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Physiological Races of *Phytophthora sojae* in Iran and Race –Specific Reactions of Some Soybean Cultivars

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ABSTRACT

Phytophthora **root and crown rot of soybean is known as a destructive disease of soybean both through out the world and in Iran. Physiologic races of *Phytophthora soja* were determined in this research and also a fast, accurate and simple method for inoculation of soybean to test race specific resistance. During the years 2001-4, infected soybean plants at different growth stages were collected from different areas and 22 isolates of *P. sojae* were recovered using PARPH medium. Physiological races of the pathogen were determined on differential seedling lines by the hypocotyl inoculation method. Ten seedlings from each differential line grown in a 10-cm pot were inoculated under greenhouse conditions ($25^{oC)}$ by a 10-14 days old fungus (LBA medium). The reaction of the seedlings was classified after 5-6 days as resistant (70% or more of seedlings alive) or susceptible (70% or more of the seedlings killed). Most isolates were identified as race one, six as race three, one as race four and one as a putative race 13. Race-specific resistance of the 60 cultivars towards race three was determined. Some of them such as 'TMS', 'Maverick and 'Williams 82' were considered as resistant cultivars. All experiments were repeated three times.**

Keywords: *Phytophthora soja*, Physiological races, Race-specific resistance.

INTRODUCTION

Phytophthora sojae M. J. Kaufmann and J. W. Gerdemann (Syn. *P. megasperma* Drechs. f. sp. *glycinea* T. Kuan and D. C. Erwin) [10], the causal agent of *Phytophthora* root and stem rot of soybean (*Glycine max* (L) Merr.), is widespread throughout soybean growing areas of the world [9, 16]. This aggressive species is race-specific to soybean and causes few or no symptoms on other hosts [2]. 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 [10]. The fungus is notable among the species of *Phytophthora* as consisting of many races of which most are built up in response to only two resistance genes in popular soybean cultivars. Soybean is unique in having many different alleles and loci for resistance to the pathogen and resistance is easy to evaluate in seedlings [9]. Schmitthenner (1985) considered this pathogen to cause pre-emergence and post-emergence damping-off, gradual killing, seed and stem rot, and infection on leaves and stems. This pathogen was first observed in Iran by Mirabolfathy *et al.* in 1998 [7]. The objectives of this investigation were to determine the frequency of races of *P. sojae* in the main soybean growing areas

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(Lorestan, Mazandaran and Golestan Provinces) and the reaction of the most common commercial soybean cultivars currently used in Iran to this pathogen.

MATERIALS AND METHODS

Isolation of *P. sojae* from Plants

Plants with symptoms of stem rot were collected from several fields in Lorestan, Mazandaran, Golestan and Ardabil Provinces from 2001 to 2004. Small sections taken from the edge of the stem lesions were placed on PARPH medium after disinfesting then with 10% housekeeping bleach. The semi-selective media (PARPH) had a corn meal agar (CMA) base and included pimaricine (10 mg/l) and quintazone (100 mg/l) for selective inhibition of nonpythiaceous fungi and ampicillin (250 mg/l) and rifampicine (10 mg/l) for bacterial control. Hymexazole (20 mg/l) was used in the medium for partial control of *Pythium* spp [11]. The hyphal tip isolates were kept on slant tubes containing CMA at 4ºC.

Race Identification

The inoculum was prepared by growing the hyphal tipped isolates on Limabean agar (LBA, Scharleau®) containing pimaricine (10 mg/l) in glass Petri plates in an unlighted germinator at 25ºC for 7-10 days. The aggressiveness of the isolates was maintained by inoculating the isolates on a susceptible cultivar 'Williams' using the hypocotyl inoculation method at 6-monthly intervals. Seeds of the differential set were supplied by D. Baretto from Argentina and H. Zeinali from the Faculty of Agriculture, Tehran University (Karaj). It included 9 differential cultivars: 'Union' (*Rps1a*), Haro 13' (*Rps1b*), Corsoy 79' (*Rps1c*), 'Haro 15' (*Rps1k*), 'Haro 16' (*Rps1d*), 'L83-570' (*Rps3*), 'L89-1581' *(Rps 6)*, 'Harosoy' (*Rps7*) and the susceptible check 'Haro (1-7)' (*rps*) (Table1). About 15 seeds per line were sown in 10-cm diameter pots containing a 2:1 pasteurized mixture of sand and farm soil. The seeds were allowed to germinate in the laboratory on filter paper, then healthy and vigorous ones planted in the 10-cm pots, covered with a thin layer of Perlite® and allowed to grow for 10 days in a greenhouse with a 25/30ºC day/night temperature and daily watering (Figure 1). At least 10 seedlings in each pot were inoculated using the wounded hypocotyl technique [1, 2, 9, 10 and 17]. In this method, a 1-cm vertical slit is made with a sharp clean scalpel just below the cotyledonary node, a small mycelial plug (2×3 mm^2) of the pathogen was placed on the slit, the inoculated point was covered with parafilm® and then incubated for 4-5 days in green-house at 25/30ºC. Hypocotyl reactions were classified resistant (70% or more of the seedlings alive) or susceptible (70% or more of the seedlings killed) (Figure 1). These experiments were repeated three times for each isolate.

