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HISTOCHEMICAL CHANGES IN PEARL MILLET PLANTS GROWN FROM Sg-TOXIN TREATED SEEDS

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ABSTRACT : Histochemical changes in the pearl millet seeds soaked in 2 per cent Sg-toxin and in the leaves of seedlings raised from treated seeds were studied. No noticeable changes occurred in the embryo and endosperm in the seed at the time of germination. However, the number of polysaccharide granules increased during the initial symptoms (stage I) in the bundle sheath cells of leaves. Subsequently at stage II, the number of PAS positive granules decreased and disappeared at stage III. The proteinoplasts were less stained at stage I, which gradually disintegrated and ultimately disappeared. From stage I to stage III of symptom development nucleic acids gradually decreased.

Keywords : Histochemical changes, Pearl millet Sg-toxin

Sclerospora graminicola is an obligate parasite causing downy mildew disease in pearl millet (*Pennisetum typhoides*). Wani and Rai (1979a, b) reported the occurrence and characterization of Sg-toxin in pearl millet plants infected by *S. graminicola*. This toxin is not host specific but group specific. Even though this disease causes devastating damage to pearl millet, the exact mechanism of syndrome initiation is not understood. However, the toxin initiates changes in the treated tissues, some of the histochemical changes are reported in this paper.

MATERIALS AND METHODS : Seeds of HB 3 variety were soaked in 2 per cent Sg-toxin solution for 12 hr. and germinated at room temperature in petri plates containing moist filter paper. The germinating seeds, the leaves from plants showing symptoms (grown from seeds soaked in toxin) and leaves from healthy plants of corresponding age were selected. Leaves showing symptoms were collected at three stages of disease

TABLE 1 : Different tests used to detect the histochemical changes in the Sg-toxin treated cells

Chemical	Test (Jensen, 1962)	Indication
Insoluble polysaccharides	Periodic acid Schiff's (PAS) Test	Magenta
Starch	Iodine-Potassium Iodide (IKI) test	Brownish violet or deep blue
Protein	Mercuric bromophenol blue test	Deep blue
Nucleic acid	Azure B method	DNA-greenish RNA–purple or deep blue
	Methyl green pyronin test	DNA–green or deep blue RNA–deep purple

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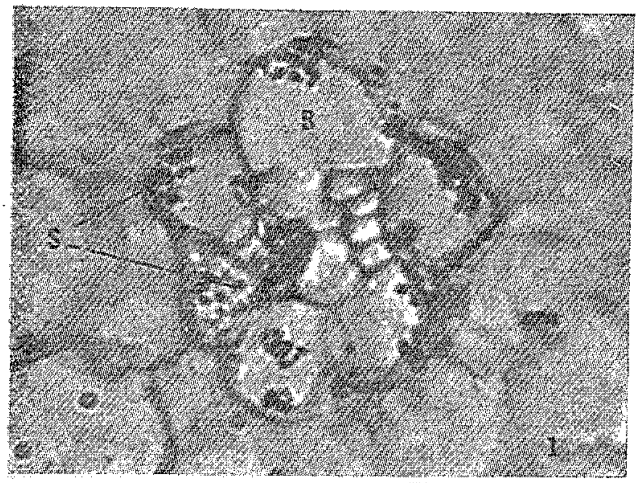
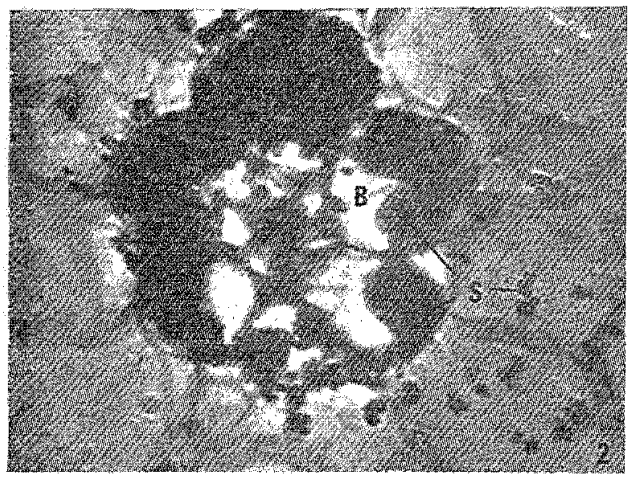
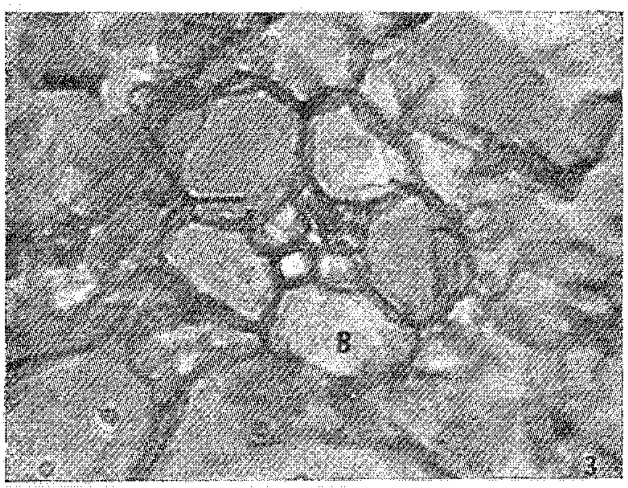
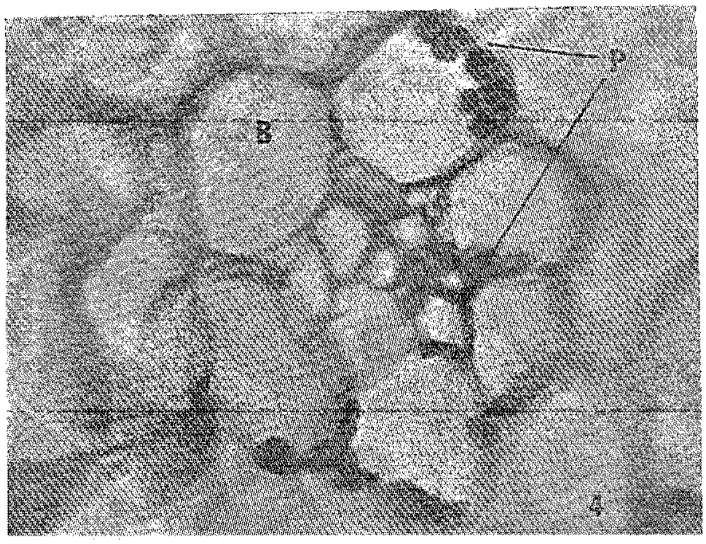
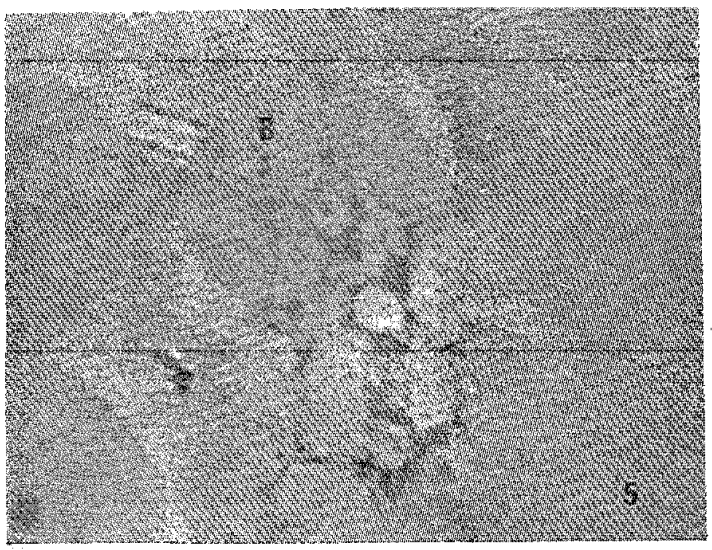






