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Full Length Research Paper

Histopathology of *Raphia hookeri* leaf Infected with *Glomerella cingulata* causing seedling blight

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The host-pathogen relationship between the *Raphia hookeri* seedling leaf and *Glomerella cingulata* showed that the host cells were macerated. This allowed penetration of the pathogen through the hypodermis to the palisade tissue which resulted in dark coloration and disruption of the transfusion tissues. Hypersensitivity reaction was observed. The xylem and phloem were left without any attachment or support. Their intact cells were darkened as a result of disease development which resulted in complete disintegration of the palisade tissues and disappearance of transfusion cells. The uninoculated seedling leaf remained healthy with glossy appearance.

Key words: *Glomerella cingulata, Raphia hookeri,* seedling blight.

INTRODUCTION

Glomerella cingulata was found to be the causal organism of seedling blight of *Raphia hookeri* (Oruade-Dimaro and Ekundayo 1992). *G. cingulata* has been reported as mulberry anthracnose fungus. The organism was isolated from leaf spot and blight symptoms on Kiwifruit (Ho, 2008). Histopathological study of leaf infected by fungus helps in elucidating how the fungus colonizes leaf surface, palisade and vascular tissues. It helps in showing the mode of entry of the pathogen (William, 1957). It is also used to study the nature of antagonism between the fungi and their hosts. Such study further enhances the formulation of most suitable means of controlling plant pathogens (Porto et al., 1988).

Literature review has shown that no work has been reported on the histopathology of *Raphia hookeri* leaf infected with *G. cingulata* in Nigeria. This study was to examine the host-pathogen relationship using histopathological method.

MATERIALS AND METHODS

The *G. cingulata* (Herbarium IMI 283849) used in this study was isolated from naturally infected *R. hookeri* seedling leaf showing symptom of seedling blights. Re-infecting healthy seedling leaf, the re-isolated fungus was *G. cingulata* which showed symptoms of seedling blight.

Sample preparations and microtome sections

Leaf samples were collected from healthy seedling leaf with no symptom which served as the control treatment (Plate 1) and healthy seedling leaf which was artificially infected with *G. cingulata* showing the symptoms of seedling blight (Plate 2). They were dehydrated, fixed, impregnated with wax, embedded, sectioned (6-8 µm thick) with a rotary microtome (Erma, Tokyo), and stained according to the methods of Sass (1958).

Observations

The mounted sections in Canada balsam from microtome sections were carefully studied under a light microscope (ZEISS West Germany). Observations were made with regards to intact cells of healthy tissues, impact of the pathogen on host tissue and histopathological changes resulting from presence of the pathogen. Photomicrographs were made where necessary with an attached camera (Motic MCCamera) to the microscope connected to a computer.

RESULTS

Microtome sections of healthy *R. hookeri* seedling leaf revealed that it was made up of transfusion tissue which is concerned with the translocation of water and food

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Plate 1. Healthy *R. hookeri* seedling leaf with no symptom of disease.

Plate 2. *R. hookeri* seedling leaf artificially infected with *G. cingulata.*

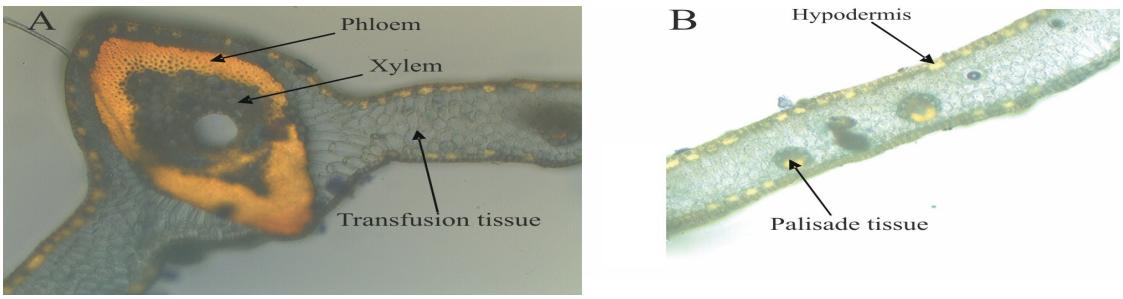

Figure 1. (×100). Microtome transverse section (TS) of healthy *R. hookeri* seedling leaf. A, mid-vein. B, blade of *R. hookeri* leaf (×100).

