Morphological, Physiological, and Genetic Evidence in Support of a Conspecific Status for *Tilletia caries*, *T. controversa*, and *T. foetida*

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ABSTRACT

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The germination properties and autofluorescence characteristics of teliospores of *Tilletia* spp. isolated from wheat grown in Oregon, Pakistan, and Turkey and electrophoretic karyotypes of monosporidial strains obtained from these teliospores were used as taxonomic characters in attempts to differentiate the teliospores that caused the bunt disease. Among the seven collections of teliospores were two that germinated at 16 C, a property of *T. caries*, but that had reticulations that autofluoresced under 485 nm UV irradiation, a characteristic of *T. controversa*. Other collections exhibited properties that suggested they could be one or the other species or *T. foetida*. The assignment of DNA fragments by hybridization to chromosomes resolved by pulsed-field gel electrophoresis revealed length polymorphisms that ranged from 8 to 11% for three chromosomes and 38% for the chromosome to which the rDNA genes map. The variability observed for the chromosomes of these diverse pathogen populations is not greater than that observed for homologous chromosomes of populations isolated from wheat grown in the western United States. Neither the autofluorescence nor the germination properties of teliospores could be used as definitive characters to distinguish these pathogens, and the karyotypes of strains from diverse populations are essentially identical for strains that cause both common and dwarf bunt of wheat.

Additional keywords: smut fungi, wheat bunt pathogens.

Tilletia caries (DC.) Tul. & C. Tul. (syn. *T. tritici* (Bjerk.) G. Wint. in Rabenh.) and *T. foetida* (Wallr.) Liro (syn. *T. laevis* Kühnin Rabenh.), which incite common bunt disease of wheat, and *T. controversa* Kühn in Rabenh., which incites dwarf bunt disease of wheat, are closely related filamentous basidiomycetes. Although both pathogens are indigenous to the United States, it is the position of the People's Republic of China that *T. controversa* is not endemic to their wheat-producing areas. In 1973, the People's Republic of China imposed a zero tolerance for the presence of *T. controversa* teliospores in imported wheat, and within a year of this quarantine, wheat shipments originating from the Pacific Northwest of the United States were banned because of their possible contamination (33). In response to that quarantine, numerous approaches have been explored to devise an irrefutable method of distinguishing the teliospores of *T. controversa* from those of *T. caries*. These approaches have included microscopic examination of the teliospores (8,34), immunological methods of detection (2), analyses of polypeptide profiles (15) and triacylglycerol profiles (3), demonstration of the presence or absence of plasmids (16,17), demonstration of differential autofluorescence properties of teliospores (27), and genetic and cytogenetic studies (23,30). However, none has been shown to be a definitive method for the identification of these pathogens.

The methods of identification presently in use rely upon discerning subtle morphological features of the teliospores as described by Duran and Fischer (4) and ascertaining the optimal conditions for their germination (9). The teliospores of *T. controversa* have been described as having deep reticulations and encompassing sheaths, whereas those of *T. caries* have less pronounced reticulations and no sheaths. *T. caries* teliospores typically germinate within 7–10 days at temperatures ranging from near 0 to 25 C (22), whereas the teliospores of *T. controversa* exhibit a low temperature-dependent phenotype and typically germinate only below approximately 10 C over a period of 3 wk or more (18,32).

The literature is replete with examples of extensive biological variation within the bunt fungi, and teliospores obtained from wheat that has either common or dwarf bunt symptoms frequently have intermediate phenotypes or characteristics of either pathogen with respect to these traits (1,10,11). Consequently, all criteria that have been established to identify the teliospores of these two pathogens have been jeopardized by the genetic variation that exists in natural populations (9,12).

The different autofluorescence properties of the teliospores of these pathogens (27) suggested a rapid and efficient method of identification because the spores can be analyzed within minutes of being detected in a wheat shipment. The *T. controversa* teliospore has been described as possessing a fluorescent, reticulated wall layer that appears spikelike in the median plane and an upper surface that appears netlike, whereas the *T. caries* teliospore lacks these features but contains fluorescing bodies in the cytoplasm.

The present study was undertaken to determine whether traits attributed to the *T. controversa* teliospore (i.e., a prominent sheath and characteristic autofluorescence and low temperature-dependent germination phenotypes) would invariably associate in teliospore samples of diverse origin. Additionally, the karyotypes and assignment of markers to chromosomes of progeny were obtained from these teliospores to measure and compare genome plasticity with that of other *Tilletia* genomes.

