한국식물보호학회지 : 제19권 제 4 호
Korean J. Pl. Prot. 19(4) : 235~240(1980)

Effect of of Predisposing Temperatures on The Histopathology of The Rice Blast Fungus, *Pyricularia oryzae*

Ⅲ. Relationship Between Hyphal Growth in The Sheath and Percent Penetration in The Leaf Epidermis

Chang Kyu KIM* and PAT CRILL**

接種前 溫度處理가 벼 稻熱病菌의 組織病理學에 미치는 影響

Ⅲ. 葉鞘의 菌糸伸展度와 잎의 侵入率과의 關係

金 章 圭*·펠 크릴**

ABSTRACT

The relationships between the mean degree of hyphal growth in the leaf sheath and percent penetration in the leaf epidermis within the same predisposing temperature regime were highly significant. A positive relationship was found between degree of hyphal growth in the leaf sheath and percent penetration in the leaf epidermis.

INTRODUCTION

The sheath inoculation method has been utilized as one of the procedures for forecasting the occurrence of leaf blast in some temperate countries because of the intimate relationship in susceptibility of the leaf sheath with the leaf blade. Ito and Sakamoto(1939~1943) developed a sheath inoculation method to study the response of living host

* 農業技術研究所 病理研究擔當官室, 水原, 韓國 170. Plant Pathology Department, Institute of Agricultural Sciences, Suweon, Korea 170.
** Plant Pathology Department, The International Rice Research Institute, P.O. Box 933, Manila Philippines.

cells within 48 hours after inoculation. Later, Sakamoto(1949, 1951) reported findings on appressorium formation, penetration and two types of cell response, and his methods were intensively used by Takahashi(1951, 1956), Doi et. al.(1952), Ohata et. al.(1963) and Kiyosawa et. al.(1975).

In the present study, effect of predisposition temperature on hyphal growth in the sheath and the relationship between hyphal growth in the sheath and percent penetration in the leaf epidermis were studied.

MATERIALS AND METHODS

Six varieties and six isolates were used throughout this experiment. Plants were grown for 35 days under four different temperature regimes similar to those for foliar inoculation(Kim et. al., 1980). However, 1g of $NH_4(SO_4)_2$ was top dressed into each tray 20 days after sowing. The sheath of a new fully expanded leaf was removed and cut into 5cm long pieces beginning at the top. Five sheath pieces/variety/isolate/temperature were placed on two paraffin coated folder stands inside a petri dish in which three sheets of wet filter paper were placed at the bottom. Spore suspension of the different isolates with a concentration of 5×10^4 spores/m*l* was injected separately with a hypodermic syringe(Figure 1). The plates containing the inoculated sheath pieces were maintained for 40 hours in a 24C incubator and then fixed in 30% alcohol for microscopic observation.

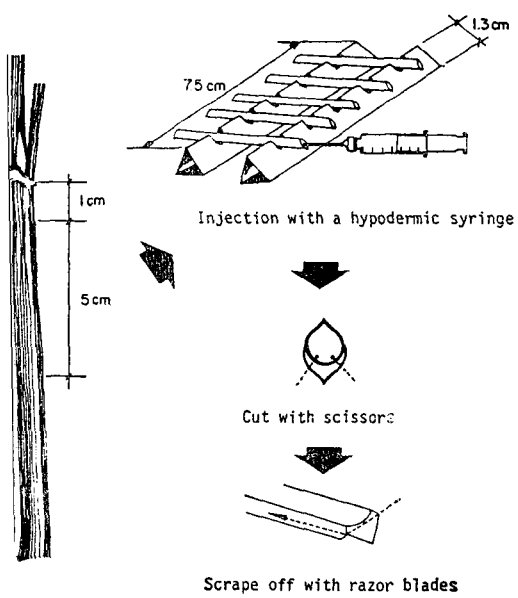

Figure 1. Procedure for sheath inoculation.

