感受性オオムギ葉におけるパピラへの蛍光性物質の集積とうどんこ病菌の侵入阻止との関係

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
巻/号	465
掲載ページ	p. 594-597
発行年月	1980年12月

日植病報 46：594-597（1980）
Ann. Phytopath. Soc. Japan 46：594-597（1980）

Correlation of Fluorescent Appearance in Papilla with Unsuccessful Penetration Attempts in Susceptible Barley Inoculated with *Erysiphe graminis* f. sp. *hordei*

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北　宜裕*・豊田秀吉*・矢野哲男**・獅山慈孝*：感受性オオムギ葉におけるパピラへの螢光性物質の集積とうどんこ病菌の侵入阻止との関係

Abstract

In susceptible barley to *Erysiphe graminis* f. sp. *hordei* race I, the relation between fluorescent appearance in papilla and fungal penetration attempts was examined using light and fluorescent microscope. In the primary penetration course, fluorescent appearance was detected in all papillae where inoculated conidia ceased the penetration into host epidermal cells, whereas the papillae which permitted the successful penetration attempts did not show any fluorescent appearance. In halo, the same fluorescent appearance was observed regardless of success or failure of fungal penetration. These cytological responses were also observed in the secondary or tertiary penetration sites 72 hr after inoculation. These results suggested that accumulation of fluorescent compound in papilla was essential for the prevention of fungal penetration in powdery mildew of barley.

(Received June 30, 1980)

Introduction

In resistant barley to *Erysiphe graminis* f. sp. *hordei*, race I, fluorescent appearance was detected in papilla, epidermal and mesophyll cells affected by fungal infection[5,6,7]. In these fluorescent sites, no appreciable development of fungi was observed. In susceptible barley, most of inoculated conidia successfully penetrated into host epidermal cells and established their infections without any fluorescing at papilla. Other conidia, however, failed to penetrate into host cells by papillar formation[2,3]. In these papillae, some compounds such as fluorescent compound[6] or basic staining material[1] were accumulated. Therefore, these chemical depositions in papilla are considered to be responsible for decreasing the successful penetration of *E. graminis* f. sp. *hordei* in both susceptible and resistant barley.

The present study was carried out to clarify the relation between fluorescent appearance in papilla and success or failure of fungal penetration attempts in susceptible barley inoculated with *E. graminis* f. sp. *hordei*.

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Materials and Methods

A susceptible barley (*Hordeum vulgare* L.), Kobinkatagi, was grown in vermiculite at 20±1C in a growth chamber with photoperiod of 12 hr. Inoculation to the primary leaves was carried out 7 days after sowing by dusting conidia of *Erysiphe graminis* f. sp. *hordei*, race I. Inoculated plants were incubated at 20±1C in a moistened chamber with 100 % r. h. for 3 hr and replaced to the ordinary conditioned chamber mentioned above. The inoculated leaves were taken randomly at various periods after inoculation and decolored by boiling alcoholic lactophenol solution for 2–3 min. For microscopic observations, the top 1–4 cm portions of decolored leaves were dipped in lactophenol–aniline–blue solution for 2–3 sec to stain the inoculated fungi and rinsed with water for several times. The staining with aniline blue did not disturb the fluorescence in leaves. Light and fluorescent microscopic observations were carried out using a light and dark field of Nikon fluorescent microscope with blue excitation (excitation filter IF410–485, and absorption filter 515W). Only a germling that attacked a single host cell was counted.

Results and Discussion

Fluorescent appearance was detected in all papillae where inoculated conidia failed to penetrate into host epidermal cells (Table 1). On the contrary, papillae which permitted successful penetration did not show any noticeable fluorescence 18, 24 and 48 hr after inoculation. Fluorescence in halo was observed at all infection sites regardless of success or failure of fungal penetration attempts.

Until 72 hr after inoculation, inoculated conidia which succeeded the primary penetration and haustorial formation tried their secondary or tertiary penetration attempts (Table 2). In papillae which prevented the secondary or tertiary penetrations, fluorescent appearance was also observed (Fig. 1. see arrow 1). When successful penetration was accomplished, any fluorescent appearance was not observed in the penetrated papilla (Fig. 1. see arrow 2). Halo was also observed regardless of success or failure of fungal penetration attempts as observed in the primary penetration (Fig. 1–B).

