A Simple Method to Observe Fungal Penetration Site on Barley Leaf

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久能 均*・白石友紀**: オオムギ葉上の糸状菌
貫穿部位を観察するための簡便法

It is often necessary to observe the exact location of fungal penetration into plants. For this purpose the staining techniques have been generally applied to inoculated leaf piece or its stripped epidermis. It has been usual to drop the dye solution onto the leaf piece on a glass slide and place a cover glass on it. However, this method often causes release of the fungal spores from the penetration site. The authors modified the microincineration which had been usually applied to observe the distribution of inorganic substances in organisms, and determined successfully the exact location of the fungal penetration site on barley leaf without using any stain.

A cultivar variety of barley, Kobinkatagi, and five fungi, *Erysiphe graminis hordei* (race I), *E. graminis tritici* (race t₂), *Alternaria kikuchiana*, *Colletotrichum lagenarium* and *Cochliobolus miyabeanus* were used in this study. The lower epidermis of the seven day old primary leaf was inoculated with conidia of the above fungi and the whole plant was incubated in a moist chamber at 20 C with 10-hr light period and 14-hr dark period. Twenty hours after inoculation, the lower epidermis was stripped and placed directly on a glass slide keeping the conidia on the upper side of the epidermis. The slide was put on an asbestos plate and heated gently with a gas burner until the entire epidermis turned light brown. After cooling, the sample was observed under a light microscope.

The penetration point of *E. graminis hordei* was characterized by a dark brown area surrounded by a bright white ring, exhibiting a sharp contrast to other area of the epidermis (Fig. 1).

The bright white ring corresponded to the heavily silicified portion in halo around the penetration point on which Kunoh *et al.*¹⁾ had reported in the previous paper. The present results suggested that the silicified portion was sensitive to incineration

Fig. 1. The penetration points of *E. graminis hordei* on barley epidermis (arrows).

c: conidium g: germ tube

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1) Kunoh, H., H. Ishizaki and F. Kondo. Ann. Phytopath. Soc. Japan. (in press.).

as compared with non-silicified portion. In addition to *E. graminis hordei*, all the other fungi employed in this study induced a clear zone around their penetration point, which was easily distinguished under a light microscope.

Overheating of the sample often led to the damage of ash structure. Furthermore, complete incineration did not cause any complication in observing silicified area around the penetration point but made it impossible to observe the fungal appressorium or hypha on the epidermis. The applications of this method to some dicotyledonous plants such as morning glory and cucumber have been unsuccessful. It is probably due to non-silicification of the leaves of those plants.

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