HISTOPATHOLOGY OF SORGHUM VARIETIES RESISTANT AND SUSCEPTIBLE TO HELMINTHOSPORIOSE INFECTION

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ABSTRACT

Histopathology and symptom expression were studied in both resistant (CSH 6 and 148) and susceptible (Neerujola and Swarna) sorghum varieties inoculated with *Exserohilum turcicum* which causes helminthosporiose disease. Aspects such as initial stages of infection, penetration and inter- and intra-cellular growth in the chlorenchyma to fungal entrance into the xylem were similar in both resistant and susceptible types of reactions. The first histological difference was observed in the xylem. Fungal growth was abundant in the xylem of susceptible plants while it was scant in the resistant plants. Rapid killing of cells resulted in a large wilt-type lesion in susceptible plants. Enlargement of lesions in the resistant plants was least and restricted to the hyphae slowly progressing in the mesophyll tissue.

INTRODUCTION

Histopathological studies are important to understand the host-pathogen relationship and the extent of host tissue damage caused by a particular disease. Histological studies of susceptible and resistant maize plants inoculated with *Helminthosporium turcicum* Pass has been conducted by Jennings and Ullstrup[1]. Resistance in maize to *H. turcicum* characterized by a chlorotic type lesion was reported by Hooker and others[2-7]. However, no attempt has yet been made to study the histopathology and tissue destruction caused by *Exserohilum turcicum* (Pass) Leo et Sug[8], the incitant of helminthosporiose disease in sorghum plants. Having found Neerujola and Swarna (cultivars) susceptible and CSH 6 and 148 (hybrids) resistant to helminthosporiose infection[9], these were chosen for the histopathological studies.

MATERIALS AND METHODS

Healthy seeds of four sorghum varieties (Neerujola, Swarna, CSH 6 and 148) were obtained from the University of Agricultural Sciences, Bangalore and Dharwar. A virulent culture of *E. turcicum* was procured from the Mycology and Plant Pathology Division, Indian Agricultural Research Institute, New Delhi. Fifteen-day-old sorghum plants of the above mentioned varieties were inoculated by atomizing a spore-cum-mycelial suspension of *E. turcicum* (1,05,000 spores/ml) and kept in a moist chamber for 24 hr. Samples of inoculated leaves were detached at two and six hourly intervals for seven days. The plants were then continuously sprayed with water and incubated in a moist chamber for 20 hr and a final sample taken[10].