Reaction of Commercial Cultivars

The responses of 60 soybean commercial cultivars currently used in Iran toward *P. sojae*, race three, were determined by the hypocotyl inoculation method. For each cultivar, at least 20 seedlings were tested. The infection percentage of hypocotyls that always lead to mortality and damping-off was recorded for each pot after 4-5 days. The soybean cultivars were classified as resistant (below 30% mortality), susceptible (more than 70% mortality), moderately resistant (30-50% mortality) or moderately susceptible (50 to 70% mortality) [12, 15]. The pots were distributed randomly in the greenhouse and cultivar 'Williams', as a susceptible control, was tested in each experiment.

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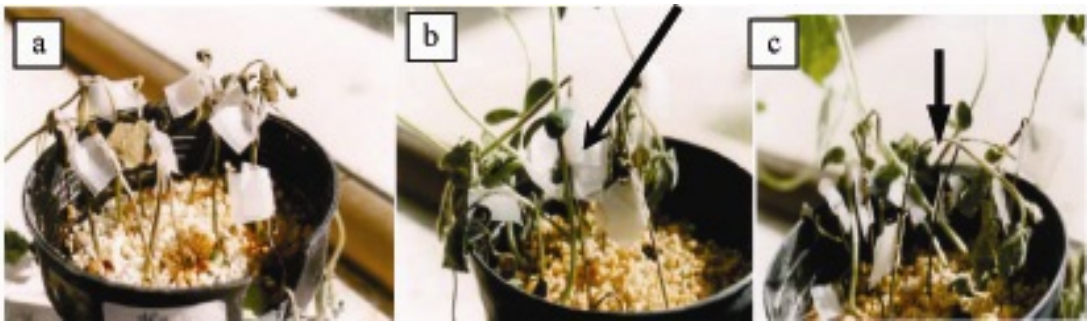

Figure 1. Inoculation of hypocotyls. a: Susceptible reaction in ' Harosoy' cultivar., b. The arrow head shows the inoculated hypocotyl that is resistant to the pathogen. c. Inoculation of hypocotyl and invasion of the pathogen to the downwards and upwards from the inoculating point.

RESULTS

The selective media couldn't inhibit abundant growth of *Pythium* spp. in rotted roots. Other fungi such as *Fusarium* spp. and *Phytophthora* spp. were also isolated, but none of them could cause disease on 'Williams' cultivar as a susceptible check by using hypocotyl inoculation method.

About 22 isolates of *P. sojae* from 35 different farms country-wide were obtained during this research. The reactions of the isolates on the differential sets using the hypocotyls inoculation method are shown in Table 1. All isolates are virulent on Harosoy and Haro (1-7). Most of them were race one (63.6%). Six isolates out of 22 (308, 328, 324, Ps-16, Ps-26 and Ps-27) were race three (27.5%), one isolate, Ps-20, was race four (4.5%) and one isolate, Ps-5, was a putative race 13. Distribution of the races was shown in Figure 2.

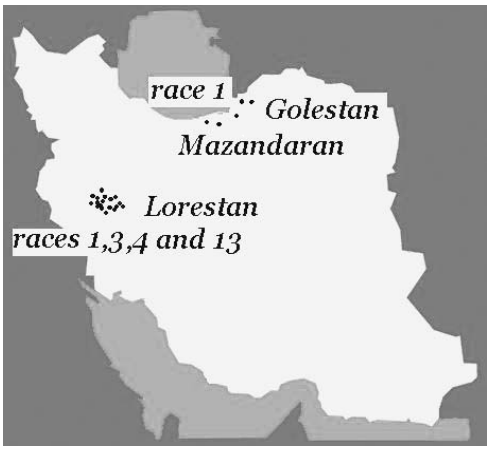

Figure 2. Distribution of the isolates and races in Lorestan, Mazandaran and Golestan Provinces.

