The Genetics of a Distinguishing Pigmentation Reaction of *Typhula idahoensis* and *T. ishikariensis*

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ABSTRACT

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A few monokaryons of *Typhula idahoensis* accept nuclei of *T. ishikariensis*, allow migration of the foreign nuclei within their hyphae, and react to the foreign nuclei by turning brown to reddish brown when cultured on Difco potato-dextrose agar. This color reaction is visible both above in the aerial mycelium and below in the matrix in the agar. Three monokaryons of *T. idahoensis* that do not become discolored with prolonged incubation, that do not develop pigment when mated with *T. idahoensis*, and that react with pigmentation with 97% of the isolates of *T. ishikariensis*, have been placed in the American Type Culture Collection. These tester monokaryons should facilitate identification of these otherwise very similar species. No reciprocal reactions (ie, monokaryons of *T. ishikariensis* paired with dikaryons of *T. idahoensis*) were observed. When mycelial transfers are made from the tester monokaryons of *T. idahoensis* after pairing with *T. ishikariensis*, growth is usually reduced, but clamp connections are present. We view the discoloration and reduced growth as symptoms of general incompatibility resulting from the presence of foreign nuclei. Staining with hematoxylin revealed that all isolates from nature were dikaryons, that the testers (basidiospore products) were monokaryons, and that nuclei migrated from dikaryotic *T. ishikariensis* into monokaryons of *T. idahoensis*.

Individual sclerotia of *Typhula idahoensis* Remsberg, *T. ishikariensis* Imai, or *T. incarnata* Lasch recovered from soil cannot be identified with certainty by any known method (1). They must be cultured and identified in culture. *T. idahoensis* and *T. ishikariensis* can only be identified with certainty by a di-mon mating system (dikaryon × known monokaryon) that requires a minimum of two monokaryons of *T. idahoensis* and two of *T. ishikariensis* and microscopic examination of hyphae of progeny for the presence of clamp connections (1). The test to be described requires only one or two di-mon matings per unknown isolate and no microscopic examinations. The tester of *T. idahoensis* monokaryons become brown after contact with *T. ishikariensis* and remain colorless after contact with *T. idahoensis*. *T. incarnata* is easily distinguished from the above two species in culture. Because all three species are common and coexist in many of our wheat field soils, an easy method of identification was needed before the ratio of the sclerotial populations of these species from soil could be determined.

MATERIALS AND METHODS

In our studies we employed 72 monokaryons of *T. idahoensis* obtained from 22 isolates and 24 monokaryons of *T. ishikariensis* obtained from 14 isolates. Field isolates (natural dikaryons) had been identified previously by the di-mon (dikaryon × known monokaryotic tester) system of identification (2). The isolates of *T. idahoensis* and *T. ishikarensis* used in the final test are listed in Table 1. The 42 isolates of *T. incarnata* were all from Washington. Difco potato-dextrose agar (PDA) and Difco corn meal agar were used in preliminary tests. All work reported was done on Difco PDA.

Transfers from fresh, vigorous colonies of the monokaryon and of the dikaryon (isolate) were spaced 55–60 mm apart in an 85-mm-diameter plastic petri dish containing 25 ml of Difco PDA. The mycelia of colonies started that far apart formed a wide zone of contact near the center of the dish. Incubation was at 10 C in the dark. Macroscopic observations of color reactions were made at least 30 days after inoculation.

Nuclear staining. Cultures to be studied for nuclear condition were grown on cellulose dialyzing membranes placed aseptically on Difco corn meal agar (4). The dialysis tubing was cut 4.5 cm^2 and autoclaved in water for 20 min prior to being placed upon the agar plates. Cultures were grown for 7–10 days at 10 C before fixation.

