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Resistance of Rice Plant to Bacterial Leaf Blight and Strains of Causal Bacteria

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Bacterial leaf blight of rice [causal bacteria : *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson] that is known to have occurred frequently since the latter part of the 19th century, increased rapidly in this century, and recently has been affecting some 300,000 or 400,000 ha. This disease, together with rice blast and sheath blight, are the three biggest diseases of rice in Japan. It has recently been recognized that bacterial leaf blight occurs in several countries in southeastern Asia, such as Formosa, the Philippines, Indonesia, Thailand, India and Ceylon. Thus, it is thought to be distributed in most of the leading rice producing countries of the world. Although no data on affected area and damage in these countries are available, injuries from this disease will become more serious with improvement in rice cultural methods and extension of fertilization.

In Japan, however, studies on bacterial leaf blight of rice have developed remarkably in recent years, clarifying to a large extent the overwintering of casual bacteria, the process of infection, the development and the spread of the disease. These results of studies have been combined with the development of new chemicals to achieve a considerable success in the control of the disease. But the Japanese control method thus developed can not necessarily be applied with the same success in other countries where the environments and conditions of rice culture are completely different. Therefore, each foreign country should study control methods with consideration of its own peculiar conditions. Most of the strains of the pathogen with different virulences and rice varieties with different resistance which have been studied so far and described in this report are Japanese ones. It should be taken for granted that many varieties completely heterogenous in resistance and various strains of the pathogen in virulence are distributed in foreign countries. Therefore, each country should study strains of bacterium and resistance of each rice variety independently from that in Japan.

Strains of bacterial leaf blight of rice

The isolates of bacterial leaf blight pathogen are somewhat different from each other in physiological properties such as production of hydrogen sulfide, liquefaction of gelation, decomposition of arabinose,[12] etc. But it is hard to classify these bacteria into strains by such different properties. Since the most conspicuous difference is usually recognized between isolates in their susceptibility to phage and virulence against rice varieties, these two properties have become the criteria for the classification of bacterial leaf blight pathogen into strains.

Table 1. Classification of bacterial leaf blight of rice distributed over Japan by their susceptibilites to phages(Wakimoto)

Strains \ Pahges	OP_1	OP_1h	OP_1h_2	OP_2
A	+	−	+	+
B	−	+	+	+
C	−	−	−	−
D	−	−	+	+
E	−	−	−	+

Classification of bacteria into strains by their susceptibilities to phages

Isolates of leaf blight bacteria can be classified into strains by their susceptibilities to phages.[22),25)] There are wide distributed 4 kinds of phages, OP_1, OP_1h, OP_1h_2 and OP_2, with different host ranges in Japan. Causal bacteria of leaf blight of rice are classified into A, B, C, D, and E strains according to their susceptibilities to these phages[22)] (Table 1). Although this classification is very simple and easy, there are no relations between virulences as mentioned below and the strains as classified above.

Classification of baceria into strains by virulences

Of all isolates of bacterial leaf blight pathogen, some have a virulence so strong as to infect almost all varieties of rice, while some are so weak as to infect only very susceptible varieties. Consequently, grouping of the isolates may be possible in comparing the virulence against various rice varieties after inoculation.

In order to compare such virulences of the isolates, the concentrated bacterial suspension (ca. 10^9/ml) of each isolate using the bacteria cultured on the slant of a PSA media (consisting of decoction of 300 g potato, 2.0 g of $Na_2HPO^4.12H_2O$, 0.5 g of $Ca(NO_3)_2.4H_2O$, 5.0 g of peptone, 20 g of sucrose, 15 g of agar, 1,000 ml of water and pH 6.8-7.0) for 3 day at the temperature of 25℃ was prepared. The inoculation was made at the central point of the leaves of young seedlings of various varieties by single needle, which were then kept in a room at a temperature of 25-30℃. The length of yellow discolored symptoms appeared and were compared each other. Seedlings to be inoculated should be with 4-5 leaves on culm and varieties for testing the virulence of the pathogen should better be those ranging from very susceptible ones to very resistant one.[11)]

According to the seedling inoculation method mentioned above, the isolates *Xanthomonas oryzae* distributed all over Japan are classified into 6 strains, AI, AII, AIII, BI, BII

Table 2. Bacterial leaf blight in Japan classified by virulences (Kusaba et al.)

