PN-AAL-228

IRRI RESEARCH PAPER SERIES
NUMBER 75 MAY 1982

# PATHOTYPES OF XANTHOMONAS CAMPESTRIS PV. ORYZAE IN ASIA

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# PATHOTYPES OF *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE* IN ASIA[1]


## ABSTRACT

Information from China, Indonesia, Japan, Korea, Philippines, and other countries indicates that there is physiological specialization of *Xanthomonas campestris* pv. *oryzae*, the rice bacterial blight pathogen, on a set of differential varieties. Based on the rice-bacterium combination, the rice varieties showed strong interaction with bacterial isolates in some countries but weak interaction in others. As a result, not all the initial set of varieties, combining those from IRRI, Japan, and Korea, was functional. They did not, therefore, differentiate isolates from some countries into pathotypes or races. It is essential to include in the set varieties that show differential response to isolates in South Asia.

Technical considerations for identification and classification of pathotypes and development of differential varieties are discussed. The implication of pathotypes and differential varieties in the rice germplasm evaluation and utilization is also elucidated. Working areas to strengthen the collaborative project are suggested.



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Submitted to the IRRI Research Paper Series Committee September 1981.

# PATHOTYPES OF XANTHOMONAS CAMPESTRIS PV. ORYZAE IN ASIA

Rice bacterial blight, caused by Xanthomonas campestris pv. oryzae, is one of the most widespread rice diseases in Asia. Following major bacterial blight epidemics in the 1960s, breeding for resistance became an integral part of rice improvement in many Asian countries. Much of the rice germplasm collection at IRRI has been tested for bacterial blight reaction and by 1980 more than 3,000 rice varieties were identified as resistant to a common strain of the bacterium in the Philippines. Although diverse resistance sources have been identified, the resistance gene Xa-4 derived from TKM6 and similar donors has, however, been the most widely used in the IRRI breeding program.

In breeding for bacterial blight resistance, as for other diseases, two independent variables play a crucial role: the host variety and the pathogen.

As indicated, there are many rice varieties resistant to bacterial blight. Whether they are resistant to all isolates or only to certain isolates -- in a country or across countries -- is important knowledge for a rice improvement program. We report briefly on the present status of knowledge on the variability of the rice bacterial blight pathogen in Asia.

## PHYSIOLOGIC SPECIALIZATION AND PATHOTYPES OF BACTERIAL BLIGHT IN ASIA

Pathogens are characterized by their host range. It is fairly easy to detect specialization within a well-defined pathogen species on different host spectra. Further specialization on the level of host varieties is common on many fungal pathogens. Such physiologic forms or physiologic races, and later pathotypes, are determined by their reaction on a set of selected test varieties (Stakman et al 1962). This appears to be common in many fungal pathogens, and especially among obligate parasites. Physiologic specialization on a set of host cultivars differing in resistance has been reported for many bacterial pathogens. X. campestris pv. malvacearum, the cotton bacterial blight pathogen, and X. campestris pv. vesicatoria, bacterial spot of pepper and tomato, are some of the few that have been shown to have distinct physiologic races (Brinkerhoff 1963, Cook and Stall 1964).

For many years, physiologic races of X. campestris pv. oryzae have not been well understood. Controversial results on whether there was physiologic specialization of the bacterium in tropical Asia were reported. However, variation in virulence from one country to another, and also from one site to another in the same country, was generally recognized. Some studies claimed that the variation pattern is continuous, others claimed that there is vertical rice-bacterium relationship (Buddenhagen and Reddy 1972, Ezuka and Horino 1974). Isolates of the pathogen from the Indian subcontinent were believed to be more virulent than those from East Asia, such as those from Japan (Buddenhagen and Reddy 1972).

Available information suggested that there was a continuum of variation in virulence across the rice growing areas in the Asian countries. Variability in virulence was shown clearly by some rice cultivars, which showed resistance in one country such as Japan, Korea, or China but not in other countries, like India or Indonesia. Tetep or Tadukan, for instance, was resistant in Japan (Ezuka and Horino 1974) but not in the Philippines. BJ1 was resistant in the Philippines or in East Asia, but susceptible in Bangladesh (S. A. Miah, Bangladesh Rice Research Institute) and at specific sites in India (Kauffman and Rao 1972). This phenomenon may have been the result of co-evolution of the rice-bacterium relationship as rice varieties of different resistance were grown in different countries. When more rice varieties and isolates from different countries were tested, evidence of a vertical rice-bacterium relationship showed (Reddy and Ou 1976).

