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Characterization of *Ustilago tritici* and *U. nuda* on wheat and barley-I. Teliospore morphology

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Morphology of teliospores of *Ustilago tritici* and *U. nuda* pathogenic on wheat and barley were studied by using light–and electron microscopy. The data on teliospore length and width subjected to ANOVA showed significant difference at 1% level between the two species. whereas UPGMA cluster analysis was not able to separate the two species. The ultrastructure of the two species was found to be substantially similar. In SEM, teliospores of *U. tritici* showed echinulation with more prominent spines than those of *U. nuda*. It is concluded that based only on teliospore morphology, it is difficult to separate two species thus, making it imperative to use approaches involving DNA polymorphism for species separation.

Key words : Teliospore morphology, *Ustilago tritici, Ustilago nuda,* wheat, barley.

INTRODUCTION

Loose smut of wheat, *Ustilago tritici* (Pers. : Pers.) Rostr., and that of barley, *U. nuda* (Jensen) Kellerm & Swingle, occur worldwide and cause damage by destroying the kernels of the infected plants and by smearing and thus reducing the quality of the grain of the healthy plants on harvest. Losses from loose smut may range from 10 to 40 per cent in certain localities in a given year (Agrios, 1997).

The cereal smuts of the genus *Ustilago,* pathogenic on wheat and barley, have been classified traditionally based on host specificity and spore morphology, but there is no general consensus regarding their taxonomic classification.

The delimitation of species in the smut fungi is mostly based on morphological characters of the teliospores such as spore measurements and spore surface ornamentation (Vánky, 1991).

The unification of the names of *U. tritici* and *U. nuda* has been suggested by Cunningham (1924), Fischer (1943) and by Ainsworth & Sampson (1950) based on growth and spore morphology. However, a strong view also exists for separating *U. tritici* and *U. nuda* at species level by Nielsen (1972, 1985) and Langdon & Fullerton (1975). Similarly Alexopoulos *et al.,* (1996) and Agrios (1997) also retained separate names for loose smut of wheat and barley as *U. tritici* and *U. nuda* respectively.

In the present investigation attempts have been made to use light and electron microscopic studies to see the differences in the teliospore morphology of these two species parasitising wheat and barley.

MATERIALS AND METHODS

Collection of isolates

Twenty samples of *U. tritici* on wheat and five of *U. nuda* on barley were collected from various parts of Northern India viz., Punjab, Haryana, Himachal Pradesh, Uttar Pradesh, Delhi and surrounding places during February and May 1999 (Table 1). The teliospores of these samples were taken from infected heads and subjeted to microscopic examination.

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Microscopical studies

I) Light microscopy (LM). Dried teliospores of each sample were rehydrated in Shear's mounting fluid (Chupp, 1940) and observed at 2000x magnification with an Olympus BX50 microscope. Measurements for length and width of one hundred teliospores were taken with the help of ocular micrometer.

Table 1 : Isolates of *Ustilago tritici* (Ut) and *U. nuda* (Un) collected from Northern India.

Isolate No.	Location	Date of collection
Ut1	**Gurgaon, Haryana**	**Mar, 99**
Ut2	Bathinda, Haryana	Mar, 99
Ut3	HAU, Hissar, Haryana	Mar, 99
Ut4	Thaska, Hissar, Haryana	Mar, 99
Ut5	Kaimri, Hissar, Haryana	Mar, 99
Ut6	Karnal, Haryana	Mar, 99
Ut7	HPKV, Palampur, U.P.	Mar, 99
Ut8	Palampur, H.P.	Mar, 99
Ut9	Shimla, H.P.	Mar, 99
Ut10	IARI, Reg. St., Shimla, H.P.	Mar, 99
Ut11	Kumargaj, Faizabad, U.P.	Mar, 99
Ut12	Kanpur, U.P.	Mar, 99
Ut13	Varanasi, U.P.	Mar, 99
Ut14	Pusa, Bihar	Mar, 99
Ut15	Pusa, Bihar	Mar, 99
Ut16	RAU, Bihar	Mar, 99
Ut17	Patna, Bihar	Mar, 99
Ut18	PAU, Ludhiana, Punjab	Mar, 99
Ut19	PAU, Ludhiana, Punjab	Mar, 99
Ut20	IARI, New Delhi	Feb, 99
Un1	**Havalbagh, Almora, U.P.**	**Apr, 99**
Un2	Almora, U.P.	Apr, 99
Un3	HPKV, Palampur, U.P.	May, 99
Un4	Palampur, U.P.	May, 99
Un5	IARI, New Delhi	May, 99

** Ustilago tritici ** Ustilago nuda*

II) Scanning Electron Microscopy (SEM). Air dried teliospores were dusted on small pieces of double sided adhesive tape, mounted on specimen stub, sputter coated with gold-palladium under vacuum, ca. 20 mm for 4.5 min, 7.5 mA. The specimens were then observed and photographed in a LEO 435 VP SEM, operated at 15kv following the procedure of Vánky (1997).