Strain	Group A			Group B		
	AI	AII	AIII	BI	BII	BIII
Koganemaru Akashinriki Nōrin No. 27 Asakaze Kōgyoku Shinzeki No. 1 Nōrin No. 12 Tosan No. 38 Ōita Mii No. 120 Nōrin No. 44 Sasashigure Nakasengoku Shinsen Nōrin No. 29 Zenshō No. 17 Taishō Akao No. 66 Jikkoku Aichi Asahi Kinmaze	To all varieties ⧺	To all varieties+	To most of varieties +	To varieties stronger than shinzeki No. 1−, Varieties weaker than Norin No. 12 ⧺	To varieties stronger than shinzeki No. 1−, Varieties weeker than Norin No. 12 +	To varieties with high resistance−, Varieties with susceptibility +
Corresponding number of isolates	37	21	20	18	13	9

and BIII as shown in Table 2.[10),11)]

No regional distribution of these bacteria with different virulences has been evidenced.[10),11)] Some reports have been published on some strains of causal bacteria whose distribution is limited to certain localities.[4),5),8),9),16),17),18),25)]

Varietal difference of rice

There is much difference between varieties of rice in their resistance against becterial leaf blight. This fact has been recognized in field surveys since the initial stage of the disease occurrence. But the true resistance of a variety may not always be ascertained in the field because it changes according to the time of its maturity, the amount of fertilizer applied, and the virulence of bacteria distributed there. Therefore, the true resistance of a rice variety will be most promptly detected from the symptoms of disease that appear through inoculation of several kinds of strains with different virulences in some growing stages. Besides, as there are several stages to pass from the infection of causal bacteria to the appearance of disease symptom, the mechanism of the resistance of varieties may be not always the same. Hence, it is very important to select an inoculation method suitable for testing the resistance of a rice variety to the bacteria. The following three methods are used for inoculation of the bacteria in Japan at present.

Dipping inoculation method[28)]

Rice seedlings whose roots have been dipped in a bacterial suspension at a density of over 10^8/ml, are transplanted in paddy field, where the occurrence of disease is investigated.

Spray inoculation method[2)]

Bacteria with high vitality are obtained by grinding infected rice plant leaves are sprayed to the plant by compressor. The plant thus inoculated is kept in a wet room for 24 hours under normal conditions. Then the occurrence of disease is investigated.

Needle inoculation method[10),11),19),20),23),24),26),27)]

Bacteria generated and grown on a PSA culture medium is made into a suspension at the density of $10^{7\sim8}$/ml which is inoculated by poly-needles (to grown rice plants) or single needle (to young seedlings) to the central point of leaves. Then the symptoms of disease appearing on them is investigated.

The first two of the above three methods may be suited to the judgment of the combined results of the resistance against invasion with the resistance to lesion enlargement of rice plants while the last one seives for the detection of resistance against lesion enlargement only. At present, however, as no remarkable difference is ascertained between rice varieties in their resistance against invasion of bacterial leaf blight pathogen, the method of above may be safely used for the judgment.

The resistance of typical rice varieties in Japan has been examined by the needle inoculation method and the results are shown in Table 3 below.[23)]

This method was also applied to some bacteria with a moderate virulence to find out the resistance of the main rice varieties in the Hokuriku region in Japan and the results are shown in Table 4 below.[27)]

The fact shown in these tables that varie-

Table 3. Resistance of rice varieties to bacterial leaf blight as identified by needle inoculation (Yoshida et al.)

With high resistance:	Nōrin No. 27, Nōrin No. 35, Kōgyoku, Koganemaru, Zenshō No. 17
With moderate resistance	
moderate:	Nōrin No. 13, Nōrin No. 31, Ōita Mii No. 120, Aikoku-Sai No. 1
escape by culture season:	Nōrin Nos. 1, 7, 21, 46, and 55.
With high susceptibility:	Nōrin Nos. 6, 8, 12, 18, 25, 32, 36, 39, 51, 53, and 57, Asahi, Senbonasahi, Aichiasahi, Nurebato

Table 4. Resistance of main rice varieties to bacterial leaf blight in Northern Japan, as identified by needle inoculation (Yoshimura et al.).

Maturity \ Grade of resistance	Resistant	Moderate	Susceptible	Extremely susceptible
Early	Nakashin No. 120	Fujiminori, Chōkai, Shin No. 2, Ootori, Sasashigure, Waseaikoku No. 3, Hōnenwase, Sawanishiki, Toyochikara	Koshijiwase, Kaguramochi, Nihonkai, Ginmasari, Ugonishiki, Hatsunishiki, Nōrin No. 21	Miyoshi, Nōrin No. 41, Oou No. 2, Nōrin No. 17, Fujisaka No.5, Towada, Akibae
Intermediate	Shirogane	Fukuminori, Yomohikari, Chikuma, Koshisakae, Senshūraku	Koshihikari, Sekiminori, Araki No. 2, Manryō	Kurobe No. 1
Late	Kōgyoku, Asakaze, Zenshō No. 26 Akashinriki, Shinzeki No. 1, Benisengoku	Nōrin no. 18, Aashi No. 1, Ooita Mii No. 120	Kinmaze, Kusabue, Jikkoku	Sampuku

Table 5. Changes in resistance of rice varieties corresponding to different virulences of inoculated bacteria.

