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Ultrastructure of the Fungus Causing Rust on Broadbean in Bangladesh

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The characteristic features of the fungus causing rust on broadbean (*Vicia faba*) were recorded and the fungus was identified as *Uromyces vicia-fabae*. Ultrastructural observation on different types of spores and infection structures of the fungus were made using scanning electron microscope. Aecia were observed on the abaxial leaf surface having smooth walled aeciospores in small groups. Uredia are amphigenous, urediospores are broadly ellipsoid to globoid, pediculate and uniformly echinulate, spines pointed and germ pores 5-7, scattered on the wall of urediospores. Telia were observed on both leaf surfaces, teliospores are obovoid with smooth wall and short pedicel. In some cases, urediospores and teliospores were found to present in the same pustule. Germinating urediospores were found on the leaf. When they germinated close to stomata, their germings formed appressoria over them and short infection pegs developed from the appressoria put forth into the stomatal aperture. Well branched intercellular mycelia were also observed in the cells of infected leaves. Cylindrical and slightly curved substomatal vesicles and bilobed haustoria were found in the cross sections of leaves.

INTRODUCTION

Rust fungi have to undergo morphological changes before they can infect a host plant. The initiation of their penetration into the hosts involves a considerable complicated process. Urediospores germinate on leaf surface, the germings go through a series of morphological events forming sequentially appressoria, infection peg, substomatal vesicles, infection hyphae and haustoria mother cells. It was demonstrated using scanning electron micrographs of artificially induced infection structures that every rust species has a characteristic, different, external morphology which is apparently identical to that seen during host infection (Paliwal and Kim, 1974 ; Wynn, 1976 ; Harder, 1984 ; Staples and Mack, 1984). *Uromyces vicia-fabae* (Pers.) Schroet is a circumglobal fungus causing rust on various species of plants (Cummins and Stevenson, 1964 ; Kapoori and Sinha, 1966 ; Talukder, 1974 ; Cummins, 1978 ; Hossain and Miah, 1988). Broadbean is a common pulse in Bangladesh. It was also found to be attacked by rust but its causal fungus yet to be reported. Detailed features of various structures of the fungus developed on broadbean leaves have not yet been studied in the country.

The present piece of work was undertaken to identify the causal fungus of the disease and to study the characteristic features of different types of spores and infection structures of the pathogen, developed on broadbean leaves under natural

conditions of Bangladesh, using scanning electron microscope (SEM).

MATERIALS AND METHODS

Symptoms of the disease were recorded observing naturally infected plants of broadbean grown at the experimental farm of Institute of Postgraduate Studies in Agriculture (IPSA), Salna, Gazipur, Bangladesh during winter 1987-88. Infected leaves of various ages were collected from the field for SEM observation. Small pieces (3 mm \times 3 mm) of infected leaves were prefixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 hr and postfixed in 2% osmium tetroxide in the same buffer for 2 hr at room temperature and dehydrated with a series of five graded ethanol viz. 50, 70, 90, 99.5 and 100% ethanol for 5, 10, 20, 40 and 60 min, respectively. The dehydrated specimens were dried in a vacuum desiccator attached to a running water aspirator for 3 hr. The specimens were mounted on SEM stub with double side adhesive tape, coated with gold (ca. 230 Å) with a JFC-1100 Ion Sputter and observed under a JEM T-220 SEM at 10 KV accelerating voltage. The important features of different types of spores and infection structures developed by the fungus were recorded. The fungus was identified following the descriptions of Cummins (1978).

RESULTS AND DISCUSSION

The disease was manifested by its characteristic symptoms. Pustules of rust were observed on the leaves and stems after blossoming. Reddish brown pustules of uredia were found on both surfaces of leaf, whereas aecia were observed only on the lower surface in small groups. The teliospores were also observed on both surfaces of leaf and they were mostly obovoid with smooth wall. The causal fungus was identified as *Uromyces vicia-fabae*.