III) Transmission Electron Microscopy (TEM). The fixing of the teliospores for TEM was done as given by Vánky (1997). Teliospores were fixed with 2% glutaraldehyde in 0.1 M Na-Cacodylate buffer at pH 7.2 for one week. After six continuous transfers in 0.1 M Na—Cacodylate buffer, the teliospores were postfixed in 1% Osmium tetraoxide in the same buffer for 1 hr. in the dark then washed in distilled water, and stained in 1% aqucous Uranyl acetate for 1 hr. in the dark. After five washings in distilled water, the material was dehydrated in acetone series, embedded in Spurr's plastic with new fuchsin and crystal violet, mounted in Entellan and studied under light microscope. Ultra thin sections were taken by ultramicrotome with glass knives and were mounted on copper slot grids, post-stained with lead citrate for 5 min and examined under a CM10 PHILIPS electron microscope at 60 kv.

Table 2 : Comparison of the mean length and width of teleutospore of different isolates of *Ustilago tritici* and *U. nuda* using Duncan's Multiple Range Test (DMRT).

Isolate No.	Length (µm)		Width (µm)	
	Mean	Rank	Mean	Rank
Ut1	5.940	I	5.270	JK
Ut2	6.050	HI	5.275	JK
Ut3	6.350	FG	5.670	EFGH
Ut4	6.690	CD	5.800	DE
Ut5	6.365	FG	5.775	DEF
Ut6	**5.540**	**J**	**5.135**	**K**
Ut7	6.470	DEF	5.940	D
Ut8	5.935	I	5.350	IJ
Ut9	7.710	C	6.170	C
Ut10	7.140	B	6.480	B
Ut11	6.420	EFG	5.575	GH
Ut12	**8.410**	**A**	**7.215**	**A**
Ut13	6.710	C	5.720	EFG
Ut14	6.730	C	5.700	EFG
Ut15	6.620	CDE	5.490	HI
Ut16	6.656	CDEF	5.635	EFGH
Ut17	6.200	GH	5.330	IJ
Ut18	6.375	FG	5.590	FGH
Ut19	6.435	EF	5.615	EFGH
Ut20	6.530	CDEF	5.590	FGH
Un1	**6.930**	**A**	**6.205**	**A**
Un2	6.655	B	6.030	A
Un3	6.600	B	5.520	B
Un4	6.650	B	5.630	BC
Un5	6.585	B	5.735	C

Data analysis

The data obtained were analysed by two methods :

I) Analysis of Variance. The Analysis of Variance (ANOVA) for length and width of *U. tritici and U. nuda* teliospores was performed by using GLM procedure of PC SAS (SAS Institute, 1989). The mean comparison of the length and width of the isolates

of *U. tritici* and *U. nuda* was separately done using Duncan's Multiple Range Test (DMRT).

II) Ilicrarchical Cluster Analysis. The dissimilarity matrix was calculated from standardized morphological characters data using Euclidean measure of distance. Unweighted pair group method using arithmetic average (UPGMA) was selected to generate grouping (Sneath & Sokal, 1973).

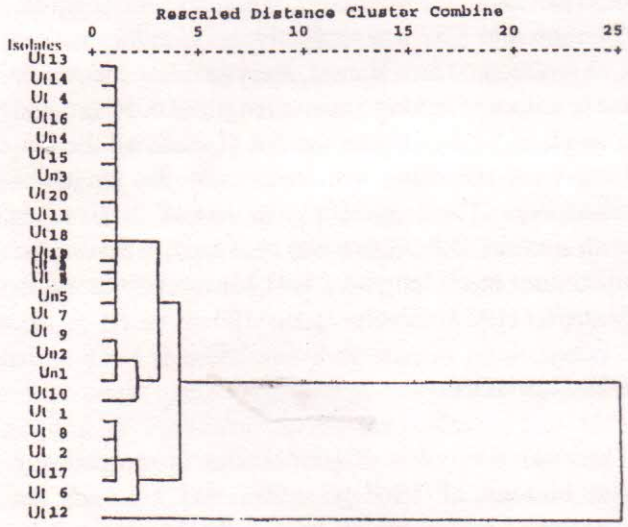
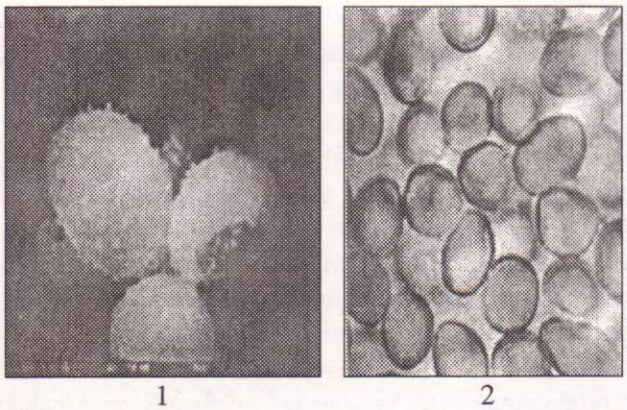


Fig. 1 : Dendrogram of *Ustilago tritici* and *U. nuda* resulting from UPGMA based on length and width of teliospores.

