Genetic Control of Pathogenicity in Rhizoctonia solani

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ABSTRACT

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Heterokaryons were synthesized among AG4 homokaryons of *Rhizoctonia solani* mutant for different stages of the infection process in flax hypocotyls. The heterokaryons were pathogenic and highly virulent. Pathogenicity of *R. solani* to flax hypocotyls is nonhost-specific and is controlled by several dominant factors. Two different genetic systems control seed rot and hypocotyl infection in the pathogen.

Previous studies of seedling blight of flax (*Linum usitatissimum* L.) caused by *Rhizoctonia solani* [*Thanatephorus cucumeris* (Frank) Donk] with isolates of Anastomosis Group (AG) 1 (9) showed that field isolates were heterokaryotic, and that virulence was controlled by several genetic factors (6, 12). Host plant resistance to these isolates has been identified (12) and studied genetically (13). Subsequent work has shown that AG4 field isolates are the most common forms of *R. solani* associated with seedling blight of flax in Minnesota and host resistance has been found (1).

The AG4 isolates of *R. solani* are heterokaryotic and homokaryons from AG4 field isolates from Australia, Britain, Canada, and the United States form heterokaryons readily (2). Approximately 30 isolates of AG4 which were isolated from soil and diseased plants have been studied (1, 2) and all of them have contained two incompatibility factors (*H* factors) per isolate. The *H* factor promotes outbreeding (2) and regulates the stability of the heterokaryon (10). The AG4 heterokaryon is multinucleate and nuclear division is conjugate (4, 11). When heterokaryons formed between field isolates of AG4 so that more than two unlike *H* factors were present in a single hypha, those heterokaryons were nonpathogenic (3).

The purpose of this study was to gain more information on the genetics of heterokaryosis in *Rhizoctonia solani* and its role in seedling blight of flax.

MATERIALS AND METHODS

Using *H* factors as well as auxotrophic markers, we looked for mutant homokaryons that could not infect flax hypocotyls of cultivar Arny. A method to study the specific steps in the infection of radish (*Raphanus sativus* L.) hypocotyls by AG2 isolates of *R. solani* had been determined (5). The steps are: (i) inhibition; ie, no fungus growth on the hypocotyl; (ii) growth, but no attachment of hyphae to hypocotyl; (iii) growth and attachment of hyphae to hypocotyl; (iv) infection cushions formed but no penetration of host cell walls; (v) hypersensitive reaction; and (vi) pathogenic reaction.

The AG4 field isolates used in this study were of the "Praticola-type" and were isolated from diseased plants in Britain and from Minnesota and Nebraska in the USA (Table 1). All field isolates were pathogenic and caused severe seedling blight of flax although none of them was originally isolated from flax. Homokaryons with naturally occurring mutations for thiamine deficiency (*Thi*) and lack of nitrate reductase (*Nar*) were detected initially by their inability to grow on Czapek's agar medium. Wild-type homokaryons grow on Czapek's medium. Nonpathogenic mutants were detected in the water agar test (Fig. 1) and the mutant stage was determined using the seedling-glass slide method described below.

The pathogenicity (ability to infect and cause disease on flax hypocotyls) and virulence (degree of pathogenic action) (14) of the AG4 isolates was tested by placing flax seeds on water-agar cultures in petri dishes (Fig. 1). A 3-mm-diameter disk was cut from the periphery of a culture of *R. solani* grown on potato-dextrose agar (PDA) and placed in the center of a 9-cm-diameter petri dish containing 20 ml of 2% water agar. The plates were incubated at 25 C for 3 days, by which time the fungus had grown over the agar surface. Twenty sound flax seeds selected under a dissecting microscope were surface disinfested with 1% sodium hypochlorite, rinsed with sterile distilled water, placed on water agar plates containing the AG4 isolates, and incubated at 22 C for 5 days. Each test was repeated twice with three replicates per test. A nonpathogenic isolate was rated 0. The virulence of the pathogenic isolates was rated on a scale of 1-10 in which 1 indicated 10% of the seedlings killed and 10 equaled 100% seedling mortality.

To study the hypocotyl infection process more precisely, the following method was used: Flax seedlings were grown for 7 days in washed sand in an environment chamber with a 12-hr day temperature of 22 C, a night

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temperature of 19 C, and a light intensity of 26,900 lux, and were watered with a nutrient solution (8). The seedlings were removed from pots and the roots were washed free of sand with distilled water. Two seedlings were attached to each microscope slide with rubber bands and inoculated with isolates of *R. solani* grown on PDA (Fig. 2). The inoculated seedlings were placed vertically in glass jars with the roots in a nutrient solution similar to that mentioned above and kept at the same temperature and light conditions used to raise the seedlings. The seedlings were observed daily under the dissecting microscope from the 2nd through the 5th day after inoculation to determine the stage reached by the isolate in the infection process. The above method also was used on 15 homokaryons from heterokaryon III which caused 100% seed rot and it was necessary to determine whether they could infect flax hypocotyls. The techniques to synthesize heterokaryons from homokaryons with two different *H* factors and to induce basidiospore formation in these isolates have been described (2).