The mycelial membranes were fixed in chromic acid-acetic acid-formalin fixative (Craf III) (5) for 3.5 hr, followed by overnight washing in running tap water. Subsequently, the cultures were

TABLE 1. Origin of isolates of *Typhula idahoensis* and of *T. ishikariensis* identified by the color reaction with three monokaryons of *T. idahoensis*

Origin		Isolates (no.) per county	
State	County	*T. idahoensis*	*T. ishikariensis*
Idaho	Bear Lake	2	0
	Blaine	2	5
	Bonneville	2	0
	Camas	2	0
	Caribou	2	1
	Cassia	1	0
	Franklin	9	4
	Idaho	0	1
	Lemhi	6	1
	Madison	1	1
	Oneida	6	0
	Power	2	0
	Teton	5	0
Montana	Flathead	1	0
	Gallatin	1	0
Utah	Box Elder	1	0
Washington	Douglas	36	6
	Grant	0	1
	Kittitas	0	3
	Lincoln	0	3
	Okanogan	14	18
	Spokane	0	4
	Stevens	2	5
Wyoming	Lincoln	3	7
Total		98	60

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placed in a 2% solution of ferric ammonium sulfate (Lang's mordant) for 3.5 hr, rinsed thoroughly, and stained overnight in a 0.5% hematoxylin solution. After staining, the cultures were differentiated in saturated picric acid for 10–30 min (depending upon the isolate) and washed thoroughly. Prior to being permanently mounted, the stained membranes with mycelia were dehydrated for 5 min successively in the following ethyl alcohol-(EtOH)-xylene series: 10% EtOH in deionized water; 30, 50, 70, 80, and 95% EtOH; absolute EtOH; absolute EtOH:xylene (1:1); and 100% xylene.

The nuclear conditions of the dikaryons, monokaryons, and of the product of paired colonies (ie, di-mons) of the *Typhula* spp. resulting from the mating of monokaryon testers C-1-48 and 70-22-26 with the tested dikaryons were determined. Methods involved with the mating of monokaryons and dikaryons have been described previously (2).

RESULTS

Seventy-two monokaryons of *T. idahoensis* were paired with three isolates of *T. ishikariensis* (DE-7354, from Norway via Drew Smith; Id 73-4-2 from Valley County, ID; and 75-96 from Okanogan County, WA). Thirty of the 72 monokaryons produced no color reaction with any of the isolates, eight turned brown on contact only with Id 73-4-2, three turned brown on contact only with 75-96, three turned brown on contact with DE-7354 and Id 73-4-2, two with Id 73-4-2 and 75-96, and 26 with all three isolates. Only six of the 26 monokaryons of *T. idahoensis* that turned brown with all three isolates were considered worth testing further. The other 20 monokaryons either responded very weakly or became pigmented whether paired with *T. ishikariensis* or not. The six monokaryons selected for further study were vigorous and did not autogenously become dark with age.

Monokaryons 6299B-12, 70-22-26, C-1-48, 75-63-5-2, 75-90-5-4, and 75-90-9-1 of *T. idahoensis*, the six monokaryons judged most promising, were paired with the isolates listed in Table 1. Color reactions of a sample of the pairings after 43 days are given in Table 2. The accuracy of monokaryons C-1-48 and 70-22-26 in this sample was 100%. Monokaryon 75-63-5-2 reacted with all isolates except I-23.

Among all pairings, the color reaction was remarkably accurate in distinguishing *T. idahoensis* from *T. ishikariensis* (Table 3).

The color in the tester monokaryon occurred in patches, wedges, solid along the edge of contact, or throughout the "monokaryon." The tester monokaryons were no longer monokaryons. These monokaryons accepted nuclei from *T. ishikariensis*, migration occurred, and the "monokaryons" were damaged by the presence of foreign nuclei.

Mycelial pieces 4 mm^2 obtained 1 cm from the union of tester 6299B-12 and the isolates of *T. ishikariensis* were transferred to fresh dishes and growth was measured after 14 days. The mycelial transfers were examined for clamp connections. The diameter of colonies of *T. ishikariensis* averaged 37.7 mm, those of tester monokaryon 6299B-12 averaged 28.0 mm, and those of the offspring of the 6299B-12 × *T. ishikariensis* cross averaged 9.7 mm. Clamp connections were present on all of the offspring and all were alive. Transfers from even very heavily pigmented mycelia grew. Migration of nuclei from isolates of *T. idahoensis* into this tester monokaryon did not result in discoloration.