Figs. 1—3. Histochemical changes in the leaves of Pearl millet brought about by toxins secreted by *S. graminicola* (Magnification 400 ×). Starch content in the bundle sheath of leaves. 1. Normal leaves grown from control seeds. 2. Stage I—starch content has increased. 3. At stage III starch has completely disappeared. B—bundle sheath s—starch.





Figs. 4—5. Histochemical changes in the leaves of Pearl millet brought about by toxins secreted by *S. graminicola* (Magnification 400 ×). Protein content in the bundle sheath of leaves. 4. Stage I—the proteinoplasts are fewer in the cells. 5. stage III—the proteinoplasts have completely disappeared. B—bundle sheath P—proteinoplasts.

progress ; Stage I–partially chlorotic, stage II–completely yellow and stage III–curled and partially dry. The materials were fixed in Carnoy's B (6 parts of ethyl alcohol+3 parts of chloroform+1 part of acetic acid) for one hour, washed in 80 per cent alcohol and dehydrated using alcohol–butanol grades. The materials were embedded in paraffin wax and serial sections of 8 μm thickness were cut and treated with various stains (Table 1). Suitable controls were kept.

RESULT : Differences were not significant between the control and Sg-toxin treated seeds during germination. However, significant morphological and histochemical changes were caused by Sg-toxin in the treated plants.

The toxin caused distinct changes on the insoluble polysaccharide content of bundle sheath cells in leaves. The number of polysaccharide grains increased in stage I (Fig. 2). This increase was followed by a gradual decrease in stage II and the polysaccharides completely disappeared in stage III (Fig. 3).

Mesophyll cells contained smaller protein positive bodies than the bundle sheath cells.

At stage I (Fig. 4) the proteinoplasts appeared distorted and took up less stain compared with the control indicating the degradation of protein material in the plastids. At stage II, further distortion in the shape of proteinoplasts and lessening in stainability occurred. In stage III, protein positive bodies completely disappeared (Fig. 5).

In control, both mesophyll and bundle sheath cells contained some RNA which decreased at stage I and II disappeared completely at stage III. Nuclei in the leaf cells of control, and those at stage I and II stained for DNA but at stage III there was hardly any staining.

DISCUSSION : The increase in number of polysaccharide grains in the bundle sheath cells of leaves at stage I of symptoms development may either be due to increased activity of polysaccharide synthesizing enzymes or due to decreased activity of catabolic enzymes. The gradual loss of polysaccharide grains in stage II and their complete disappearance in stage III indicated that the synthesized polysaccharides were completely utilised for cellular metabolism. In plants affected by obligate parasites, starch accumulates in the photosynthetic tissues (Akai *et al.*, 1967) and in the cytoplasm of non-photosynthetic tissues (Williams *et al.*, 1968). However, the mechanism of increase in starch in the initial stages of infection followed by reduction in the subsequent stages is not understood. Whether Sg-toxin activates polysaccharide degrading enzymes or directly interferes with polysaccharide accumulation is not clear. Sg-toxin equally affected the proteinoplasts and nucleic acids in the treated cells. The mechanism is not known.

Admittedly treatment of pearl millet tissue with Sg-toxin causes drastic changes in polysaccharides, proteins and nucleic acids. How these changes are caused by toxin treatment is open for experimentation.

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