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Histopathological Study of Soybean Rust and Anthurium Leaf Blight in the Philippines

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Abstract: Histopathology of soybean rust (*Phakopsora pachyrhizi* Sydow) and anthurium leaf blight (*Xanthomonas campestris* pv. *Dieffenbachiae*) were studied. The stage uredia were found in soybean rust infected sample containing urediospores of round to oval shaped with yellowish brown or hyaline in colour. Disintegration of plant cells, presence of bacterial cells, and clogging of xylem vessels were observed in the anthurium leaf blight infected samples.

Key words: Histopathology, soybean rust and anthurium leaf blight

Introduction

Studies on the host anatomy after infection by a pathogen is sometimes important for correct identification of causal organism. A histological symptoms produced internally can be observed after dissection of diseased tissues and examination under microscope. The enlargement of cells, presence of pathogen's spores and discolouration of the vascular bundles are said to be histologic symptoms (Ilag, 1987).

The obligate parasite like, rust fungi are host specific and they have different races. Soybean (*Glycine max* L.) rust caused by *Phakopsora pachyrhizi* Sydow is most important and it is a major constraint in soybean production in the Philippines (Quebral, 1988). It has wide host range. Yeh (1981) reported 87 hosts of soybean rust. *Pueraria pulcherrima* is reported to be important source of initial infection of soybean by the fungus in Philippines (De La Cueva, 1994).

Anthurium andreanum Lind. is an important ornamental plants, its production is limiting due to bacterial disease commonly referred to as anthurium leaf blight caused by *Xanthomonas campestris* pv. *Dieffenbachiae* (Natural *et al.*, 1990). The disease was observed in early 1980's in the Philippines (Divinagracia, 1983). The typical symptoms of the disease are the yellowing of the leaf margins of infected leaf area. Initially minute water-soaked 1-2 mm in diameter lesion appears, with the progress of time the affected areas develop necrosis, surrounded by bright yellow, water-soaked border (Natural *et al.*, 1990).

This experiment was conducted to study the histopathology of soybean rust (obligate parasite) and anthurium leaf blight (facultative parasite) for practical experience on the internal symptoms produced by these pathogens.

Materials and Methods

Soybean rust infected leaves (variety TK-5) with typical symptoms were collected from the field of the Institute of Plant Breeding (IPB), Los Banos, Laguna, Philippines and anthurium leaf blight infected leaves were obtained from the department of Plant Pathology, UPLB, Philippines. Diseased specimens were cut into small pieces (5 mm sq. size) and were preserved in Rawlin's FAA # II (50% ethyl alcohol 100 ml, formalin 10 ml and glacial acetic acid 10 ml) solution for 24 h. The glass slides were kept in the solution of potassium dichromate (20 g), concentrated sulfuric acid (100 ml) and water (100 ml) for 2 days in order to clean them and then preserved in ethyl alcohol. Haupt's adhesive were used on the slides (gelatin 1 g in 100 ml water at 90°C and after cooling at 30°C added 15 ml glycerin and 2 g of phenol) and then stored the slides in the refrigerator.

Both soybean rust and anthurium leaf blight infected samples were moved through a series of ethyl alcohol (EA) and tertiary butyl alcohol (TBA) solutions which contained progressively higher concentrations of alcohol and lower concentration of water for dehydration. Tertiary butyl alcohol (TBA) is reported to be the best dehydration reagent for plant materials and for anatomical studies (Natural, 1994), as described below:

Tertiary butyl alcohol dehydration schedule:

Solution	**Time of exposure**
Solution No. 1 (Distilled water 50 ml, 95% EA 40 ml , TBA 10 ml)	2 h
Solution No. 2 (Distilled water 30 ml, 95% EA 50 ml , TBA 20 ml)	overnight
Solution No. 3 (Distilled water 15 ml, 95 EA 50 ml, TBA 35 ml)	1 h
Solution No. 4 (95% EA 45 ml and TBA 55 ml)	1 h
Solution No. 5 (TBA 75 ml and Absolute alcohol 25 ml)	1 h
Solution No. 6 (100% TBA)	1 h
Solution No. 7 (pure TBA)	1 h
Solution No. 8 (100% TBA)	Overnight
Solution No. 9 (TBA 50 ml and paraffin oil 50 ml)	1 h

