Tilletia indica: A Heterothallic Wheat Bunt Fungus with Multiple Alleles Controlling Incompatibility

Rubén Durán and Robert Cromarty

Professor and former Research Technologist, respectively, Department of Plant Pathology, Washington State University, Pullman, WA 99163. Present address of junior author: 4225 Sturdivant Drive, Klamath Falls, OR 97601.

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ABSTRACT

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To elucidate the relation between incompatibility alleles which govern heterothallism and pathogenicity, paired and nonpaired monosporidial lines of the Karnal bunt fungus *(Tilletia indica)* were used to inoculate wheat heads in the boot stage or during anthesis. Paired lines which proved pathogenic were postulated to be of different mating types, whereas nonpathogenic pairs were postulated to consist of the same mating type. In all cases, nonpaired lines were nonpathogenic. From results obtained in testing 93 monosporidial lines, it is suggested that heterothallism and pathogenicity are controlled by multiple alleles at one locus; four such alleles were demonstrated in the material studied. Consistent with the bipolar incompatibility system posulated for the fungus, basidia yielded only monosporidial lines of two mating types.

Partial or Karnal bunt of wheat, caused by *Tilletia indica* (Mitra) Mund. was reported by Mitra in 1931 when he discovered the disease at Karnal, Punjab, in northwestern India (9). The disease since has spread to Uttar Pradesh and West Pakistan, but apparently has not become widespread in India or other parts of the world.

In 1943, Mundkur demonstrated that the disease could be reproduced by spraying heads of susceptible wheat cultivars during anthesis with sporidial suspensions (10). His work clearly showed that individual kernels are infected by airborne sporidia. Since Mundkur's study, little has been added to our knowledge of the disease or pathogen. In fact, until 1972, the disease was known only from India. That year, Durán (5) reproduced the disease on Siete Cerros wheat using sporidia of the fungus isolated from smutted wheat kernels from Sonora, México where the disease is now endemic. Although its presence in México cannot be readily explained, apparently the fungus either was recently introduced there (possibly on infected seed), or the disease simply escaped notice and was not detected until 1972. In any event, its presence in México poses a potentially serious threat to wheat-producing areas of North and South America where environmental conditions may be favorable for disease development and spread of the fungus.

The disease can easily be produced experimentally; yet, insofar as we know, incompatibility between monosporidial lines as it relates to heterothallism and pathogenicity has not been previously studied.

It is important to note that although incompatibility in some smut fungi can be determined in vitro; e. g., by the Bauch test (1, 2, 3, 4, 6), it cannot in *T. indica*, since apparently neither dikaryons nor infection hyphae develop on artificial media, even when pairs of monosporidial lines known to be pathogenic are paired on a wide variety of media. Consequently, incompatibility between monosporidial lines was determined by pathogenicity tests using paired and unpaired lines to inoculate wheat.

MATERIALS AND METHODS

Following germination of teliospores on 2% soil-extract agar at 23 C, individual primary sporidia were transferred to 1 cm^2 blocks of potato-dextrose agar (PDA) by micromanipulation. Sporidia that germinated were transferred to PDA slants after 3 days to promote growth and for subsequent use as sources of inoculum. Since primary sporidia are produced in large numbers upon individual promycelia (Fig. 1), and because they frequently become desiccated during micromanipulation, attempts to culture complete pedigreed sets of sporidia from single teliospores proved impractical. Instead, individual primary sporidia were randomly isolated from different teliospores, or sporidia were isolated in series from specific teliospores and their identity was maintained. All isolates were obtained from two collections of naturally infected kernels of Siete Cerros wheat received from Sonora, México in 1969-1970.

Inoculum Production.—Monosporidial lines of the fungus were grown in shake culture in 250-ml flasks containing 50 ml of Difco potato extract solution to

which mycelia and secondary sporidia were added from the PDA slants. Secondary sporidia developed in profuse numbers following 7 days of continuous shaking at 24 C. These were pelleted by centrifugation and the pellets were resuspended in 100 ml of dilute potato extract. Immediately thereafter, portions of the various sporidial suspensions were paired and used as inocula. In all tests suspensions of unpaired monosporidial lines served as controls.

