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BREEDING FOR RESISTANCE TO RICE BLAST (*Pyricularia oryzae* CAV.) DISEASE IN BRAZIL

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Yoshitaka Tanaka
Research Specialist for Rice Pathology and Genetics, Ph.D.
CNPAF/EMBRAPA/IICA
GOIÂNIA - BRAZIL

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BREEDING FOR RESISTANCE TO RICE BLAST (*Pyricularia oryzae* Cav.) DISEASE IN BRAZIL

IV. The reaction of Brazilian blast fungus isolates obtained from the main upland varieties of five states to Kiyosawa's and international differential varieties.

V. The reaction of the isolates obtained from the main lowland varieties and breeding materials from the state of Santa Catarina.

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I. Preface

I joined the Rice Project of CNPAF/EMBRAPA through IICA in October 1980, to streghten and support to the blast resistant breeding program.

During the first year, I have spent considerable time to obtain general information on rice cultivation in Brazil and in collecting the blast fungus materials.

The area under upland rice has been increasing. I acquired adequate knowledge on rice production and its the cerrados region.

The researches on rice production is on progress at a rapid pace.

However, with the expansion of cultivated area the problems of diseases and pests on rice cultivation are increasing.

At the moment, the basic information about rice blast disease and resistant breeding is lacking.

We recognized the importance of the accumulation of basic data for resistant breeding on blast.

Many rice cultivating countries have spent a great deal of time in repeating the trials and errors of resistant breeding. The past failtures in successful breeding of resistant varieties has led to the need for accumulating the basic data for future breeding program in Brazil.

I do not have enough data to discuss about blast resistant breeding. However, I would like to gather latest information and cooperation from other advanced part of the wo~~u~~rld concerning blast resistant breeding.

I will very much appreciate recieving your suggestions and advice in this respect.

I would like to thank Dr. Almiro Blumenschein (Director, CNPAF/EMBRAPA) for giving me the opportunity to join the Rice Project in CNPAF/EMBRAPA through IICA.

Thanks are also to IICA especially Dr. J.C. Scarsi (Coordinator IICA/CNPAF/EMBRAPA) for coordinating the project between IICA and CNPAF/EMBRAPA. I would like to thank the coordinators at different experimental stations of EMBRAPA for their help in collecting the materials.

BASIC STUDY OF BREEDING FOR RESISTANCE TO RICE BLAST (*Pyricularia oryzae* CAV.) DISEASE IN BRAZIL

II. Introduction

Brazil is the biggest rice producing country except the Asian countries and it is the biggest upland rice producer in the world.

Blast disease of rice is a major problem in Brazil as well as in all rice growing countries. It seriously affects upland, paddy and all other cultivared conditions.

The disease, however, occurs in greater portions with well water management. Blast disease becoming a serious problem at the present time coincident with the expansion of rice cultivation both upland an lowland. Losses caused by rice blast disease are variable.

Under such circumstances, it is very important that these be basic research for resistance breeding to control blast disease to obtain higher and more stable yields.

In the past, vertical (major R. gene, gene-specific) resistance breeding was common, the resistance gene being introduced from sources different from those for vertical resistance. Vertical resistance to blast, however, has been shifly and unpredictable with varieties previonsly considered as resistance becoming susceptible.

The phenomenon, "breadown" of a resistance variety, is due to the expansion of a strain of blast pathogen which was indetected in the original development of the variety.

To overcome those difficulties caused by breakdown of vertical resistance, various methods for breeding have been reported by many researchers, as follows:

1) Utilization of horizontal resistance (gene non-specific) alone.
2) The combination of horizontal resistance and vertical resistance.
3) Mixture cultivation of varieties with different vertical resistance genes (multiline).
4) Rotation of varieties with different vertical resistance genes.
5) Accumulation of many vertical resistance genes in a single variety (Pyramidation of resistance gene).

Recently, genetic studies have greatly advanced and the mechanisms for blast resistance as a function of individual genes have been studied.

However the knowledge of variety of resistance genes in tropical areas is not available.

In Brazil, according available literatures, Amaral (1971, 1979), Ribeiro (1971, 1980), race differential were done by use of a set of international differential varieties.

Results showed that there is a high pathogenic variability in the fungus proportions in the state of São Paulo and Rio Grande do Sul.

Yunoki and Igarashi (1980) identified and classified 10 races from 45 isolates based on a set of international differentials, which had been collected from different varieties in different locations in the state of Paraná.

