

HISTOCHEMICAL STUDIES ON PATHOGENESIS IN SEEDLING BLIGHT OF
JUTE INCITED BY *MACROPHOMINA PHASEOLINA* (TOSSI) GOID

BY

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Histopathological and histochemical studies on pathogenesis of seedling blight of jute incited by *Macrophomina phaseolina* indicate that the penetration was mechanical through infection pegs. Infection was confined upto cortical cells, and phloem in some cases. Pectin and cellulose were degraded, while lignin was not affected. Breakdown of proteins into amino acids was noticed in the infected cells. Accumulation of polyphenols in the healthy cells neighbouring the infected ones was observed.

INTRODUCTION

Macrophomina phaseolina is a facultative parasite which grows both intracellular and intercellular inside the host tissue. The pathogen in course of its entry inside the host tissue and establishment in the host tissue primarily depends on enzymes which affect cell wall. Hence in the progress of the disease from initiation of infection to expression of symptoms, changes in cell walls have been studied by histo-chemical methods to have a greater insight into mechanism of pathogenesis.

MATERIALS AND METHODS

The samples for the study were harvested from affected jute plant at three stages of disease development mainly first stage—browning, 2nd stage drooping and the third stage—wilting.

Fixing: The samples were collected each day from the inoculated plants and fixed in formalin-Acetic acid-alcohol.

Study on Pectin: Free hand transverse sections were made through the stem containing both healthy and diseased tissues. They were placed in 10 per cent ferric chloride solution for 5-10 minutes in a watch glass, then washed in distilled water with 3-4 changes, each for 15 minutes. These sections were then transferred to 1-2 droops of 2 percent potassium ferrocyanide solution on a glass-slide. After 2 minutes a drop of 2 percent hydrochloric acid was added to ferrocyanide solution and the sections were mounted in glycerine and observed

under microscope. The cell wall with intact pectic materials will show *Prussian blue* colour, while those where pectin has been degraded by the pathogen will either remain unstained or will be stained lightly.

Study on cellulose : Free hand sections were placed in several drops of Zinc-chlor-iodine solution in a watch glass for 1-3 minutes. These were then mounted in glycerine and observed under microscope. Cellulose show *blue* colouration on staining with zinc-chlor-iodine solution.

Study on Protein : Free hand sections were made for FAA fixed diseased material. They were then placed in 0.5 percent ninhydrin solution in absolute alcohol in a small glass tube, enclosed with rubber cocks and incubated at 37°C for 20-24 hours. Sections were then used in 2 changes of absolute alcohol and then in distilled water.

These were placed in Schiff's reagent in a watch glass covered by Petridish for 3-10 minutes, washed in water for 10-20 minutes mounted in glycerine and examined under microscope. Protein degradation, if any, in the diseased tissues would be indicated by dark red colouration of cells due to presence of more free amino acid during disease development.

Study on lignin : Free hand transverse sections were made from freshly harvested materials and were placed in a few drops of phloroglucinol solution for 2-3 minutes on glass-slides, then removed from the solution, mounted in glycerine and observed under microscope. Presence of lignin in the cell-walls would be detected by *red colouration* of the cell wall. The process of penetration was studied from the peeling of epidermis. Study of colonization of the pathogen inside the host tissue was made on transverse sections of the infected seedling. In the present study, inoculated soil constituted the source of infection.

RESULTS AND DISCUSSION

It was observed that hyphae growing in the soil on coming in contact with surface of the host first colonized there. Hyphal growth was found to follow the lines of junction of the underlying epidermal cells. Appresoria formation was noted of hyphal growth at a later stage after formation. Appressora were formed from the side branches and were not very conspicuous. Penetration of the hyphae through the epidermis was found to take place at the junction of the two epidermal cells. Infection hyphae was observed to enter into the host cells through the side wall of the epidermis.

No visible damage to the epidermal cells was noted at this stage, though the pathogen evidently after penetration secreted enzymes, during course of entry into the host tissues.