TABLE 1. The origin and pathogenicity of AG4 field isolates of *Rhizoctonia solani* and the auxotrophy and pathogenic ability of mutant homokaryons derived from them

Isolate number	Origin: Host and Locality	Auxotrophy	Pathogenicity on flax
42	*Beta* sp., Slough, U.K.	W-t[a]	W-t[a]
42-2	F_1 spore of Isolate 42	W-t[a]	Stage IV mutant [b]
134	*Beta vulgaris*, Nebraska, USA	W-t[a]	W-t[a]
134-9	F_1 spore of Isolate 134	*Nar*-[d]	Stage II mutant[c]
141	*Amaranthus* sp., Nebraska, USA	W-t[a]	W-t[a]
141-7	F_1 spore of Isolate 141	*Thi*-[e]	W-t[a]
127	*Pisum sativum*, Minnesota, USA	W-t[a]	W-t[a]
127-11	F_1 spore of Isolate 127	*Nar*-[d]	Stage II mutant[c]

[a]Wild-type.
[b]Infection cushion development only; no penetration.
[c]Hyphal growth on stems, but no attachment of hyphae to hypocotyl.
[d]*Nar* = nitrate reductase.
[e]*Thi* = thiamine.

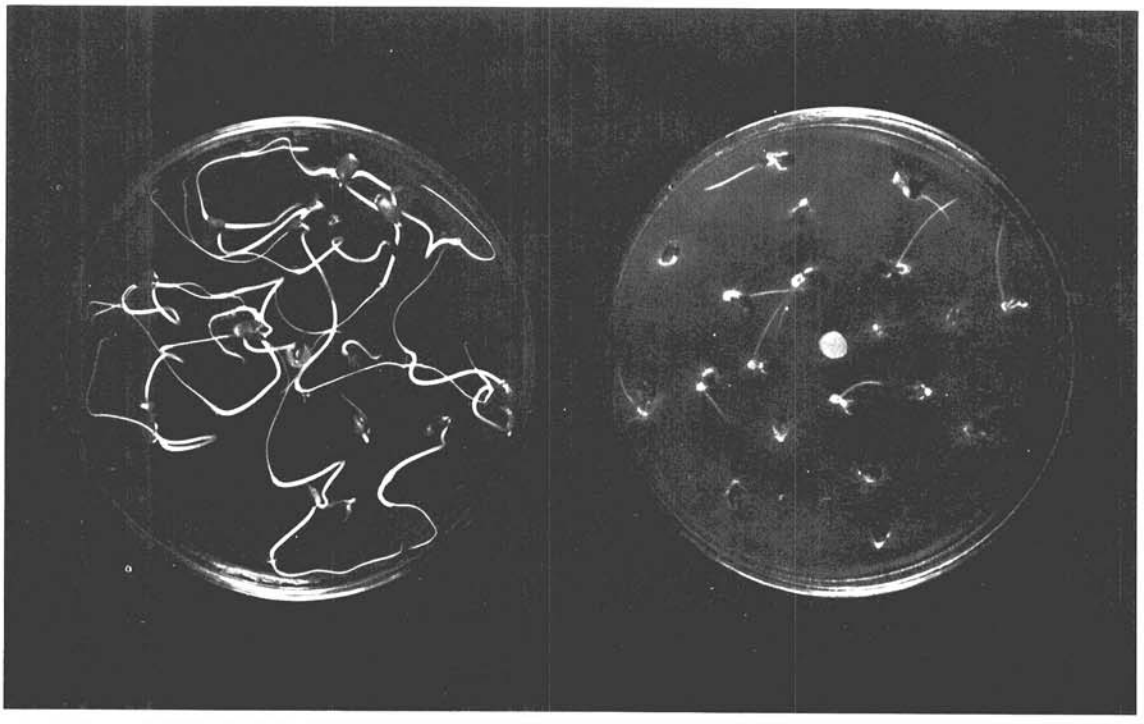

Fig. 1. Pathogenicity on germinating flax seed of two homokaryons from heterokaryon III of *Rhizoctonia solani*: left, a homokaryon which caused neither seed rot nor hypocotyl infection; and right, a homokaryon which caused both seed rot and hypocotyl infection.

