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Histopathology of infected tomato fruits with reference to the external symptoms of the disease

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Tomato is one of the most popular and important vegetables not only in India but also around the world. During the survey of vegetable market for determining the nature and extent of fungi associated with the decay of tomato fruit, a large number of fruits were found to be rotted by *Alternaria tenuis*. It was observed that *A tenuis* was highly pathogenic to ripe tomato fruits. Experiments were carried out in the site of infection as well as with healthy tomato fruits. The progress of invaded hyphae inside the fruit tissue by Scanning Electron Microscope (SEM)was studied. It was found that invaded hyphae of *A. tenuis* entered the stroma on the fruit cuticle and mycelia spread well over the fruit surface. The hyphal mass colonized the mesocarp (cortex) of the fruit and after that the colour and texture of the cell deteriorated. The fungal hyphae growth both intra and inter cellulary and within 3 to 4 days of inoculation conidia appeared on the fruit surface by rupturing the fruit cuticle.

Key words: *Alternaria tenuis,* histopathology, tomato, Scanning Electron Microscope

INTRODUCTION

The sequence of structure and biochemical events which occur during infection may be unique to the particular host and parasite interaction under consideration. There is a series of events leading to the establishment of the fungus in the host tissues. Even before penetration occurs there are number of interactions between the pathogen and its host. When pathogens could not penetrate directly the outer protective layer of host, they enter through the wounds or by natural opening, such as lenticels, stomata etc. Among natural opening through which parasites enter plants, stomato are the most important. A number of *Septoria* sp. enter through stomata. Occasionally germ tubes grow over stomata without penetrating them (Cooper, 1993, Kurtun and Ali 1979). The intracellular and intercellular development of the fungus resulted in the rapid degradation of cell wall and membrane structural integrity, although cells are not killed in advance of invasion during these early stages of colonization.

Therefore the anatomy of healthy and artificially infected tomato fruits are carried out during the investigation with a view to study the nature of disease, the cause of pentration and disintegration of fruit by *Alternaria tenuis.*

MATERIALS AND METHODS

Fruit inoculation

Alternaria tenuis was isolated from infected tomato fruits. The pure culture of seven days old fungal pathogen were taken for the investigation. Tomato fruits were surface sterilized with mercuric chloride (0.01%), washed repeatedly with distilled water and inoculated by placing spore suspension within a circled area (10 mm in diameter) on the fruit surface. Inoculated fruits were incubated at 27°C in moist chambers.

Tissue preparation for SEM

Scanning Electron Microscope (SEM) was used as a tool in the study of the progress of fungal growth on fruit surface and inside the fruit. The procedure adopted by Hyat (1981) was followed. Fruit tissue about 5 × 5x 2 mm was excised from the margin of the rotted areas or directly from the lesion of tomato fruits when white lession typical of a rot had formed. Sample was fixed for 4 hrs. with 3% glutaralhehyde in 0.1M Cacodalite buffer (pH. 7.2) and post fixed with 1% OSO_4 in phosphate buffer for 12 hrs. at 4°C and washed with Cacodalite buffer. The fixed materials were dehydrated in an acetone series. Samples were then critical point dried (CPD) for

surface studies and mounted on brass stubs using colloidal graphite (dotite) and then sputter coated with gold and viewed in a JEOL JSM 35. CF Scanning Electron Microscope with 15 KV accelerating voltage. Conidia and fungal mycelia from culture plates were post fixed with OSO4 in phosphate buffer for 4 hrs. and then mounted on specimen stub using double-sided adhesive tape coated with gold and examined under SEM at 15KV.

RESULTS AND DISCUSSION

Histology of the healthy fruits

Tomato fruits are fleshy, many seeded barry. The examination of the ultramicrotome thin sections of healthy tomato fruits under Scanning Electron Microscope (SEM) revealed the following structures. The fruits showed a thin outermost cuticle and fine smooth layered epicarp (epidermis) followed by fleshy succulent mesocarp of thin walled parenchymatous cells. Usally the cells of the epidermis and peripheral mesocarp layers were small compared with those of the middle fleshy layers. At the peripheral mesocarp tissue few, round, large, empty cavity enclosed by cell wall were present. In the whole mesocarp scatteredly arranged single or closely packed stone cells were found. Endocarp was richer in parenchymatous cells in healthy ripe tomato fruits (Fig.1)

Histopathology of infected fruits

The infected tomato fruits pieces were examined with SEM. A thorough examination of the infection process of *A tenuis* in tomato fruits revealed that the infection hyphae directly developed from the conidia within 24 hrs. on the surface of the tomato fruits. The infected tomato fruit covered by white mycelial growth of pathogen was clearly visible on the surface. Mycelial growth of *A tenuis* was greater forming a mat on the fruit surface. Once the infection hyphae entered into the cells of epidermal layer, they penetrated other cells of epidermis (epicarp) and inner tissue. Hyphae branched out and traversed further into the parenchyma cells of the fruit cortex (mesocarp). After penetration the mycelium had spread rapidly in all directions and at this time the typical symptoms of the disease, round, white spots appeared on the fruit surface at the point of infection. This microscopically visible symptoms soon develop into a definite lesion that progressively extended. The mycelial growth was intense beneath the epidermis and displaced the epidermal cell and cuticle giving swollen structures in infected tomato fruits (Fig.2). The fungal growth was not associated with marked changes in the structure of host cell walls because signs of swelling or disintegration were seldom observed. The massive colonization occurred in tomato fruits after inoculation and resulted in marked alternations including disintegration, cell collapse and wall break down. The infected tissue turned watery. The fungus frequently sporulated soon after mycelium had established throughout surface layers of host cells. Conidiophores frequently forced their way through the epidermis of infected tomato fruits (Fig.3) and as a result host tissue was observed. *Alternaria tenuis* contained black slimy mass of conidia and infected tuissue turned blackish brown, dry and rotten photographs of mycelium of the fungus from culture plate were were taken by SEM (Fig.4) exhibiting stout branched mycelium of