After that the 10 races were classified into 12 races based on the reaction to the 12 Japanese differentials.

It is concluded that further studies on the choice of differential varieties and the identifying race are needed in the future. The present research will be carried out with the main purpose of accumulating basic and genetic data for resistant breeding as follows:

First Stage:

1. Establishment of the method to classify the Brazilian isolate according to race.
2. Identification of the prevalent race in the main upland and lowland rice cultivating areas.
3. Observation of monthly and annual frequencies of the prevalent race at main rice cultivating areas to establish the system of forcasting of epidemics of blast disease.
4. The reaction of Brazilian isolates to Kiyosawa's, Japanese and international differenatial varieties.

Second Stage:

5. Establishment of the Brazilian differential races.
6. Establishment of the Brazilian differential varieties.

Third Stage:

7. Genetic analysis - Identification of the major resistance

gene.

a) Genetic analysis of the resistance gene of the main upland varieties including IAC 47, IAC 25 and breeding materials.

b) Genetic analysis of the resistance gene of the main lowland varieties including IRGA and EMPASC materials.

8. Blast resistance breeding

a) The effect of accumulation of major R. gene in one cultivar using Japanese varieties.

b) Evaluation of horizontal resistance in the Brazilian materials.

c) The new approach to control the blast such as mixed varieties: mixture cultivation of varieties with different major R. genes.

As mentioned above these basic studies require a long term project for the success of resistance breeding.

We know that even in well developed rice breeding countries there have been repeated trials and errors with reference to horizontal or vertical resistance breeding.

The breadkdown of varietal resistance makes breeders and pathologists reconsider and reevaluate horizontal resistance and vertical resistance breeding.

Though screening from a blast nursery and from multilocation trials are one of the easiest selection methods of varietal resistance if the knowledge of the genetic base of the variety is lacking, breakdown of varietal resistance inevitably occurs as a natural phenomenon.

So far, we realized that horizontal resistance breeding is important as well as vertical resistance to obtain higher and more stable yields. However, to evaluate horizontal resistance it is necessary to exclude the effect of vertical resistance.

On the other hand, to evaluate vertical resistance it is necessary to know the relationships between races and resistance gene constitutions of varieties.

The progress in genetic studies on blast resistance has greatly influenced the field of plant pathology. The differential system of blast fungus races established by many researchers has been widely used in Japan.

The comparative studies between the resistance gene found in Japan and those found other countries has not started yet. Rice varieties vary from country to country as do blast races. It is difficult to compare the genetic studies with different varieties and races in different countries.

Kiyosawa (1974,b) proposed a procedure of comparing the genes found independently in different countries.

The first step is to obtain suitable differential varieties which have singly different genes found in each country. Then differential varieties are exchanged according to the reaction of Japanese or Kiyosawa's differentials among the countries and tested for their resistance to the differential races in each country.

If an exchanged variety showed the same reaction as any one of the domestic differential varieties, the resistance genes of both varieties may be (sometimes, many not be) identical.

The conclusion should be drawn from the gene analysis using the hybridization method.

According, systematic studies are essential for resistance breeding on blast disease.

III. Materials and Methods

In this chapter, the materials and methods used in the following chapters of this Annual Report are summarized. Following materials, methods and criteria are established based on the results of the trials of last year.

It consists of differential varieties, isolation, inoculation criteria for rating the pathogenicity of isolates, sampling, differential races and individual reaction of the differentials to Brazilian blast fungus isolates.

It is one of the most important points to determine the suitable material, method and criteria to identify the pathogenicity of blast fungus isolates and to estimate the major R. genes by genetic analysis under different climatic conditions. In addition comments were made about the applicability and reaction performance of Kiyosawa's and international differential varieties to the Brazilian blast fungus isolates.

A) Differential varieties: 12 Kiyosawa's (Japanese) and 8 international differentials are normally used for race identification. The Kiyosawa's and international differential varieties used in this research are shown in Table 1. The details and characteristics of these varieties are mentioned in the following section.

Differentials were planted in plastic trays, 28x16x12 cm in the greenhouse to avoid contamination. Each variety was

TABLE 1. A set of Japanese (Kiyosawa's)differential varieties to identify the Brazilian blast fungus strains.