A vial was filled 3/4 full of melted paraffin and allowed to solidify but not cooled completely 5 min before changing the solution No.9 above. Samples were placed to be embedded on top of solidified paraffin and just covered with the tertiary butyl alcohol-paraffin mixture. The vial was placed in the oven with the specimens for a period of 1 h until the material has sunk to the

bottom of the vial. Poured off entire mixture of paraffin oil and a trace of alcohol remain and replaced it with pure melted paraffin as follows:

	Time
Pure melted paraffin	2 h
Pure melted paraffin	2 h
Pure melted paraffin	Overnight
Pure melted paraplast	1 h

Embedded in paraplast

Embedding

The specimens were taken from oven and placed in the small embedding dish containing melted paraffin (four samples were placed in a dish). Before placing the samples the inside of the dish were coated with glycerin. A warm needle was used for arranging and orientation of the tissues such a way that individual pieces can be cut easily from the finished block and placed the dish on the surface of a ice water immediately after placing the tissues in the melted paraffin and then solidified. After the block has completely cooled, removed it from the ice and placed it in the refrigerator. The block was removed from the dish and placed individual tissue with paraffin on a wooden block by carefully heating the bottom of paraffin and the top of the wooden block. A scalpel was used to built up a mound of paraffin at the base of the piece to add strength. The block was placed in the refrigerator for cooling the paraffin.

Microtome sectioning

The block was taken out from the refrigerator and was trimed with sharp scalpel and the excess paraffin was removed from around the tissue leaving the base strong. Before sectioning in the microtome the block was placed in the refrigerator for few minutes. The block was fixed in the microtome so that the edge of the block is just near and parallel with the blade. The razor blade was tilted about 8° from vertical so only the edge touched the block. The thickness of the ribbon was selected and turned in a clockwise direction with a steady, even stroke. A fine hairbrush was used to hold the ribbon so that it can not curled. After ribbon started with tissues used a needle for remove it from the microtome and placed it on the slide (Haupt's adhesive was used on the slide and dried). The razor blade was cleaned during sectioning by cotton moistened with xylene very gently for preparing good ribbon.

Ribbon mounting

Small pieces ribbon as the length of the slide (2 pieces in each slide) were placed and the slide was flooded before placing the ribbon on the slide, then it was placed on the slide warmer at 40°C for overnight. Twenty slides were prepared for each of the disease sample (Soybean rust and anthurium leaf blight).

Slide processing and staining

After drying the slides on the slide warming tray they were passed through a series of chemicals and stainer following the Sass-Safranin-Fast Green methods (Natural, 1994).

After staining the objects were smeared with canada balsam and covered with cover slips and placed on the slide warming tray at 40°C for a period until the canada balsam spread evenly thoroughly under the cover slip. Then the slides were examined under the microscope (low and high power) for observing fungal fruiting structures (Soybean rust) and bacterial cells in the xylem vessels (anthurium leaf blight). Photographs were taken for some slides using photomicroscope showing anatomical structure of both specimens (40x magnification).

Results and Discussion

Urediospores of soybean rust fungus (*Phakopsora pachyrhizi)* were observed under microscope. Urediospores in the uredium are hyaline to yellowish brown, oblong; the uredium are short and light brown in colour (Fig. 1, 2, 3). In Fig. 1 uredium of soybean rust with several urediospores are presented. In Fig. 2, dispersal of urediospores from uredium and in Fig. 3, it was shown that 2 uredia of soybean rust observed under same field. The fruiting bodies are yellowish brown and the host tissues are green colored. Some urediospores were observed inside the uredium and some were in dispersed from uredia. Similar structures of uredia and urediospores of soybean rust were reported earlier (Bromfield, 1976; Quebral, 1988).

Fig. 1: Uredium of soybean rust (*Phakopsora pachyrhizi* Sydow) containing several urediospores

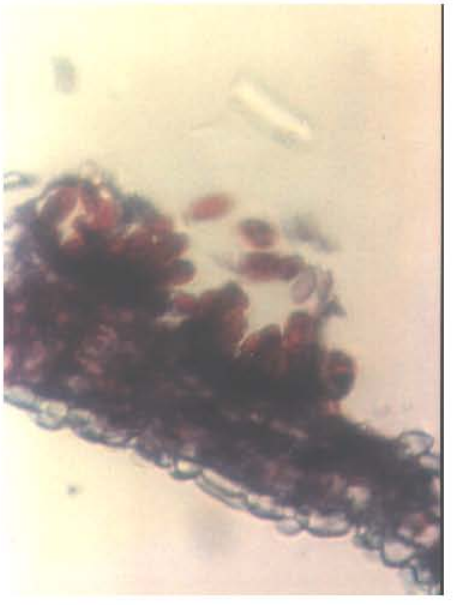

Fig. 2: Urediospores of soybean rust (*Phakopsora pachyrhizi* Sydow) dispersed from uredia