Young and developed uredia were observed underneath the leaf cuticle. Growing urediospores push the cuticle upward (Fig. 1A). At maturity the uredia were observed to break through the leaf cuticle and densely populated urediospores appear in the pustules which are surrounded by fragments of ruptured host cuticle (Fig. 1B and C). Matured urediospores are broadly ellipsoid, uniformly echinulate with 5-7 germ pores on the wall (Fig. 1D). Pedicel of urediospores is short and looks like the jointed femur and tibia of an insect (Fig. 2A). The spines are conical, pear-shaped with pointed and slightly curved tip. They are uniformly distributed on the spore wall. The spines reside in small polygonal depression with raised annuli (Fig. 2B). Uredia and urediospores are comparatively larger than aecia and aeciospores respectively (Fig. 2C). In some cases, urediospores and teliospores were found in the same pustules (Fig. 2D).

Germinating urediospores were observed over stomata and also away from them. The germings formed over the protruding stomatal lips gave rise to appressoria (Fig. 3A). The appressoria developed over stomata were found to form short infection pegs which put forth into the stomatal aperture (Fig. 3B). The process of appressorium and infection peg development indicates the ability of the fungus to enter the host through the stomatal aperture, as recorded in other rust fungi (Wynn, 1976 ; Harder, 1984). When urediospores were found to germinate away from stomatal aperture or the

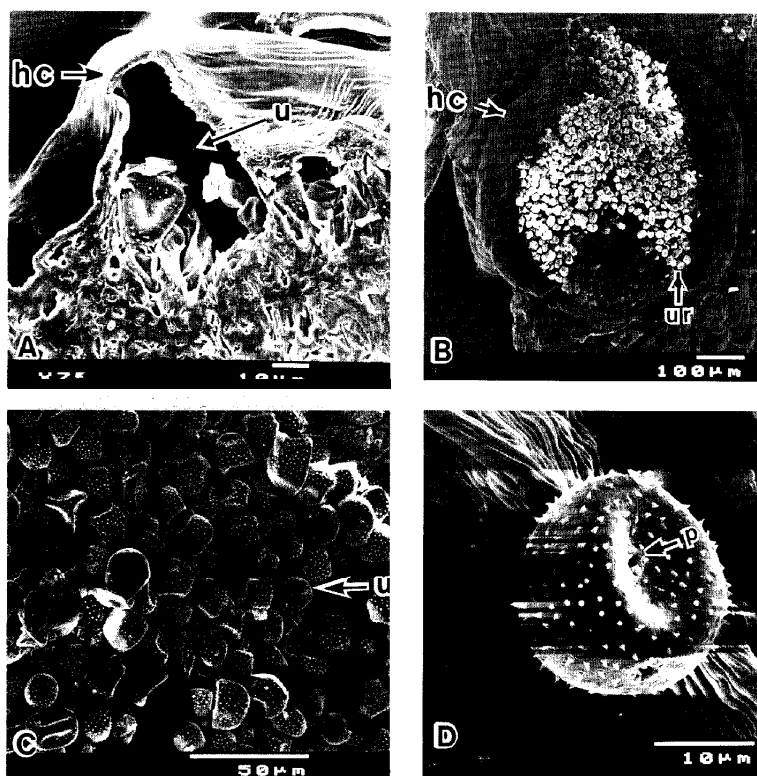


Fig. 1. Scanning electron micrographs of different structures of *Vromyces vicia-fabae* on broadbean.

(A) Developing urediospores (u) pushing the leaf cuticle (hc).

(B) Uredial pustule breaks through the host cuticle (ur) Urediospores, (hc) fragments of ruptured host cuticle.

(C) Mass of urediospores (u).

(D) Urediospores with germ pores (p).

sporelings failed to find an open stoma the appressorium became extremely larger, flattened and bulky (Fig. 3C). Wynn (1976) also found similar types of appressoria when the urediospores of *U. phaseoli typica* germinated over closed stomata and on intact artificial membranes. It was also observed that urediospores of *U. vicia-fabae* can germinate on dead and dried leaves of broadbean under natural conditions but appressoria are formed only when the leaf surface was wavy with large ridges (Fig. 3C). The germling developed from a urediospore germinated on older leaf having smooth surface and no stomatal lip was found to be elongated, cylindrical and comparatively narrow, and it did not form appressorium (Fig. 4D). The present findings indicate that *U. vicia-fabae* needs protruding stomatal lips or wavy leaf surface with