Inoculation.—Initially, atomizers were used to spray heads in anthesis with the sporidial suspensions. In later experiments, wheat heads were inoculated in the boot stage using sterile hypodermic syringes with 0.124 cm diameter (No. 18) needles because this technique increased percent infection. Furthermore, heads in the boot stage were less subject to aerial contamination following inoculation and humidity around the developing heads requisite for infection was maintained. Following inoculation, plants were shaded for 2 wk and thereafter were grown to maturity in a greenhouse maintained at 18-20 C during the day and 5-7 C at night.

Ninety-three isolates were used as inocula. Each was used singly and/or in pairs to inoculate wheat plants grown in groups of five or six in pots 20.3 cm (8 inches) in diameter. In the initial experiments, inocula consisted of isolates paired in all possible combinations. In later experiments, isolates in which incompatibility alleles had previously been determined were used as "maters" to determine incompatibility of additional untested lines. Wheat cultivar Siete Cerros, a Mexican cultivar of spring wheat, was used in all inoculations.

Interpretation of the data from the inoculations presented here is based on the assumptions that (i) only paired lines are pathogenic and (ii) the incompatibility system in *T. indica* is homogenic as in other smut fungi (7).

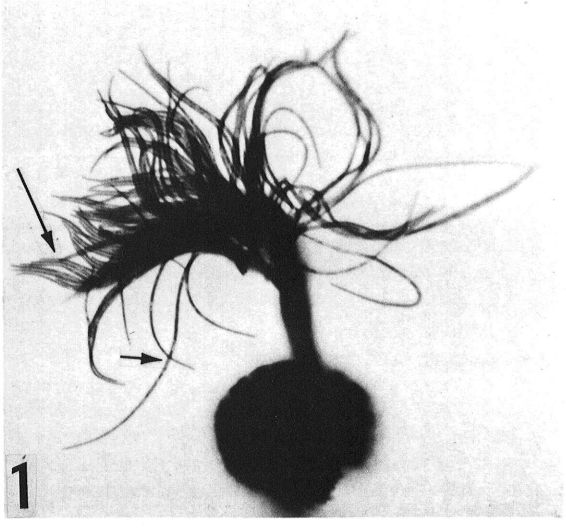


Fig. 1. Germinated teliospore of *Tilletia indica*, on soil extract agar showing the characteristically large numbers of primary sporidia attached to the promycelium. Note the typically filiform sporidia (arrows) which intertwine and superimpose, but do not fuse. Stained with trypan-blue and lactophenol to increase contrast. (× 1000).

RESULTS AND DISCUSSION

The data summarized in Table 1 consistently indicate the heterothallic nature of the fungus, since only certain paired lines were pathogenic. Additional evidence of heterothallism was obtained when isolates 1, 2, and 21 were paired with 84 other lines (Table 2). In some preliminary pathogenicity tests, only two mating types were detected, since only lines isolated from single teliospores were paired and used as inocula. However, as lines from single teliospores were paired with lines isolated from others, additional mating types were detected. Thus, in pairing nine lines in all combinations isolated from different teliospores, three mating types were hypothesized (a_1, a_2, and a_3) following interpretation of results of the pathogenicity tests (Table 1). But when lines 1, 2, and 21 (of a_1, a_2, and a_3 incompatibility, respectively) were paired with 84 other lines, additional a_1, a_2, and a_3 lines were identified and also a_4 lines not encountered in earlier experiments (Table 2). Of the 93

TABLE 1. *Tilletia indica*: numbers of bunted heads following inoculation of wheat with nine monosporidial lines paired in all possible combinations and postulated incompatibility alleles

Monosporidial lines	Postulated alleles	Monosporidial lines								
		1	2	3	21	31	32	33	34	35
		Postulated alleles								
		a_1	a_2	a_1	a_3	a_2	a_1	a_2	a_2	a_2
1	a_1	0/38[z]	25/38	0/35	28/36	23/38	0/40	26/44	22/35	19/33
2	a_2		0/45	15/42	25/44	0/40	21/44	0/40	0/42	0/38
3	a_1			0/46	27/43	26/34	0/41	26/35	28/41	21/28
21	a_3				0/43	30/40	23/41	26/42	24/44	14/30
31	a_2					0/45	21/38	0/38	0/40	0/42
32	a_1						0/42	22/40	25/35	21/32
33	a_2							0/45	0/40	0/39
34	a_2								0/45	0/38
35	a_2									0/34

[z]Numerators and denominators indicate bunted heads and the total number of heads inoculated, respectively, in separate tests. (In the first test 36% smut resulted when plants were inoculated with lines of different incompatibility; in the second 62%; and in the third 82%).

lines tested, 51 proved to be a_1; 25 a_2; 14 a_3; and three a_4.