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MATERIALS AND METHODS

Strains and culture conditions. The monokaryotic strains used in this study were isolated from teliospore collections kindly provided by R. J. Metzger (U.S. Department of Agriculture, Oregon State University, Corvallis; retired). The preliminary relevant characteristics of each spore collection are listed in Table 1. Teliospores were germinated at either 5 or 16 C on 3% water agar, and monokaryotic strains of each collection were obtained as single, primary basidiospores isolated from germinated teliospores. Basidiospores were germinated on agar-solidified T-19 medium (31), and the ensuing cultures were transferred after approximately 3-4 wk to potato-dextrose agar (Difco Laboratories, Detroit, MI) for maintenance. All cultures were grown at 16 C and stored on potato-dextrose agar slants at either 5 or −80 C.

Germination tests. Teliospores from each collection were surface sterilized with a solution of 0.16% sodium hypochlorite for 3 min at room temperature. The spores were rinsed three times with sterile water and suspended in 1 ml of sterile water. A 250-μl aliquot of the spore suspension was spread onto each of two plates containing 3% water agar, and an area containing approximately 100 spores was identified for further observation. One of the plates was incubated at 5 C and the other at 16 C, and the spores were monitored daily for evidence of germination. A teliospore was considered to have germinated when the promycelium was equal to the diameter of the teliospore. Data were collected until the ungerminated teliospores were either overgrown with mycelia or the remaining spores were deemed unable to germinate.

Autofluorescence test. Teliospores suspended in water were placed on a microscope slide and allowed to air dry. The spores were then covered with a small amount of nonfluorescing immersion oil and a cover slip. Autofluorescence was observed with an epifluorescence microscope (Carl Zeiss, Inc., New York, NY) with a 50 W mercury lamp and equipped with the Zeiss filter set 487709 (485-nm excitation, 520-nm barrier filter) as described by Stockwell and Trione (27). For visualizing the hyaline sheaths, teliospores were mounted in water and photographed with background lighting.

TABLE 1. List of *Tilletia* teliospore collections and strains used in this study

Collection or strain	Relevant characteristics[a]	Origin
Collection		
M1	Germination typical of *T. controversa*, required 6 wk at 5 C	Flora, Oregon, 1987
M2	Ridges autofluoresce	Karimabad, Pakistan, 1986
M3	Ridges autofluoresce	Danyur, Pakistan, 1986
M4	Ridges autofluoresce, rapid germination, pathogenic to Heines VII[b] and wheat expressing *Bt7*[c]	Balistain, Pakistan, 1986
M5	Pathogenic to Heines VII	Turkey, 1983
M6	Pathogenic to Heines VII and wheat expressing *Bt4*, *Bt6*, *Bt7*	Eshirsehir, Turkey
M7	Pathogenic to Heines VII	Eshirsehir, Turkey
Strain		
M1.1	Monokaryotic strain of M1	This study
M2.5	Monokaryotic strain of M2	This study
M3.10	Monokaryotic strain of M3	This study
M4.1	Monokaryotic strain of M4	This study
M5.4	Monokaryotic strain of M5	This study
M6.3	Monokaryotic strain of M6	This study
M7.7	Monokaryotic strain of M7	This study

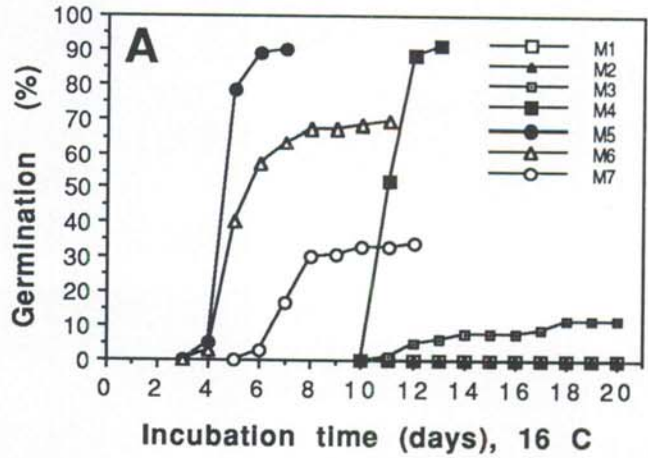
[a] Preliminary characteristics of the teliospore collections were determined by R. Metzger (Department of Crop Science, Oregon State University; retired).

[b] The universally susceptible wheat cultivar.

[c] Spore collection determined to be virulent on hosts that carry these bunt resistant (*Bt*) genes (20).

