Inheritance of a Morphological Factor Limiting Infection by Ustilago hordei

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ABSTRACT

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Matings of compatible sporidial cultures from ordered tetrads from single teliospores of race 8, of *Ustilago hordei* sometimes failed to infect the highly susceptible barley cultivar, Odessa (C.I. 934). When the infection failed on Odessa, no infection was found on any other barley cultivars inoculated with the same culture. Matings of sporidial cultures from ordered tetrads of single teliospores which were derived from a single sporidial mating of race 8 wild type resulted in a ratio of three virulent to one avirulent dikaryon. Pathogenicity was always associated with the production of large numbers of infection hyphae of normal length and growth rate. Teliospores were selected from each virulent line, and sporidial cultures from their ordered tetrads were mated. Matings from these F_3 tetrads segregated either in a 3:1 or a 4:0 ratio with virulence dominant. Testcrosses of sporidial cultures of tetrads from F_2 teliospores confirmed that a single recessive gene conditioned the development of infection hyphae. Microscopic examination of the avirulent matings showed a decrease in both length and number of the infection hyphae compared with those of pathogenic matings from the same tetrad. The gene pair, *ihi ihi*, conditions poor development of infection hyphae.

Ustilago hordei (Pers.) Lagerh. causes covered smut of barley. Teliospores germinated on an agar medium produce four haploid sporidia (basidiospores). Compatibility is bipolar; thus, four compatible matings may be made with the sporidia produced from each teliospore (3). Odessa (C.I. 934) barley (*Hordeum vulgare* L.), is susceptible to all 13 races of *U. hordei* (8). However, Lade (4) and Jensen (2) reported some dikaryons avirulent on Odessa and attributed lack of infection to escape, a low level of virulence, or to poorly developed infection hyphae. Production of infection hyphae after the mating of sporidial cultures is correlated with compatibility and infection of susceptible hosts (1).

This study was conducted to investigate the lack of virulence by certain compatible, sporidial matings on the susceptible cultivar Odessa.

MATERIALS AND METHODS

Teliospores from isolate 448, an inbred product of race 8, *U. hordei*, are known to be heterozygous for a recessive factor which inhibits the development of infection hyphae in compatible, virulent matings. This factor has been assigned the symbol *IHI* (dominant) or *ihi* (recessive). When teliospores were germinated on 3% potato-dextrose agar (2, 6, 7), each of the four primary sporidia were separated in order from the promycelium using a Leitz micromanipulator. These primary sporidia are subsequently referred to as an ordered tetrad. The sporidia were allowed to form individual colonies and then were transferred to V-8 juice agar plates (9). This medium was used for mating and storage. Compatability of the sporidial cultures was determined by the modified Bauch test (5). Selfing involved the mating of four sporidial cultures from one teliospore in all compatible combinations. Crossing involved the mating of a sporidial culture from one teliospore with a sporidial culture from a different teliospore. Sporidial cultures from four ordered tetrads of isolate 448 were selfed and pathogenicity was tested on the highly susceptible barley cultivar, Odessa (C.I. 934). These four tetrads were designated 448-1,2,3,and 4. Seedlings of the eight barley covered-smut differential cultivars and Jet (C.I. 967) were inoculated using the following procedure.

(i) V-8 juice agar in petri dishes was streaked with two sporidial cultures of opposite mating type. The cultures were streaked on opposite sides of the plates and incubated at 20 C for 72 hr.
(ii) Odessa barley seeds were hand dehulled, soaked in distilled water for 2 hr, and placed in petri dishes lined with moist filter paper. The petri dishes were wrapped with aluminum foil and incubated at 20 C for 24 hr.
(iii) The sporidial cultures were mixed on the V-8 juice agar plate using a sterilized artist's paint brush. A small amount of the mixture was then brushed on the base of the coleoptile of a 24-hr-old seedling.
(iv) The inoculated seedlings were again placed in petri dishes lined with moist filter paper, wrapped in aluminum foil, and incubated at 20 C for 48 hr.

The inoculated seedlings then were transplanted into

TABLE 1. The pathogenicity on Odessa barley and the proposed genotypes of four selfed tetrads from isolate 448, race 8, *Ustilago hordei*

Tetrad	Cross	Pathogenicity[a]	Proposed genotype	Isolate accession number
448-1	1 × 2	V	ihi IHI	5057
	1 × 3	A	ihi ihi	5058
	4 × 2	V	IHI IHI	5061
	4 × 3	V	IHI ihi	5062
448-2	1 × 2	V	IHI IHI	5063
	1 × 4	V	IHI ihi	5065
	3 × 2	V	ihi IHI	5066
	3 × 4	A	ihi ihi	5068
448-3	1 × 2	V	ihi IHI	5078
	1 × 3	A	ihi ihi	5079
	4 × 2	V	IHI IHI	5082
	4 × 3	V	IHI ihi	5083
448-4	1 × 2	V	ihi IHI	5084
	1 × 3	A	ihi ihi	5085
	4 × 2	V	IHI IHI	5088
	4 × 3	V	IHI ihi	5089

[a]Symbols: V = virulent, A = avirulent.