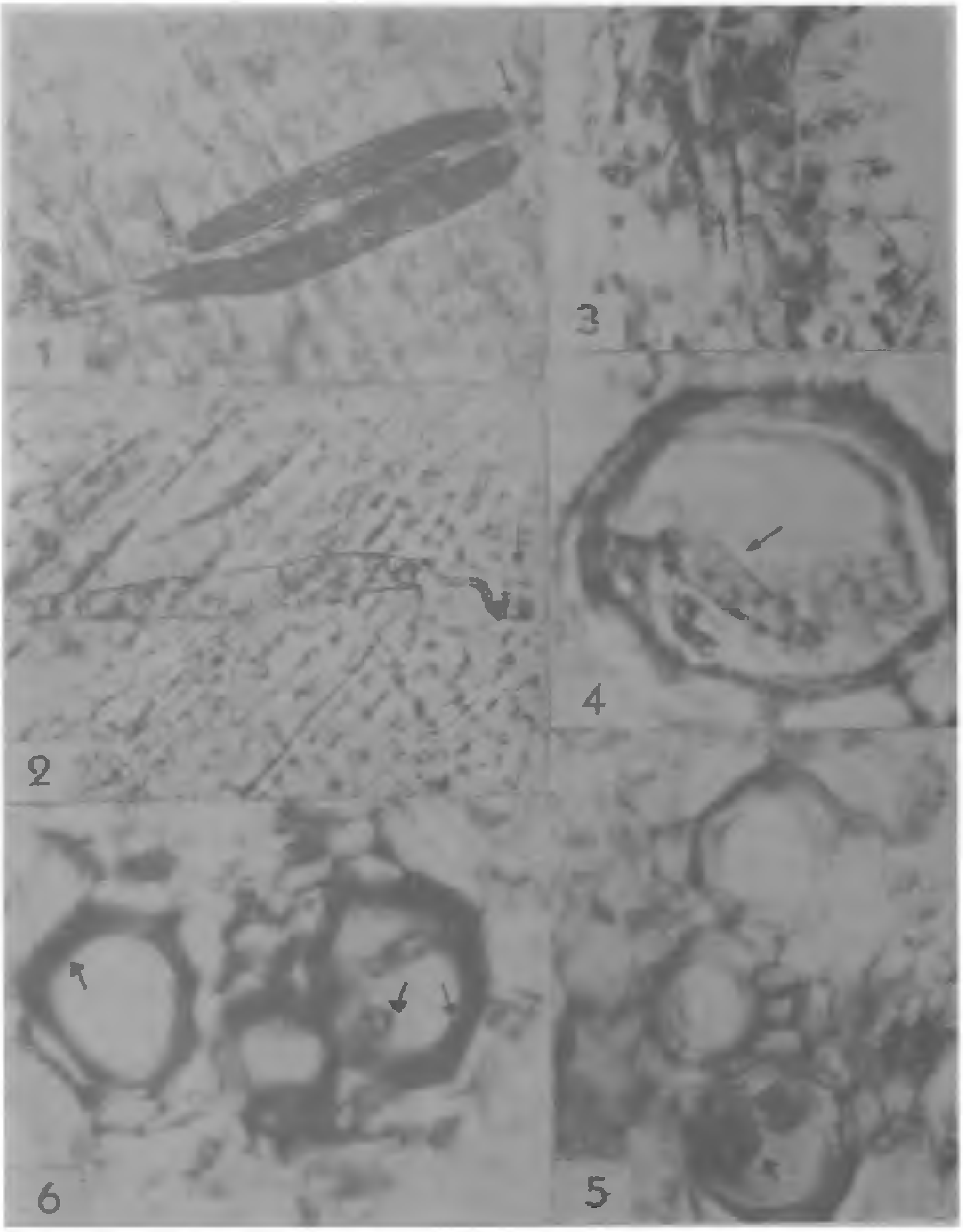
Whole mounts were cleared in pyridine, stained in a mixture of 0.1% methylene blue and 0.2% acid fuchsin and mounted in lactophenol. For serial sections, leaf segments were fixed in formalin-aceto-alcohol, dehydrated in ethyl alcohol series and embedded in paraffin wax. Sections (12 μ thick) were cut and stained in aqueous safranin and fast green[10].

RESULTS

Susceptible reaction:

Penetration and infection: Spore germination was mostly bipolar (figure 1) and occurred 8 to 12 hr after inoculation. More germ tubes developed from the apical, than the basal cells of spores and produced simple terminal appressoria (figure 2) from which the penetration pegs developed. Penetration was usually direct and rarely occurred through stomata. Infection pegs grew into or between epidermal cells of both sides of the leaf. While penetration was sometimes observed within 8 hr it occured mostly after 14 to 20 hr of inoculation.

Following penetration, the fungus produced a vesicle-like structure (15 to 30 μm in diameter) in or between the epidermal cells. This structure gave rise



Figures 1–6. (×493) Stages of infection in susceptible and resistant sorghum leaves inoculated with *Exserohilum turcicum*. **1.** Germinating spores; **2.** A germinating spore with appressorium; **3.** A hypha in cross-vessel; **4.** Hyphal growth from xylem to adjacent tissue; **5.** Abundant hyphae in vessels; **6.** Scant fungal growth in xylem with thickened vessel walls.

to secondary hyphae which grew intercellularly as well as intracellularly in the mesophyll tissue in various directions, some growing towards the xylem. After 48 hr of inoculation, first macroscopic symptoms appreared as minute light brown flecks on the upper surface of leaves. Hyphae grew slowly in the mesophyll cells of these flecks and in the cells beyond the necrotic tissue of the fleck. Other hyphae grew rapidly penetrating the xylem elements within three to six days after inoculation and either before or after the initial appearance of symptoms. The hyphae were found to spread laterally from one vein to another through the cross connections (figure 3).