Pulsed-field gel electrophoresis and assignment of DNA fragments and genes to chromosomes. The culturing and preparation of mycelial samples from liquid shake cultures for contour-clamped homogeneous electric field (CHEF) pulsed-field gel electrophoresis (PFGE) have been previously described (23). The CHEF DR-II electrophoresis system (Bio-Rad Laboratories, Richmond, CA) was used for separation of chromosome-sized DNA molecules in ultrapure agarose (IBI, New Haven, CT). Chromosomes smaller than 2,200 kilobase pairs (kb) were resolved in 1% agarose in 0.5× Tris-borate-EDTA buffer (19), which was maintained at 12 C. The electrophoresis conditions consisted of an initial 20-h period of 480-s pulses at 100 V (3 V/cm) followed by 26 h of ramped pulses from 480 to 240 s at 100 V and ending with 26 h of ramped pulses from 240 to 120 s at 150 V (4.5 V/cm). Chromosomes larger than 2,000 kb were resolved in 0.87% agarose in 1× Tris-acetate-EDTA buffer (19), which was maintained at 15 C. Electrophoresis conditions consisted of an initial 48-h period of 480-s pulses at 100 V (3 V/cm) followed by 24-h period of 1,800-s pulses at 50 V (1.5 V/cm). The bands were stained with ethidium bromide (3 μg/ml) in electrophoresis buffer for 30 min and photographed after destaining overnight in electrophoresis buffer. The lengths of the chromosome-sized DNAs were estimated with Cricket Graph (Cricket Graphics, Inc., Philadelphia, PA) to configure a standard curve derived from migration distances of molecular size markers that included chromosomes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* purchased from Bio-Rad Laboratories.

Inserts from the plasmids pOSU1003, pOSU1006, and pOSU1101, which contain anonymous fragments from *T. caries* and *T. controversa* (23), and pSF8, which contains the conserved



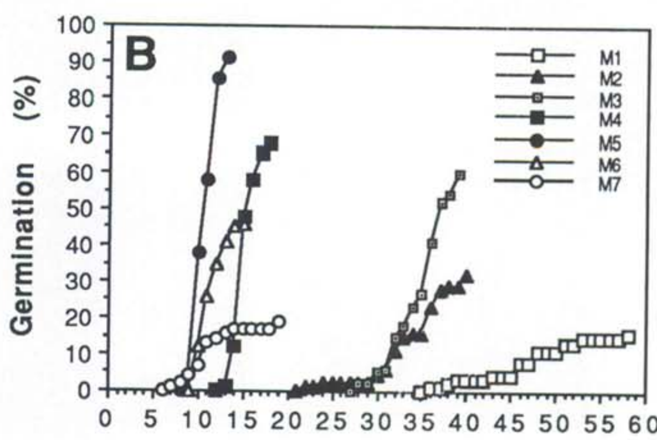


Fig. 1. The germination responses of teliospores from collections M1-7 isolated from Oregon, Pakistan, and Turkey when incubated at **A,** 16 C or **B,** 5 C. The teliospores were surface sterilized with sodium hypochlorite and incubated on 3% water agar.