Nº	VARIETY	RESISTANCE GENE	CODE Nº
1	Shin 2	Pi - k^{s}	1
2	Aichi-Asahi	Pi - a	2
3	Fujisaka 5	Pi - i	4
4	Kusabue	Pi - k	10
5	Tsuyuake	Pi - k^{m}	20
6	Fukunishiki	Pi - z	40
7	K 1	Pi - ta	100
8	Pi nº 4	Pi - ta^{2}	200
9	Toride 1	Pi - z^{t}	400
10	K 60	Pi - k^{p}	.1
11	BL 1	Pi - b	.2
12	K 59	Pi - t	.4

A set of international differential varieties

Nº	VARIETY	RESISTANCE GENE	~~CODE Nº~~ Origin
1	Raminad Str. 3		Philippines
2	Zenith	Pi-a, Pi-z	U.S.A.
3	NP-125		India
4	Usen	Pi-a	China
5	Dular	Pi-k	Pakistan
6	Kanto 51	Pi-k	Japan
7	Shao-tiao-tsao	Pi-k^{s}	China
8	Caloro	Pi-k^{s}	U.S.A.

planted at 3 cm between rows and there were 10 seedlings per variety. Before planting, seeds were coated and treated with Benlate (to avoid seed transmissible disease). 8 g (N.P.K. + Zn = 5:30:15, 0,3%) of fertilizer and excess nitrogen (3 g) was applied. Trays were kept under upland conditions.

B) Isolation: Single spore isolates were made directly from leaf lesions under the microscope after they had been kept for 24 hrs in a moist condition.

Isolates were incubated by the following procedure, single spore isolates were cultured on a PDA medium at room temperature; spore formations were plate-cultured on an oatmeal-dextose-agar medium at 25°C for about 10 days; after this, the aerial hyphae were removed with a small amount of sterilized water using a paint brush and kept for 48 hrs under a fluorecent light to promote spore formation. The inoculum spore suspension was prepared at $5x10^4$ spores per ml of water. When spore suspension was less than $2x10^4$ spores, a small amount of Tween 80 (about 1/5000) was added.

C) Inoculation: Inoculation of spore suspension was at 4.0 ~ 4.5 leaf stage of the seedlings using a small hand compressor (Bullcraft 85 W, Japan) and attached spraygun. The amount of spore suspension was 50 ml per tray.

After inoculation seedlings were incubated in a moisture chamber for 24 hrs at 20^{o} - 25^{o}C, and transferred to a greenhouse kept at 20^{o}- 35^{o}C. Observation and recording were made 7 days after transfer to the greenhouse.

D) Race Identification: Pathogenicity of the isolates was rated by the following criteria. They were modified for the spray inoculation based on the criteria developed for the injection inoculation (Yamasaki & Kiyosawa 1966).

I. Number of plants with lesions in a given variety $\leq$ 1/3 (Number of plants with lesions in the variety with the largest number of lesions)............................R^{h}

II. Number of plants with lesions in a given variety > 1/3 (Number of plants with lesions in the variety with the largest number of lesions:

1. Mode in b type lesions

a. $b > 2(bg + bG + pG)$ .... R

b. $(bg + bG + pG) \leq b \quad 2(bg + bG + pG)$ α......... R

β.........MR

c. $b < (bg + bG \% pG)$ α.........MR

β......... M

2. Mode in bg type lesions

a. $(b + bg) \geq (bG + pG)$ α.........MR

β......... M

b. $(b + bg) < (bG \% pG)$

α....... M

β.......MS

3. Mode in bG type lesions

a. $(b + bg) \geq (bG + pG)$

α.......MR

β....... M

b. $(b + bg) < (bG + pG)$

α....... M

β.......MS

4. Mode in pG type lesions

a. $(b + bg + bG) \geq pG$

α....... M

β.......MS

b. $(b + bg + bG) < pG$

α.......MS

β....... S

α: The average number of lesions is less than one - fifth of the average number of lesions on the variety having the largest average number of lesions.

β: The average number of lesions on the variety having the largest average number of lesions.

To evaluate the differential ability of the varieties individual plants were rated according to the criteria given above, and figures from zero to five were given to reactions from R^h to S, respectively, to calculate means and standard deviations.

Lesions on the inoculated plants were divided into 4 types, b (brown spot), bg (small white or gray lesion with brown margin), bG (large white or gray lesion with brown margin) and pG (large white or gray lesion with purple or uncoloured margin), and the number of each type of lesion was counted about 7 days after inoculation.