Spread of the fungus and lesion development: Flecks enlarged either singly or coalesced to form lesions. Lesions developed during the first 4–6 days after inoculation by the action of slowly advancing hyphae inside the chlorenchyma tissue. The number of necrotic cells increased as a lesion developed. Some cells in a fleck died without any direct contact with the hyphae. Cellular host response was observed initially by the lighter staining of the chloroplasts which later lost their identity. Cells in the vicinity of a lesion eventually died, having become disorganized and lost the cytoplasm. In some cases, the wrinkled walls adhered to each other and stained deep red.

A lesion occupied an area between 4–8 small veins, 4–6 days after inoculation. At this stage, most of the xylem elements in the region were found to contain the fungus hyphae (figure 5). Enlargement of a lesion was mainly due to the growth of hyphae from the xylem into the surrounding healthy tissues (figure 4). A lesion contained many air spaces between the cells of the epidermis.

Conidiphore and spore development: Spraying plants in a moist chamber enhanced the fungal growth in the lesions. Mycelial strands were found to form pseudoparenchymatous structures in the substomatal chamber. Conidiophores produced from these structures emerged through the stomata in most cases and produced abundant conidia (figure 7).

Resistant reaction:

Penetration and infection: The pathological histology from fungal penetration to its entrance into the xylem was similar to that in the susceptile reaction. However, the appearance of flecks was earlier by 6–8 hr than in susceptible reaction.

Spread of the fungus and lesion development: Hyphae gained access to xylem parenchyma and later to xylem elements at the same rate as in susceptible plants but the fungal growth was sparse in the resistant plants (figure 6) compared with abundant growth in the vessels of susceptible plants.