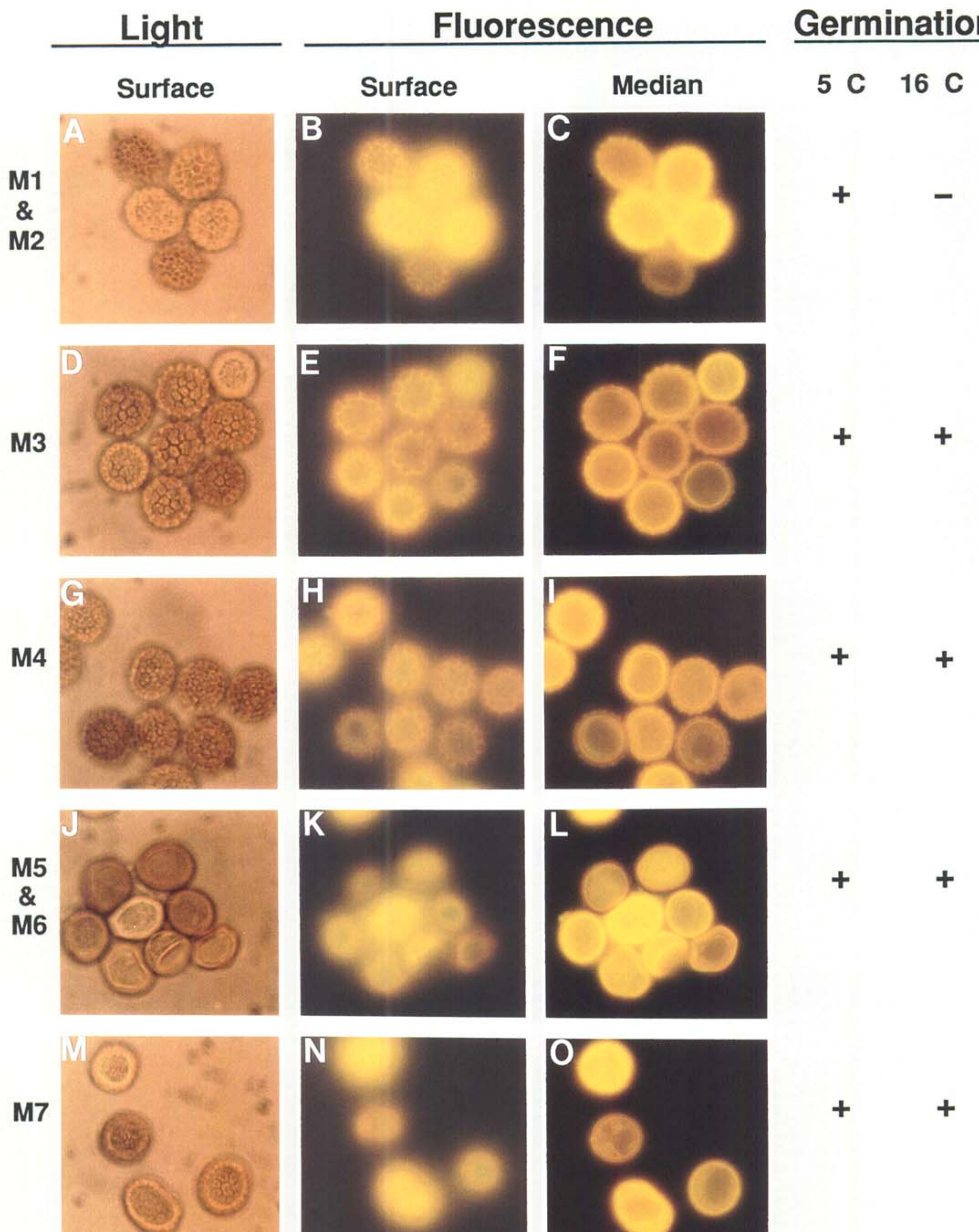


Fig. 2. A, D, G, J, and M, Upper teliospore surfaces when viewed with transmitted light; **B, E, H, K, and N,** the upper teliospore surfaces when viewed with UV irradiation; **C, F, I, L, and O,** the median planes of teliospores when viewed with UV irradiation. Representative samples of teliospores from collections M1–7 are presented. The teliospores were mounted in immersion oil and viewed at 600× and 485-nm excitation; a 520-nm barrier filter was used for UV irradiation. The germination responses of the teliospore collections at 5 and 16 C (+ = germination and – = no germination) are shown in the far right column.

actin gene from *Aspergillus nidulans* (5), were used as hybridization probes. Southern hybridization to assign probes to specific chromosome bands has been previously described (23).

RESULTS

Analysis of teliospore germination. At 16 C, the onset of teliospore germination occurred between 3 and 11 days for collections M3–7 (Table 1 and Fig. 1A), a phenotype attributed to spores of *T. caries*. The teliospores from collections M4–7 reached maximum germination percentages (30–90%) within 3–4 days after the onset of germination. In contrast, teliospores from collection M3 initiated germination on day 11 and attained their maximum percent germination (15%) 8 days thereafter. None of the teliospores from collections M1 and M2 (Table 1) germinated at 16 C over a period of 6 wk (Fig. 1A and data not shown), which is characteristic of *T. controversa*.

The teliospores from all the collections were, however, competent to germinate at 5 C, although the onset of germination varied greatly (Fig. 1B). Teliospores from collections M4–7 germinated within 6–13 days and attained maximum percent germination (15–90%) within 3–7 days thereafter. In contrast, the germination of teliospores from collections M1–3 began at 36, 28, and 22 days, respectively. The kinetics of germination was prolonged for collections M1 and M2, and the maximum percentages of germination observed after approximately 3 wk were only 15 and 30%, respectively. However, the kinetics of germination of teliospores from the M3 collection were more synchronous, and within 1 wk of the onset, approximately 60% had germinated. Of interest was the observation that a higher percentage of these spores (M3 collection) were competent to germinate at 5 C (65%) than at 16 C (15%). In contrast, the maximum percentage of germinated spores was similar at either temperature for the M5 collection, but it was reduced for collections M4, M6, and M7 at 5 C. Had a taxonomic classification scheme (which focuses solely on the germination properties of these teliospores) been used, collections M1 and M2 would have been assigned to *T. controversa* because they failed to germinate at 16 C, and collections M3–7 would have been designated *T. caries*.

