III. PLANT PATHOLOGY

a. INVESTIGATION OF PHYSIOLOGICAL RACES OF THE BLAST FUNGUS

Chang-shiung Hung and Chin-chung Chien

(Department of Plant Pathology, TARI)

1. Isolation and culture of rice blast fungus

In order to demonstrate the existence of physilogical races of blast fungus, which is assumably an important factor that leads the resistance of a variety to differ in different locaïities, 151 cultures of *Piricularia oryzae* were isolated from diseased plants of various rice varieties collected from Kaoshung, Chiayi, Taichung, and Iran areas in 1959. The cultures were primarily classified into seven groups according to the coloration substrate, spore formation and other cultural characteristics of colonies. No sporulation was found in some cultures. The cultures which appeared to be more stable were selected for single-spore isolation. They were classified into four distinct groups on the basis of their morphological characteristics as shown in Table 1. In addition to these, two cultures, G-2, G-7, were isolated from diseased weeds collected from a field near our Institute. The cultural characteristics of G-2 were similar to those of Group 4, while G-7 differed from any of the four groups.

Table 1. Characteristics of the groups of isolates of *Piricularia oryzae*

Group	Colony appearance		Isolate No.*
	colour of colony	aerial hyphae	
1	dark-green	2 mm height, abundant	886, 111, K67
2	white	1 mm height, sparse	891, 1155
3	brown-grey	1-1.5 mm height, velvet	288, 1134
4	grey-green	1 mm height, rather abundant	1127, 1124, 529

* Isolate No.	Original variety	Place of collection
886	Taichung line No. 33	Chiayi
111	Chianung No. 242	Kaoshung
K67	Chianung No. 242	Kaoshung
891	Cutsugulcule	Chiayi
1155	Chianung No. 242	Tungshu Taichung
233	Taichung line No. 33	Chiayi
1134	Kwang-Fu No. 1	Tungshu Taichung
1129	Kwang-Fu No. 1	Tungshu Taichung
1124	Kwang-Fu No. 1	Tungshu Taichung
529	Kwang-Fu No. 1	Tungshu Taichung

Some isolates showed different reactions to temperatures, nutrients in medium and pH values, as shown in Tables 2, 3 and 4.

Table 2. Cultural characteristics upon rice-polished agar medium of *Piricularia oryzae* influenced by temperature

Isolate	Temperature				
	13°C	19°C	25°C	28°C	34°C
886	d. 42 mm, black-green, hyphae radial	d. 60.5 mm, black-green, hyphae radial	d. 69 mm, black-green, hyphae radial	d. 84.5 mm, black-green, hyphae radial	d. 10.7 mm, black-green, hyphae radial
891	d. 50.3 mm, white, hyphae minute	d. 69.2 mm, white, hyphae minute	d. 75 mm, white, hyphae minute	d. 84.0 mm, white, hyphae rather sparse	d. 11.5 mm, white, hyphae rather sparse
288	d. 45.5 mm, grey-brown, hyphae velvet	d. 63.3 mm, grey-brown, hyphae velvet	d. 69.5 mm, grey-brown, hyphae velvet	d. 83.5 mm, grey-brown, hyphae velvet	d. 10.9 mm, grey-brown, hyphae velvet
1124	d. 43.5 mm, green-gray, with a rim of 0.5 cm wide white hyphae	d. 63.1 mm, green-gray, with a rim of 0.5 cm wide white hyphae	d. 68.9 mm, green-gray	d. 81.5 mm, green-gray	d. 10.9 mm, green-gray
G-7	d. 29.5 mm, grassy-green, center rather dark	d. 45.5 mm, grassy-green, center rather dark	d. 51.0 mm, grassy-green, center rather dark	d. 58.5 mm, grassy-green, center rather dark	d. 5.1 mm, grassy-green, center rather dark

Note: 1. 10 days culturing.
2. "d" represents the diameter of colony.

