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RHIZOCTONIA STEM CANKER DISEASE OF MUNGBEAN (PHASEOLUS AUREUS) IN IRAN[1]

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Abstract

A damping-off and stem canker disease of mungbean (Phaseolus aureus) incited by Rhizoctonia solani reduced plant populations by 4-57% in experimental field plots at Karaj, Iran. Mortality of seedlings resulted when hypocotyl lesions enlarged or coalesced to girdle the stem. In fields with a high incidence of disease the inoculum potential of R. solani was highest in the top 10 cm of soil and decreased rapidly with depth. The fungus could not be detected below 40 cm. No resistance was exhibited in 40 mungbean types in greenhouse tests.

Mungbeans (Phaseolus aureus) comprise one of the high protein edible legumes (pulses) which are grown as a food crop in Iran and other countries in the Middle East. The Regional Pulse Improvement Project (RPIP) is striving to increase the production and improve the quality of pulses grown in Iran and the Near East-South Asian countries through plant selection and breeding, soil and crop management, and plant disease and insect control.

Young mungbean plants in the 1967 field trials at Karaj, Iran were severely damaged by a canker disease which girdled the stems at the soil surface, resulting in reduced vigor or death of seedlings. This study was undertaken to determine the cause of the disease, the nature and host range of the pathogen, its distribution in the soil, and the relative susceptibility of different mungbean selections to infection.

MATERIALS AND METHODS

Diseased and healthy mungbean seedlings were collected from field plots and isolations were made from the washed roots and stems on water agar (WA), potato-dextrose agar (PDA), or acidified potato-dextrose agar (APDA) (acidified to pH 4.0-4.5 with 25% lactic acid) after surface sterilizing the tissue in 0.5% sodium hypochlorite (10% clorox) for 5 minutes. Pure cultures of all fungi were maintained on slants of PDA.

Fungus isolates used in pathogenicity tests were grown in a sterile mixture of cornmeal-sand (5%-95% v/v) in 250-ml flasks for 5-7 weeks at 25°C. One-hundred cc of inoculum was incorporated in 900 cc of pasteurized greenhouse soil (composed of 3 parts clay loam, 3 parts sand, and 1 part cow manure), and the mixture was placed in sterile 8-inch clay pots partially filled with 1500 cc of pasteurized soil. Mungbean seeds were planted on the surface of the inoculated soil and covered with approximately 1 cm of pasteurized soil. The same procedure was followed in all tests requiring the use of infested soil. Control treatments were identical except that the sterile cornmeal-sand mixture was added to pasteurized soil. Greenhouse temperatures varied from 20 to 30°C.

Several isolates of R. solani from different hosts and localities were used in the pathogenicity studies. These, with the original host, were as follows: isolate 1, bean (Phaseolus vulgaris); 2, bean; 3, bean; 4, chickpea (Cicer arietinum); 5, chickpea; 6, cowpea (Vigna sinensis); 7, lentil (Lens esculenta); 8, mungbean (Phaseolus aureus); 9, mungbean. All isolates are from Iran, except isolate 3 from Turkey.

RESULTS

Disease symptoms: The first symptoms were small reddish-brown lesions in the cortex of the hypocotyl at or below the soil surface. The cankers became darker and larger with age

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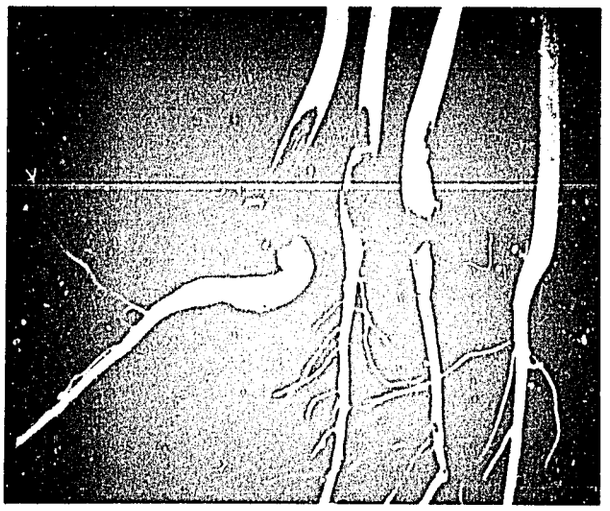