Teliospore morphology and autofluorescence. The reticulations (Fig. 2A) and hyaline sheaths (Fig. 3) that encompass some teliospores can be readily visualized on spores viewed with transmitted light. Teliospores from collections M1–4 had conspicuous reticulations (Fig. 2A, D, and G), spikes in the median planes (Fig. 2C, F, and I), and hyaline sheaths (Fig. 3). Teliospores from

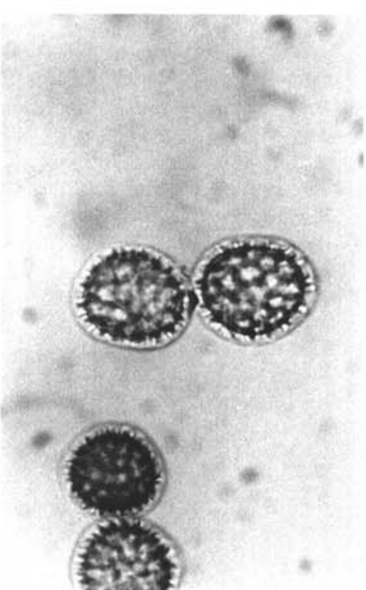


Fig. 3. Teliospores from the M4 collection that have conspicuous hyaline sheaths. The hyaline sheath is visualized as the opaque outer ring encompassing each spore (600×).

collections M5 and M6 appeared devoid of reticulations (Fig. 2J), spikes in the median planes (Fig. 2L), or hyaline sheaths (data not shown). The teliospores from the M7 collection appeared devoid of sheaths (data not shown) but had small reticulations (Fig. 2M). Teliospores from collections M1–4 appeared mostly spherical and reticulated, whereas teliospores from M5 and M6 were primarily aspherical and smooth, a characteristic of another wheat bunt pathogen, *T. foetida* (4). The teliospores from M7 were frequently aspherical and slightly reticulated (Fig. 2). If the presence of conspicuous reticulations and hyaline sheaths is used for taxonomic consideration, the teliospores from collections M1–4 are from *T. controversa* and those from M5–7 are from either *T. caries* or *T. foetida*.

Teliospores mounted in immersion oil and viewed by epifluorescence microscopy can be grouped on the basis of autofluorescence as described by Stockwell and Trione (27). Teliospores from collections M1–4 had reticulated wall layers, spikelike protrusions that fluoresced orange yellow when viewed in the median planes (Fig. 2C, F, and I), and a netlike appearance on the surfaces (Fig. 2B, E, and H). These are characteristics that were described for teliospores of *T. controversa* (27). However, some of the teliospores also contained autofluorescing cytoplasmic bodies (Fig. 2I), which were previously described for *T. caries* (27). The number and intensity of these cytoplasmic bodies varied considerably and appeared to be correlated with the amount of pigmentation in the teliospore wall. Teliospores that appeared darker when viewed with transmitted light had autofluorescence of lower intensity. Many of the teliospores from the M1 and M4 collections were darkly pigmented, and these fluoresced less intensely than did spores from other collections. Teliospores from the M5–7 collections were typically devoid of autofluorescing reticulations, but occasionally nonfluorescent reticulations were observed (Fig. 2M). Smooth, yellowish fluorescent bands encircled these teliospores when viewed in the median planes (Fig. 2L and O), and yellowish cytoplasmic fluorescing bodies were also observed in some spores of these collections (Fig. 2O). If fluorescing ornamentations and cytoplasmic bodies are used as taxonomic criteria, collections M1–4 are *T. controversa* and collections M5–7 are either *T. caries* or *T. foetida*.

Electrophoretic karyotypes and genome sizes. Electrophoretic karyotypes were obtained with CHEF PFGE for at least two randomly selected monokaryotic strains from each collection, and there appeared to be only infrequent chromosome length polymorphisms for strains from the same collection. Therefore, the karyotype of only one strain from each of the seven spore collections is presented in Figure 4. The number of chromosome bands varied from 14 to 17 for the seven strains, and the chromosomes ranged in size from approximately 850 to 4,300 kb (Fig. 4 and Table 2). Bands of similar size that fluoresced relatively more intensely with ethidium bromide were assumed to contain at least two chromosomes and are listed as doublets in Table 2. The minimum estimated number of chromosomes in the karyotypes of the seven strains ranged from 16 to 20, and the minimum estimated genome size varied from approximately 34 to 42 megabase pairs (Table 2).

Assignment of DNA fragments and genes to chromosomes. To obtain a measure of the amount of chromosome variability in these strains of diverse origins, four hybridization probes, previously shown to be unlinked in strains isolated from wheat grown in the western United States (23), were used to probe Southern blots (19) of CHEF gels containing chromosomes of strains M1–7. The probes were made of two cloned, single copy, anonymous restriction fragments from *T. caries* (pOSU1003 and pOSU1006), the actin gene from *A. nidulans* (pSF8), and a restriction fragment from *T. controversa* that has homology with an internal 1-kb region of the 17S rDNA gene from *Neurospora crassa* (pOSU1101). The anonymous fragments and the actin gene each hybridized to a single chromosome band in each of the seven strains, and these nonhomologous chromosomes had maximum length polymorphisms of 4–11% (Table 3). The maximum variability observed for these three chromosomes among strains isolated from the western United States was 2–10% (Table 3, 23). Conversely, the

rDNA probe hybridized with one to three chromosomes in the seven strains, which exhibited 38% maximum length variability. This also compares favorably with the strains from the United States (Table 3, 23).