Research at IRRI in the past 5 years has shown evidence of specialization in the pathogenicity of X. campestris pv. oryzae on selected rice varieties. There were similar findings in Japan after Asakase, a resistant rice variety, became susceptible to bacterial blight in a farmer's field (Kuhara et al 1963). A strain isolated from this variety showed high virulence not only to a known susceptible variety, such as Jukkoku, but also to the resistant variety Asakase. It was concluded that the unexpected epidemic of bacterial blight in 1957 on Asakase, in the Beniya area of Kyushu, was probably caused by a new virulent strain. Since then scientists in Japan have become aware of a distinct relationship between rice and its bacterial pathogen.

Many attempts have been made to classify rice varieties and bacterial isolates in Japan (Kusaba et al 1966, Sakaguchi et al 1968, Washio et al 1966). Later, Kozaka (1969), on the basis of differential reactions, presented a combined system of varietal classification made by others (Sakaguchi et al 1968, Washio et al 1966). In this system, rice varieties are divided into four groups: Kinmaze, Kogyoku, Rantai-emas, and Aikoku. The bacterial isolates are divided into three groups: I, II, and III. These varietal differentials are now commonly used in Japan (Ezuka and Horino 1974).

Based on this system, Indonesian isolates were compared and grouped accordingly (Yamamoto et al 1977), and it was concluded that most of the Indonesian isolates were comparable to group III in Japan. In addition two different groups were distinguished and designated as IV and V. The results provide good evidence of physiologic specialization and a strong interactive effect of the differential varieties and bacterial isolates. Later, another bacterial group was identified as virulent to Kinmaze and Tetep and avirulent to Kogyoku and Wase Aikoku 3 in Indonesia.

In Thailand, Eamchit and Mew (198) identified, on the basis of virulence, four groups of bacteria on a set of differential varieties. One group was

less virulent, even to a susceptible check cultivar, one was virulent only to IR8 and RD9, another was virulent to RD7 but not to IR20, and another was virulent to both RD7 and IR20. All were virulent to IR8 and RD9 and avirulent to DV85.

Similar studies were conducted in Korea (Choi et al 1977). Different bacterial groups were identified on the Japanese differential varieties and later a set of differential rices was proposed to include such popular varieties as Milyang 23, Tongil, Yushin, and Suweon 281.

Information from China has shown a weak interaction between some isolates from Asia and rice varieties that serve as differentials identified in China (Wu et al 1981). The bacterial groups have been classified into five groups according to virulence to differential varieties. IR26 was highly resistant to four bacterial groups and Jing-gong 30 was susceptible to all.

At IRRI, differential interaction was shown first on four rice varieties and three bacterial groups (Mew and Vera Cruz 1979). The differential response of the varieties to the bacterium was clear. The rice-bacterium interactions were rather strong, when Cas 209 was identified and included as one of the differential varieties (Fig. 1). A more comprehensive study compares the two sets of differential rices developed independently in Japan and those at IRRI on bacterial isolates in Japan and in the Philippines, (Horino et al 1980). Based on infection of flag leaves by a double-needle pin-prick method of inoculation, it was evident that the nine bacterial groups were distinct in vertical virulence. As to the differential varieties, each had a major gene different from the others for vertical resistance. It appears that a gene-for-gene relationship between the resistance in rice and the virulence in the bacterial blight pathogen exists.