Even though cultures of *T. incarnata* are easy to distinguish from those of *T. idahoensis* and *T. ishikariensis*, the six tester monokaryons of the previous experiments were paired with 42 isolates of *T. incarnata*. After 30 days, no pronounced color reaction had occurred, either in the monokaryons or in the isolates of *T. incarnata*. Four of the *T. idahoensis* tester monokaryons were antagonistic to *T. incarnata*, one (75-90-5-4) was weakly antagonistic to most, and tester 75-90-9-1 overgrew most isolates of *T. incarnata*.

The ability of monokaryons of *T. idahoensis* to react macroscopically to isolates of *T. ishikariensis* led to exploration of the reciprocal possibility. Twenty-four monokaryons of *T. ishikariensis* obtained from 14 field dikaryons were paired with 10 isolates of *T. idahoensis* and with 10 of *T. ishikariensis*. After 34 days at 10 C, the cultures were examined. These pairings resulted in no useful macroscopic reactions.

Results of staining. The cells of all dikaryotic isolates (Tables 4 and 5) were typically binucleate with the nuclei generally occurring in conjugate pairs (Tables 4 and 5); however, cells occasionally contained three to five nuclei. The hyphae of all dikaryons formed clamp connections. All clamps did not form opposite hyphal branches. Generally, branches arose at an acute angle opposite clamp connections.

Each cell of the monokaryotic tester was typically uninucleate; however, a few cells, usually near the hyphal tip, contained two nuclei. The nuclei appeared to be larger in the monokaryons than in the dikaryons or in the products of di-mon matings. Furthermore, the cells of the di-mons and the dikaryons were shorter than those of the monokaryons. No clamp connections were formed on the hyphae of monokaryons.

There were no differences in nuclear condition, presence and location of clamp connections, or in the degree of hyphal branching when di-mons resulting from matings between dikaryons of *T. idahoensis* and monokaryons of *T. idahoensis* were compared with natural dikaryons of *T. idahoensis*. Except in four cases, there were no differences in microscopic characteristics of di-mons resulting from matings between dikaryons of *T. ishikariensis* and monokaryons of *T. idahoensis*, as compared to natural dikaryons (Tables 4 and 5). Di-mons resulting from the matings of 1-4 and 1-36 with C-1-48 exhibited both uninucleate and binucleate conditions, with the binucleate condition prevailing. Excessive

TABLE 2. The production of color in monokaryons of *Typhula idahoensis* C-1-48 (No. 1), 70-22-26 (No. 2), and 75-63-5-2 (No. 3) when paired with isolates of *T. idahoensis* and *T. ishikariensis* on Difco potato-dextrose agar for 43 days at 10 C

Dikaryons			Monokaryon		
Species	Isolate	Origin	1	2	3
T. idahoensis	I-1	Douglas Co., WA	0[a]	0	0
T. idahoensis	I-2	Douglas Co., WA	0	0	0
T. ishikariensis	I-4	Okanogan Co., WA	S	S	S
T. ishikariensis	I-6	Stevens, Co., WA	S	S	S
T. idahoensis	I-13	Douglas, Co., WA	0	0	0
T. idahoensis	I-15	Okanogan Co., WA	0	0	0
T. ishikariensis	I-18	Spokane Co., WA	S	S	S
T. ishikariensis	I-19	Spokane Co., WA	S	S	S
T. ishikariensis	I-23	Idaho Co., ID	L	M	0
T. ishikariensis	I-25	Lemhi Co., ID	S	S	M
T. idahoensis	I-26	Lemhi Co., ID	0	0	0
T. idahoensis	I-31	Bear Lake Co., ID	0	0	0
T. ishikariensis	I-36	Franklin Co., ID	S	S	S
T. idahoensis	I-41	Oneida Co., ID	0	0	0
T. idahoensis	I-46	Flathead Co., MO	0	0	0
T. ishikariensis	I-70	Kittitas Co., WA	S	S	S
T. idahoensis	I-73	Box Elder Co., UT	0	0	0
T. ishikariensis	I-77	Blaine Co., ID	S	S	M

[a] 0 = no color reaction in the tester monokaryon. S = strong, M = moderate, and L = light color reaction.