DISCUSSION

Seven teliospore collections obtained from bunted wheat grown in Oregon, Pakistan, and Turkey were examined for characteristics currently used in differentiating *T. caries* from *T. controversa,* and a summary of their characteristics is presented in Table 4. These include the presence or absence of a prominent hyaline sheath (4), the temperature at which the teliospores germinated (9), and the autofluorescence properties of the teliospores (27).

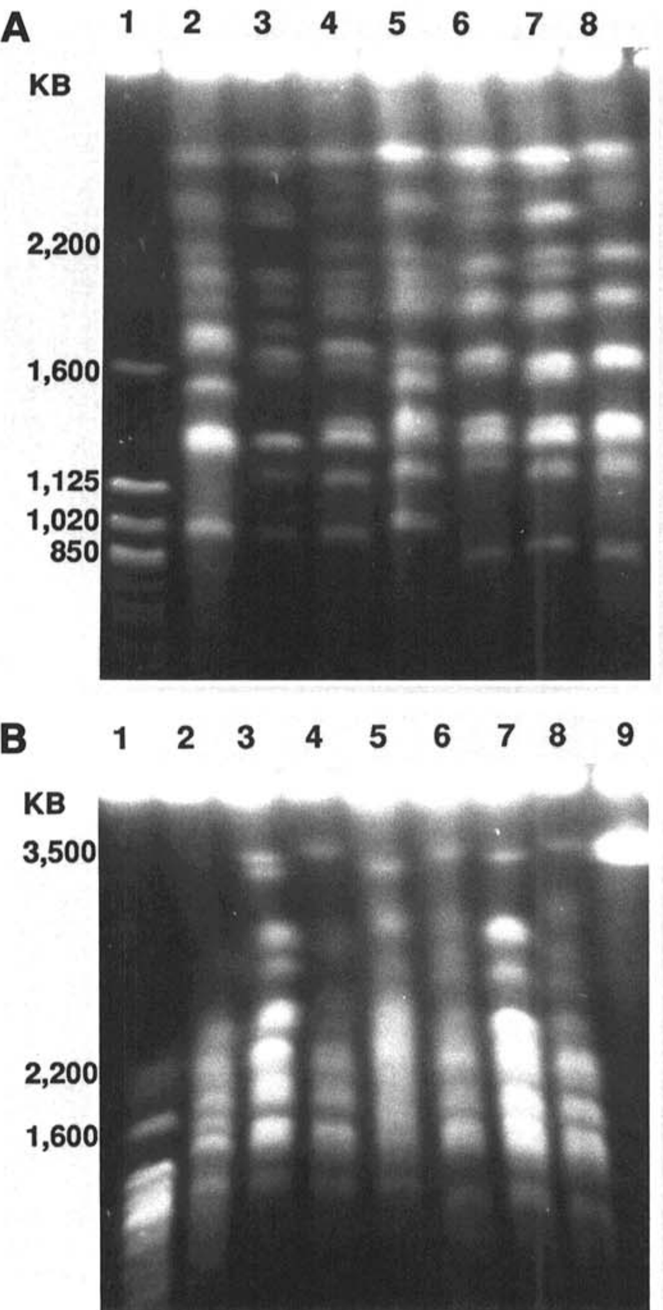


Fig. 4. Electrophoretic karyotypes of seven monokaryotic strains of *Tilletia* spp. representing the seven teliospore samples M1–7. **A,** Resolution of chromosomes between 850 and 2,200 kb. **B,** Resolution of chromosomes between 2,200 and 5,000 kb. Lanes 2–8 represent strains M1–7, respectively, in both **A** and **B.** Lanes 1 and 9 are molecular size markers of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* chromosomes, respectively.

Additionally, electrophoretic karyotyping and the assignment of genes and cloned anonymous fragments to Southern blotted chromosomes were employed both in this study and in a recent comparison of the genomes of *T. caries* and *T. controversa* (23). This kind of genome analysis has also provided an addendum for species identification of *Kluyveromyces marxianus* var. *marxianus* and var. *lactis* (26). Our results clearly demonstrate that traits that have been traditionally ascribed to the teliospores of *T. controversa*, and used in taxonomic schemes for the identification of this pathogen, may also be features of the teliospores of *T. caries.* Exceptions are frequently encountered when taxonomic schemes are based on teliospore morphology (13) and germination temperature (Fig. 4). The genes that confer different teliospore morphologies and germination phenotypes are likely to be alternate alleles that can arise through mutation and recombination in natural populations.