FIGURE 1. Dark brown, sunken cankers developing on the hypocotyls of mungbean inoculated with Rhizoctonia solani. Death frequently resulted when cankers enlarged or coalesced and girdled the stem. Healthy seedling at right.

(Fig. 1). Seedlings collapsed and died when cankers enlarged or coalesced to girdle the stem. Prior to collapse of seedlings, the foliage became yellowish-green and the leaves drooped. Roots of infected seedlings were generally not discolored and appeared healthy.

Disease incidence in the field: Seeds of 13 mungbean selections were planted in the field in mid-June 1967, in single-row plots. Plant mortality caused by stem girdling ranged from 0 to 51%, 7 weeks after planting, and from 4 to 57% by 15 weeks after planting (Table 1).

For greenhouse work, soil was collected from the surface 10-12 cm of field plots with a high incidence of disease. Emergence of seedlings of two mungbean varieties in this soil in clay pots ranged from 44-52% and 32-48%, 3 and 7 weeks after planting, respectively (Table 2). Emergence of seedlings after 7 weeks in autoclaved field soil was 90-97%. Isolations from rotted seed or hypocotyl cankers usually yielded pure cultures of a fungus similar to R. solani.

The pathogen: A fungus resembling R. solani in cultural and morphological characteristics was consistently isolated from stem cankers, although this or other fungi were seldom isolated from the roots of diseased seedlings. All isolates were identified by J. R. Parmeter, Jr. as typical, multinucleate R. solani, the mycelial state of Thanatephorus cucumeris (Frank) Donk (5).

Pathogenicity studies: All except one isolate of R. solani from different legume hosts were highly pathogenic to mungbean in the greenhouse pathogenicity tests (Table 3). Only isolate 4 from chickpea proved to be nonpathogenic. R. solani was reisolated from seedlings with crown cankers or from rotted, discolored seeds which were killed prior to emergence. I was unable to isolate Rhizoctonia from plants in the controls. Symptoms of the disease reproduced in greenhouse inoculation tests were similar to those produced under field conditions.

Susceptibility of mungbean seedlings of different ages to infection by R. solani: Mungbean seed was planted in pasteurized sand, and seed or seedlings were transplanted at intervals to pasteurized soil inoculated with R. solani. Disease incidence decreased as seedlings became older (Table 4). All ungerminated seeds (0 days) planted in infested soil were destroyed by Rhizoctonia prior to emergence while disease incidence was only 20% in seedlings transplanted to infested soil when 19 days old. R. solani was recovered in all isolations made from ungerminated seed or from cankers formed in the crown area of seedlings planted in the infested soil. The control treatments remained free of the disease.

Vertical distribution of R. solani in naturally infested field soil: Soil samples were taken from four locations at 10-cm intervals from the soil surface to a depth of 60 cm in field plots with a high incidence of canker disease. Care was taken not to contaminate the lower soil samples with surface soil. After planting with a susceptible mungbean line, pots were placed on greenhouse benches where observations were made on seedling emergence and canker formation (Table 5).

The highest incidence of disease occurred in the soil from the top 10 cm where seedling emergence was reduced by 50% and canker formation on hypocotyls of surviving plants exceeded

Table 1. Effect of Rhizoctonia canker disease on mortality of plants in 13 mungbean (Phaseolus aureus) lines grown in single-row plots at Karaj, Iran.