RESULTS

Heterokaryon I, which was synthesized from two nonpathogenic homokaryons, mutant for the 2nd and 4th stages of the infection process, was rated 10 for virulence in the water agar test. The segregation pattern for *H* factor and auxotrophy (Table 2) indicated that genetic recombination occurred; of 50 homokaryons from heterokaryon I, 36 were nonpathogenic and 14 were pathogenic. The 14 pathogenic homokaryons varied in virulence from 3 to 10. The ratio of nonpathogenic:pathogenic homokaryons approximates a 3:1 ratio ($\chi^2 = 0.24$, $P = 0.7$-0.5) if the two mutants used in this heterokaryon were single-gene controlled and

TABLE 2. Parental genotypes and segregation patterns of basidiospore progenies of three AG4 heterokaryons of *Rhizoctonia solani*

Het.[a]	Homokaryon	Genotype	Segregation pattern			
I	42-2	*H12*[b], *Nar+*[c]	*H2Nar+*:	*H2Nar−*:	*H12Nar−*:	*H12Nar+*
	127-11	*H2*, *Nar−*	15	10	13	11
			$\chi^2 = 1.204$, $P = .95 - .5$			
II	42-2	*H12*, *Nar+*	*H3Nar+*:	*H3Nar−*:	*H12Nar+*:	*H12Nar−*:
	134-9	*H3*, *Nar−*	34	23	27	12
			$\chi^2 = 10.582$, $P = .05 - .01$			
III	42-2	*H12*, *Thi+*[d]	*H12Thi+*:	*H12Thi−*:	*H8Thi+*:	*H8Thi−*
	141-7	*H8*, *Thi−*	19	20	18	11
			$\chi^2 = 2.941$, $P = .5 - .3$			

[a]Abbreviation Het. = heterokaryon.
[b]Abbreviation *H* = H factor.
[c]Nitrate reductase auxotrophic marker.
[d]Thiamine auxotrophic marker.