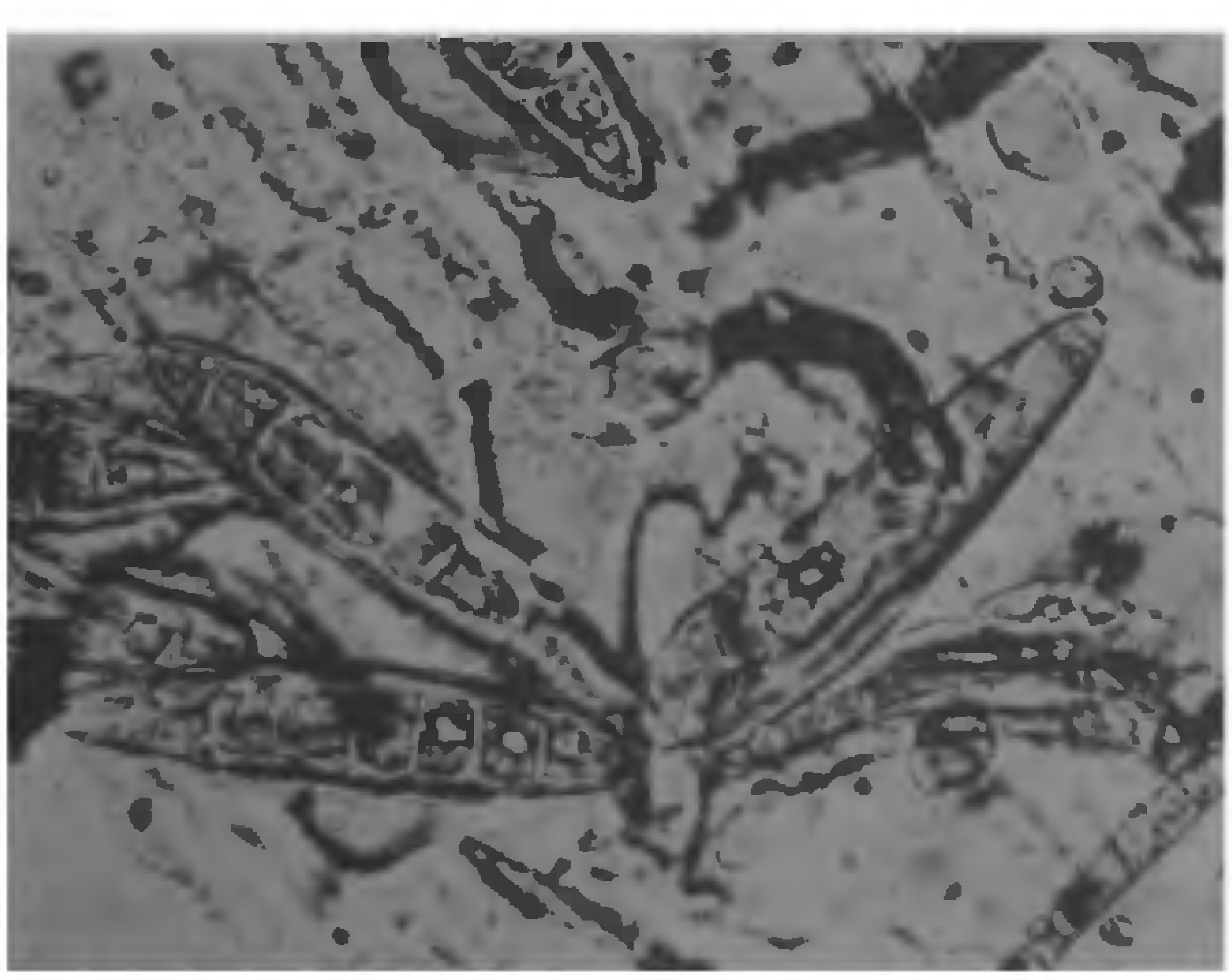


Figure 7. Spores of *E. turcicum* (×613).

A cross-section of an infected susceptible leaf 4–6 days after inoculation revealed that most xylem elements contain hyphae. In addition to the reduction in number, the hyphae were thinner in the xylem of resistant plants than in the susceptible plants.

Lesion enlargement was mainly caused by the slow spread of hyphae originally growing in mesophyll cells of fleck. Rapid killing of cells by the growth of hyphae from vessels into the surrounding regions noticed in the susceptible plants was completely lacking in the resistant plants.

Cellular host response was similar to the response described for susceptible plants. Initial response elicited disorganization of cell contents. Empty wrinkled walls disintegrated into disorganized masses. In contrast to the susceptible reaction, older lesions were devoid of large air-spaces and the epidermal layer disintegrated. Cells adjacent to a lesion significantly showed enlarged nuclei which persisted longer in these cells than in comparable cells of susceptible plants. Also, a unique cellular response observed in infected resistant plants was the thickening of vessel walls, laid in thin separate lignified layers (figure 6) which stained deep red.

Conidiophore and spore development: Rapid hyphal growth as observed in susceptible reaction did not occur in the lesions of resistant plants sprayed with water and incubated in moist chamber. Also, hyphae failed to aggregate in the substomatal chamber and initiate the asexual reproduction of the pathogen.

DISCUSSION

An adequate understanding of the host-pathogen relationship of a disease is desirable to gather further knowledge of parasitism. Observation on pathological histology in susceptible and resistant plants used in this study agree, for the most part, with the observations reported by Jennings and Ullstrup[1] and Hilu and Hooker[10]. The fungus *H. turcicum* was reported to leave the xylem tissues only after the death of the surrounding tissues in both the susceptible and resistant maize varieties[1]. This observation supports the theory that wilting is caused by mechanical plugging of the xylem. In contrast, Hilu and Hooker[10], while studying another susceptible maize hybrid observed the hyphae of *H. turcicum* leaving the xylem and growing into the apparently normal bundle sheath and chlorenchyma. Rapid death of the cells follows and results in typical wilt-type lesions. This agrees with the present investigations. The susceptible and resistant sorghum varieties exhibited distinct differences in their reaction towards helminthosporiose infection caused by *E. turcicum*. The hybrids which were resistant to attack by *E. turcicum* not only suppressed the growth of the fungus but also significantly inhibited asexual spore production, thereby reducing the level of potential inoculum of the pathogen. This in turn checks the further spread of the disease.

ACKNOWLEDGEMENT

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ANNOUNCEMENT

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