TABLE 3. The percent of positive color reactions observed in pairings of six monokaryons of *Typhula idahoensis* with 98 isolates of *T. idahoensis* and 60 isolates of *T. ishikariensis* after 43 days on Difco potato-dextrose agar at 10 C. Light to strong color reactions were read *T. ishikariensis*; no or questionable color reactions were read *T. idahoensis*

Species of the dikaryon	Accuracy of tester (%)					
	1[a]	2	3	4	5	6
T. idahoensis	93	82	100	100	100	99
T. ishikariensis	100	98	98	100	97	75
Total sample	96	89	99	100	99	90

[a] Tester 1 = monokaryon 6299B-12; 2 = 75-90-9-1; 3 = C-1-48; 4 = 70-22-26; 5 = 75-63-5-2; and 6 = 75-90-5-4.

hyphal branching occurred with branches arising either at an acute angle or at right angles from the parent hyphae. Clamp connections were more numerous than in the natural dikaryons. In contrast, hyphae resulting from the di-mon matings of 1-70 with 70-22-26 and of 1-23 with C-1-48 exhibited both uninucleate and binucleate conditions, with the monokaryotic condition prevailing. Furthermore, clamps were less abundant than in dikaryons. However, hyphal branching was similar to that described for the above di-mons, ie, excessive. Various degrees of anastomoses were observed within mycelia of the four di-mon matings noted above. No anastamoses were observed within dikaryons, monokaryons, or other synthesized dikaryons.

The diameter of colonies of *T. ishikariensis* (natural dikaryons) averaged 34.3 mm, whereas the diameter of interspecific mycelia resulting from di-mon matings involving *T. ishikariensis* as a parent was reduced (ie, 9.6 mm) (Tables 4 and 5). In addition, all matings involving a dikaryon of *T. ishikariensis* and a monokaryon of *T. idahoensis* resulted in the formation of a brown color reaction in the monokaryon. The diameter of natural colonies of *T. idahoensis* (dikaryons) averaged 23.7 mm. The diameter of synthesized di-mon colonies involving monokaryons of *T. idahoensis* × isolates of *T. idahoensis* averaged 24.2 mm (Tables 4 and 5). No brown color reactions occurred in monokaryons upon mating dikaryons of *T. idahoensis* with these monokaryons of *T. idahoensis*.

DISCUSSION

No significant color reactions occurred in *T. idahoensis* monokaryons 70-22-26, 75-63-5-2, and C-1-48 after contact with 98 different isolates of *T. idahoensis*. The inheritance of mycelial color in this species is not known, but no color developed following all these matings. In contrast, when these monokaryons contacted 60 *T. ishikariensis* isolates, color developed in 59, 60, and 58 of the matings with each of the tester monokaryons, respectively. These *T. idahoensis* monokaryons are essentially universal acceptors of nuclei of *T. ishikariensis*. This statement was made in an earlier paper (2): "Nuclear migration was more effectively prevented within incompatible matings of *T. idahoensis* and *T. ishikariensis* (intraspecific) than between (interspecific) the two species." This study reinforced that statement. The incompatibility factors in these testers (C-1-48 = A8B4, 70-22-26 = A2B6, 75-63-5-2 = A7B1) apparently played no role in the interspecific pairings.

The discoloration of the receptor hyphae (the *T. idahoensis* tester monokaryons) was visible below (matrical) and above (aerial) the surface of the medium. The aerial hyphae appeared to collapse, yet the reaction was not fatal in any combination, even though growth of the offspring was often greatly reduced. The color reaction and poor growth is interpreted as a result of general interspecies incompatibility. The vast majority of the offspring *T. idahoensis* × *T. ishikariensis* cross certainly do not exhibit hybrid vigor (3).