Holton and Kendrick (13) noted that morphologic characteristics of teliospores are highly variable and constitute inadequate criteria to establish species delimitation of these two pathogens. Moreover, Hoffmann (9) acknowledged that extreme variation in the depth of the reticulations and the presence or absence of a prominent hyaline sheath are also characteristics of the teliospores of other species that infect grasses, which makes it difficult, if not impossible, to use these morphologic criteria for species identification. Induced and natural hybrids readily form between strains of *T. controversa* and *T. caries,* and the teliospores that are produced have properties of either parent as well as intermediate phenotypes (24,25,30). An examination of wheat heads with symptoms typical of either common or dwarf bunt revealed spores with features of either pathogen as well as spores with intermediate phenotypes (13).

In the Ustilaginales, the echinulate and smooth teliospores of *Ustilago nigra* and *U. hordei,* respectively, were phenotypes determined by only two genes (14). Subsequently, ornamentation of *U. kolleri* and *U. avenae* teliospores (21,28,29) was shown also to involve two genes. Genetic analysis of the wheat bunt pathogens may reveal that alternate alleles at a few loci also control the phenotypic variability observed in the prominence of the reticulations, the presence or absence of a sheath, the autofluorescence properties, and the germination of teliospores. Subsequent analyses of other physiologic properties of the teliospores of *T. controversa* and *T. caries,* including surface antigens (2), phenol-soluble extracted polypeptides (15), and triacylglycerol profiles (3), revealed no significant differences and suggest that homologous genes control these traits in the two pathogens.

Recent molecular genetic evidence suggests that the genomes of these pathogens have homologous chromosomes (23). Electrophoretic karyotypes of four unrelated strains of each of these pathogens and five hybrid progeny revealed no obvious characteristics that could be used to differentiate strains of these two pathogens. The chromosomes in the seven strains, to which four genetic markers could be assigned by Southern hybridization (Table 3), are of similar size and have the same relative length variability as the homologous chromosomes from unrelated strains isolated in the western United States and ascribed to either *T. controversa* or *T. caries* (23, Table 3). Moreover, when compared with the unrelated strains of *T. controversa* and *T. caries,* the karyotypes of strains M1–7 have the same relative number of chromosomes and the same relative genome size and contain chromosomes of similar sizes regardless of worldwide geographic location. These results strongly suggest that the genomes of these pathogens have homologous genes that are organized similarly in the chromosomes; this raises serious questions regarding the validity of using morphologic and physiologic taxonomic schemes to differentiate these and other closely related bunt pathogens.

The onset, kinetics, and maximum attainable level of germination of teliospores are temperature-dependent traits that are known to show considerable variation (33). In this study, the teliospores from five collections (M3–7) germinated at 16 C (Fig. 1A) and, by convention, would have been classified as the spores of *T. caries,* although those from the M3 collection exhibited poor germination (15%). The teliospores from collections M1 and

TABLE 2. Estimated sizes (kb), number of chromosomes, and genome sizes of seven strains representing the seven teliospore samples M1–7

Band number	M1.1	M2.5	M3.10	M4.1	M5.4	M6.3	M7.7
17	...	...	4,300	...	...	...	...
16	...	...	4,000	...	...	...	...
15	...	...	2,040	...	4,160	...	...
14	3,870	3,870	2,890	3,300[a]	3,780	3,550	4,300
13	3,240	3,240	2,480	3,110[a]	3,150	2,980[a]	4,000
12	2,820	2,980[a]	2,370	2,780	2,820	2,670	3,110
11	2,300	2,780	2,110	2,440	2,670	2,290[a]	2,820
10	2,220	2,440	2,130	2,030	2,330	2,120	2,590
9	2,130	2,330	2,030	2,000	2,200	2,030	2,260
8	2,030	2,030	1,940	1,920	2,090[a]	1,920	2,200
7	1,900	1,900	1,860	1,880	1,900[a]	1,850	2,110[a]
6	1,790[a]	1,810	1,730[a]	1,700	1,680[a]	1,660[a]	1,940[a]
5	1,700	1,700	1,630	1,580[a]	1,600	1,590	1,680[a]
4	1,560	1,590	1,400	1,420	1,400[a]	1,400[a]	1,400[a]
3	1,350[a]	1,330[a]	1,350[a]	1,350	1,350[a]	1,330[a]	1,350
2	1,310[a]	1,190	1,170	1,230	1,250	1,230	1,210
1	970	940	940	990	860	900	840
Chromosome number[b]	17	16	19	17	20	19	18
Total[c]	33.6	34.4	39.4	35.7	41.7	37.2	38.9

[a]Bands are presumed to be doublets and to contain twice the amount of DNA.
[b]Values represent the estimated number of chromosomes in each strain.
[c]Estimated genome size in megabase pairs of each strain.