E) Sampling for estimation of race frequency from main cultivars: Single spore isolations are obtained from the main cultivar at each location. A total of 50-60 different sizes of lesions collected from at least 5 locations at one place were incubated in BDA; then 20-39 single spore isolations were isolated as the representatives at each location.

Isolates from these locations are used for estimation of the frequency of predominant races.

To estimate the frequency of the prodominant race at each location is not easy because of the influence by the cultivars which have different resistance genes sorrounding the main cultivars.

The location where a single cultivar is cultivated in as nearly monocultural condition in a large area (such as IAC 47 and IAC 25 at Jataī, Goiās state or Cuiabā, Mato Grosso) estimation of the race frequencies may be possible. On the other hand, the location where many varieties are cultivated it is difficult to estimate the race frequencies. In such a place a great number of isolations are needed to be collected for estimation of the race frequencies. Observation of the frequency of the predominant race gives much information not only to select a suitable resistance variety at a certain location but also may give information for future breeding programs.

F) Differential races: Table 2 shows the origin and reaction patterns of six different pathogenic races to Kiyosawa's differential varieties. The set of six different races is used for temporary differential races in brief screening of major R. gene in Brazilian varieties and breeding materials.

The details of individual components of differential varieties are mentioned in following section.

The characteristics of pathogenicity of each race are summarized as follows:

1) Race 11 (Code nº 157.5) can match to both Fukinishiki (Pi-z) and Kusabue, Kanto 51 (Pi-k). Race 11 was obtained one of maior upland variety, IAC 25 at CNPAF/ EMBRAPA, Goiânia.

   This is one of the most common race as well as Race 39 and Race 162 in main upland rice cultivating regions.
2) Race 39 (code nº 147.5) shows R reaction to Kusabue and Kanto 51 (Pi-k).

   Both Race 11 and Race 39 can match to Zenith (Pi-a, Pi-z), however Race 39 shows MR reaction to Fukunishiki (Pi-z).

3) Race 162 (code nº 117.5) can match to Fukunishiki (Pi-z) beside S reaction to Kusabue and Kanto 51 (Pi-K). From the combination of above three races, it can distinguish the difference of the major R. gene Pi-k and Pi-z.

4) Race 240 (code nº 37.4) was isolated from one of induced foreign breeding material SR 2041-50-1 from Korea. Same race can be identified from the pedigrees of cross between SR 2041-50-1 and IAC 47. Race 240 can match to Tsuyuake (Pi-k^m) with typical susceptible reaction. It shows R^h reaction to K 1 (Pi-ta) and K 60 (Pi-k^p). Race 240 is supposed to distingiush the major R. genes Pi-ta and Pi-k^p.

5) Race 303 (code nº 307.1) was isolated from one of main lowland varieties, IRGA 408 in the state of Santa Catarina. This race could not obtained from blast fungus collections from main upland varieties in five main upland cultivating states (Goiás, Minas Gerais, Mato Grosso, São Paulo and Paraná).
Race 303 can match to Pi nº 4 (Pi-ta^2). As Tadukan carrys Pi-ta^2 and Pi-ta, Pi-ta^2 major R. gene is so-called Tadukan type major R. gene.
Race 303 showed R^h reaction to Kusabue, Kanto 51 (Pi-k) and K 59 (Pi-t).

6) Race 305 (code nº 7.3) was obtained from one of main lowland varieties in the state of Santa Catarina as well as Race 303.

The detail of Race 303 and 305 will be mentioned in chapter V.

Race 305 can match to BL 1 (Pi-b) however can not match to Pi Nº 4 (Pi-ta^2).

Depending on the combination of Race 303 and Race 305, it is able to distinguish the difference of major R. genes Pi-ta^2 and Pi-b.

From these results, a set of six differential races that maybe able to identify the six major R. genes as follows: Pi-k, Pi-z, Pi-k^m, Pi-ta^2, Pi-b and Pi-k^p.

Since three of thirteen major R. genes, Pi-a, Pi-i and Pi-k^s are supposed to be inadequate donars of resistance to blast, the existing set of six differential races are quite sufficient to identify the major R. gene or genes in Brazilian materials.

TABLE 2. Reaction patterns of six different pathogenic races to the Kiyosawa's differential varieties.