Table 3. Influence of nutrient on the growth of *Piricularia oryzae*

Isolate No.	Media			
	Rice-polished Agar	Sorghum-agar	Czapek-agar	Corn-meal-agar
886	7.5×7.5 cm, black-green, hyphae radial	7.9×7.9 cm, black-green, hyphae radial	6.0×6.5 cm, black-green, hyphae rather sparse	7.0×7.0 cm, grey-green
891	9.0×8.9 cm, white	9.0×9.0 cm, white	6.0×6.5 cm, grey-white hyphae sparse	8.0×9.0 cm, white
288	8.5×8.0 cm, grey-brown, hyphae fine velvet	8.9×8.5 cm, grey-brown, hyphae fine velvet	7.8×6.5 cm, grey-brown, hyphae rather sparse, coarse	8.0×7.5 cm, light-brown, hyphae sparse
1124	8.5×8.5 cm, grey-green, with a rim of 0.5 cm wide white hyphae	8.0×7.5 cm, grey-green, with a rim of 0.5 cm wide white hyphae	6.0×6.0 cm, deep-grey-green, hyphae rather coarse	8.0×8.0 cm, with a rim of 0.5 cm wide white hyphae
G-7	6.0×6.0 cm, grassy-green, with a rim of 1 cm wide white hyphae	6.5×6.5 cm, grassy-green, with a rim of 1 cm wide white hyphae	5.0×5.0 cm, grassy-green, with a rim of 1 cm wide white hyphae	6.0×6.0 cm, grassy-green, with a rin of 1 cm wide white hyphae

Note: The fungi were cultured at 28°C for 2 weeks.

Table 4. Influence of pH values upon cultural characteristics of *Piricularia oryzae* (On Czapek agar at 28°C)

Isolate	pH value				
	4.5	5.5	6.5	7.5	8.5
886	4×4.5 cm, dark-olive, center rather dark, rim few hyphae	4.8×4.5 cm, dark-olive, center rather dark, rim few hyphae	5.3×5.3 cm, dark-olive, hyphae rather coarse, center rather dark, rim few hyphae	5.0×5.2 cm, dark-olive, hyphae rather coarse, center rather dark, rim few hyphae	4.2×4.0 cm, dark-olive, hyphae gery coarse, center rather dark, rim few hyphae
891	6.2×6.0 cm, white-grey, hyphae minute	5.8×6.0 cm, white-grey, hyphae minute	5.6×5.8 cm, white-grey, hyphae minute	5.5×5.3 cm, white-grey, hyphae minute	5.0×5.0 cm, grey-brown, aerial hyphae velvel, fine
288	5.0×5.2 cm, grey-brown, aerial hyphae velvet, fine	5.6×6.0 cm, grey-brown, aerial hyphae velvet, fine	6.5×6.5 cm, grey-brown, aerial hyphae velvet, fine	5.5×5.5 cm, grey-brown, aerial hyphae velvet, fine	5.0×5.0 cm, grey-brown, aerial hyphae velvet, fine
1124	4.5×4.5 cm, green-grey, with a rim of 0.5 cm wide white hyphae	5.2×5.2 cm, green-grey, with a rim of 0.5 cm wide white hyphae	5.0×5.0 cm, green-grey, with a rim of 0.5 cm wide white hyphae	5.0×5.0 cm, green-grey, with a rim of 0.5 cm wide white hyphae	5.0×5.0 cm, green-grey, with a rim of 0.5 cm wide white hyphae
G-7	5.5×5.5 cm, grassy-green	4.5×4.5 cm, grassy-green, outer hyphae rather coarse	4.0×4.3 cm, rather deep, grassy-green	3.3×3.3 cm, grassy-green, aerial hyphae rather coase	2×2.5 cm, grassy-green

Note: 10 days culturing.

2. Reactions of sixteen standard varieties of rice to "rice blast" pathogenes

(*1*) *Inoculum Supply*

Spore inocula used for inoculation were obtained from the culture of rice grain medium. The fungi performed a certain amount of vegetative growth but formed few conidia. This phenomenon may be due to the inhibitiory action of ammonia produced by the fungi as staling product, as reported previously. Then in order to improve this situation, airiation equipments were employed. After 9-10 days culture at 25°C, the conidia were gathered from the substrate by washing the whole culture in distilled water and spore suspension containing a large quantity of conidia was obtained by this method the amount of conidia was increased for example, in isolate 886, from the initial 1,600 per ml. to 870,000 per ml. The sporulation was affected by temperatures. Isolate 886 produced less than 1,000 per ml. at temperatures bellow 20°C. When the temperature was higher than 30°C, it showed relatively fine vegetative growth but little sporulation and produced less than 10,000 spores per ml.