Mungbean line	Source	Plant population	Mortality 7 weeks	Mortality 15 weeks
			%	%
1	U.S.A.	315	16	34
2	Brazil	541	16	24
3	Iran	329	19	27
4	Iran	121	23	31
5	Iran	319	--	4
6	Iran	188	4	24
7	Iran	426	51	57
8	W. Pakistan	529	20	41
9	Brazil	368	3	16
10	Iran	264	38	47
10	Iran	568	18	24
11	Iran	582	14	23
11	Iran	825	17	34
12	Iran	528	6	18
12	Iran	513	0	19
13	Iran	586	12	32
13	Iran	499	16	23

Table 2. Emergence of seedlings of two mungbean varieties in natural and sterilized field soil collected from plots with a high incidence of stem canker disease.

Treatment	% Emergence[a] Variety 1 3 weeks	Variety 1 7 weeks	Variety 2 3 weeks	Variety 2 7 weeks
Natural field soil[b]	52	48	44	32
Sterilized field soil[c]	95	90	97	97

[a]Results are the average of five replications of 10 plants each for natural field soil, and the average of three replications of 10 plants each for sterilized field soil.
[b]Soil samples collected from different plots were thoroughly mixed before being placed in 8-inch clay pots.
[c]Soil was autoclaved for 4 hours at 15 psi.

Table 3. Pathogenicity of nine isolates of Rhizoctonia solani from various crops grown in Iran and Turkey to mungbean in greenhouse inoculation tests.

Fungus isolate	Source	% Dead[a]	% Diseased	% Healthy
1	Bean	100	0	0
2	Bean	100	0	0
3	Bean	82	18	0
4	Chickpea	0	0	100
5	Chickpea	100	0	0
6	Cowpea	100	0	0
7	Lentil	92	8	0
8	Mungbean	98	2	0
9	Mungbean	100	0	0
Healthy check		0	0	100

[a]Average of five replications of 10 plants each. Test terminated 24 days after planting.

Table 4. Susceptibility of mungbean seedlings of different ages to infection by Rhizoctonia solani.

Days after planting	Seedling height (cm)	Inoculated[a]		Control	
		% Healthy	% Diseased	% Healthy	% Diseased
0	Ungerminated seed	0	100[b]	100	0
3	Germinating seed[c]	2	98	100	0
6	5-8	12	88	100	0
10	7-10	34	66	100	0
15	13-15	64	36	100	0
19	14-16	80	20	100	0

[a]Results are the average of five replications (inoculated) or two replications (control) of 10 plants each.
[b]All seeds damped-off prior to emergence.
[c]Seeds began germinating in 1 day.

Table 5. Vertical distribution of Rhizoctonia solani in mungbean planting at Karaj, Iran, where the stem canker disease was prevalent. Soil samples were taken at 10-cm increments from four sites.

Soil Depth (cm)	% Seedling emergence[a]		% Crown cankers on surviving plants 5 weeks after planting
	2 weeks	5 weeks	
0-10	62 a[b]	50 a	71.1 a
10-20	88 b	83 b	7.5 b
20-30	89 b	90 bc	0.7 b
30-40	93 b	96 c	0.6 b
40-50	94 b	95 c	0.0 b
50-60	94 b	94 c	0.0 b

[a]Average of four replications of 40 plants each.
[b]Within each column, all percentages not followed by the same alphabetical letter are significantly different at the 1% level.

70%. Few stem cankers were found on seedlings exposed to soil from depths of 10-40 cm, and no evidence of infection occurred on plants exposed to soil from deeper than 40 cm.

When the experiment was terminated after 5 weeks isolations were made from 20 surviving plants from soil at each depth, including those with crown cankers. R. solani was isolated from the hypocotyls of diseased plants growing in soil from a depth of 20-30 cm and was recovered from many rotted seeds in the top 10 cm of soil.

