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Full Length Research Paper

Effect of anthracnose and powdery mildew pathogens on plant morphology

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Chilli (*Capsicum annum*) crop suffers very severely due to the infection of Anthracnose (*Colletotrichum capsici*) and powdery mildew (*Leveillula taurica*) leading to malformation of plant parts with heavy yield losses. Hence, it was aimed to find out the anatomical changes occur due to the infection by these pathogens. The observations on the microtomy sections of leaves and fruits infected by *C. capsici* revealed thickening of cell wall with dense and granular cytoplasm. The epidermal cells and cortical parenchyma were collapsed and larger cavities were also seen. Large numbers of small vacuoles were also observed in the cytoplasam. Infected cells become black and both inter cellular fungal cells were present in the infected cells. The xylem vessels were also thickened and both proto and metaxylem were plugged with mycelial network. The microtomy sections of plant samples infected with *L. taurica* showed the collapse of epidermal cells, disruption of mesophyll and guard cells. The infected section of the plant showed more fungal invasion which was absent in the healthy.

Key words: Anthracnose, chilli, histopathology, powdery mildew.

INTRODUCTION

Chilli (*Capsicum annum*) is the fourth most important vegetable crops in the world and first in Asia, with world production approximately 122.34 million tonnes of fresh chilli and 2.8 tonnes of dry chilli in 2010 (Indian Horticultural Database). The most important producers and exporters of chilli include China, India, Mexico, Morocco, Pakistan, Thailand and Turkey. Demand for chilli in the world is increasing every year. Chilli is a very remunerative spice crop of the Indian subcontinent (Sharma et al., 2005) and occupies an area of about 0.81 million ha (Suthin Raj and Christopher, 2009) which accounts for 25% of the world production (Chandra et al., 2009). In Tamil Nadu, chilli is cultivated on 49.0 thousand hectares with 31.8 thousand tonnes of production. Chilli not only meets domestic consumption but also helps in earning foreign exchange. Unlike other chilli-producing countries, about 90 per cent of the production (estimated over 10 lakh tonnes of chilli) in India is absorbed by the huge domestic market. India exports only about 1.5 lakh tonnes of chilli out of the total production of 7.5 lakh tonnes (Anon, 2008).

Chilli is attacked by several fungal, bacterial and viral diseases among them; anthracnose and powdery mildew are found to be the major diseases incurring heavy losses, if not cared. Anthracnose (fruit rot and die back) caused by *Colletotrichum capsici* (Syd. Butler and Bisby) is prevalent throughout the chilli growing areas of India (Jeyalakshmi, 1996). The powdery mildew caused by *Leveillula taurica* (Lev.) Arn is a major constraint in chilli production in India causing heavy yield loss ranging from

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Table 1. Concentration of Different alcohol levels for histopathology analysis of Powdery mildew and anthracnose infected chilli leaves.

Alcohol percentage %	TBA (ml)	Ethanol (95%) (ml)	Distilled water (ml)	Duration
50	1.0	4.00	5.0	2
70	2.0	5.00	3.0	12
85	3.0	5.00	1.5	12
95	4.5	5.00	1.0	12
100	7.5	2.5	0.0	12

14 to 20%, due to severe defoliation and reduction in photosynthesis, size and number of fruits per plant (Mathur et al., 1972, Sivaprakasam et al., 1976 and Gohokar and Peshney, 1981).

The objective of the present study is to find out the effect of infection of anthracnose and powdery mildew pathogens on the plant morphology, which in turn helps in elucidating how the fungus colonizes leaf surface, palisade and vascular tissues.

It helps in showing the mode of entry of the pathogen. It is also used to study the nature of antagonism between the fungi and the host.

This type of study further enhances means to develop newer and the most suitable means of managing the plant pathogens.

MATERIALS AND METHODS

Microtome

Microtome sectioning of healthy as well as diseased leaves were carried out as described by Johnson (1940). For this purpose chilli leaves from healthy and infected plants were taken for powdery mildew and fruit samples were taken for *C. capsici.*

Fixation and washing

The leaf bits were fixed in parts of 30% formalin, 5 parts of glacial acetic acid and 90 parts ethanol for 29 h at 4°C. The leaf bits were then transferred successfully to 50, 60 and 70% ethanol for one hour.