The failure of monokaryons of *T. ishikariensis* to react in matings with isolates of *T. idahoensis* is probably due to a lack of nuclear migration. Christen and Bruehl (3) reported that monokaryons of *T. idahoensis* commonly accepted nuclei of *T. ishikariensis*, but that monokaryons of *T. ishikariensis* seldom permitted much migration by nuclei of *T. idahoensis*.

Useful tester monokaryons are white or nearly so, remain colorless for long periods of time, accept nuclei of *T. ishikariensis*, and react to the presence of these nuclei macroscopically. The use of corn meal agar in this laboratory, upon which the color reaction is absent, delayed this discovery for years. The choice of medium is important.

It is now possible to identify cultures of *T. idahoensis* and *T. ishikariensis*, the two major species with black sclerotia on winter cereals, without resorting to the normal di-mon test and without the use of a microscope. The reactions are so pronounced in most instances that a quick glance is all that is required to classify the cultures. The method requires incubation for at least 30 days after inoculation, but it requires little labor. It facilitates identification of cultures obtained from sclerotia screened from soil, making meaningful population studies possible.

Testers C-1-48, 70-22-26, and 75-63-5-2 have been deposited in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 as accessions ATCC 44102, ATCC 44144, and ATCC 44103, respectively.

TABLE 4. Radial growth and nuclear condition of di-mons from matings between monokaryon C-1-48 of *Typhula idahoensis* and dikaryons of *T. idahoensis* and *T. ishikariensis*

Isolate (dikaryon)	Radial growth (mm) and nuclear condition (D,M)[a]		
	Dikaryon	Monokaryon	Di-mon
T. idahoensis			
1-1	21 D	21 M	27 D
1-2	26 D	22 M	31 D
1-13	27 D	18 M	15 D
1-15	30 D	20 M	25 D
1-26	31 D	21 M	30 D
1-31	29 D	19 M	26 D
1-41	14 D	18 M	20 D
1-46	10 D	19 M	12 D
1-73	25 D	20 M	25 D
Average	23.7	19.8	23.4
T. ishikariensis			
1-4	37 D	19 M	6 D+M[b]
1-6	38 D	20 M	5 D
1-18	36 D	20 M	7 D
1-19	33 D	19 M	6 D
1-23	36 D	17 M	12 M+D[c]
1-25	42 D	23 M	5 D
1-36	33 D	22 M	6 D+M[b]
1-70	31 D	19 M	5 D
1-77	24 D	20 M	15 D
Average	34.4	19.9	7.4

[a] D = binucleate cells and M = uninucleate cells.
[b] D+M = both binucleate and uninucleate cells with those in the binucleate condition prevailing.
[c] M+D = both binucleate and uninucleate cells with uninucleate condition prevailing.

TABLE 5. Radial growth and nuclear condition of di-mons from matings between monokaryon 70-22-6 of *Typhula idahoensis* and dikaryons of *T. idahoensis* and *T. ishikariensis*

Isolate (dikaryon)	Radial growth (mm) and nuclear condition (D,M)[a]		
	Dikaryon	Monokaryon	Di-mon
T. idahoensis			
1-1	20 D	23 M	31 D
1-2	28 D	23 M	32 D
1-13	25 D	23 M	15 D
1-15	28 D	20 M	27 D
1-26	33 D	21 M	31 D
1-31	28 D	22 M	25 D
1-41	15 D	23 M	16 D
1-46	15 D	25 M	26 D
1-73	22 D	23 M	21 M[b]
Average	23.8	22.6	24.9
T. ishikariensis			
1-4	37 D	24 M	22 D
1-6	32 D	21 M	6 D
1-18	32 D	25 M	23 M[b]
1-19	37 D	22 M	14 D
1-23	35 D	25 M	7 D
1-25	40 D	24 M	6 D
1-36	34 D	23 M	13 D
1-70	35 D	25 M	7 M+D[c]
1-77	26 D	22 M	7 D
Average	34.2	23.5	11.7

[a] D = binucleate cells and M = uninucleate cells.
[b] Nuclear migration from the dikaryon had not advanced into the monokaryon to the site of sampling (1 cm from the union) at the time of sampling for staining.
[c] M+D = both binucleate and uninucleate cells with uninucleate condition prevailing.

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