TABLE 3. Average chromosome length and maximum variability of chromosomes identified by single copy anonymous DNA fragments and conserved genes

Probe	M1–7			Maximum variability of homologous chromosomes[b,c]	
	Bands identified	Chromosome length[a] (kb)	Maximum variability[b] (kb)	*Tilletia controversa* (kb)	*T. caries* (kb)
pOSU1003	1	1,690	70 (4)	140 (8)	90 (5)
pOSU1006	1	2,260	170 (8)	40 (2)	190 (9)
pOSU1101[d]	1–3	3,650	1,630 (38)	1,790 (40)	1,770 (39)
pSF8[e]	1	1,230	140 (11)	130 (10)	50 (4)

[a]The average size of chromosomes identified by the probe.
[b]Numbers in parentheses represent maximum percent variability (calculated by dividing the difference between the largest and the smallest chromosomes identified and the average of those chromosomes identified by the probe).
[c]Data are for presumptive homologs in strains isolated from the western United States (23).
[d]Probe DNA shares homology with an internal fragment from the 17S rDNA of *Neurospora crassa*.
[e]The single copy actin gene from *Aspergillus nidulans*.

M2 failed to germinate at 16 C and, by this criterion, would have been spores of *T. controversa*. Clearly, these latter spores have a temperature-dependent phenotype; and it could be argued that spores from the M3 collection, which germinate to 60% at 5 C, also exhibit this phenotype, but they are leaky at 16 C (Fig. 1). The variation in the optimum temperature at which collections of teliospores germinate could readily be a manifestation of single-gene mutations that either reduce or abolish the activities of enzymes that are required for germination. These naturally occurring mutations would be analogous to the conditional lethal, temperature-sensitive *cdc* mutants of *S. cerevisiae*, which may be blocked in the synthesis of protein, DNA, and RNA and arrested at different steps in the cell cycle (6,7). If conditional lethal mutations are affecting the germination process, it could be strongly argued that teliospores that express this phenotype are merely mutant forms of *T. caries*, as suggested by Young (35), rather than spores of a different species.

The presence of prominent autofluorescent reticulations on teliospores from collections M3 and M4 (Fig. 2E and H) is a phenotype attributable to *T. controversa* teliospores (27), but their germination at 16 C (Fig. 1A) is a phenotype of *T. caries*. Teliospores that germinate at temperatures ranging from near 0 to 25 C are acknowledged to be from *T. caries* (22). The elevated temperature at which *T. controversa* teliospores had been bioassayed for germination incompetence was 10 C in different studies (18,33). More recently, Hoffmann (9) redefined the cardinal temperatures for germination of *T. controversa* teliospores and established the optimum as 3–8 C and the maximum temperature as 15 C. Although there is no compelling reason to select a particular elevated temperature to screen for the temperature-dependent phenotype, the choice of temperature becomes extremely important in terms of wheat exports to foreign markets. For example, if a temperature of 10 C were chosen to assay germination of teliospores from the M3 collection, they most certainly would have been identified as *T. caries*. However, these

TABLE 4. Summary of the sheath, germination, and autofluorescence characteristics of seven *Tilletia* teliospore samples and their species determinations on the basis of germination and autofluorescence

Teliospore collection	Sheath	Germination temperature (C)		Teliospore auto-fluorescence	Species determination[a]
		5	16		
M1	+	+	−	+	*T. controversa*
M2	+	+	−	+	*T. controversa*
M3	+	+	+	+	Ambiguous[b]
M4	+	+	+	+	Ambiguous
M5	−	+	+	−	*T. foetida*
M6	−	+	+	−	*T. foetida*
M7	−	+	+	−	*T. caries*

[a]Using current taxonomic criteria (i.e., spore germination, autofluorescence properties, and presence or absence of a sheath).
[b]Teliospores have characteristics attributable to both *T. controversa* and *T. caries*.

spores germinate poorly at 16 C (Fig. 1A), and under less favorable conditions of moisture content of the agar (18,22) or pH, they may have been incompetent to germinate at temperatures of 15–17 C and would have been classified as *T. controversa.* Presently, teliospores that germinate at 5 C but not above 15 C are considered to be spores of *T. controversa,* and grain containing such spores is rejected by the People's Republic of China.

A substantial body of information from genetic, molecular genetic, biochemical, physiologic, and morphologic studies suggests that invoking separate binomials for these bunt pathogens is unfounded. Although dwarf and common bunt are acknowledged to be epidemiologically distinct diseases, the etiologic agents appear to be essentially indistinct. Genetic and biochemical analyses provide strong evidence that *T. caries, T. controversa,* and *T. foetida* are variant forms of one species, which occur because of genetic variation present in natural populations. The classification schemes presently used to differentiate *T. caries* and *T. controversa* rely on phenotypes that are common to both pathogens.

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