Dehydration

The leaf bits were gradually dehydrated in tertiary butyl alcohol (TBA) series furnished in Table 1. Later the samples were washed in running tap water for 10 -15 min followed by washing in ethanol 10, 50, 70 and 100% for 5 min respectively. Then the samples were transferred to xylene I, Xylene II for 5 min each and stained with touline blue 1 g/100 ml. The slides were mounted in DPX and left undisturbed for 24 h and photographed in an image analyser.

RESULTS AND DISCUSSION

Histopathological studies on pathogens

In order to study the infection of *C. capsici* in chilli plants, samples healthy and infected leaves were collected. The microtomy sections were taken and the slides prepared as per the method shown in the chapter of Material and Methods and observed under image analyser and photographs were taken.

Histological studies with *C. capsici.*

Microtomy sections of healthy chilli leaves showed regular arrangement of epidermal cells, cortical parenchyma cells, conducting vessels and pith cells. The observations showed that infection by *C. capsici* lead to several histological changes in the infected leaf tissue of chilli plant. In the infected leaf, thickening of cell wall was pronounced and cytoplasm was dense and granular. The epidermal cells and cortical parenchyma were collapsed and larger cavities were also seen. Large numbers of small vacuoles were also observed in the cytoplasam. Infected cells become black and both inter cellular fungal cells were present in the infected cells. The xylem vessels were also thickened and both proto and metaxylem were plugged with mycelia net work.

Histological studies with *Leveillula taurica*

The microtome sections of healthy leaves of chilli revealed the presence of thick and intact epidermal layer with cylindrical palisade and round spongy parenchyma cells, whereas the sections of diseased leaves showed more damage to leaf structures. The infection by *L. taurica* leads to collapse of epidermal cells, disruption of mesophyll and guard cells. In addition large cavities were also found in the cytoplasm (Figures 1 and 2). The studies on histological observations of diseases and healthy leaf tissues revealed that microtome sections of healthy leaves showed thick and intact epidermal layer with cylindrical palisade and parenchyma cells. Whereas, in diseased leaf microtome sections, the upper and lower epidermis were disintegrated and but not intact. The cells of leaves were completely destroyed, no demarcation of

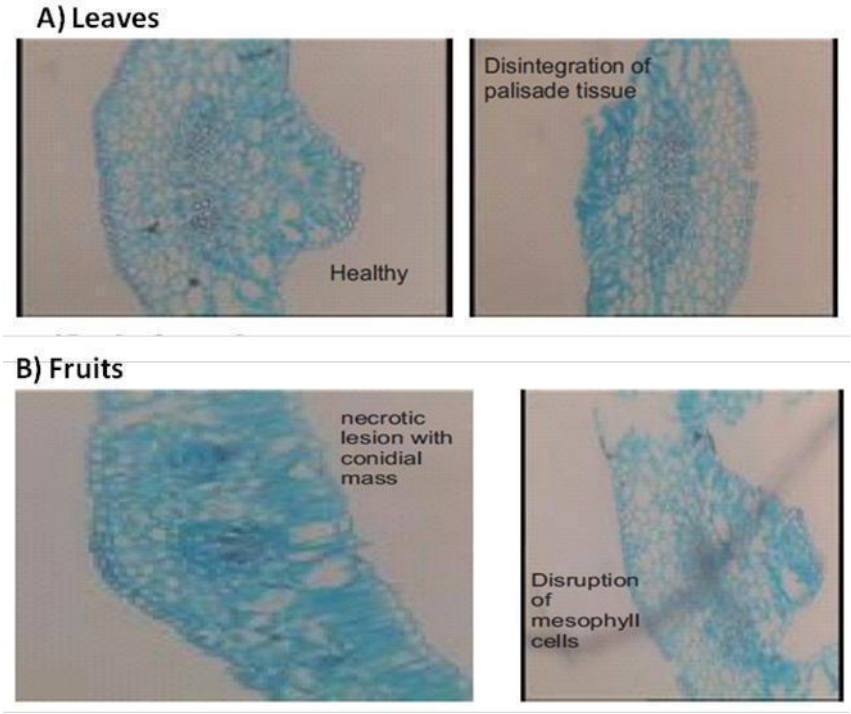

Fig. 1. Histopathological studies of powdery mildew infected chilli.