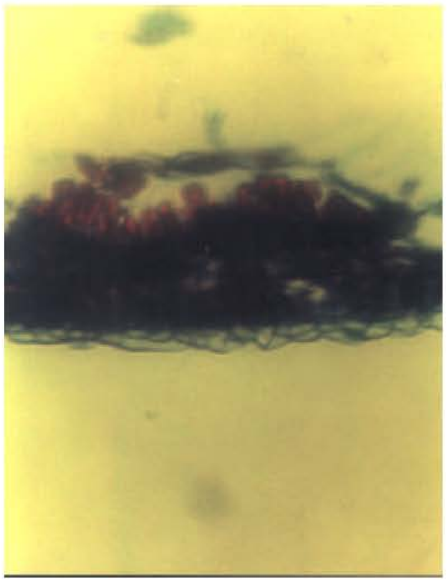

Fig. 3: Two uredia of soybean rust (*Phakopsora pachyrhizi* Sydow) observed under same field

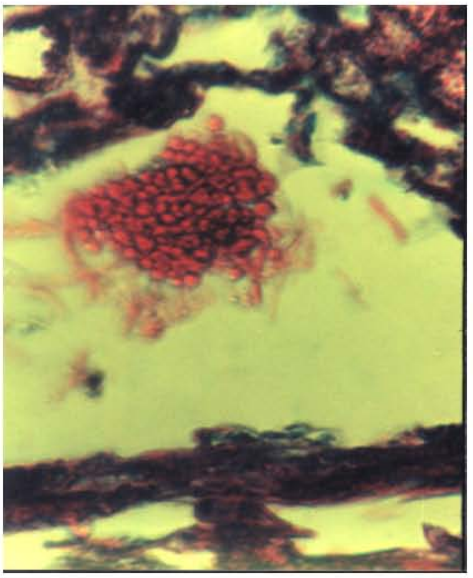

Fig. 4: Presence of bacterial cells of *Xanthomonas campestris* pv. *Dieffenbachiae* (anthurium leaf blight) and disrupted plant cells

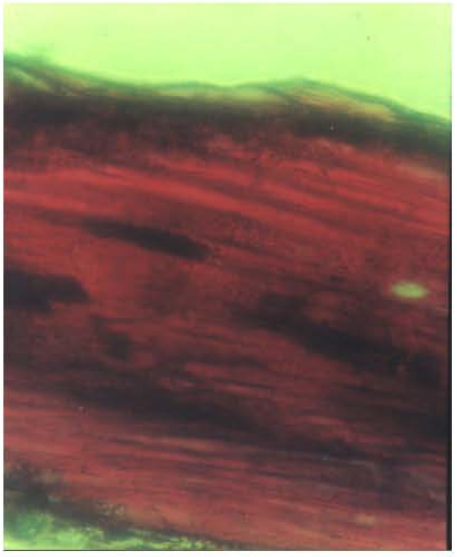

Fig. 5: Clogging of xylem vessels due to bacterial infection (*Xanthomonas campestris* pv. *Dieffenbachiae)*

The histopathology of *Xanthomonas campestris pv. dieffenbachiae* of the anthurium leaf blight is presented in Fig. 4, 5. Host anatomical changes due to the bacteria has been observed.

Presence of bacterial cells and disintegration of cells were observed under microscope (Fig. 4). The discoloration and or blocking of xylem vessels also observed (Fig. 5). Earlier it was reported that bacteria have been systemically translocated from infected leaves to the stem and other parts and bacteria were found in the xylem vessels; they prevent the translocation of water and mineral materials to the leaves (Natural ***et al.***, 1990). Normal host anatomy is modified, disrupted cells, occluded intercellular spaces by ***X. campestris pv. citri*** in ***Citrus aurantifolia*** was reported by Lawson ***et al.*** (1989). The findings of the results will be helpful for further research activities.

The results of the histopathology of both pathogens using the protocol mentioned above showed useful for proper identification of the pathogen. For proper identification of the pathogen, study on the histopathology plays an important role in plant pathology.

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