Inoculated Bacteria \ Resistance	Slightly resistant	Susceptible
H5809 (moderately virulent)	Ginbōzu Nakate, Ginbōzu, Rikuu No. 132, Koshisakae, Yomohikari, Yachikogane, Koshihikari	Nōrin No. 29, Nōrin No. 17, Shinmasari, Fujisaka No. 5, Kinmaze, Moritawase
H5905 (extremely virulent)	Ginbōzu	Nōrin No. 8, Shin No. 7, Rikuu No. 132, Nōrin No. 21, Hatsunishiki, Koshijiwase, Sasashigure, Yachikogane, Ginbōzu Nakate, Fujisaka No. 5, Nōrin No. 17, Moritawase, Kinmaze, Yomohikari, Yamakogane, Shinmasari, Koshisakae

ties belonging to a resistant group fell to disease if strong bacteria were inoculated, indicates that the resistance of varieties is relative and not independent from the strains of bacteria. Table 5 shows the results of an experiment in which the resistance varied with inoculation of strains of bacterium.[26]

Strains of X. oryzae in foreign countries

In the Philippines and India, there are distributed phages with host ranges foreign to Japan and reactions of causal bacteria to phages are very different from those in Japan. In consequence, the above strains from A to E that are applied to Japanese bacteria are not adequate for classifying foreign bacteria, for which, therefore, many more phages will be required. The bacteria isolated in the Philippines once were classified into strains according to the criteria applied in Japan.[3] The

Table 6. Difference in bacteriophage susceptibility of the isolates of X. *oryzae* isolated from Japan and the Philippines.

Strain	Corresponding no. of isolates	
	Philippines*	Japan**
A	0	46
B	3	20
C	24	4
D	36	9
E	3	3
Total	66	82

Note: * Goto[3] ** Wakimoto[22]

results are as shown in Table 6, in which A strain common in Japan is not found while D and C strains rarely seen in Japan are prevalent in the Philippines.

A similar fact is recognized regarding Indian bacteria. (Report not yet published.)

It is reported that some bacteria which have been isolated from infected leaves of rice plant in foreign countries have much more virulence than that found in Japan, and the ones in India seems to be especially virulent. (Report not published yet.)

Resistance of foregin rice varieties against bacterial leaf blight

A test to ascertain the resistance of foreign rice varieties to bacterial leaf blight was recently performed with a view to introducing resistant genes to Japanese varieties.[29] A test in which causal bacteria of three different strains with strong, medium and weak virulent pathogenisity were inoculated in young rice seedlings of foreign varieties, showed the following results: of 101 varieties tested, Lead rice (Burma), TKM 6 (Ceylon), and Nigeria 5 (Ceylon) are most resistant, while Pachchaiperumarl, Chiem Chank 198-A, Chen Chia Tao No. 3, H 5, Rantaj-emas 2, Tadukan, Pe-bi-Fen, P'in Pu Ming Hsüan Hsing Ch'an, Wan Hsien Chü Yung Ch'an, Kuan Yin Hsien, G.E. B. 24 x Di 325, Nagadami, Blue Rose, Prelude, T 1031, Lu Chien, Ts'ai Yüan Chung, Hsia Chih Pai, etc. follow as a group with comparatively resistance.

Future problems

In foreign countries, there are distributed not only many kinds of phage of X. *oryzae* that have different properties from those found in Japan, but also the causal bacteria that are foreign to Japan as mentioned above. The resistance of foreign rice varieties to the disease is also completely different from that of the Japanese. It should be studied in relation to bacteria distributed in respective countries. The detailed study has been slowly proceeding in Japan under many difficulties such as in import of isolates of X. *oryzae*, culture of foreign rice varieties in Japan and so on. In order to overcome these difficulties and to establish complete control systems against bacterial leaf blight of rice which may be different according to the countries, it is vitally needed to advance the study not only on resistance of varieties and strains of causal bacteria, but also on every aspect of the disease in every country concerned.

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Toxoplasmosis in Animal and Laboratory Diagnosis

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The protozoa known as *Toxoplasma gondii* was discovered by Nicolle and Manceaux in 1908 in the North African rodent *Ctenodactylus gondi*. Since then morphologically identical parasite have been discovered in many mammals, birds and reptiles in all parts of the world. Sabin (1939), however, demonstrated that the morphological and immunol-