Detection of bunt and smut pathogens of wheat through scanning electron microscopy

RASHMI AGGARWAL, K.D. SRIVASTAVA and D.V. SINGH
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012

Key words: Teliospore, wheat, bunt, smut, morphology, SEM

The reticulations of teliospore wall of smut fungi and sheath configurations are critical characters for their identification and taxonomic differentiation. In these spores, it is often difficult to resolve these structural details by light microscopy, particularly because of the presence of dark pigments in spore wall. Teliospores of two Neovossia species, viz., *N. indica* (Mitra) Mundkur (Syn. *Tilletia indica* Mitra), the incitant of Karnal bunt of wheat and *N. horrida* (Tak.) Padwick and Azmatullah Khan (syn. *Tilletia barclayana* Takahashi), causing kernel smut of rice, look similar morphologically under light microscope. *Tilletia caries* (DC) Tul. & C. Tul. (Syn. *T. tritici* (Bjerk) G. Wint.) and *T. foetida* (Wallr.) Liro (Syn. *T. laevis* Kuhn) which incite hill bunt of wheat are also closely related filamentous basidiomycetes belonging to

![Fig. 1. Light and scanning electron micrographs of teliospores of *N. indica* (a) light micrograph showing thick sheath 45X; (b) SEM of teliospores showing episporium 2,000X; (c) teliospore with ruptured sheath 3,000X; and (d) a part of teliospore showing fused projections of episporium 7,500X.](image-url)
Ustilaginales. On the other hand, the chlamydospores of loose smut pathogen *Ustilago segetum* (Pers.) Roussel *tritici* Jensen being small and smooth walled match with conidia of many hyphomycetes and pose difficulty in distinguishing by light microscopy. Therefore, in such cases, surface ornamentations as resolved by scanning electron microscopy (SEM) is especially valuable in characterisation of smut fungi (Kozakiewicz et al., 1994).

The teliospores of *N. indica* (Mitra) Mundkur, *N. horrida*, *Tilletia caries* and *T. foetida*, *U. segetum* *tritici* and *Urocystis agropyri* (Preuss) Schroet were collected from infected grains and dried at room temperature for 24 h in a vacuum desiccator. Subsequently, the size of teliospores of the pathogens were measured under light microscope in lactophenol. One hundred observations taken for each sample were statistically analysed. The morphological characteristics of teliospores were also recorded and photomicrographs were taken. In order to study the ultrastructural surface ornamentations of teliospores, all the samples were mounted separately on copper stubs over double adhesive tape. The mounted stubs were dried over silica gel for 24 h in desiccator and then coated with gold in sputter coater JFC 1100 of 300Å thickness. The material was scanned in JEOL JSM-5200 electron microscope and photographs were taken at 15KV.

The size of teliospores of *N. indica* and *N. horrida* differed significantly under light microscope, but *T. caries* and *T. foetida* teliospores were of the same size (23.0-22.5 μm). Chlamydospores of *U. segetum* var. *tritici* were smallest ranging from 4.8 to 6 μm. Average size of spore balls of *U. agropyri* was 36.2 μm. Teliospores of *N. indica* under light microscope appeared dark brown to black, ellipsoid having thick hyaline sheath of 4.4-4.6 μm thickness, while *N. horrida* teliospores were light brown to brown, opaque with comparatively thinner sheath of 1.5-2.3 μm (Fig. 1a, 2a). However, morphological observations of teliospores of *N. indica* under SEM showed very prominent thick projections of episporium which were irregular with blunt margins due to which the surface of the spore looked rough. The stretchability of perisporium did not keep pace with the advancing maturity and increase in size of the spore projections resulting in its rupturing at a few places (Fig. 1b).

In most of the spores, surface remained covered with perisporium while in some, projections became visible due to rupturing of the perisporium (Fig. 1c). The surface projections were 3.1-4.0 μm thick in mature teliospores, and appeared fused with each other (Fig. 1d). In comparison to *N. indica*, teliospores of *N. horrida* showed ornamented episporium, projections being more regularly arranged and compact having blunt tips (Fig. 2b). These projections were more conspicuous and smaller in size than those in *N. indica*. Earlier, Gardener et al. (1983) and Aggarwal et al. (1990) also observed *N. indica* under SEM and reported very prominent irregular thick projections of episporium.

Teliospores of *T. caries* and *T. foetida* were spherical to ellipsoidal with thick perisporium and smooth wall (Fig. 3a,c) but under SEM, spores of the two species differed with respect to surface ornamentation of episporium. In *T. caries*, the episporium was variously reticulated showing minute indentations to wide meshes. The reticulations resulted in the formation of areolae which vary in size and shape. The size varies

---

**Fig. 2.** Light and scanning electron micrographs of teliospores of *N. horrida* (a) light micrograph showing sheath 45X; and (b) SEM showing projections of episporium 2,000X.
Fig. 3. Light and scanning electron micrographs of teliospores of Tilletia spp. (a) light micrograph of T. caries 45X; (b) SEM of T. caries showing reticulations 2,000X; (c) light micrograph of T. foetida 45X; and (d) SEM of T. foetida showing smooth wall 2,000X.

from 1.5 - 3.16 × 1.66 - 3.66 μm and the shape was hexagonal though pentagons were also seen (Fig. 3b). In T. foetida, the episporium wall was smooth (Fig. 3d), showing germ tube depressions. Earlier, Khanna et al. (1966), and Hess and Weber (1971) have also reported that teliospores of T. caries have wall with distinctive reticulate surface patterns.

Under light microscope, the chlamydospores of U. segetum var. tritici were yellow to light brown, spherical, rarely oval showing thin smooth episporium, while teliospores of U. agropyri were seen in spore balls surrounded by sterile cells. The spore balls were dark brown to black and irregular in shape (Fig. 4a, 4c). SEM observations of loose smut spores showed depressions on the inner side, where the episporium was thick walled and lightly echinulated while on outer side it was thin and heavily echinulated. The echinulations were small, pointed, regularly spaced (approx. 0.2 μm) without annular rings (Fig. 4b).

Echinulations on exosporium of chlamydospores of U. segetum tritici were also noticed by Khanna et al. (1971). Description of the ultrastructure of U. agropyri is not available so far. The observations taken through SEM showed that peripheral sterile cells were ring shaped, spherical, more often elliptical, compactly joined together forming a sac like structure. Sterile cells were 11-20 μm in size and were cemented together tightly enclosing the chlamydospores inside. The margins of the individual cells were raised and gave irregular shape to the spores (Fig. 4d).

ACKNOWLEDGEMENTS

The authors are thankful to the Head, Division of Plant Pathology for providing guidance and necessary facilities.
Fig. 4. Light and scanning electron micrographs of teliospores of (a) *Ustilago segetum tritici* 45X; (b) SEM of *U. segetum tritici* 5,000X showing echinulations; (c) light micrograph of chlamydospore of *Urocystis agropyri* 45X; and (d) SEM of *U. agropyri* showing ring like cells.

REFERENCES


Received for publication May 21, 1997.