Histopathological method utilized for observation and determination of the degree of hyphal growth following leaf sheath inoculation was as follows:

Sample fixation: 30% alcohol 40 hours after inoculation

Staining : 5 minutes in 0.25% Rose Bengal solution

Washing : Saturated Boric acid solution followed by distilled water

The formula used in this study was as follows:

Degree of hyphal growth $=\frac{\Sigma df}{n}$, where

d is the standard class value, as defined by Takahashi (1958, Figure 2),

f is the frequency of appressoria showing the same standard value,

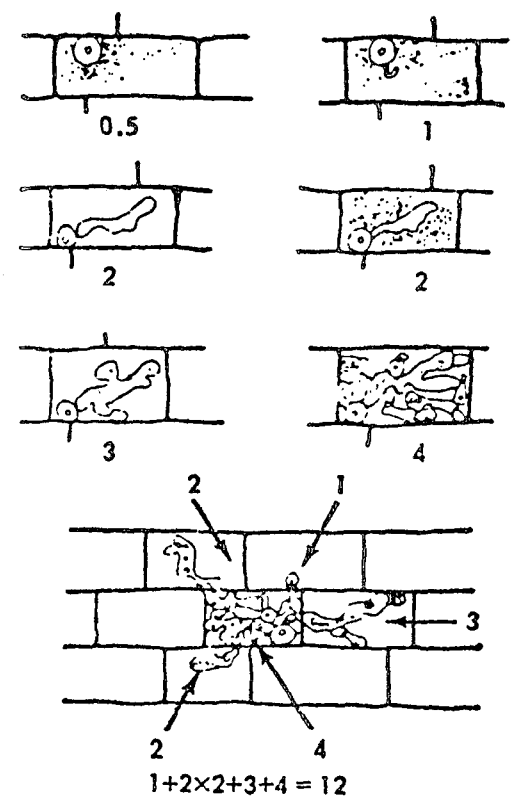

Figure 2. Standard used for calculating the degree of hyphal growth in the sheath adaxial cells.

n is the total number of appressoria showing hyphal growth.

RESULTS AND DISCUSSION

The relationship between susceptibility of the leaf sheath and leaf blade has been documented by Ono(1965) who concluded the reaction of the adaxial cell of a leaf blade to the blast fungus was similar to that of the leaf or neck. In northern Japan, a high positive correlation between the degree of hyphal growth in the sheath and lesion development has been observed and the results utilized for forecasting the occurrence of blast (Yamaguchi, 1970).

In the present study, leaf sheaths detached from rice plants grown at four different predisposing temperature regimes were inoculated with six blast fungus isolates. Hyphal growth was observed on all varieties by all isolates at all predisposing temperature regimes except for isolate I-4702 which did not grow on Tetep at GH and 35/27C or on IR36 at 32/24C and 35/27C(Table 1). Likewise, penetration of the leaf epidermis was not observed for isolate I-4702 on Tetep at GH and 35/27C or on IR36 at 35/27C(Table 2). However, penetration of IR36 by isolate I-4702 was observed at 32/24C. The degree of hyphal growth in the sheath by isolate I-750678-1 was higher than for other isolates(Table 1).

Highly significant differences were found between degree of hyphal growth in the sheath and percent peretration in the leaf epidermis for isolates 1509, 750678-1 and L-1441 but only significant differences for isolates 2137, 4702 and 2017 (Table 3). The

Table 1. Mean degree of hyphal growth on the sheath adaxial epidermis of six *P. oryzae* isolates on six rice varieties under four different predisposing temperature regimes at 40 hours after inoculation and maintenance at 25±1C temperature.