materials between the vascular bundles. The xylem is concerned with water conduction, storage and support while the phloem is a complex tissue which contains parenchyma cells. It is also concerned with conduction, storage of food and with support. The palisade tissue occurred on the lower side, arranged on the axis of the leaf, while the hypodermis occurred on the upper side (Figure 1b).

Sections of infected *R. hookeri* seedling leaf revealed that disease development started from the hypodermis with brown coloration which extended to the palisade tissue. The coloration is deepened, showing hypersensitivity reaction (Figure 2b and c). Complete disintergration of palisade and disappearance of transfusion tissues were seen, leaving the xylem and phloem without any attachment or support. The intact cells in the xylem and phloem were darkened as a result of disease development (Figure 2d) when compared with

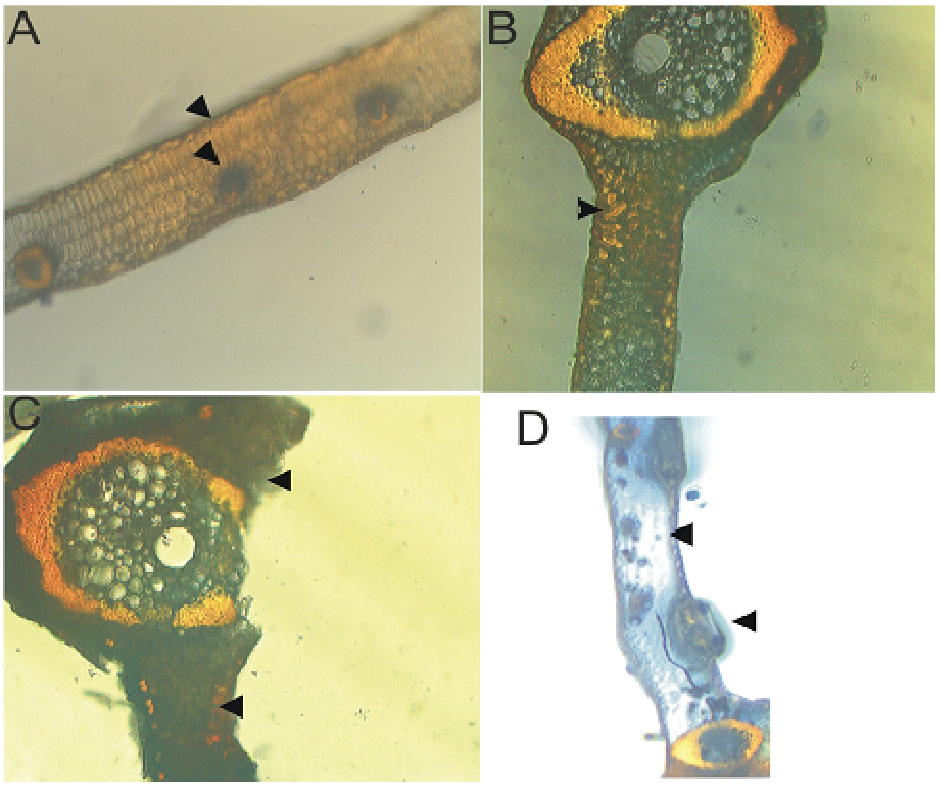

Figure 2. Microtome transverse sections of *Raphia hookeri* seedling leaf infected with *G. cingulata* (x100). (A. Arrow heads show initial infection points from the hypodermis to the palisade tissue; B. Arrow head shows progression of invasion and coloration of hypodermis, and part of the transfusion tissues; C. Arrow head shows deepened coloration. D. Arrow heads show disintegration of palisade tissue and disappearance of transfusion cells eight days after inoculation.

the control (Figure 1a).