The definition of physiologic races of *Phytophthora sojae* based on their interaction with *Rps* alleles in differential lines was adapted from Ward (1990). Those reactions in table 1 were repeated at least three times except for the specific reaction of isolate Ps-5 on L89-1581(*Rps 6*) which was repeated two times. When an isolate showed infection percent between 30-70 %, we repeated inoculation to get infection lower than 30% or more than 70%, showing resistance or susceptibility, respectively. If it didn't show such a reaction, it was omitted and we didn't include it in the race determination experiments.

In race-specific resistance experiments, we used the same method for inoculation. Most cases showed resistance or susceptibility reactions. However we could see some intermediate cases in which the infection percentage was in the range between 30 to 70 %. These cases were considered as moderate (Table 2).

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Table 1. Seedling reactions of differential lines (df_s) to hypocotyl inoculation with different isolates of *Phytophthora sojae* .

df_s Alleles / Isoltes	Haro (1-7) *rps*	Union *Rps1a*	Haro 13 *Rps1b*	Corsoy 79 *Rps1c*	Haro15 *Rps1k*	Haro16 *Rps1d*	L83-570 *Rps3*	L89-1581 *Rps 6*	Harosoy *Rps7*	Race type
186	S[a]	R	R	R	R	R	R	R	S	1
187	S	R	R	R	R	R	R	R	S	1
196	S	R	R	R	R	R	R	R	S	1
201	S	R	R	R	R	R	R	R	S	1
300	S	R	R	R	R	R	R	R	S	1
303	S	R	R	R	R	R	R	R	S	1
308	S	S	R	R	R	R	R	R	S	3
324	S	S	R	R	R	R	R	R	S	3
328	S	S	R	R	R	R	R	R	S	3
330	S	R	R	R	R	R	R	R	S	1
343	S	R	R	R	R	R	R	R	S	1
Ps-3	S	R	R	R	R	R	R	R	S	1
Ps-5	S	R	R	R	R	R	R	S	S	13
Ps-11	S	R	R	R	R	R	R	R	S	1
Ps-15	S	R	R	R	R	R	R	R	S	1
Ps-16	S	S	R	R	R	R	R	R	S	3
Ps-17	S	R	R	R	R	R	R	R	S	1
Ps-18	S	R	R	R	R	R	R	R	S	1
Ps-19	S	R	R	R	R	R	R	R	S	1
Ps-20	S	S	R	S	R	R	R	R	S	4
Ps-26	S	S	R	R	R	R	R	R	S	3
Ps-27	S	S	R	R	R	R	R	R	S	3

[a] R and S indicate to the resistance and susceptible reaction, respectively.

DISCUSSION

Thirteen alleles of resistance genes to *P. sojae* in seven different loci have been identified in different cultivars and lines of soybean (*Glycine max*). The main alleles used in race determination by most researchers all over the world are *Rps1a, Rps1b, Rps1c, Rps1k, Rps1d, Rps3, Rps6* and *Rps7* [9,11 and 14]. Evaluation of the virulence formulas for the *P. sojae* isolates performed was based on the lines and cultivars having those alleles. *Rps1-b, Rps1-k or Rps1-d* and *Rps3* could be used to control a majority of the races causing root and stem rot (Table 1). So these alleles especially *Rps1-k* [1, 10] have been used by plant breeders for introducing to the high yielding soybean cultivars.

In the first two years of this research we recognized race one and three. So some high yielding cultivars were tested with isolate 324 (race three) and 'TMS' was introduced to the farmers in Lorestan Province as a resistant cultivar (Table 2). Many farmers used this cultivar in Lorestan for getting rid of this disease in the second and third year, but we were able to distinguish new races (four and putative race 13) in our disease samples in the third year. Diversity of the races can be increased by using race-specific resistant cultivars in infected areas. Theoretically, there must be 256 races (2^8, 8 main resistance genes which are listed in Table 1) in the infected areas and using race specific resistant cultivars suppressed distribution of the prevailing races (races one and three) in that area and new races, such as race four or race 13 that had a different virulence formula, could escape the resistance and have occupied the ecological niches that the previous races are no longer able to use. So, determination of the races must be done every year to know the prevailing virulence in the farms. Race four was the most virulent isolate in this research (Table 1). So it must be used for future disease resistance breeding programs. However we found this race in the last year of this research and the race-specific resistance shown in Table 2 are

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Table 2. Race-specific resistance of 60 soybean cultivars to *Phytophthora sojae* race 3.