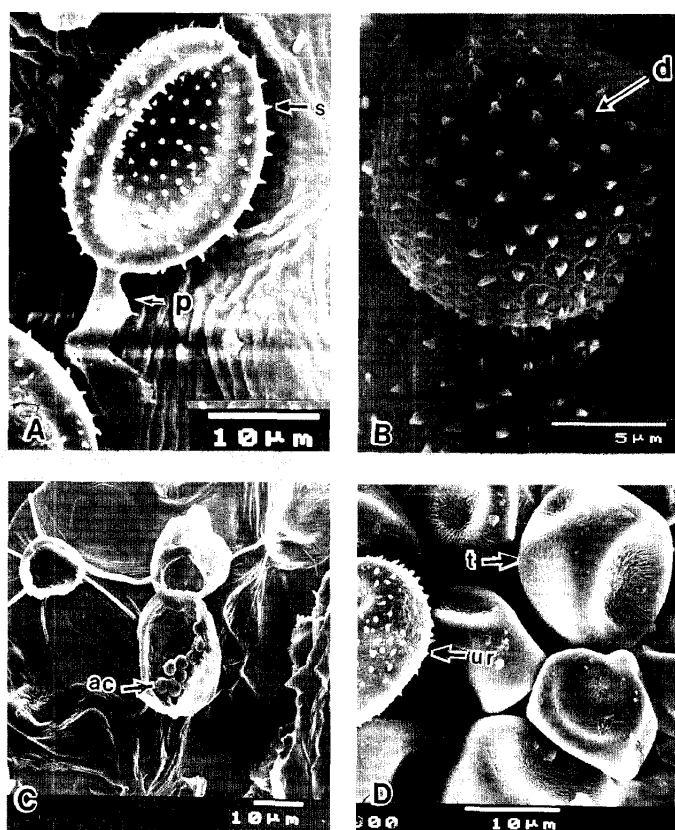


Fig. 2. Scanning electron micrographs of urediospores, aeciospores and teliospores of *Uromyces vicia-fabae*.

(A) Urediospores with spines (s) and pedicel (p).

(B) Spines on the urediospore reside in small polygonal depression (d).

(C) Aecium with aeciospores (ac).

(D) Teliospores (t) and urediospores (ur) in the same pustule.

large ridges to form appressoria, like *U. phaseoli typica*. Using scanning electron microscopy of artificial membranes, it was shown that *U. phaseoli typica* forms appressoria only when their urediosporelings grow over the relatively large ridges of craters and scratches. The investigator mentioned that similar natural stimuli might be the protruding stomatal lips (Wynn, 1976).

Cylindrical and slightly curved vesicles (Fig. 4A) and bilobed haustoria (Fig. 4B) were found in the epidermal cells of leaves. Well branched intercellular mycelia were observed in the cells of infected leaves (Fig. 4C). In some cases, tips of growing hyphae were found to push out through the stomata of the lower surface of leaves (Fig. 4D).

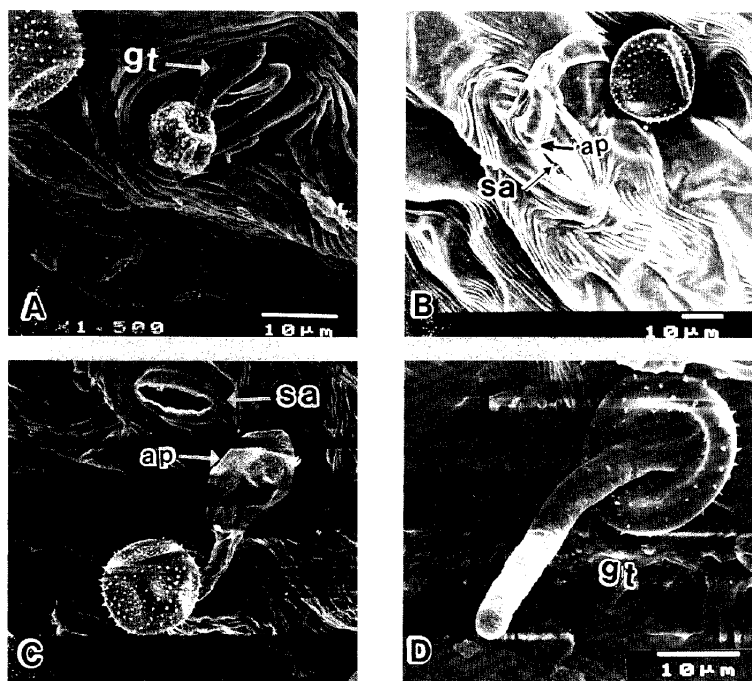


Fig. 3. Scanning electron micrographs of germinating urediospores of *Uromyces vicia-fabae* on broadbean.