DISCUSSION

The host cell showed hypersensitive reaction in the presence of *G. cingulata.* A similar observation was made by Eziashi et al. (2007). Interaction between the host and the pathogen also showed that the cell was macerated. It was observed that disease development from the epidermis gave rise to maceration in and around the palisade tissue. The maceration of cells may have resulted from the action of hydrolytic enzymes produced by *G. cingulata.* Products of the enzymatic breakdown of cell components will most probably serve as a source of nutrients to the pathogen. Extracellular hydrolytic enzymes have previously been found to be produced by rot pathogens (Nwufo and Fajola, 1988; Adisa and Fajola, 1983).

In advanced stage of *G. cingulata* infection, the infected tissues turned black. The black coloration is still not fully understood, but Clark et al. (1981) reported the accumulation of furanoterpenoids in sweet potato following inoculation with *Botryodiplodia theobromae*. The mode of penetration of *G. cingulata* into cells was not investigated. It might have been mechanical, enzymatic or a combination of both. Enzymatic penetration will mean that cellulase may have been produced by this pathogen. Adisa and Fajola (1983), Turner and Ogundana (1983) and Arinze et al. (1976) have reported production of cell wall-degrading enzymes by *B. theobromae*. The total disappearance of the intact cells confirmed invasion of the host by the pathogen. However, the enzymes involved in this study were not determined. The result of this investigation could be exploited in the management of folia disease associated with *G. cingulata.*

REFERENCES

Adisa VA, Fajola AO (1983). Cellulolytic enzymes associated with the fruit rots of *Citrus sinensis* caused by *Aspergillus aculeatus* and *Botryodiplodia theobromae.* Mycopathologia 82(1): 23-27.

Arinze AE, Naqvi SHZ, Ekundayo JA (1976). Production of extracellular cellulolytic and pectolytic enzymes by *Lasiodiplodia theobromae* on sweet potato (*Ipomoea batatus*) tubers. Int. Biodeterioration Bull., 12(1): 15-18.

Clark CA, Lawrence A, Martin FA (1981). Accumulation of furano terpenoids in sweet potato tissues following inoculation with

pathogens. Phytopathology 71: 708-711.
Eziashi EI, Omamor IB, Oruade-Dimaro EA (2007). Histopathology of *Ceratocystis paradoxa* causing Black Seed Rot on Oil Palm Sprouted Seeds. Advances in Food Sciences 29(4): 224-227.
Ho J (2008). http://www.riceblast.snu.ac.kr/file/58/57371 04-24 (2)-06.pdf.
Nwufo ML, Fajola AO (1988). Production of amylolytic enzymes in culture by *Botryodiplodia theobromae* and *Sclerotium rolfsii* associated with corn rots of *Colocasia esculenta*. Acta Microbiologica Hungaria 35: 371-378.
Oruade-Dimaro EA, Ekundayo CA (1992). Seedling blight of *Raphia hookeri* Man and Wendl. caused by *Glomerella cingulata* (Stonem). Sprauld and Shrunk in Nigeria. Princepes J. Int. Palm Soc. USA. 35(4): 41-44.
Porto MD, Grau CR, De Zoeten GA, Gaard G (1988). Histopathology of *Collectotrichum trifolii* on alfalfa. Phytopathology 78: 345-349.
Sass JE (1958). Botanical Microtechnique Jowa State University Press, Jowa p. 229.
Turner JG, Ogundana SK (1983). Production of yam cell-wall degrading enzymes by *Bothryodiplodia theobromae*. Transactions of British Mycological Society 80 (1): 163-166
William RJ (1957). The control of cowpea diseases in the IITA grain leagume improvement program. In: Tropical diseases of Legumes (ed. Bird, J. and Marancrosch, K.). Academic Press Inc. New York p. 172.