Pectin : The lateral cell walls of epidermis and cell walls of cortical cells of

Table 1. Observation on histochemical changes in the jute seedlings due to infection of *M. phaseolina*

Different histo-chemicals	First stage browning (a)	Second stage drooping (b)	Third stage wilting (c)
Pectin	The epidermal cells become infected and the lateral walls of epidermal cells did not take any Prussian blue colour thereby showing degradation of pectin.	The cortical cells and epidermal cells did not show any staining, reaction for pectin. The tissues were disorganised.	Tissues were found to be collapsed.
Cellulose	The infected host epidermal and one or to layers of cortical tissues did not take the stain.	The entire cortical tissues of infected host did not take any blue stain. Tissues were disorganised.	Do
Protein	The epidermal cells and cortical tissues were more intensely stained.	The cortical tissues and cambium tissues of conductive tissues stained more intensely than pith cells showing the presence of more amino acids.	Do
Lignin	No positive result was obtained i. e. there was no change in lignin.	No change noticed.	No change noticed
Polyphenols	The surrounding cells of the diseased host tissues in cortical layers showed the presence of polyphenols	Slight appearance of pink colour in surrounding cells of the infected host tissues.	No demarkation. The tissues were collapsed.

a) Browning-Brown water soaked lesion on the cotyledons; b) Drooping-Enlargement of brown lesion turns into black and drooping tendency of the infected host; c) Wilting: Collapse of the entire host leads to wilting of the host.

Table 2. Presenting the summarised observations on histochemical changes in different tissues infected host plant

List of histo-chemicals	Cuticle	Epidermal tissues	Cortex	Fibre tissues	Phloem vessels	Cambium tissues	Xylem vessel	Pith cell	Healthy host tissues
Wax	—	*	*	—	—	—	—	—	Stained
Cutin	—	*	*	—	—	—	—	—	—
Suberin	—	*	*	—	—	—	—	—	—
Lignin	—	*	*	—	Stained	Stained	Stained	—	Stained
Protein	More stain	* More stain	—	—	—	Stained	—	Stained	Stained
Cellulose	No blue stain	* No stain	* No stain	—	* No stain	—	—	—	Blue stained
Pectin	No stain	* No stain	* No stain	—	—	—	—	—	Prussian blue stain present.
Polyphenols	—	*	* Stain	—	—	—	—	—	No stain

(*) The tissues were infected

(—) Not observed

diseased tissues did not show any reaction of presence of pectin on being stained with ferric chloride, potassium ferrocyanide, while corresponding healthy cells showed positive staining reaction. This denoted that in the diseased tissues the pectic materials had been rapidly degraded by the pathogen.

Cellulose : Staining studies with zinc-chlor-iodine solution revealed that cells of cortical tissues and of phloem of diseased host plants were not stained *blue*, while the cells of corresponding healthy plants were stained *blue*, showing presence of cellulose. Changes were noted only in cells of cortex and phloem and not in pith.

Protein : The infected tissues of epidermis and cortical tissues and cambium layer of conductive tissues were stained more intensely with Schiff's reagent than other cells of the host, while the cells from the infected host were found to be stained lightly.

Polyphenols : The parenchymatous cells, surrounding the infected tissues, showed the presence of polyphenol compounds, by red colour on being stained with 10 per cent ferric chloride solution.

Lignin : Lignin was found to be unaffected.

Histopathological as well as histochemical studies show that lignified tissues are not affected. These observations together with role of pectinolytic and cellulolytic enzymes tend to explain the two distinct phases of the disease, namely, (a) seedling blight and mortality at stages when lignified tissues are not properly developed and the attack on cells causes collapse and death of seedlings, and (b) adult phase wherein no mortality takes place, but secondary bast fibres which are primarily of cellulose are affected without cells with lignified or lignocellulose cell walls being involved resulting in shredding of fibres with consequent effect on yield and quality of fibres.

Histopathological studies also show that infection is located mainly in the cortex and to some extent in phloem. Previous studies by Chattopadhyay and Bhattacharya (1968) on guava wilt caused by *Rhizoctonia bataticola* (sclerotial stage of *M. phaseolina*) also show the same results.

Histochemical observations show the presence of phenolic substances in the cells bordering the infected ones. Evidently presence of these phenolic substances indicate the limitation of invasion due to reaction of the host. In recent years, polyphenols and polyphenols oxidase system have been found to play a very significant role in delimiting the attack of pathogens on the host plant and host plant resistance.

In case of attack of *Macrophomina phaseolina* on jute such a situation is likely to play an important part. As phenolic substances have been found in the areas neighbouring infection court. detailed study of role of polyphenols oxidase on delimitation of attack and resistance as a whole in adult phase may be of worth investigation.

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