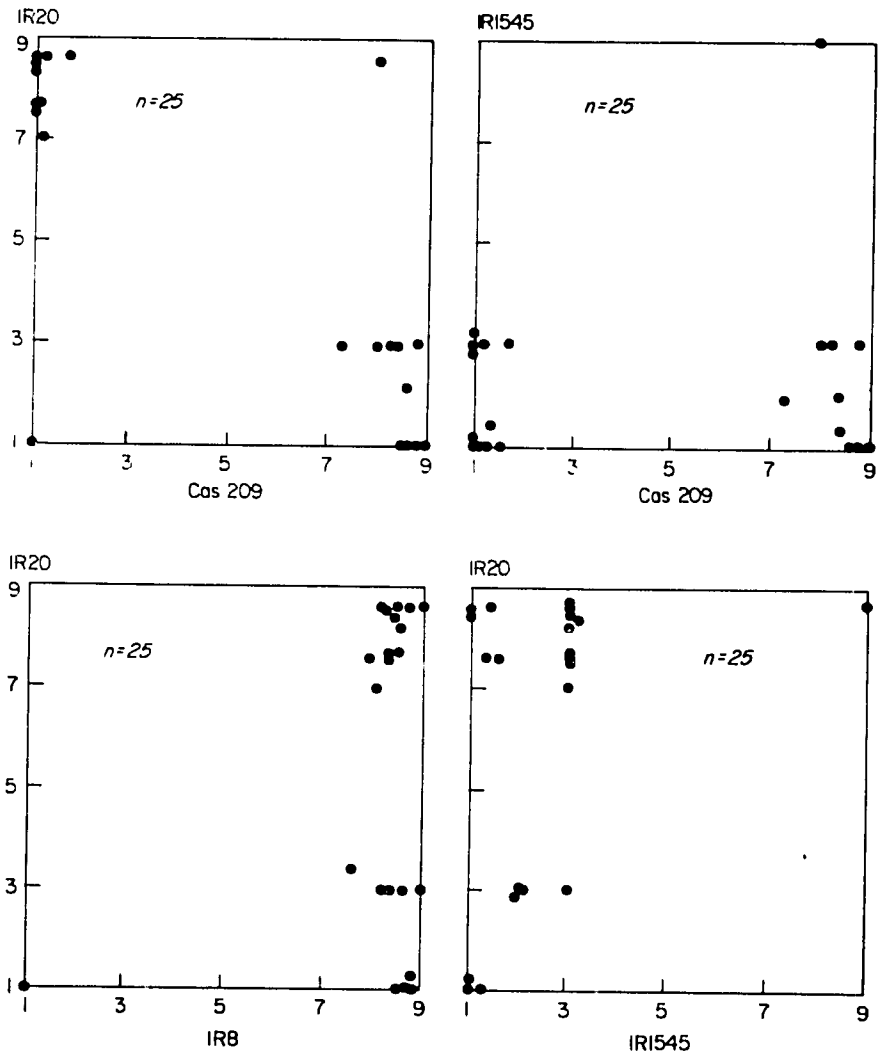


Fig. 1. Response of 4 rice varieties to 25 isolates of Xanthomonas oryzae on a 0-9 disease scale (Source: Mew et al 1981).

From this information, it became certain that there are physiologic races, or pathotypes, of the rice bacterial blight pathogen. The questions asked are:

- Are there different races in different rice-growing countries in Asia?
- If there are, how different are they in virulence to rices that have genes for resistance identified at IRRI or other national programs?
- How can they be classified and named according to the resistance of the differential varieties?
- What set of rice differential varieties, conditioned by major genes for resistance, will be acceptable and usable in identifying the races?

An international collaborative project on bacterial blight was initiated in 1979. The differential varieties with different resistance developed at IRRI, Japan, and Korea were combined and seeds were sent to interested participants in the project. The results have shown diverse reactions on the rice differentials, from which distinct pathotypes could be identified. It was also obvious that not all the varieties could serve as differentials to the isolates.

It is, therefore, suggested that additional differentials for isolates in each country be identified and tested in other countries. An international set of differential varieties may then be developed. Collaborative effort in this aspect should, therefore, be strengthened. Because the monogenic differentials are more efficient, and ideal, it is imperative to develop and assemble them as international differentials.

## TECHNICAL CONSIDERATIONS FOR IDENTIFICATION AND CLASSIFICATION OF PATHOTYPES AND DIFFERENTIAL VARIETIES

Stakman et al (1962) indicated that all races of certain pathogens probably can never be cataloged at any given time for two reasons -- the practical difficulty of obtaining adequate worldwide samples from the thousands of cultivars and wild biotypes of the host plants, and the difficulty of being sure that the methods of identification are reliable at any given time. The last point of consideration relates well to the uncertainty of various attempts to verify the pathogenic specialization of X. campestris pv. oryzae and the rice varieties.

There is difficulty in making sure the methods of identification of races are based on rice-bacterium interactions. Interaction, as pointed out by Zadoks and Schein (1979), is the mathematical way of describing the biologic phenomenon called physiologic specialization. With a two-variety, two-pathogen isolate scheme, the response may be classified into classes, i.e. presence versus absence of diseases, or several classes according to the scales. The host-pathogen interactions could, however, result in 16 possible combinations. To summarize the points concerning our interest, Zadoks and Schein stated that "when there is interaction, some C's (cultivars) differentiate between the P's (pathogen isolates) and likewise these P's differentiate between the C's." Figure 2 shows that physiologic specialization may be identified based on rice-bacterium interactions.