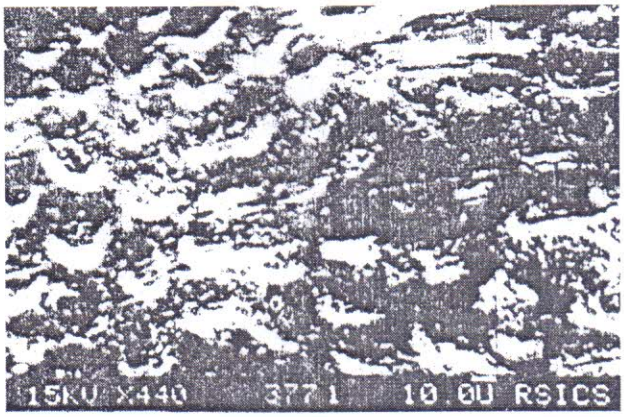

Fig. 1 : SEM of section healthy ripe tomato fruits showing internal tissue system

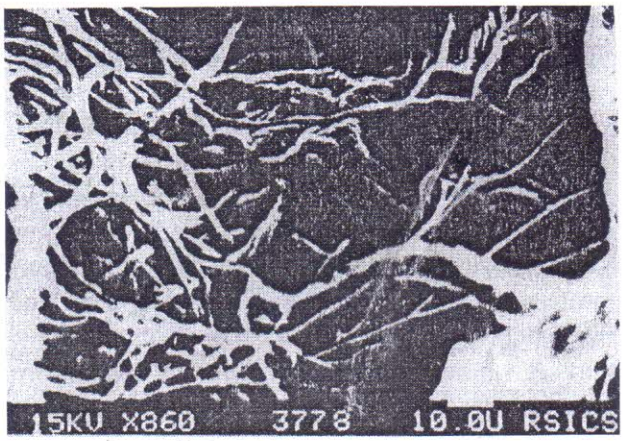

Fig. 2 : SEM of infection of hyphae of *A.tenuis* entering through stomata

A.tenuis.

The formation of germ tube and entrance through stroma or cuticle as a whole resemble the

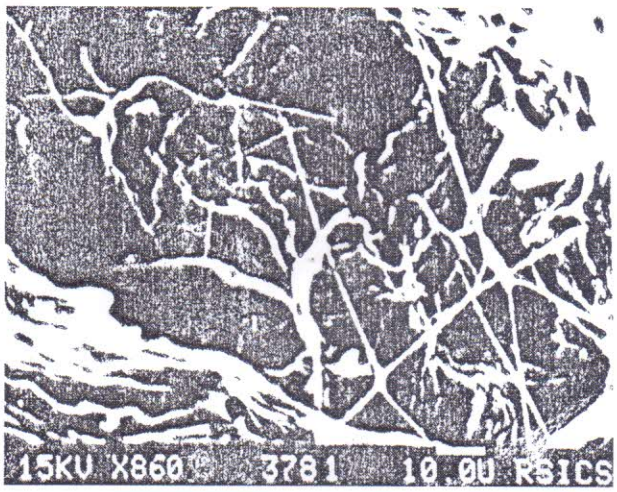

Fig. 3 : SEM of conidiophores opening through the epidermis infected tomato fruits

description of Sharma *et al* (1986) in *Glomerella cingulata* in fruit rot of pear and found that infection hyphae entered the fruit within 48 hrs. of inoculation. This was followed by colonization of mycelia in between the cells after 96 hrs. After 10 days numerous cup shaped acervuli having conidiophores and conidia developed below the

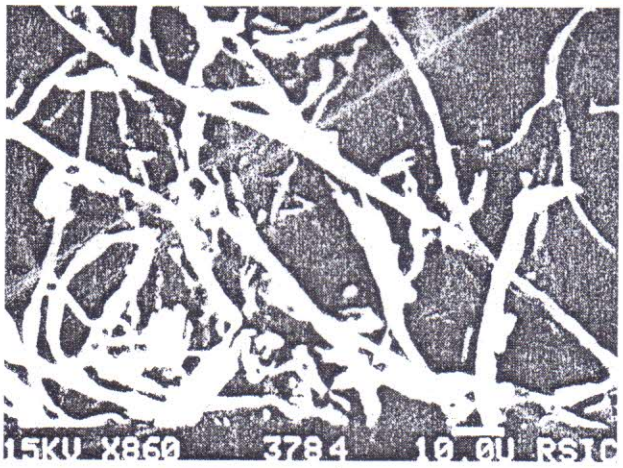

Fig. 4 : SEM of *A tenuis mycelia* from culture plate

epidermis. Ilker *et al* (1977) observed the anatomical changes associated with the developing of gold fleck and fruit pox symtoms on tomato fruit. According to Brown (1977) the histopathology of diseased guava fruit by *Colletotrichum acutatum* and *Phytophthora versicolar* resembled the anthracnose of *Colletotrichum gloeosporioides* on Robinsons tangerines, *C gloeosporioides* on carica papaya fruit (Chau and Alvarez, 1983). A scanning electron microscopic (SEM) study of *Peronospora parasitica* in *Brassica oleracea* has been made by Anchar (1995). It has been noted that the presence of single germ tube in a conidium was common, occassionally two germ tubes arose from a single conidium. It has been reported that the germ tubes may enter the tissues through a stroma but usually penetrated directly through the cuticle.

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