Table 1. Relation between penetration attempts and fluorescent appearance in papilla in susceptible barley infected with *E. graminis* f. sp. *hordei*, race I

Time after inoculation (hr)	Number of conidia			
	unsuccessful penetration at		successful penetration at	
	f-pap[a)]	non-f-pap[b)]	f-pap	non-f-pap
18	331	0	0	857(7[c)])
24	200	0	0	779(28)
48	171	0	0	774(32)

a) fluorescent papilla
b) non-fluorescent papilla
c) partialy or slightly fluorescent papilla

Table 2. Relation between secondary or tertiary penetration attempts and fluorescent appearance in papilla in susceptible barley infected with *E. graminis* f. sp. *hordei*, race I

Averaged number of penetration sites per pustule			
unsuccessful penetration at		successful penetration at	
f-pap[a)]	non-f-pap[b)]	f-pap	non-f-pap
1.8	0	0	8.2(0.2[c)])

a), b) and c) Refer to Table 1
Penetration sites were observed 72 hr after inoculation

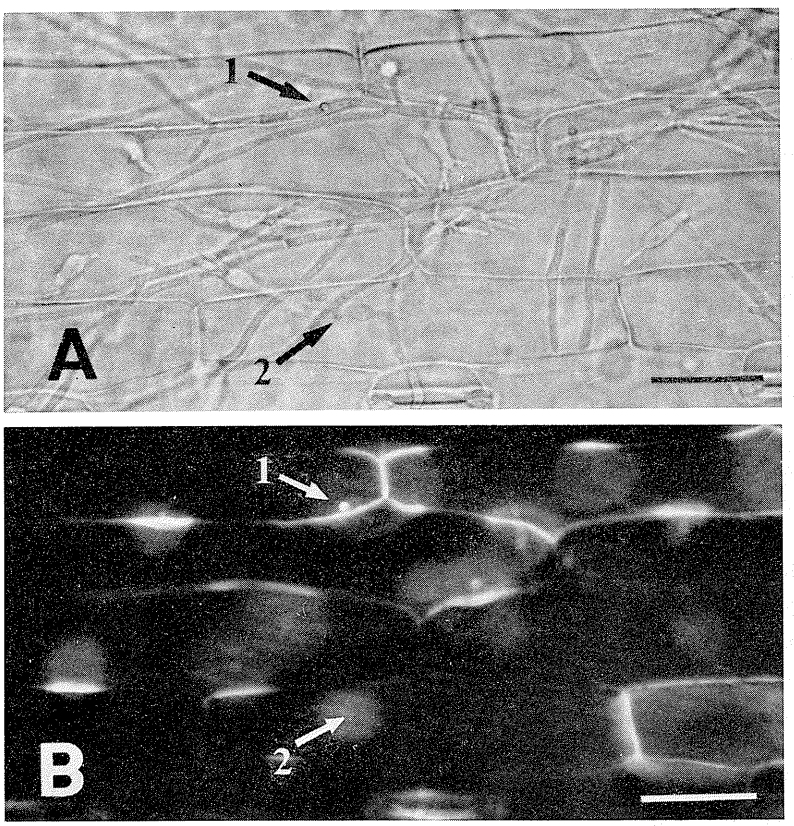

Fig. 1. Correlation of fluorescent appearance in papillae with unsuccessful or successful penetartion attempts at various infection sites of *E. graminis* f. sp. *hordei*, race I, on susceptible cultivar of barley 72 hr after inoculation

A: Papillar and haustorial formation at various infection sites of a pustule under light microscope.

B: Fluorescent micrograph of A. Fluorescent appearance was observed in papilla (arrow 1) where fungal penetration was prevented. No fluorescence, however, was observed at papilla (arrow 2) where fungal penetration and haustorial formation were observed.

Scales in the micrographs represent 100 μm.

In powdery-mildewed barley leaves, it has been postulated that papillar resistance to fungal penetration was attributed either to the accumulation of antifungal compounds in papilla or to the mechanical function of papilla[1,4,8]. The present study indicated that the accumulation of fluorescent compound in papilla was highly correlated with unsuccessful penetration attempts of pathogen even in susceptible leaves. These results suggested that the production or accumulation of fluorescent compound in papilla was essential for prevention of fungal penetration rather than mechanical function of papilla.

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和文摘要

感受性オオムギ葉におけるパピラへの螢光性物質の集積とうどんこ病菌の侵入阻止との関係

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うどんこ病菌，*Erysiphe graminis* f. sp. *hordei*，レースⅠを接種した感受性オオムギ品種コビンカタギを用いて，パピラにおける螢光化とうどんこ病菌侵入の成否との関係を，接種18，24，48および72時間後に調べた。第一次侵入部のパピラに螢光化が認められた場合には，菌の侵入は抑えられたが，菌がパピラを貫通し吸器を形成した場合には，そのパピラに螢光化は起こらなかった。接種72時間後の第二次以後の侵入部においても同様ことが観察された。一方，侵入部周辺のハローには，貫穿の成否にかかわらず，螢光化が認められた。以上のことから，オオムギうどんこ病菌の侵入阻止には，パピラへの螢光性物質の集積が重要な役割を果していると推論した。