Cultivar	Infection percent	Reaction	Cultivar	Infection percent*	Reaction
TMS	4	R[a]	Cook	70	S
Clark	81	S	Faur	92	S
LWK	100	S	Maccal	100	S
LBK	82	S	Chippewa	60	MS
Hobbit	53	MS	Cattler	100	S
Clifford	66.7	MS	Franklin	83.4	S
Stressland	10	R	Tiffin	10	R
Probsen	0	R	Appolo	10	R
NSMB 149	72	S	L75-6141	70	S
Haueri	70	S	NE-3297	80	S
Jack	72.7	S	Graham	81.8	S
LD3	45	MR	Darby	0	R
Iriquis	75	S	K1410	0	R
Maverick	27	R	Rend	90.9	S
LD10	70	S	L85-3059	12.5	R
NSMB5779	10	R	L92-7857	0	R
Delsoy476	36	MR	Loda	60	MS
Essex	100	S	L91-8347	0	R
Crawford	63.7	MS	L88-570	0	R
Colombus	100	S	Hatcheson	87.5	S
Elgon	90	S	L89-1581	0	R
Union	89	S	KS-3494	90.9	S
Williams	100	S	L91-8915	92.9	S
SRF	90	S	Olympus	72.8	S
Monark	27	R	K-1380	20	R
Lindarin	81.8	S	Doles	67	MS
Calland	62.5	MS	L93-3258	72.7	S
Douglas	100	S	Kottaman	0	R
Bonus	100	S	Savoy	0	R
Kenwood	0	R	L88-3488	0	R

[a] R, S, MR and MS indicate to the resistant, susceptible, moderately resistant and moderately susceptible, respectively.

tested on the basis of race three. Cultivars resistant to race four were also resistant to both race one and three.

This disease is known as a good model as gene for gene hypothesis, so we expect to have only a resistant or susceptible reaction, but race-nonspecific resistance toward *P. sojae* in soybean germplasm is another trait that is being used in controlling *Phytophthora* root rot [4, 5, and 13]. In some cultivars such as Zane (data not shown), LD3 or Delsoy 476 we found that kind of resistance.

A number of methods to screen soybean genotypes for tolerance to *Phytophthora* rot have been reported in literature [3, 5, 8 and 13]. Although field screening has the advantages of measuring full-season effects and is relatively cheap, it shows several disadvantages. These include: (i) Non-Uniformity of the field in *P. sojae* density and soil conditions favouring the pathogen; (ii) Non-Uniformity in frequency of races within the field; (iii) The possibility that the test field does not represent the soybean production area in race frequency; and (iv) Limitation to a single screening experiment for year [6]. These problems have led researchers to develop laboratory and greenhouse resistance screening methods. Since zoospores of the pathogen enter the plants through hypocotyls [9], we used hypocotyl inoculation that is known as a worldwide and standard way to establish disease in the seedlings. This

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method allows us to test single gene resistance easily and effectively. Single-gene resistance is also easy to incorporate and it will continue to be popular among soybean breeders. Several years may be required to incorporate new *Rps* alleles into high yielding cultivars. So planting blends of lower yielding resistant and higher yielding susceptible cultivars may help to optimize production until high-yielding resistant strains are available.