Proper infection may occur by the application of spore suspension which contains spores at the rate of 50 million per sq. ft or more. Spore suspensions were prepared from various isolates, and they were adjusted so as to keep the same density of

conidia, because an excessive amount of conidia in suspension will induce a severe infection and make the observation difficult.

(*2*) ***Inoculation***

In order to identify physilogical races of *Piricularia oryzae*, seedlings of sixteen standard varieties of rice were inoculated with the spore suspensions in a greenhouse. After the inoculation the plants were placed in a chamber with controlled humidity and temperature for 12 to 24 hours. Investigation was made as soon as the infected leaves could be distinquished, usually 4 to 9 days after inoculation.

The isolates used and their origines are as follows:

Isolate No.	Original variety	Place of collection
529	Kwang-fu No. 1	Tungshu, Taichung
886	Taichung Line No. 33	Chiayi
891	Custugulcule	Chiayi
1124	Kwang-fu No. 1	Tungshu, Taichung
1127	Kwang-fu No. 1	Tungshu, Taichung
1125	Chianung No. 242	Tungshu, Taichung

Infection types were determined according to the following varietal reaction types:

S: (Susceptible): Characterized by a rapid enlargement of large and spindle-shaped lesions.

The color of lesion center was grey, being rimmed by a brownish colored inner edge, and also surrounded by a yellow-brownish outer edge. Small lesions of acute infection type as the whole grey in colour, also belonged to this type.

M: Various types of brown lesions, slowly expanding, restricted in size.

R: (Resistant): Infection is readily established but the lesion remain as black-brown flecks on the leaves.

I: (Immune): No lesions appear.

Reactions of sixteen rice varieties to the isolates used are shown in Table 5.

Comparing the reactions of sixteen rice varieties, five kinds of isolates could be distinguished. They were designated as A, B, C, D, E types and were considered to be different physiological races. They are described as follows:

(A) The isolate 886 gave the most serious infection to eight rice varieties, i. e, Taichung No. 65, Kun-shau-wu-shan-ken, Chianung No. 242, Custugulcule, Chianung-yu 280, Taichung No. 171, Taichung Line No. 33, Kwan-fu No. 1. Kaoshung Ta-li-chen-yu, Nung-lin No. 21, and Sensho appeared to be immune, while other varieties, Kao-chio-lin-chou, Pai-kan-tao, Taichung-ti-chio-chien, Natala were moderately susceptible to this isolate.

(B) The isolate 1127 gave the second serious infection to the test varieties. Four rice varieties, Taichung No. 65, Kun-shan-wu-shan-keng, Custugulcule, Nung-lin No.

Table 5. Reactions of sixteen Standard varieties of rice to seven isolates of rice blast pathogen

Variety	Isolate						
	529	886	891	1124	1127	1155	G-11
Taichung No. 65	M	S	M	I	S	I	I
Kao-chio-lin-chou	I	M	M	I	M	I	I
Kun-shan-wu-shan-keng	R	S	S	M	S	M	I
Kaoshung-ta-li-chen-yu	I	I	I	I	I	I	I
Pai-kan-tao	I	M	I	I	I	I	I
Kanto No. 51	I	I	I	I	I	I	I
Chianung-yu No. 280	I	S	I	I	I	I	I
Taichung No. 171	I	S	I	I	I	I	I
Taichung Line No. 33	I	S	I	I	I	I	I
Kwang-fu No. 1	I	S	I	I	I	I	I
Chianung No. 242	I	S	I	I	I	I	I
Custugulcule	R	S	I	I	S	I	I
Taichung-tichio-wu-chien	I	M	I	I	Sr	I	I
Nung-lin No. 21	I	I	I	I	S	I	I
Sensho	I	I	I	I	I	I	I
Natala	M	M	I	I	M	I	I

Note: Temperature 28°C.

21, were susceptible to this. Taichung-ti-chio-wu-chien, also showed susceptible reaction. Natala and Kao-chio-lin-chou showed the same intermediate reaction as that found after the inoculation with (A) race. All other test varieties showed immune types to this race.

(C) The isolates 1,124, 1,155 showed the weakest infecting ability to the test varieties. Only Kung-shan-wn-keng showed medium degrees of reaction, while all other fifteen test were immune.