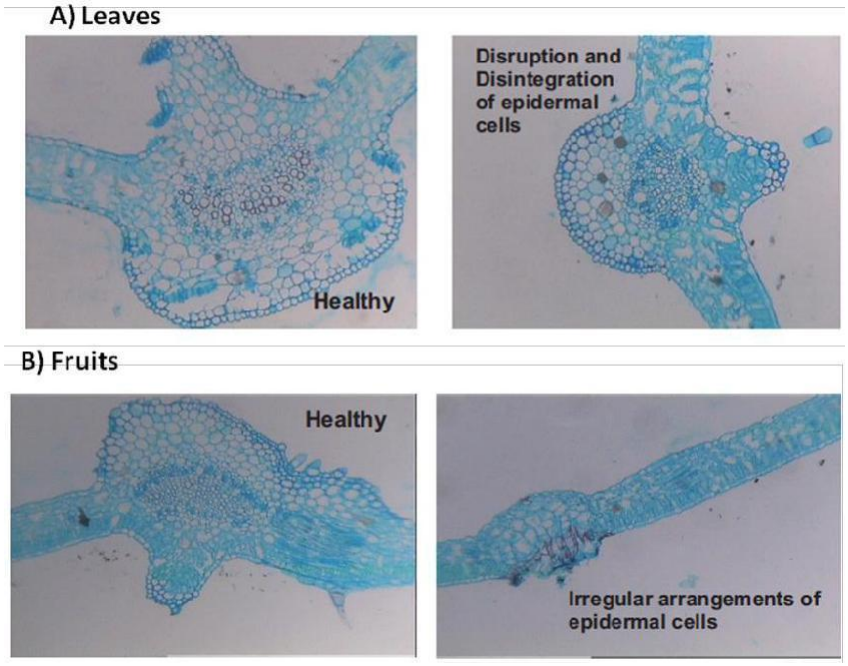

Fig. 2. Histopathological studies of anthracnose mildew infected chilli.

palisade tissue and spongy parenchyma.

The cells were irregularly distributed, and disintegration of stomata and damaged cuticle were also observed. Increase in air space in infected leaf tissue attributed to disintegration of tissue, hence more number of air spaces with reduced size of palisade and spongy parenchyma cells with reduction in number of destruction in shape and size of plastids.

Similar type of observations were made by Prameela et al. (1990) in case of pigeon pea infected by powdery mildew, where infection results in reduction of leaf and epidermal cells thickness, palisade cell length, number of plastids and nucleic acid contents of leaves.

Histochemical studies revealed that the concentration of polysaccharides, proteins and nucleic acids differed between healthy and diseased leaf tissues. Healthy tissues exhibited medium to rich concentration of polysaccharides, total proteins and nucleic acids while the reduction of insoluble polysaccharides, total proteins and nucleic acids in diseased leaf tissues was observed during the present study under report. The pathogen depleted the host leaf tissues of these important metabolites due to fast degradation that is, varied metabolism.

The histochemical localization revealed gradual depletion of polysaccharides, proteins and nucleic acids from the pigeon pea host tissue infected by powdery mildew (Prameela et al., 1990) and from the groundnut host tissue infected by early and late leaf spot (Kaur and Dhillon, 1988; Vijaykumar, 1990).

Conclusion

The infection of the both the pathogens showed a greater changes in the leaves as well as fruits. The observations on the sections of leaves and fruits infected by anthracnose revealed thickening of cell wall with dense and granular cytoplasm. The epidermal cells and cortical parenchyma were collapsed and larger cavities were also seen. Large numbers of small vacuoles were also observed in the cytoplasam. Infected cells become black and both inter cellular fungal cells were present in the infected cells. The xylem vessels were also thickened and both proto and metaxylem were plugged with mycelial network. In the case of powdery mildew infection, the sections showed the collapse of epidermal cells, disruption of mesophyll and guard cells.

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