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REFERENCE

1. Abney, T. S., Meglar, J. C., Richards, T. L., Scott, D. H., Grogan, J. and Young, J. 1997. New Races of *Phytophthora sojae* with *Rps1-d* Virulence. *Plant Dis*., **81:** 653-655.
2. Barreto, D., Stegman de Gurfinkel, B. and Fortugno, C. 1995. Races of *Phytophthora sojae* in Argentina and Reaction of Soybean Cultivars. *Plant Dis*., **79:** 599-600.
3. Buzzell, R. I. and Anderson T. R. 1982. Plant Loss Response of Soybean Cultivars to *Phytophthora megasperma* var. *sojae* under Field Condition. *Plant Dis.*, **66:** 1146-1148.
4. Franks, R. K. and Schmitthenner, A. F. 1978. Factors Affecting Tolerance in Soybean to *Phytophthora megasperma* var. *sojae* under Greenhouse Conditions. (Abstr.). *Phytopathol. News*., **12(9):** 182.
5. Jimenez, B. and Lockwood, J. L. 1980. Laboratory Method for Assessing Field Tolerance of Soybean Seedlings to *Phytophthora megasperma* var. *sojae*. *Plant Dis*., **64:** 775-778.
6. Mc Blain, B. A., Hacker, J. A., Zimmerly, M. M. and Schmitthenner, A. F. 1991. Tolerance to *Phytophthora* Rot in Soybean. II. Evaluation of the Three Tolerance Screening Methods. *Crop Sci*., **31:** 1412-1417.
7. Mirabolfathy, M., Alizadeh, A. A. and Ershad, D. 1998. *Phytophthora* Root and Stem Rot of Soybean (*Glycine max*) in Iran. *Proc. 13th Iranian Plant Protection Congress.* p.107.
8. Olah, A. F. and Schmitthenner, A.F. 1985. A Growth Chamber Test for Measuring *Phytophthora* Root Rot Tolerance in Soybean Seedlings. Phytopathol., **75:** 546-548.
9. Schmitthenner, A. F. 1985. Problems and Progress in Control of *Phytophthora* Root Rot of Soybean. *Plant Dis*., **69:** 362-368.
10. Schmitthenner, A. F., Hobe, M. and Bhat, R. G. 1994. *Phytophthora sojae* Races in Ohio Over a 10-year Interval. *Plant Dis*., **78:** 269-276.
11. Sinclair, J. B. and Backman, P. A. 1999. *Compendium of Soybean Diseases* (4th ed.). *APS Press*. 121 pp.
12. Tooley, P. W. and Grau-Craig, R. 1982. Identification and Quantitative Characterization of Rate Reducing Resistance to *Phytophthora megasperma* var. *sojae* in Soybean Seedlings. *Phytopathol*., **72:** 727-733.
13. Walker, A. F. and Schmitthener, A. F. 1984. Comparison of Field and Greenhouse Evaluations for Tolerance to *Phytophthora* Rot in Soybean. *Crop Sci*., **24:** 487-489.
14. Ward, E. W. B. 1990. The Interaction of Soya Beans with *Phytophthora megasperma* f.sp. *glycinea*. In: "*Biological Control of Soil-borne Plant Pathogens*". (Ed.) Hornby, D., C. A. B. International, UK. pp. 299-310.
15. Wilcox, J. R. and St. Martin, S. K. 1998. Soybean Genotypes Resistant to *Phytophthora sojae* and Compensation for Yield losses of Susceptible Isolines. *Plant Dis.*, **82:** 303-306.
16. Wrather, J. A., Anderson, T. R., Arsyad, D. M., Gai, J., Ploper, L. D., Porta Puglia, A., Ram, H. H. and Yorinri, J. T. 1997. Soybean Disease Loss Estimates for the Top 10 Soybean Producing Countries in 1994. *Plant Dis*., **81:** 107-110.
17. Yang, X. B., Ruff, R. L., Meng, X. G. and Workneh, F. 1996. Races of *Phytophthora sojae* in Iowa Soybean Fields. *Plant Dis*., **80:** 1418-1420.

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نژادهای فیزیولوژیکی قارچ عامل پوسیدگی ساقه و ریشه سویا *Phytophthora sojae* در ایران و ارزیابی مقاومت اختصاصی برخی ارقام سویا

ح. صادقی گرمارودی, م. ابوالفتحی, ح. بابایی و ح. زینالی

چکیده

بیماری پوسیدگی ریشه و طوقه سویا ناشی از قارچ فیتوفترا یک بیماری مخرب درجهان و ایران است. در این تحقیق نژادهای فیزیولوژیک قارچ *Phytophthora sojae* درایران مشخص شد و یک روش سریع- ساده و دقیق جهت آلوده‌سازی گیاه سویا و تعیین مقاومت اختصاصی ارقام و لاینهای سویا بکار گرفته شد. طی سالهای ۸۳-۱۳۸۰ نمونه‌های متعدد سویا از مناطق مختلف کشور جمع‌آوری و ۲۲ جدایه قارچ *P. soja*با استفاده از محیط کشت PARPH بدست آمد. نژادهای فیزیولوژیک بیمارگر بر روی گیاهچه‌های ارقام افتراقی با روش تلقیح در هیپوکوتیل تعیین شدند. ۱۰ گیاهچه از هر رقم افتراقی، با قارچ ۱۴-۱۰ روزه که روی محیط کشت LBA رشد یافته بود تلقیح شدند. واکنش گیاهچه ها، ۶-۵ روز بعد بصورت مقاوم (۷۰٪ یا بیشتر زنده) ، یا حساس (۷۰٪ یا بیشتر می‌میرند) یادداشت برداری گردید. اغلب جدایه‌ها نژاد ۱ ، ۶ جدایه نژاد۳ یک جدایه نژاد۴ و یک جدایه نژاد۱۳ بودند. مقاومت اختصاصی ۶۰ رقم سویا با نژاد ۳ تعیین شدند. برخی از ارقام مثل 'TMS' ، 'Maverick' و 'Williams' '82مقاومت بالایی از خود نشان دادند.همهٔ آزمایشها ۳ بار تکرار شدند.

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