PREDISPOSING[a] TEMPERATURE REGIME	VARIETY	ISOLATES					
		2137	L-1441	2017	4702	1509	750678-1
GH	Carreon	1.50	1.69	1.38	2.16	1.35	1.66
	IR36	1.59	1.38	1.46	1.10	1.29	1.63
	KTH	1.73	1.97	1.60	1.63	2.38	2.89
	Peta	1.66	1.64	1.18	1.30	1.88	2.80
	Sensho	1.44	1.33	1.13	1.56	1.68	2.36
	Tetep	1.29	1.33	1.46	0	1.54	1.69
29/21C	Carreon	1.61	1.46	1.42	1.45	1.27	1.94
	IR36	1.65	1.52	1.50	1.38	1.32	2.08
	KTH	1.94	2.74	1.77	1.57	2.79	3.68
	Peta	1.78	1.80	1.70	1.65	2.36	3.32
	Sensho	1.44	1.20	1.33	2.41	1.61	2.56
	Tetep	1.29	1.00	1.52	1.63	1.15	2.46
32/24C	Carreon	1.31	1.18	1.48	1.12	1.15	2.25
	IR36	1.78	1.47	1.18	0	1.05	2.10
	KTH	1.50	1.79	1.38	2.03	1.86	2.95
	Peta	1.82	1.73	1.38	1.80	1.74	3.30
	Sensho	1.43	1.29	1.30	2.36	1.30	2.05
	Tetep	1.18	1.20	1.29	1.00	1.15	2.04
35/27C	Carreon	1.40	1.31	1.20	1.67	1.10	2.10
	IR36	1.50	1.57	1.00	0	1.07	1.69
	KTH	1.43	1.72	1.41	1.40	1.82	2.69
	Peta	1.54	1.60	1.63	1.00	1.50	2.87
	Sensho	1.50	1.67	1.43	1.40	1.00	2.25
	Tetep	1.25	1.00	1.40	0	1.21	1.94

[a]GH refers to greenhouse where temperatures were variable and fluctuated from 33 to 23C. The fir number refers to a constant day temperature and the second to a constant night temperature, each and 16 hours in duration.

Table 2. Percent penetration of six rice varieties by six *P. oryzae* isolates under four different predisposing temperature regimes at 72 hours after inoculation and maintenance at 25C temperature.[a]

PREDISPOSING TEMPERATURE REGIME[b]	ISOLATE	VARIETIES					
		CARREON	IR36	KTH	PETA	SENSHO	TETEP
GH	2137	0.725a[c]	0.535a	3.210ab	1.660a	0.558ab	0.074b
	L-1441	0.497a	2.042a	1.001c	2.475a	0.091bc	0 b
	2017	0.295ab	0 b	2.010bc	1.280ab	0 c	0.997a
	4702	0 b	0.468a	1.408c	0.789b	1.022a	0 b
	1509	1.234a	1.179a	4.528a	2.008a	0.493ab	0.201b
	750678-1	0.275ab	0.814a	3.946ab	2.286a	1.164a	0.294b
29/21C	2137	0.415ab	0.582b	2.112c	1.342b	0.075b	0.108a
	L-1441	0.199b	0.514b	4.120ab	2.061b	0.196b	0.146a
	2017	0.663ab	0.201b	1.215c	1.168b	0.210b	0.804a
	4702	0.268b	0.100b	1.461c	1.137b	0.676b	0.097a
	1509	0.505ab	0.253b	2.701bc	2.122b	0.332b	0.283a
	750678-1	1.485a	2.453a	5.875a	5.251a	2.759a	0.501a
32/24C	2137	0.335a	0 b	1.926ab	1.067bcd	0 b	0.132bc
	L-1441	0.196a	1.583a	3.037a	2.652a	0 b	0 c
	2017	0.100a	0 b	0.672bc	0.199d	0.101b	1.027a
	4702	0.241a	0.111b	0.587bc	0.412cd	0.095b	0 c
	1509	0 a	0 b	2.081a	1.227abc	0.087b	0.099bc
	750678-1	0.159a	1.232a	3.658a	1.617ab	1.343a	0.613ab
35/27C	2137	0.253a	0.183ab	1.311a	0.324ab	0.098a	0 b
	L-1441	0.097a	0.647a	1.370a	0.746a	0 a	0 b
	2017	0 a	0 b	0.190b	0.312ab	0.067a	0.542a
	4702	0.095a	0 b	0.108b	0 b	0.102a	0 b
	1509	0.097a	0 b	1.551a	1.286a	0 a	0.130ab
	750678-1	0.149a	0.580a	1.819a	1.330a	0.297a	0.097ab

[a]The plants were maintained 24 hours inside the inoculation chamber at a temperature of 25±1C and transferred to a separate incubation room.