(D) The isolate 891 gave a weak infection to the test varieties, Kung-shan-wu-shan-keng was susceptible to this isolate and Taichung No. 65 and Kao-liu-chou were moderately susceptible, while all other test varieties were immune to this race.

(E) The isolate 529 gave the weaker infection. Even Kung-shan-wu-shan-keng, the most susceptible variety, showed a resistance. Custugulcle was also. Natale and Taichung No. 65 were intermediate, while all of others test varieties were immune.

(3) *Differential reactions of rice varieties affected by temperatures*

In order to find the effect of temperatures on the inoculation reaction of rice varieties, the inoculation trial were made under different temperatures. The result are shown in Table 6.

As Table 6 shows, the reaction of rice varieties to the isolates seems to be modified by temperatures. At 25°C, Kaoshung-ta-li-chen-yu Kanto 51 and Nung-lin No. 21

Table 6. Reactions of test rice varieties to four isolates of blast pathogen under different temperature

Variety	Isolate No.							
	886		K67		891		G-7	
	25°C	20°C	25°C	20°C	25°C	20°C	25°C	20°C
Taichung No. 65	S	I	S	I	R	R	I	I
Kao-chio-liu-chou	S	M	S	S	I	I	I	I
Kun-shan-wu-shan-keng	S	S	S	S	I	I	I	I
Kaoshung-ta-li-chen-yu	R	R	S	M	I	I	I	I
Pai-kan-tao	S	I	S	S	I	I	I	I
Chianung-yu No. 280	S	S	M	M	R	R	I	I
Kanto No. 51	R	I	M	I	I	I	I	I
Taichung No. 171	S	I	R	R	I	I	I	I
Taichung Line No. 33	S	R	I	I	I	I	I	I
Kwang-fu No. 1	S	I	M	M	I	I	I	I
Chianung No. 242	S	I	M	R	R	I	I	I
Custugulcule	S	I	M	R	I	I	I	I
Taichung-tichio-wu-chien	S	I	S	S	R	I	I	I
Nung-line No. 21	R	R	S	S	I	R	I	I
Sensho	M	I	M	M	I	R	I	I
Natala	S	R	M	S	R	I	I	I
Weeds	I	I	I	I	I	I	I	I

Isolate No.	Original variety	Place of collection
886	Taichung line No. 33	Chiayi
K-67	Chianung No. 242	Kaoshung
891	Cutsugulcule	Chiayi
G-7	Weed	Taipei

were resistant. Sensho was moderately resistant, and all other test varieties were susceptible to isolate 886. At 20°C however, only Kun-shan-wu-shan-keng was susceptible, and Taichung No. 65 and other seven varieties were resistant or immune. It is apparent that the infection ability of isolate 886 decreases at low temperature. The reaction of test varieties to the isolates K67 and 891 however, showed small differences between the temperature treatments.

DISCUSSION

Hsu: What is the object of your experiment?

Hung: The prevalence of blast disease in Taiwan markedly differ from year to year. Besides environmental factors, physiological races in the pathogene may also play an important role in this phenomenon. The number of races, the relation between their occurrence and environment, and the reaction of rice varieties to different races should be investigated. These have been our main object of experiment.

Oka: When two or more races were inoculated in mixture, different races may compete each other. Such may be a method of study if the races can be distinguished from each other by some characteristcs. How do you think about that?

Hung: I think it is a very good idea.

Wu: The prevalence of blast disease is closely related to the chemical component, of rice plants. I think you should make chemical analysis of rice plants.

Hung: Yes, but owing to limited activity, this line of study cannot be done at this time.

Hsu: How about the optimum temperature for the growth of blast pathogenes?

Hung: Usually 28°C.

Hsieh: Pathogenic races which attack leaves may differ from those for neck and node blasts. Have you conducted any such experiment to clarify this point?

Hung: We don't know yet whether the blast pathogen on leaves is the same as that on neck and node. We are isolating single spore cultures from different parts of plant to answer this problem.

Ku: In wheat, pathogenic reactions to seedlings and adult plants are different how about the blast disease in rice.

Chien: It's different.

Oka: How about the statistical methods to look into the physiological races?

Hung: It is to be tried in our future studies.