Resistance of *Medicago* Species Accessions to *Phytophthora megasperma* f. sp. *medicaginis*

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ABSTRACT

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Resistance to six isolates of *Phytophthora megasperma* f. sp. *medicaginis* was evaluated in 25 accessions, all closely related to *Medicago sativa*. *M. coerulea* (PI 299046) and *M. falcata* (PI 377727) were most resistant, with 26 and 21% resistant plants, respectively. Many accessions contained relatively few resistant plants.

Alfalfa plants resistant to *Phytophthora megasperma* Drechs. f. sp. *medicaginis* have been found, usually at low frequencies, in most dormant and nondormant cultivars that have been evaluated (2–5,9). Since the release in 1973 of Agate, the first cultivar resistant to Phytophthora root rot (1), no isolates of *P. megasperma* f. sp. *medicaginis* that are virulent on the currently used resistant cultivars have been reported. New races of the fungus virulent on these resistant cultivars may develop, however, and prior identification of new sources of resistance in accessions of *Medicago sativa* L. and closely related species could be of considerable benefit.

Accessions of diploid and tetraploid *Medicago* spp., all closely related to *M. sativa*, were tested for their mature plant root reaction to *P. megasperma* f. sp. *medicaginis*. Our studies identified possible new sources of resistance and also provided material used in inheritance studies that will be reported later.

MATERIALS AND METHODS

The 25 accessions (Table 1) evaluated, including both diploid and tetraploid *Medicago* spp., were obtained from the North Central Regional Plant Introduction Station at Ames, Iowa. The cultivars Saranac (susceptible) and Apollo (moderately resistant) and North American Plant Breeders breeding line 0310 (resistant) were included as controls.

Isolates of *P. megasperma* f. sp. *medicaginis* were supplied by S. A. Miller, University of Wisconsin, Madison. These isolates were obtained by baiting from soil (7) from the Phytophthora root rot nurseries in St. Paul, Minnesota (isolates 5b, 5c, 7b, and 7x) and Ames, Iowa (isolate 20) and by direct isolation from diseased alfalfa roots collected in Wisconsin (isolate 21).

The six pathogenic isolates of *P. megasperma* f. sp. *medicaginis* were grown as still cultures in 100 ml of liquid V-8 juice medium (100 ml of V-8 juice and 2 g of $CaCO_3$/L of deionized water) in 500-ml Erlenmeyer flasks at 25 C on the laboratory bench. After 10 days, mycelial mats were harvested, washed, and pressed between sterilized brown paper towels to 75–80% moisture (dry weights were determined on a subsample of the mycelial mats). The mats were then chopped in distilled water in a Waring Blendor for 10 sec. Equal weights of each of the six isolates were used to prepare a composite inoculum. Four isolates failed to produce oospores in the culture medium, and isolates 5b and 7x produced only a few oospores.

Four germinated seeds of each alfalfa line were sown into a 946-ml watertight plastic cup containing about 650 g (dry weight) of a peat and sand (1:1, v/v) mixture. The mixture, pH 6.4, had 78, 36, 35, and 30% (w/w) water retention at 0, −0.1, −0.33, and −1.0 bars matric potential, respectively. Pots were fertilized once each week with a 20-20-20 water-soluble fertilizer (Peters soluble general purpose fertilizer; R. B. Peters Co., Inc.).

Ten-wk-old plants were inoculated with the composite inoculum at 0.31 g dry weight of mycelium per cup. Forty milliliters of the mycelial suspension was poured over the surface of the mixture in each cup and then incorporated into the top 2 cm. One control (uninoculated) cup of each accession was included.

Immediately after inoculation, the cups were saturated by adding water until 1 mm remained on the surface. Saturation was maintained for 4 days before holes were punched in the bottom of each cup to allow drainage. After 21 days of twice-daily heavy watering, severity of disease on plant roots was rated. The test was done in a greenhouse at 18–20 C.

RESULTS AND DISCUSSION

Considerable variation existed in the percentage of plants highly resistant to *P. megasperma* f. sp. *medicaginis* (disease severity index [DSI] = 1 or 2) in the 25 plant accessions, two cultivars, and one breeding line (Table 1). Ninety-one percent of the plants of the susceptible cultivar Saranac were highly susceptible (DSI = 4 or 5). This indicated that the test was effective in allowing differentiation of resistant and susceptible lines. Breeding line 0310, with 64% of plants resistant to Phytophthora root rot, was the most resistant line tested. It is difficult to draw conclusions about the presence of resistance in *M. pironae* (PI 253449 and PI 253450) and *M. falcata* (PI 228152), because only a few plants were tested. No resistant plants were detected in accessions such as PI 325384 (*M. falcata*, USSR), however, even though 60 plants were evaluated. Such accessions were presumed unlikely to provide resistant clones.