(A) A urediospore germinating over a stomatal lip : (gt) developing germ tube and its tip passed away the opening.

(B) An appressorium developed over a stomatal lip and an infection peg formed by it entered into stomata : (ap) appressorium, (sa) stomatal aperture.

(C) Large and flattened appressorium formed by a germ tube which failed to find a stomatal aperture : (ap) appressorium, (sa) stomatal aperture.

(D) A germinated urediospore on a older leaf having smooth surface and no stomata. The germ tube fails to form appressorium : (gt) germ tube.

The characteristic features of various structures and their activities recorded in the present study were in accordance with the findings of other investigators who studied with other rust fungi (Dickson, 1970 ; Paliwal and Kim, 1974, Wynn, 1976). Findings of the present study also indicate that the urediosporeling of *U.vicia-fabae* goes through a series of complicated morphological events on broadbaen under natural conditions of Bangladesh forming appressoria, infection pegs, substomatal vesicles and haustoria.

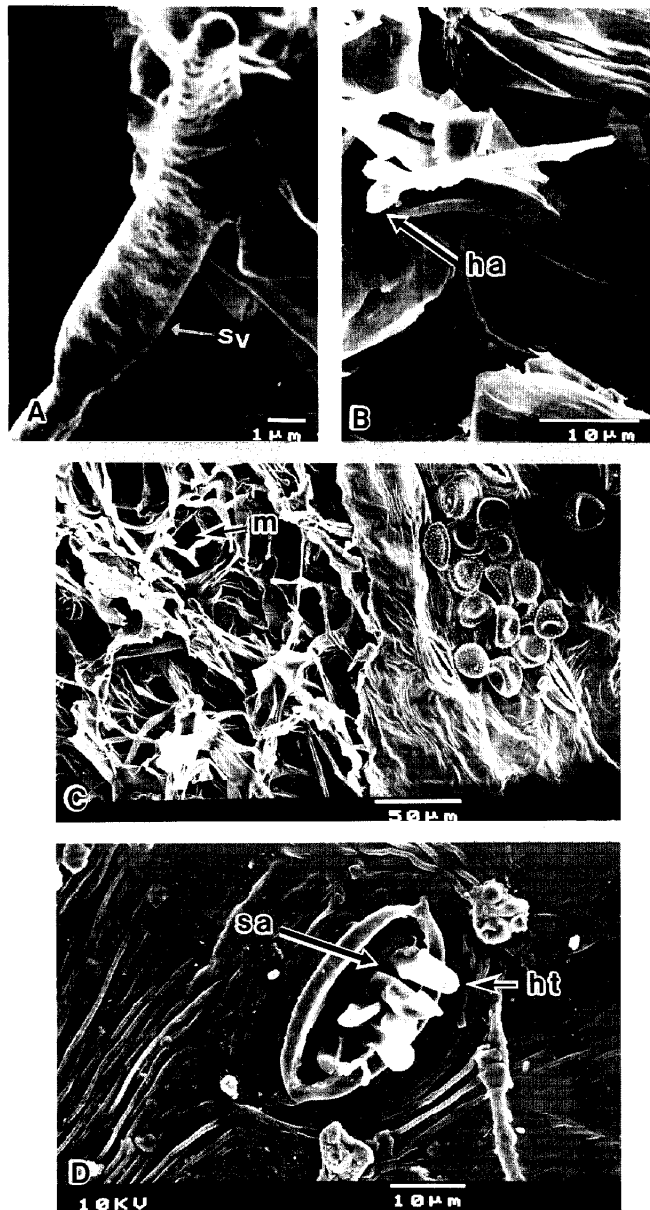


Fig. 4. Scanning electron micrographs of infection structures of *Uromyces vicia-fabae* on broadbean.

(A) A cylindrical and slightly curved substomatal vesicle (sv).

(B) Bilobed haustorium (ha).

(C) Intercellular mycelia in leaf tissues : (m) mycelia.

(D) Intercellular mycelium growing out through the stomatal aperture: (ht) tip of growing hyphae, (sa) stomatal aperture.

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