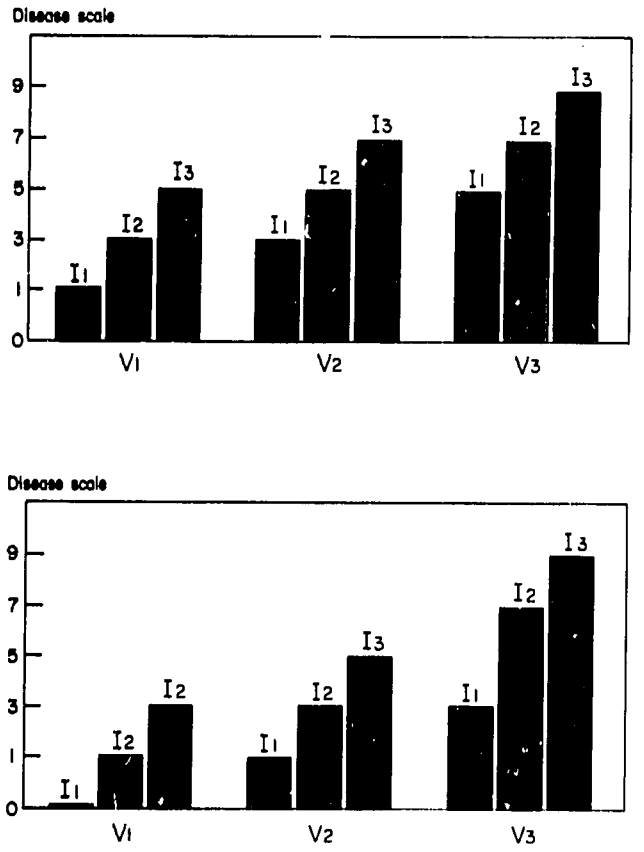


Fig. 2. With a 3-variety (V), 3-isolate (I) scheme, weak V-I interactions are shown on a 0-9 disease scale (IRRI 1980).

There are other difficulties in working on rice bacterial blight. An important one is the variety's response to the isolates. Ideally, a rice variety's response to an isolate of X. campestris pv. oryzae can be divided into two classes -- presence versus absence of disease. The former can be divided into a scale qualifying the class. The Standard Evaluation System (SES) for Rice (IRRI 1980) is a good reference for this purpose.

In Japan, rice-bacterium interaction is rated on a disease scale of 0 to 7: a scale below 2 indicates resistance and above 2 susceptibility (Ezuka and Horino 1974). At IRRI, based on the SES, 0-3 indicates resistance and 7-9 indicates susceptibility. Varieties chosen to be differentials ideally should show a yes-or-no response to a race of vertical virulence. Varieties showing an intermediate

response, or with adult plant resistance, are not suitable for differentials. Therefore, it is suggested that only varieties with an overall resistance, i.e. resistance at the seedling (tillering and adult plant (booting with flag leaf) stages, are considered. Practically, as indicated previously, rice does not always show a yes-or-no response to X. campestris pv. oryzae. It is, therefore, suggested that the SES scale of 0-3 could be considered resistant and 7-9 susceptible. With a three-variety, three-isolate scheme, strong V-I interactions are presented in Figure 3 based on the SES 0-9 scale on bacterial blight.