Although we were unable to determine incompatibility by inoculating with complete sets of monosporidial lines, the large numbers of heads inoculated with stock cultures established by isolating sporidia at random from different teliospores yielded results over a 4-year period which suggest that *T. indica* is heterothallic and bipolar, and that incompatibility is controlled by multiple alleles at one locus.

Bipolar heterothallism in *T. indica* is further indicated inasmuch as isolates from each basidium represented only two mating types. This was demonstrated in a single

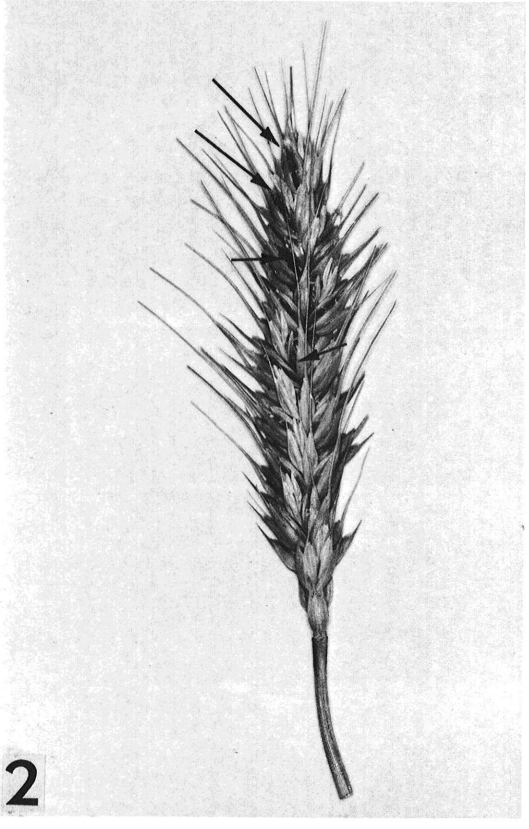
TABLE 2. *Tilletia indica*: numbers of bunted heads and incompatibility alleles of randomly isolated monosporidial lines postulated by pairing them with lines 1, 2, and 21, and subsequently using them to inoculate wheat[x]

Monosporidial lines tested	Mater lines[y]			Postulated alleles following results of pathogenicity tests
	$1a_1$	$2a_2$	$21a_3$	
116	0/9[z]	7/8	7/8	a_1
128	0/10	6/10	9/9	a_1
67	10/14	0/13	11/14	a_2
73	9/12	0/14	8/15	a_2
40	10/14	3/11	0/15	a_3
44	12/15	5/12	0/14	a_3
176	5/12	6/9	4/12	a_4
178	2/9	4/13	3/14	a_4

[x]Data shown for eight lines are representative of results obtained in testing 84 lines. Of these, 48 proved to be a_1; 20 a_2; 13 a_3; and 3 a_4.

[y]Alleles indicated are for lines 1, 2, and 21 postulated previously (Table 1). In this test, these lines were again paired in all possible combinations and used as inocula with the following results: $1 \times 1 = 0/14$; $2 \times 2 = 0/10$; $21 \times 21 = 0/15$; $1 \times 2 = 7/11$; $1 \times 21 = 10/12$; and $2 \times 21 = 3/11$; however, none of the 84 lines was used singly as inocula.

[z]Numerators and denominators = bunted heads and the total number of heads inoculated, respectively.