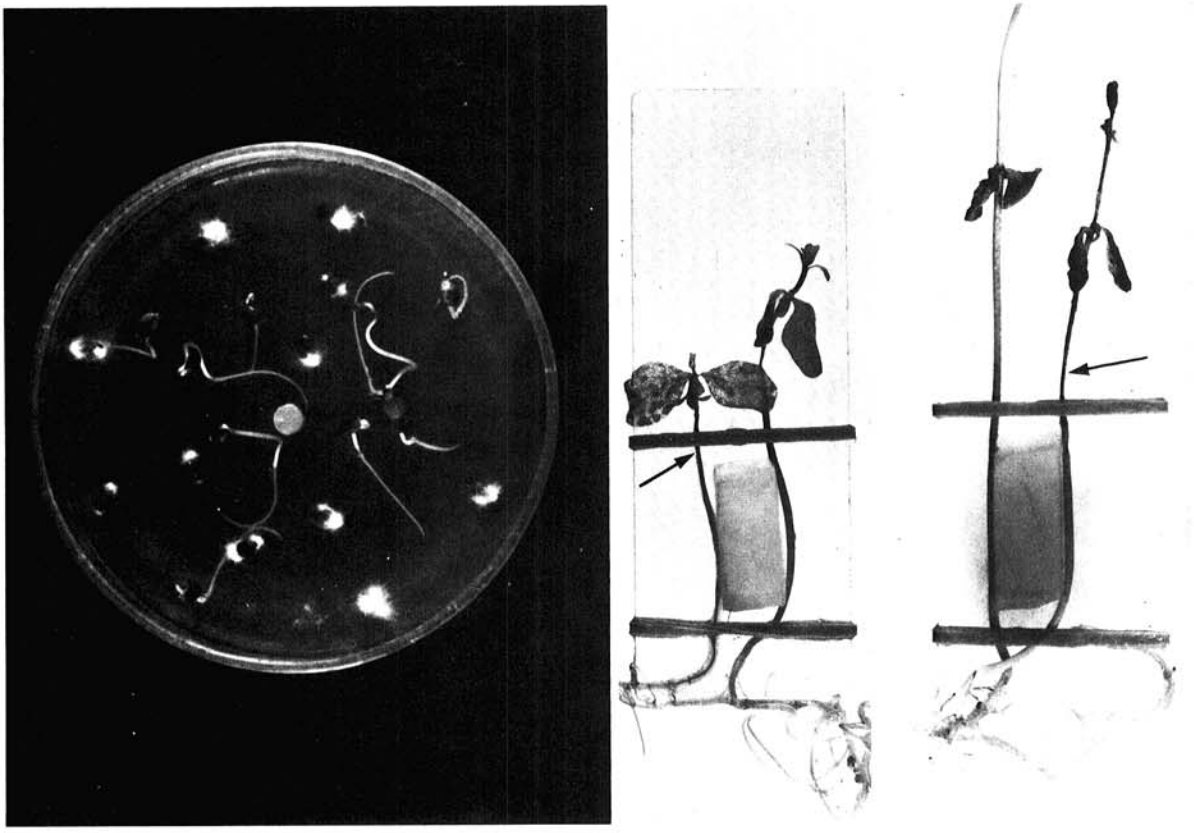

Fig. 2. Tests to study hypocotyl infection by three homokaryons of *Rhizoctonia solani* from heterokaryon III: left, a homokaryon which caused seed rot but did not infect flax hypocotyls; and right, method used to study the infection process or to determine the mutant stage of isolates unable to infect flax hypocotyls. In this test both homokaryons have infected the hypocotyls (arrows).

independent. One homokaryon among the above 50 progeny had a recombinant *H* factor. It formed a mycelial tuft when paired with both the *H2* and *H12* parental homokaryons, was able to utilize nitrate, but did not infect the hypocotyls of flax seedlings.

Heterokaryon II, synthesized from nonpathogenic homokaryons mutant at the 2nd and 4th stages of the hypocotyl infection process, was rated 10 for virulence. The segregation pattern for *H* factor and auxotrophy is shown in Table 2. Of 77 homokaryons from heterokaryon II, 55 were nonpathogenic, 22 were pathogenic ($x2 = .628$, $P = 0.5 - 0.3$). This ratio suggests that the two mutants used in this heterokaryon involved single genes that were recessive, and not linked. Fifteen of the above 77 homokaryons caused 100% seed decay in the water agar test. To determine if they could infect flax hypocotyls they were observed on the glass slide-seedling test; five were pathogenic and 10 nonpathogenic on flax hypocotyls.

Heterokaryon III was derived from a homokaryon mutant for the 4th stage in the hypocotyl infection process, plus a pathogenic, highly virulent, thiamine-deficient homokaryon. In the water agar test this heterokaryon was rated 10 for virulence. The segregation pattern for *H* factors and auxotrophy is presented in Table 2. The ratio of nonpathogenic:pathogenic homokaryons on flax hypocotyls in the water agar test was 39:29 ($\chi^2 = 1.470$, $P = 0.3$-0.2) and suggests that the stage-4 mutant used in this heterokaryon segregated as a single recessive factor. Seventeen homokaryotic progenies from heterokaryon III did not infect flax hypocotyls, but caused seed decay. One homokaryon had a recombinant *H* factor, required thiamine, and did not infect flax hypocotyls or cause seed rot.

A heterokaryon was synthesized from two stage-2 mutant homokaryons 127-11 and 134-9 involving the *H2* and *H3* factors. This heterokaryon was pathogenic on Arny flax hypocotyls using the water agar test and indicated that the two stage-2 mutant genes were not alleles. Had they been alleles, the heterokaryon would not have been pathogenic. Thus more than one factor is involved in stage-2 of the hypocotyl infection process.

DISCUSSION

Both the infection process and the relative virulence of isolates of *R. solani* are affected by heterokaryosis. In this study the infection process was controlled by several dominant genes and resembled hypocotyl infection of radish by AG2 isolates (5). The virulence of a number of AG1 heterokaryons was rated 10 for hypocotyl infection when these heterokaryons were synthesized from two homokaryons each with a low virulence rating (12). This suggested that virulence was controlled by a number of factors and that both epistatic and additive gene action were involved (12). The effect of heterokaryosis on seed rot has not been determined.

The virulence of AG4 field isolates was altered by various nutrients (15). In this study, pathogen nutrition did not affect pathogenicity. Homokaryons unable to infect flax hypocotyls in the water agar test were also nonpathogenic on the glass slide-seedling test where PDA was the nutrient base for fungus growth.

Seedling blight of flax caused by AG1 and AG4 isolates is due to seed rot and hypocotyl infection. Isolates of *R. solani* in AG's 2 and 3 caused seed rot but not hypocotyl infection and were not thought to be important pathogens of flax seedlings in Minnesota (1). In the present study, seed rot and hypocotyl infection were controlled by different genetic factors in the pathogen. Some homokaryons could not complete the hypocotyl infection process but caused seed rot. It was noted earlier that yellow-seeded flax cultivars were extremely susceptible to seedling blight and this was due chiefly to increased susceptibility to seed rot (7).

Some 30 different AG4 isolates have been isolated from diseased plants and soil and all of them caused moderate to high (50 - 100%) mortality on Arny flax seedlings. Resistance to the AG4 isolates has been found and it appears to prevent penetration of hypocotyl cells by the pathogen (1).

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