[b]GH=refers to greenhouse where temperatures were variable and fluctuated from 33 to 23C. The first number refers to a constant day temperature and the second to a constant night temperature, each 8 and 16 hours in duration.

In a column, means followed by a common letter are not significantly different at the .05 level by DMRT.

·elationships between the degree of hyphal growth n the sheath and percent penetration within four ›redisposing temperature regimes were highly sig-ificant (Table 4) and a positive relationship was ound (Figure 3~6).

摘 要

벼 稻熱病菌의 葉鞘內菌糸伸展度와 잎表皮細胞內의 侵入率과는 寄主가 接種前 같은 溫度條件下에서 자랐을 境遇 高度의 有意性이 있었으며 또한 이들 두 要因

Table 3. Simple linear regression between the degree of hyphal growth in the sheath and percent penetration in the leaf epidermis by six blast isolates on six rice varieties.

ISOLATES	CORRELATION COEFFICIENT	REGRESSION EQUATION
2137	.497*[a]	$Y=2.093X-2.441$
L-1441	.759**	$Y=2.437X-2.730$
2017	.474*	$Y=1.412X-1.469$
4702	.496*	$Y=0.337X-0.009$
1509	.853**	$Y=2.042X-2.179$
750678-1	.779**	$Y=2.205X-3.607$

[a]** : Significantly different at 1% level.
* : Significantly different at 5% level.

間에는 正의 相關關係가 있었다.

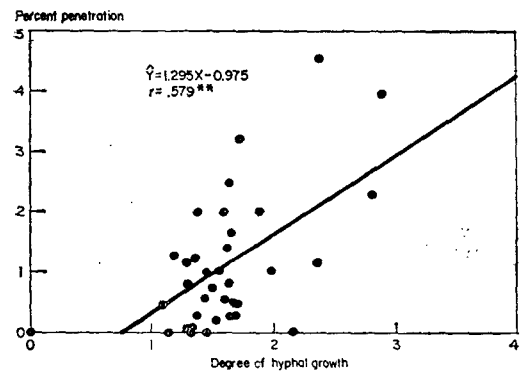

Figure 3. The relationship between the degree of hyphal growth in the leaf sheath and percent penetration in the leaf epidermis by six blast fungus isolates on six rice varieties predisposed in the greenhouse.

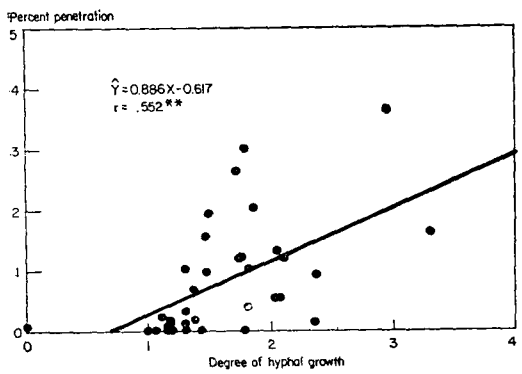

Figure 5. The relationship between the degree of hyphal growth in the leaf sheath and percent penetration in the leaf epidermis by six blast fungus isolates on six rice varieties at the 32/24C (day/night) predisposing temperature regime.

Table 4. Simple linear regression between the degree of hyphal growth in the sheath and percent penetration in the leaf epidermis by six blast isolates on six rice varieties within the same predisposing temperature regimes.

TEMPERATURE[a]	CORRELATION COEFFICIENT	REGRESSION EQUATION
GH	.579**[b]	$Y=1.295X-0.975$
29/21C	.891**	$Y=2.093X-2.563$
32/24C	.552**	$Y=0.886X-0.617$
35/27C	.581**	$Y=0.555X-0.339$

[a]GH=Uncontrolled and variable greenhouse conditions; first number refers to 8 hours constant day temperature, second number 16 hours constant night temperature.
[b]**=Significantly different at 1% level.