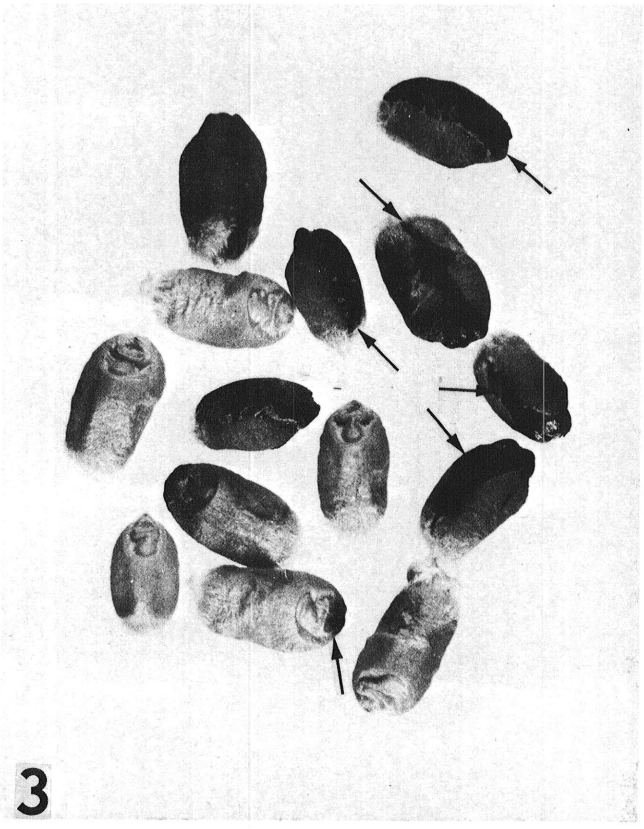


Fig. 2-3. Karnal bunt on Siete Cerros wheat following boot inoculation with paired lines of *Tilletia indica*. **2)** An infected head, with the sori partially concealed by the glumes (arrows) (× 1), and **3)** kernels from a bunted head showing the localized nature of the sori (arrows) (× 5).

experiment by the isolation of 13 a_1 and nine a_2 isolates from one basidium and six a_1 and one a_3 isolates from another.

Demonstration of four alleles governing heterothallism suggests that sporidia from six teliospore classes may have been sampled: (i) a_1a_2, (ii) a_1a_3, (iii) a_1a_4, (iv) a_2a_3, (v) a_2a_4, and (vi) a_3a_4. Although only the first two classes were demonstrated by isolations from single basidia, the remaining classes can be inferred since isolates of a_4 incompatibility also were obtained at random from different basidia. However, the high frequency of isolates of a_1 incompatibility also suggest that sporidia may have been sampled only from the first three classes indicated. It is reasonable to assume, moreover, that additional alleles will be demonstrated when additional collections are studied.

Evidence that results of incompatibility studies in vitro can be used to accurately predict pathogenicity of paired monosporidial lines has been categorically demonstrated in *Sorosporium consanguineum* (6). Although this was not possible with *T. indica*, extensive studies of *S. consanguineum* serve to indicate that, at least in this species, there is a direct correlation between compatible lines (i.e., those which form dikaryons when paired on artificial media) and those which are pathogenic.

In interpreting our results we concluded that heads which became smutted were inoculated in all cases with compatible lines; i.e., those which form a parasitic dikaryophase somewhere in the life cycle. That incompatibility in *T. indica* is controlled by multiple alleles is supported by the fact that heads were consistently smutted so long as they were inoculated with any combination of the four mating types postulated (Fig. 2-3).

Although the available data suggest otherwise, we do not deny the possibility that *T. indica* may be tetrapolar since relatively few lines were tested in proportion to the large number of sporidia characteristically produced by individual basidia.

This and other accounts of complex heterothallism in cereal and grass smuts (4, 6, 8) make untenable previous claims that heterothallism in the smuts always is controlled by one pair of alleles (11, 12). However, to determine whether incompatibility and pathogenicity are controlled by similar or different alleles is a question which will require study of many species before it can be answered for the smuts as a whole.

Interestingly, in one experiment when primary sporidia were isolated from different teliospores from the same sorus, sporidia of a_1, a_2, and a_3 incompatibility were obtained. This suggests that sori of the fungus form following multiple sporidial infections and that fusions between monokaryons of different incompatibility type may occur within the host. Whether in nature dikaryons form before or after host penetration, however, is unknown.

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