Resistance of mungbean lines to infection by R. solani: Included in the resistance studies were 40 mungbean lines which had performed well in 1966 yield trials. There was no apparent resistance to R. solani in the 40 lines when grown under greenhouse conditions favoring disease development. Seedling emergence in all lines was reduced 92-100% and cankers had formed on the hypocotyls of most emerged seedlings.

DISCUSSION

Diseases limiting the cultivation of mungbeans in Iran are caused by bean common mosaic virus and root rot fungi (3, 4). This study has identified R. solani as a pathogen of mungbean in Iran inciting a damping-off and stem canker disease in experimental mungbean plantings located near Tehran (3, 4). R. solani is generally considered to be a pathogen of seedlings and young plants (2). In naturally infested field soil mungbeans were very susceptible to infection in the seedling stage and several isolates of the fungus were highly pathogenic to germinating seed and young seedlings in greenhouse studies. These isolates were less pathogenic on older seedlings. However, death of older plants occurred in field trials when hypocotyl lesions, apparently initiated in the seedling stage, enlarged or coalesced and girdled the stem. The effect of infection on plant growth and yields was not determined, but both appeared to be adversely affected. Yield reductions attributable to R. solani causing stem canker diseases of other crops have been reported in the literature (6, 7).

The high mortality in some experimental mungbean plots indicated that the inoculum potential of R. solani in these field soils was high. Although Rhizoctonia could be isolated occasionally from diseased seedlings grown in field soil from depths of 20 to 30 cm, the pathogen was concentrated in the top 10 cm of soil. Soil-inhabiting fungi are capable of prolonged survival in field soils as soil saprophytes colonizing dead plant or animal tissues (2). It is, therefore, not surprising to find R. solani concentrated in the upper 10 cm of soil where accumulation of organic matter is highest and CO_2 is at its lowest concentration.

Rhizoctonia solani is an important root and stem pathogen of many crops grown in Iran, including several pulses (4). Most isolates of R. solani from five pulse crops were highly pathogenic to mungbean in greenhouse trials, resulting in pre-emergence damping-off or lesion formation on the hypocotyls of emerged seedlings. The fungus appeared to be confined to hypocotyl lesions, since in repeated isolations from the roots of diseased seedlings I failed to recover Rhizoctonia. Confinement of the pathogen to the canker region may only occur during the seedling stage, since R. solani has been isolated from discolored, necrotic roots of mature root rot-infected mungbeans from various areas of Iran (4).

Canker formation and death of mungbean seedlings caused by R. solani decreased with an increase in age of the plants. Increased resistance with age of mungbean hypocotyl tissue may be related to the conversion of pectins to calcium pectate which coincides with hypocotyl maturation (1).

Field resistance to R. solani may be present in a few mungbean lines, particularly line No. 5, which had a low incidence of disease in the Karaj single row field tests. In the absence of replicated trials, however, an uneven distribution of fungus inoculum in the field could produce a similar effect. In greenhouse tests with one isolate of R. solani, no resistance was observed in 40 mungbean lines, including those with a low disease incidence under natural field conditions. Mungbean lines with some resistance to the pathogen in the field may have been eliminated in the greenhouse trials if the inoculum potential of the pathogen were higher than normally encountered in nature.

Control of plant diseases in the developing nations is becoming increasingly more important, especially with serious diseases that affect essential food crops, like wheat, rice, corn, and pulses. In these countries the cultivation of varieties that incorporate resistance to one or more major plant diseases is highly desirable because it eliminates the necessity of using chemical control measures that are often more costly and difficult to obtain or apply, and which may require special training or precautions in their proper use. If Rhizoctonia becomes a limiting factor in mungbean cultivation before resistant varieties are developed and available to the farmer, it may be necessary to implement other control measures, including use of chemicals, before this crop can be grown successfully in Iran. While continuing our search for Rhizoctonia-resistant mungbeans, numerous fungicides are being screened for their effectiveness in controlling the disease. Encouraging results have been obtained in preliminary greenhouse and field tests with some of the newer organic, seed-treatment fungicides.

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