Some accessions showed promise for selection of resistant phenotypes. *M. coerulea* (PI 299046) and *M. falcata* (PI 377727) had 26 and 21% resistant plants, respectively. These results and the large standard deviations indicate a large genetic variation for reaction to *P. megasperma* f. sp. *medicaginis*. Both of these accessions could be useful sources of resistance for future breeding programs.

Since almost all plants from *M. coerulea* (PI 299046) are diploid, resistant clones from this line were retained for inheritance studies, which have shown that the resistance factors are inherited quantitatively (*unpublished data*). Lu et al (6) suggested that susceptibility to *P. megasperma* f. sp. *medicaginis* in tetraploid *M. sativa* is conditioned by one tetrasomic gene, Pm, with incomplete dominance. Thus, *M. coerulea* will provide a new source of resistance factors for control of *P. megasperma* f. sp. *medicaginis*.

Many of the accessions contained relatively few resistant plants. Gene transfer from most accessions tested to the cultivated tetraploid *M. sativa* should be achieved with relative ease. Ploidy

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Table 1. Disease reactions of *Medicago* spp. inoculated with *Phytophthora megasperma* f. sp. *medicaginis*

PI number	Species	Origin	Plants tested (no.)	Mean DSI[a] ± S.D.	Resistant plants[b] (%)
179370	*M. sativa*	Turkey	55	4.25 ± 1.09	11
228152	*M. falcata*	USSR	9	5.00 ± 0.00	0
234815	*M. falcata*	Switzerland	46	4.30 ± 0.66	0
234817	*M. hybrid*	Switzerland	35	3.94 ± 1.03	9
234818	*M. hybrid*	Switzerland	57	4.02 ± 0.92	9
239951	*M. sativa*	Iran	57	4.28 ± 0.96	9
251205	*M. falcata*	Yugoslavia	60	4.17 ± 0.87	7
253443	*M. falcata*	Yugoslavia	52	4.25 ± 0.93	6
253446	*M. carstiensis*	West Germany	56	4.60 ± 0.71	2
253449	*M. pironae*	West Germany	5	5.00 ± 0.00	0
253450	*M. pironae*	West Germany	5	5.00 ± 0.00	0
262532	*M. falcata*	Israel	22	4.91 ± 0.29	0
299046	*M. coerulea*	Minnesota	39	3.33 ± 1.13	26
312458	*M. coerulea*	USSR	24	4.92 ± 0.28	0
315462	*M. coerulea*	USSR	49	4.63 ± 0.70	2
315473	*M. falcata*	USSR	48	4.63 ± 0.53	0
315481	*M. hemicycla*	USSR	57	3.72 ± 1.03	9
325384	*M. falcata*	USSR	60	4.53 ± 0.65	0
325385	*M. falcata*	USSR	53	3.74 ± 0.76	8
325408	*M. romanica*	USSR	48	4.75 ± 0.44	0
325416	*M. falcata*	USSR	56	3.57 ± 0.76	11
377724	*M. falcata*	USSR	60	3.55 ± 0.85	12
377727	*M. falcata*	USSR	56	3.30 ± 0.93	21
379581	*M. falcata*	USSR	13	3.00 ± 1.23	23
403962	*M. falcata*	USSR	61	3.67 ± 1.03	15
	M. sativa 'Saranac'[c]	USA	57	4.04 ± 0.98	9
	M. sativa 'Apollo'[d]	USA	57	3.30 ± 1.21	33
	M. sativa NAPB 0310[e]	USA	58	2.38 ± 0.88	64

[a] Disease severity index: 1 = Taproot, secondary roots, and fine feeder roots white (healthy). 2 = Small lesions not encompassing more than 0.2 of the circumference of the taproot (present mainly at the junction of the taproot and lateral roots); girdling lesions permissible on all roots up to 1 mm in diameter. 3 = Lesions on the taproot encompassing 0.2–0.5 of the circumference; girdling lesion permissible on all roots up to 2 mm in diameter. 4 = Lesions completely girdling the taproot and/or larger lateral roots; almost all smaller secondary roots destroyed. 5 = Entire taproot rotted, aboveground parts dead.

[b] Plants with a score of 1 or 2 were considered resistant.

[c] U.S. commercial cultivar (susceptible).

[d] U.S. commercial cultivar (moderately resistant).

[e] Breeding line (highly resistant) developed by North American Plant Breeders, Ames, Iowa.

number should not be a barrier to gene transfer, since diploid clones could be tetraploidized with colchicine, as outlined by Obajimi and Bingham (8), before appropriate crosses are performed.

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