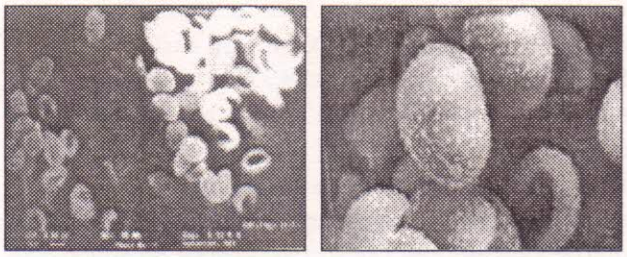
Plate 1 : ১



1 2

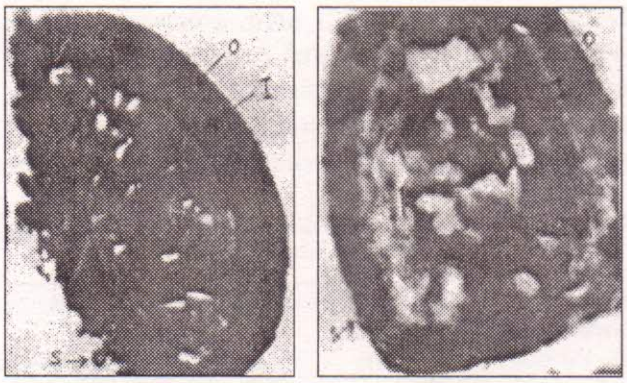
1 and 2 Teliospores of *Ustilago tritici* (Ut?) and *U. nuda* (Un?) seen by LM. Bar = ?? µm.

Plate 1 : ১



3 4

3 and 4 SEM micrographs of surface ornamentation of teliospores of *U. tritici* (Ut?) and *U. nuda* (Un?). Bars = ?? µm.



5 6

5 and 6 Ultrathin sections of teliospores of *U. tritici* (Ut?) and *U. nuda* (Un?) in TEM, showing two wall layers, outer layer (OL) and inner layer (IL) and spines (Sp). Bar = µm.

The size varying from 5—12 µm (6.50 ± 0.91) × 4—9 µm (5.71 ± 0.73). Teliospores of *U. nuda* are olivaceous brown, lighter coloured on one side, globose to subglobose, sometimes elongate and the light coloured side of the teliospore in thin and flattened while the slightly convex, darker side is thickened. SEM showed that teliospores in *U. nuda* are minutely echinulate ANOVA showed significant differences at 1% level between the two species and among isolates within the speies for both length and width of *U. tritici* and *U. nuda* teliospores (Table 3).

Based on DMRT data, results showed that there were 10 classes in case of *U. tritici* and 2 in *U. nuda* with respect to width of teliospores. Ut12 is separated from all other isolates by DMRT. The

length and width data of the teliospores of various isolates of *U. tritici* and *U. nuda* were subjected to cluster analysis using UPGMA on distance matrix based on Eulidean measure. The cluster analysis also separates isolate Ut12 from all other isolates (Figure 1). The remaining isolates were subdivided into 2 groups, one comprising Ut1, Ut8, Ut12, Ut17 and Ut6 and the other comprising the rest. This second big cluster comprised two groups of which one was a large group having 15 isolates including Ut13, Ut14, Ut4, Ut16, Un4, Ut15, Un3, Ut20, Ut11, Ut18, Ut19, Un2, Un1, Ut10. According to DMRT, Un1 and Un2 were grouped together whereas Un3, Un4 and Un5 belonged to different rank. The cluster analysis based on teliospore length and width however could not separate *U. tritici* and *U. nuda.*

Table 3 : ANOVA of isolates of *Ustilago tritici* and *U. nuda* for length and width of the teliospores.

Source of Variance (S. V.)	Degree of Freedom (df)	Mean Square (MS)* Length	Width
Species	1	9.579	4.752
Isolates/species	23	27.281	19.891
Iso. (*U. tritici*)	19	32.461	22.365
Iso. (*U. nuda*)	4	2.679	8.139
Error	2475	0.5317	0.3505
Coefficient of Variation (%)		11.15	10.32

* All sources of variance are significant at 1% level.

