SOJAE* IN IRAN BETWEEN 1998 AND 2005

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Abstract: Phytophthora root and stem rot of soybean is a destructive disease of soybean in Iran. During 1998–2005, 142 isolates from soil and diseased soybean plants were collected and tested. Race identification was made possible by inoculating *Rps* differential soybean cultivars and lines. Of the 142 isolates tested, 110 isolates belonged to race 1 and 32 isolates belonged to race 3. Race 1 was dominant in soil and diseased plant samples. There was no variability in virulence of *Phytophthora sojae* between the areas surveyed.

Key words: *Phytophthora sojae*, soybean, race, differentials, hypocotyl inoculation

INTRODUCTION

Phytophthora root and stem rot of soybean [*Glycine max* (L.) Merr.] caused by *Phytophthora sojae*, is widespread throughout soybean growing areas of the world (Doupnik 1993). This species, formerly named *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D.C. Erwin (Kuan and Erwin 1980) exhibits aggressive, race-specific pathogenicity to soybean and causes few or no symptoms on other hosts (Hansen and Maxwell 1991). The population of this pathogen is made up of numerous pathogenic or physiological races described by their virulence on a set of differential soybean varieties (Keeling 1982; Layton and Kuhn 1988; Schmitthenner et al. 1994).

Since *P. sojae* was recorded in the United States in 1955 (Suhovecky and Schmitthenner 1955), 50 races have been classified on differential soybean genotypes (Abney et al. 1997) and many more isolates with unique virulence patterns have been identified but not given a race number (Schmitthenner et al. 1994).

Control of this disease has been achieved largely through breeding resistant cultivars (Athow et al. 1980; Schmitthenner 1985). At present, 13 resistance genes (*Rps* gene) have been characterized in soybean (May et al. 2002; Burnham et al. 2003).

However, *P. sojae* has been found to overcome these resistance genes by generating new races (Keeling 1984).

The disease was first recorded in Iran by Rezaee an Alizadeh (1998). They studied the biology of the pathogen and reaction of commercial cultivars to *P. sojae* in Iran (Alizadeh and Rezaee 1999). The objectives of this work were to determine races of *P. sojae* in Iran and its population diversity.

MATERIALS AND METHODS

Isolation of *P. sojae* from plants and soil. Plants with symptoms of stem rot and suspected infested soils were collected from several fields in the Lorestan, Golestan, Mazandaran and Ardabil provinces (40, 20, 20 and 20 fields respectively) in Iran from 1998 through 2005. Isolations of *Phytophthora* from stems with symptoms were made by surface sterilizing a 20 mm sections of stems at the advancing margin of a lesion with sodium hypochlorite for 1 min. After surface sterilization, small parts of these stem pieces were submerged in freshly preparated CMA-PARPH (100 ppm PCNB, 500 ppm ampicillin, 10 ppm rifampin, 10 ppm pimarcin, 50 ppm hymexcazol) medium (Schmitthenner et al. 1994).

The pathogen was isolated from soil by modification of the soybean leaf baiting technique (Schmitthenner et al. 1994). Air-dried, naturally infected soybean field soils were moistened in a flask and were pre-incubated at 25°C for 2–4 weeks, flooded with 5–10 mm of distilled water and then baited with soybean leaf discs for 2–6 h. Distilled water was adopted to incubate the baited leaf discs. Sporangia emerging from the edge of the infected leaf discs were observed under stereo microscopy after 72 h incubation in distilled water. For pure isolations, zoospore solution was spread on 1.5 per cent water agar containing anti-bacterial antibiotics, and 24 h later the germinated zoospores were isolated and pure cultures could be obtained.

Race identification. Inoculum for race identification was prepared by growing isolates on OA (oatmeal agar) in Petri plates at 25°C for 2 to 4 weeks. Inoculations were performed by the standard hypocotyls method (Laviolette and Athow 1981), using 2 x 2 mm pieces of mycelia with abundant oospores, and the wound was covered to prevent desiccation of the inoculum and host tissue. Ten 7-day-old seedlings of soybean differentials (Athow et al. 1980; Anderson and Buzzell 1992; Schmitthenner et al. 1994) representing different *Rps* alleles (Table 1) were inoculated with each isolate and grown in the greenhouse at 25 ± 2°C with supplemental fluorescent and incandescent light. Race determination was repeated twice with each isolate.

