SEM studies on spore morphology and infection process of spot blotch pathogen in wheat

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Drechslera sorokiniana (Syn.=Bipolaris sorokiniana, teleomorph: Cochliobolus sativus) causing spot blotch is one of the most important foliar pathogens of wheat causing severe losses in wheat production in warm and humid conditions (6). The pathogen has a worldwide distribution, but is particularly aggressive under conditions of high relative humidity and temperature. It is capable of causing damage from the primary leaf stage, though the plant tends to become more susceptible after flowering. The small spots on leaf coalesce and the leaf prematurely dries up, reducing the photosynthetic area of the plant. Studies have shown that foliar blights can cause up to 20% yield loss in farmers fields and close to 30% in experiments in South Asia (4, 5). In India the disease can cause 2.7 to 36.2% loss in yield (3). A critical step in breeding for spot blotch resistance is to understand host pathogen interaction and very little information is available on this aspect. The present studies highlight the information on ultra structure of pathogen, its germination and establishment of infection.

Pure culture of most virulent isolate OS-3 of D. sorokiniana was maintained on PDA for studies on pathogen and interaction with host. The spore size and morphology was studied through light and scanning electron microscopy. For host pathogen interaction studies, leaves of susceptible cultivar Agra local, inoculated under green house conditions were processed for light and scanning electron microscopy.

Inoculated leaf samples were placed on filter paper, one end of which was dipped in fixative (3:1::absolute ethanol:glacial acetic acid, v/v) for 48 hrs. These leaf samples were transferred to other filter paper moistened with (1:2:: lactophenol:ethanol, v/v) for 24 hrs. Thereafter, samples were transferred to other filter paper moistened with trypan blue stain (0.1% in lactophenol-ethanol) and kept for 24 hrs. Mounted the samples on glass slide, observed under light microscope and photographed.

Inoculated leaf bits 3 x 3 mm were fixed in 5 per cent glutaraldehyde prepared in phosphate buffer pH 6.4 for 24 hrs and mounted on copper stubs over double adhesive tape. The samples were exposed to 0.1% osmium tetroxide vapours for one hr. and the gold coating was done in sputter coater JFC 1100 of 300 Å thickness. The stubs were placed in specimen chamber and scanned in JEOL JSM 5200 and photography done using B/W 120mm, 120ASA photographic film.

Light microscopic studies have shown that the conidia are slightly curved or sometimes straight, fusiform to broadly ellipsoidal, dark olivaceous brown, smooth, thick walled, 3-12 (mostly 6) pseudoseptate. They were about 40-120 μm long

Fig.1. Light micrograph of conidia of Drechslera sorokiniana showing germ pore (10X)
and 17-28 μm thick (Fig.1). The basal cell had a sub-hyaline terminal portion with a clear scar. Earlier, Zillinsky (7) reported that under low magnification, conidia appeared black and shiny but under higher magnification they were dark olive brown.

Scanning electron microscopy of conidia showed that they are smooth walled showing thickenings at the places where pseudosepta are there (Fig.2a). Terminal germ pores are visible in some conidia (Fig.2b). At the point of attachment with conidiophores, a dark scar is visible on the conidia.

In host pathogen interaction studies it was observed that conidia on germination on the host surface produced germ tubes from one or both the terminal cells (Fig. 3a, b). The germ tube elongated and produced long hyphae over the leaf surface running across the veins. Under light microscope hyphae were seen penetrating through stomata. Small appressoria like structures were observed in

Fig.2. Scanning electron micrographs of conidia of *Drechslera sorokiniana* showing thickenings at places of pseudosepta 2000X (a); apical germ pores on conidia 1000X (b)

Fig.3. Light micrograph showing (a, b) germinating conidia of *Drechslera sorokiniana* on leaf surface of wheat cv. Agra local (20X). (c) Scanning electron micrograph showing infection hypha of *D. sorokiniana* penetrating through stomata (1500X)
sub stomatal cavity. Scanning electron microscopic observations revealed that conidia on germination produced infection hyphae which formed appressoria over stomata (Fig. 3c). The mechanism of pathogenesis included germination from one or both terminal cells of conidia, formation of appressoria from the germ tube that supported the penetration by infection hyphae through the stomata. Bidari and Govindu (1) also reported formation of appressoria from the germ tube, which penetrated through the cuticle of the host plant.

Recently Das et al. (2) observed that the conidia of pathogen, *D. sorokiniana* germinated and hyphal penetration and ramification was observed on susceptible host, Agra local, while on resistant host, abortive germination was observed without showing penetration. They established the role of leaf surface waxes in imparting resistance.

The present investigations have revealed that conidia of *D. sorokiniana* under SEM are smooth walled with polar germ pores. On coming in contact with susceptible host they germinated from the polar cells and infection hyphae penetrated the host through stomata by forming appressoria like structures.

REFERENCES