TABLE 2. The pathogenicity on Odessa barley and the proposed genotypes of eight selfed tetrads from selfed tetrad 448-1 or 448-3, race 8, *Ustilago hordei*

Tetrad	Cross	Pathogenicity[a]	Proposed genotype
5057	1 × 2	A	ihi ihi
	1 × 3	V	ihi IHI
	4 × 2	V	IHI ihi
	4 × 3	V	IHI IHI
5058	(Nonpathogenic mating)		
5061	1 × 2	V	IHI IHI
	1 × 3	V	IHI IHI
	4 × 2	V	IHI IHI
	4 × 3	V	IHI IHI
5062	1 × 2	V	ihi IHI
	1 × 3	A	ihi ihi
	4 × 2	V	IHI IHI
	4 × 3	V	IHI ihi
5078	1 × 2	A	ihi ihi
	1 × 4	V	ihi IHI
	3 × 2	V	IHI ihi
	3 × 4	V	IHI IHI
5079	(Nonpathogenic mating)		
5082	1 × 2	V	IHI IHI
	1 × 3	V	IHI IHI
	4 × 2	V	IHI IHI
	4 × 3	V	IHI IHI
5083	1 × 2	V	ihi IHI
	1 × 3	A	ihi ihi
	4 × 2	V	IHI IHI
	4 × 3	V	IHI ihi

[a]Symbols: V = virulent, A = avirulent.

15 cm-diameter clay pots filled with an autoclaved mixture of sand, soil, and peat moss. The plants were maintained in a greenhouse at 22 ± 3 C until maturity. When harvested, any plant having an infected tiller was considered to be infected. The percentage of infection was determined by dividing the number of infected plants by the number of plants inoculated.

TABLE 3. The pathogenicity on Odessa barley and the proposed genotypes of the testcross matings between cultures of six ordered tetrads and cultures of sporidia 1 or 3 from tetrad 448-1, race 8, *Ustilago hordei* known to carry the *ihi* factor

Testcross		Pathogenicity[a]	Proposed genotype
5057,1 × 448-1,3		A	ihi ihi
2	1	A	ihi ihi
3	1	V	IHI ihi
4	3	V	IHI ihi
5061,1 × 448-1,1		V	IHI ihi
2	3	V	IHI ihi
3	3	V	IHI ihi
4	1	V	IHI ihi
5062,1 × 448-1,1		A	ihi ihi
2	3	V	IHI ihi
3	3	A	ihi ihi
4	1	V	IHI ihi
5078,1 × 448-1,3		A	ihi ihi
2	1	A	ihi ihi
3	3	V	IHI ihi
4	1	V	IHI ihi
5082,1 × 448-1,1		V	IHI ihi
2	3	V	IHI ihi
3	3	V	IHI ihi
4	1	V	IHI ihi
5083,1 × 448-1,1		A	ihi ihi
2	3	V	IHI ihi
3	3	A	ihi ihi
4	1	V	IHI ihi

[a]Symbols: V = virulent, A = avirulent

Cultures from ordered tetrads of teliospores derived from the virulent matings of tetrads 448-1 (designated 5057, 5061, and 5062) and 448-3 (designated 5078, 5082,

TABLE 4. Summary of the mean length and number of infection hyphae from compatible matings of tetrad 448-1, *Ustilago hordei*

Mating	Proposed genotype	Infection hyphae	
		Length (μm)[a]	Number per mm^2[a]
448-1			
1 × 2	ihi IHI	56.2	18.12
1 × 3	ihi ihi	4.1	0.63
4 × 2	IHI IHI	42.9	14.26
4 × 3	IHI ihi	45.3	12.65

[a]The mean length and number were obtained by measuring and counting the length and number of infection hyphae at ten different locations per mating. Each mating was replicated five times.

and 5083) were selfed and pathogenicity was tested on Odessa. Cultures from these tetrads also were testcrossed to sporidia that possessed the infection hyphae inhibitor (*ihi*).

The number and length of the infection hyphae from the four matings from tetrad 448-1 were determined after 24 hr. Nuclear staining of the infection hyphae also was conducted using the procedure of Jensen and Kiesling (3). Inoculated seedlings were examined during incubation and the depth of penetration of coleoptile was determined.

RESULTS

When the four tetrads, 448-1,2,3, and 4, were selfed, one compatible mating from each tetrad was avirulent on Odessa (Table 1). The matings from selfed tetrads 5057, 5062, 5078, and 5083 (derived from 448-1 and 448-3) segregated virulent to avirulent in 3:1 ratios (Table 2). However, the matings from tetrads 5061 and 5082 were all virulent on Odessa. When cultures of the same tetrads were testcrossed to sporidia having the *ihi* character, the matings from 5057, 5062, 5078, and 5083 segregated in 1:1 ratios (Table 3). All testcross matings from tetrads 5061 and 5082 were virulent on Odessa (Table 3).

Microscopic examination of the *ihi* matings and normal matings confirmed Jensen's (2) observation that there was a decrease in number and length of infection hyphae (Table 4). Plasmogamy occurred between + and − sporidia in all matings. Nuclear staining of infection hyphae from *ihi* and normal matings did not show any cytological differences. Penetration of the coleoptile by the *ihi* infection hyphae occurred. At the end of the incubation period, the *IHI*–matings had penetrated deeply into coleoptile tissue, but the homozygous *ihi* matings had penetrated only to a depth of two or three cells.

DISCUSSION

The data from selfed tetrads and test crosses establishes that the *ihi* factor, which slowed infection of the susceptible cultivar Odessa, was controlled by a single recessive gene pair. No teliospores were recovered from Odessa inoculated with matings homozygous for the *ihi* factor. Microscopic examination of the *ihi* matings proved that dikaryotic infection hyphae were present but were reduced in both number and length.

This is not a recessive gene for avirulence, but rather a gene that restricts the formation of infection hyphae and causes the fungus to grow slowly. This slows normal penetration of the host by infection hyphae and thus prevents infection of the crown meristematic tissues.

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