Disease reaction of the differential cultivars was recorded 7 days after inoculation (Schmitthenner et al. 1994). Soybean seedling hypocotyls reaction was classified as resistant where 70 per cent or more of seedlings,were alive and as susceptible where 70 per cent or more of seedlings were killed (Schmitthenner et al. 1994; Barreto et al. 1995).

RESULTS AND DISCUSSION

From plants and soil samples 142 isolates were obtained and identified as *P. sojae.* The 110 isolates were virulent on harosoy which carries the *Rps*7 genes, therefore they were determined as race 1. Other 32 isolates were virulent on Harosoy (*Rps*7) and Union (*Rps*1-a), therefore they were determined as race 3 (Table 1).

Table 1. Seedling reaction of differential soybean cultivars to hypocotyls inoculation with two races of *Phytophthora sojae*

Rps gene	Cultivars/Lines	Seedling reaction	
		(race 1)	(race 3)
Rps	Williams	S	S
*Rps*1-a	Union	R	S
*Rps*1-b	Haro13	R	R
*Rps*1-c	Corsoy	R	R
*Rps*1-d	Haro16	R	R
*Rps*1-k	Haro15	R	R
*Rps*3a	L83-570	R	R
*Rps*6	L89-1581	R	R
*Rps*7	Harosoy	S	S

R – resistance, S – susceptible

Apparently the composition of *P. sojae* races in Iran did not change during the years of this study. This research demonstrates the presence of at least 2 physiologic races of *P. sojae* that attack soybeans in Iran. Race 1 and race 3 were the only two isolated in all areas of the study and race 1 was dominant (Table 2).

Table 2. *Phytophthora sojae* races isolated from diseased plants and soil samples

Region	Race 1	Race 3	Total
Lorestan	50	15	65
Golestan & Mazandaran	41	9	50
Ardabil	19	8	27
	110	32	142

There was no variability in virulence of the pathogen between the areas surveyed. The frequency of occurrence of *P. sojae* races in Iran is similar to their occurrence in some regions of other countries (Barreto et al. 1995; Ryley et al. 1992). Barreto et al. (1995) reported only one races of *P. sojae* from Argentina. Other studies have shown different physiological races in specifics areas. Keeling (1979) reported several races in lower Mississippi River Valley area. Ryley et al. (1992) determined that until 1989, there were only two races in Australia, race 1 and race 15. However, new races have been recovered recently from Australia (Ryley and Obst 1998). Anderson and Buzzll (1992) observed that shift in frequency of races could be partially explained by changes of compatible-incompatible genotypes recommended as cultivars.

Race composition is affected by several factors such as farming history, diversity of cultivated varieties, weather and irrigation system. Population diversity increases with introducing new races of *P. sojae* or recombination in stabilized population (Leitz et al. 2000). Races of *P. sojae* isolates from the United States (Schmitthnner et al. 1994) or Australia (Ryley et al. 1998) had more diversity than the Iranian isolates. In the United States 54 races of *P. sojae* had been found so far (Schmitthnner et al. 1994).

Current investigations showed a very low diversity of *P. sojae* population in Iran.

This is due to restriction in new variety import, absence of other hosts of the pathogen such as *lupines*, restriction of the area under culture and absence of *Rps* genes in commercial varieties in Iran. Soybean cultivars/var. Williams and Clark are cultured in Lorestan and Ardabil respectively, which are susceptible to all races of *P. sojae* (Alizadeh and Rezaee 1999) and did not have any selection pressure to the appearance of new races and race 1 remains as a dominant race in Iranian soybean farms. Introducing one or two resistance gene to gene pool of Iranian commercial varieties can reduce the disease severity. Some *Rps* genes are linked together and (Rezaee and Alizadeh 1998) can transfer several genes together to new lines during gene transformation that caused resistance to several races of pathogen.

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POLISH SUMMARY

ZMIANY W SKŁADZIE RAS *PHYTOPHTHORA SOJAE* W IRANIE W LATACH OD 1998 DO 2005

Zgnilizna korzeni i pedów soi jest destruktywną chorobą w Iranie. W latach 1998–2005 wyosobniono i przetestowano 142 izolaty pochodzące z gleby oraz chorych roślin. Rasy patogena oznaczano przeprowadzając inokulację odmian i linii soi wchodzących w skład zestawu różnicującego. Ze 142 przetestowanych izolatów – 110 zaliczono do rasy 1, a 32 izolaty do rasy 3. W glebie oraz próbach materiału roślinnego dominowały izolaty rasy 1. Na badanym terenie nie stwierdzono zmienności wirulencji *Phytophthora sojae*.