Teliospores of *U. tritici* are dark brown, lighter coloured on one side, globose to subglobose, to ovoid. Under SEM they are minutely echinulate. Echinulation was observed to be more on the lighter side of the teliospores with spares spines but in *U. tritici* the spines are more prominent than in *U. nuda.* Here the size varied between 5.50—10 μm (6.66 ± 0.75) × 5—8 μm (5.82 ± 0.68). In both the species the cell wall has two distinct layers. The outer layer is electron dense and dark and the inner layer exhibits uniform electron density, which has corky texture. The spines which are highly electron dense, are appended on the surface of the outer layer. In *U. tritici* both cell wall layers appear uniformly thickened but in *U. nuda* the inner layer is thicker under the thinner part of the outer layer. Width of the outer layer is similar in both species and protoplasm membrane is distinct from the cytoplasm, which is granular in nature (Plate 1). From the results obtained, we conclude that the ultrastructurs of the two species is substantially similar.

RESULTS

The length of the teliospores of *Ustilago tritici* varied from 5 to 12 μm and the width from 4 to 9 μm. for *U. nuda* the length varied from 5.5 to 10 μm and width from 5 to 8 μm. In case of *U. tritici* the average mean length and the width of teliospores was 6.50 μm and 5.71 μm respectively, and for *U. nuda,* 6.66 μm and 5.82 μm respectively. *U. tritici* isolate Ut6 (collected from Karnal, Haryana) has the smallest teliospores having a mean length of 5.54 μm and a width of 5.13 μm, whereas for *U. nuda* all the isolates were the same with respect to the length of teliospores. The biggest teliospore size in *U. nuda* with a mean of 6.93 μm was that Un1, whereas the maximum mean length of 8.41 μm was observed in *U. tritici* (Ut12) (Table 2).

DISCUSSION

There are a number of similartities in morphology and biology of *Ustilago tritici* and *U. nuda* on wheat and barley which have led to various taxonomic proposals : the two smuts are synonymous, only specialized varieties or forms of the same species, or really two different species. Criteria accepted by some mycologists are rejected by others. Review of the literature showed that the single-species concept is very far from being universally accepted by mycologists and plant pathologists all over the world. Some are probably reluctant to give up the long-standing use of separate names for each smut, whereas others find the arguments for the single—species concept unconvincing. These arguments are mainly due to the fact that in the loose smut of wheat and barley the infection type, spore morphology and morphological characteristics after germination of the spores are identical.

Cunningham (1924) suggested unification under the name *U. tritici,* because the two forms differ only in that each in confined to its host, but as they are identical in morphological characters, they must be considered as the same species. Fischer (1943) united the two species under the name *U. tritici,* with "*Specialized varieties*" on wheat and barley

However, Fischer & Hirschhorn (1945), Ainsworth & Sampson (1950) and Fischer (1953) adopted and used the name *U. nuda* with "*Specialized races*" on wheat and barley. Nannfeldt in Lindeberg (1959) united them under *U. tritici.*

Popp (1955) considered that a) *U. tritici* produces growth only from individual cells of the promycelium, whereas *U. nuda* develops it only from conjugate cells. b) The promycelial branches of *U. tritici* are monokaryotic, whereas those of *U. nuda* are predominantly dikaryotic. He concluded that *U. tritici* and *U. nuda* are separable at the species level. Nielsen (1972) concluded that both mating types of *U. tritici* grow readily on a minimal medium, in contrast to *U. nuda,* whose mating type a is proline-deficient. This fundamental physiological difference, supported by previously known morphological differences, was used as a evidence to retain *U. tritici* and *U. nuda* as separte species and chaims were rejected that they should be included under one name. Similarly, observations of Langdon & Fullerton (1975) have shown that *U. tritici* destroys the epidermis of the host but *U. nuda* dose not. Khanna *et al.,* (1971) studied the ultrastructure of *U. tritici* and *U. nuda* and found that the ultrastructure of both species is substantially similar.

Nielsen (1985) rejected the use of trinomial system for *U. tritici* because of the multitude of possible *formae speciales* and the overlapping of pathogenicity by races on several host species, although Hawksworth *et al.,* (1995) in the 8th edition of the Dictionary of the fungi considered loose smut of wheat and barley as *U. segetum* var. *tritici* which was accepted by Mordue & Ainsworth (1984) but cautioned that the causal organisms of loose smuts of cereals are biologically distinct but morphologically close and variously treated as species, varieties or even physiologic races.

Studies undertaken by us on the teliospore morphology could not differentiate *U. tritici* from *U. nuda* based on teliospore length and width but minute differences were observed under SEM with reference to the echinulation on the teliospore wall. This has led us to investigate the use of molecular fingerprinting techniques in order to detect vriability amongst *U. tritici* and *U. nuda* as well as to justify their separation at species level.

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