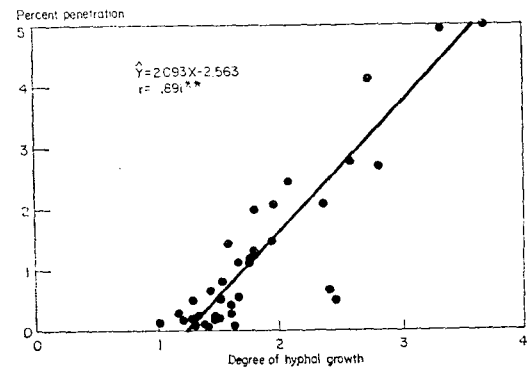

Figure 4. The relationship between the degree of hyphal growth in the leaf sheath and percent penetration in the leaf epidermis by six blast fungus isolates on six rice varieties at the 29/21C (day/night) predisposing temperature regime.

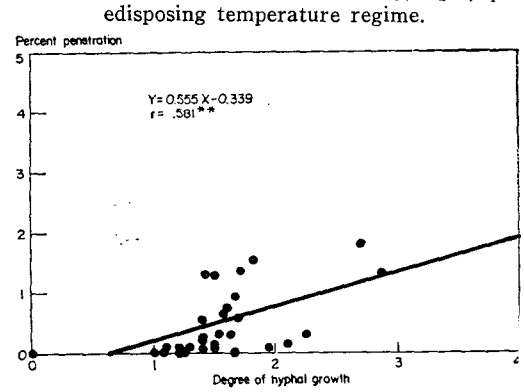

Figure 6. The relationship between the degree of hyphal growth in the leaf sheath and percent penetration in the leaf epidermis by six blast fungus isolates on six rice varieties at the 35/27C (day/night) predisposing temperature regime.

LITERATURE CITED

Doi, Y. and N. Suzuki. 1952. Histochemical studies on rice blast leaions. (In Japanese); Preliminary report of 1951 work in Section of Plant Pathology, Inst. Agr. Sci. 268-286.

Ito, S. and M. Sakamoto. 1939~1943. Studies requested by Min. Agr. Forestry, Japan. Ann. Repts. on rice blast. Hokkaido Univ. Botan. Lab. Fac. Agr. (In Japanese).

Kim, C.K. and Pat Crill. 1980. Effect of predisposing temperatures on the histopathology of the rice blast fungus, *Pyricularia oryzae*. I. Effect of blast fungus isolates on penetration of rice varieties at different predisposing temperature regimes. Korean J. Pl. Prot. 19 : 11-20.

Kiyosawa, S. and E.J. Lee. 1975. Expression of genes for true and field resistance to blast disease in cells of leaf sheath of rice plant. Japan J. Breed. 25 : 145-154.

Ohata, K., K. Goto and T. Kozaka. 1963. Observations on the reaction of rice cells to the infection of different races of *Pyricularia oryzae*. (In Japanese, English summary) : Ann. Phytopath. Soc. Japan. 28 : 24-30.

Ono, K. 1965. Principles, methods, and organization of blast disease forecasting. *In* the Rice Blast Disease. The Johns Hopkins Press, Baltimore, Maryland. 174-194.

Sakamoto, M. 1949. On inoculation of the leaf sheath of rice with the blast fungus. (In Japanese, English summary); Tohoku Univ. Inst. Agr. Res. Bull. 1 : 120-129.

Sakamoto, M. 1951. On the new method of inoculation of rice plants with the blast fungus, *Pyricularia oryzae* Cav. Tohoku Univ. Inst. Agr. Res. Rept. 1-2 : 15-23.

Takahashi, Y. 1951. Phytopathological and plant breeding investigation to determine degrees of blast resistance. (In Japancese); Hokkaido Pref. Agr. Expt. Sta. Rept. 3 : 1-65.

Takahashi, Y. 1956. Studies on the mechanism of resistance of rice plants to *Piricularia oryzae*. Ⅱ. Pathological changes microscopically observed in host cells in which fungus hyphae do not grow well. (In Japanese, English summary); Yamagata Univ. Agr. Sci. Bull. 2 : 37-51.

Takahashi, Y. 1958. A method for forecasting blast disease using the leaf sheath inoculation technique (In Japanese) : Plant Prot. Japan. 12 : 339-345.

Yamaguchi, T. 1970. Forecasting techniques of rice blast. Japan Agricultural Research Quarterly. 5(4) : 26-30.