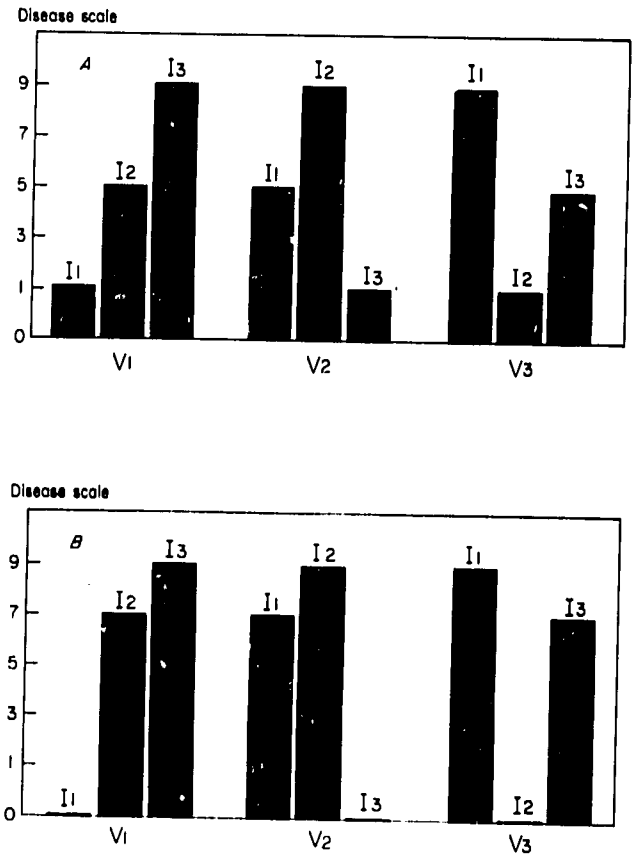


Fig. 3. With a 3-variety (V), 3-isolate (I) scheme, strong V-I interactions are shown on a 0-9 disease scale (IRRI 1980).

The other difficulty that scientists may encounter in work with bacterial blight is the inoculation method. Because bacterial blight is a vascular disease, a lesion develops when bacteria multiply in the vascular elements. It has been demonstrated that there is a high correlation between pin-pricking and clipping methods of inoculation (Horino et al 1980, Mew et al 1981). According to Mew et al (1982), there was a dose response even in a highly compatible variety-isolate combination. To elucidate the rice-bacterium interaction, the inoculum density should be high -- about $10^8$ to $10^9$ cells/ml. With the pin-pricking method, density of actual inoculum deposited at the infection site is generally about 1,000-fold less than with clipping (Mew, unpubl.).

Other considerations that are equally important are:

- experiments on rice-bacterium interaction should be done at optimum conditions for plant growth, and
- isolates should be properly maintained for their virulence.

Based on these considerations, we suggest the following for the collaborative project:

1. The IRRI and Japanese differentials could be used as the initial set of international differential varieties. TN1 should be included as susceptible check by all collaborators. One to two resistant varieties from South Asia are badly needed and, once identified, should be included.
2. The Standard Evaluation System for Rice (IRRI 1980) should be used in scoring bacterial blight reactions.
3. Inoculum density for the bacterial suspension should not be less than $10^6$ cells/ml, and should preferably be $10^9$ cells/ml.
4. Conditions for growing rice should be optimal.

## APPLICATION IN RESISTANCE BREEDING

Once physiologic specialization of a pathogen is shown in the infection of host varieties that have different genes for resistance, one of the handicaps to the development of disease-resistant varieties is the presence of numerous physiologic races (Stakman and Christensen 1960). When the pathogen variation is simple, the strategy for resistance breeding may not appear too complicated if resistance donors can be identified and used. With races of the pathogen present, the mistakes of omission and of commission in connection with the races must not be repeated in a breeding program if resistant varieties are to be developed and to have practical use.

For many years, we have not known how variable X. campestris pv. oryzae is. Because there have been different resistance donors, with diverse major genes identified and used, it has not been clear whether there is a distinct rice-bacterium relationship.

Systematic studies of rice varieties resistant to bacterial blight in the tropics was initiated in the 1960s. The lack of detailed information on the variability of the bacterial pathogen has sometimes hampered progress. It is often assumed in resistance breeding that varieties that respond identically to pathogen isolates have common genes for resistance. On the other hand, isolates of the pathogen, which have similar genes for virulence, infect all varieties identical in resistance.

On the basis of the bacterial blight collaborative project, we have learned that varieties that have the Xa-5 gene for resistance are susceptible to isolates in Bangladesh (S. A. Miah, BRRI, pers. comm.). This means that for Xa-5 resistance there is a matching virulence gene in most of the isolates of X. campestris pv. oryzae in Bangladesh. Similarly, varieties carrying Xa-4 are susceptible in Indonesia (Horino et al, unpubl. data). It is, therefore, concluded that there is a matching gene for Xa-4 in virulence in most of the Indonesian isolates. The gene or genes for virulence of these isolates are similar to pathotypes II and III in the Philippines.

Analyzing the pathogenic specialization, and thus the pathotypes, will lead to a good understanding of resistance needed in resistance breeding in different regions or countries; and the difference or shift of virulence of the bacterium, if it occurs, can then be detected.

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