Nº	Variety	Resistance gene	Code Nº	RACE					
				11	39	162	240	303	305
1	Shin 2	Pi-k^s	1	S	S	S	S	S	S
2	Aichi Asahi	Pi-a	2	S	S	S	S	S	S
3	Fujisaka 5	Pi-i	4	S	S	S	S	S	S
4	Kusabue	Pi-k	10	S	R	S	S	R^h	R^h
5	T̄suyuake	Pi-k^m	20	R^h	R^h	R^h	S	R^h	R^h
6	Fukunishiki	Pi-z	40	M	MR	R^h	R^h	R^h	R^h
7	K 1	Pi-ta	100	S	S	S	R^h	S	R^h
8	Pi Nº 4	Pi-ta^2	200	R^h	R^h	R^h	R^h	M	R^h
9	Toride 1	Pi-z^t	400	R^h	R^h	R^h	R^h	R^h	R^h
10	Zenith	Pi-a,Pi-z		S	S	R^h	R^h	R^h	R^h
11	Kanto 51	Pi-k	10	S	R	S	S	R^h	R^h
12	K 60	Pi-k^p	0.1	S	S	S	R^h	S	S
13	BL 1	Pi-b	0.2	R^h	R^h	R^h	R^h	R^h	M
14	K 59	Pi-t	0.4	S	S	S	S	R^h	R^h
	CODE Nº			157.5	147.5	117.5	37.4	307.1	7.3
	ORIGIN	Variety		IAC 25	IAC 25	IAC 47	SR2041-50-1	IRGA 408	BR IRGA 409
		Location		CNPAF GOIĀNIA GOIĀS	CNPAF GOIĀNIA GOIĀS	JATAĪ GOIĀS	CNPAF GOIĀNIA GOIĀS	URUSSANGA SANTA CATARINA	URUSSANGA SANTA CATARINA

G) Characteristics of reaction pattern of Kiyosawa's and international differential varieties to Brazilian blast fungus isolates: It is very important to distinguish the characteristics of the reaction between races and individual components of differential varieties to initiate the blast study on resistance.

Under following circumstances, more careful observations of reaction between races and differentials are needed.

1) Non existence of suitable differential varieties
2) Using the differentials in which the gene constitution on resistance have not been identified
3) Observations will be carred out under different circumstances such as race frequency and constitution and climatic condition.

Otherwise it leads to the production of many races by the misreading of reaction.

From the results of race identification using the Kiyosawa's and international differentials to Brazilian blast fungus isolates, characteristics of individual component of differentials were summarized as follows.

1) Individual reaction of Kiyosawa's differential varieties to Brazilian isolates.
   (1) Shin 2 (Pi-k^s): showed two different types of reaction: one is a typical susceptible reaction with pG type lesions, another is one of resistant reaction with b and bg type lesions. Shin 2 showed typical susceptible symptoms to many isolates but

sometimes showed an ambiguous reaction according to the resistance reaction.
Soil condition and temperature may have influenced the appearance of the reaction. This variety does not show similar reaction with Caloro, Sha-tiao-tsao which are supposed to have the Pi-k^s resistance gene.

(2) Aichi-Asahi (Pi-a): Aichi-Asahi always showed the typical susceptible reaction to all isolates obtained from all of locations to date. Reaction showed severe symptoms by both the spray and injection methods. No isolate showed ambiguous reaction to Aichi-Asahi.

(3) Fujisaka 5 (Pi-i): always showed the typical susceptible symptoms as did Aichi-Asahi but the number of susceptible lesions was less than that of Aichi-Asahi. Fujisaka 5 showed the typical susceptible reaction to all the isolates to date.

(4) Kusabue (Pi-k): showed two different reactions: one is the typical susceptible reaction with PG lesions, another is one of resistant reaction with b and bg type lesions. Kusabue showed resistant reaction to many isolates but showed typical susceptible reaction to some isolates. Kusabue showed a quite different reaction from that of Kanto 51 (Pi-k). The difference in reaction between the two varieties will be distinguished by genetic analysis.

(5) Tsuyuake (Pi-k^m): showed typical susceptible symptoms to the isolates obtained from variety SR 2041 and its progenies. On the other hand this variety showed a resistant reaction with a few b or bg type lesions. It appears to be the one of the characteristics of this variety.

(6) Fukunishiki (Pi-z): Fukunishiki did not show typical susceptible reaction of Aichi-Asahi.
Reactions of this variety are always MR - M type with several pG type. Based on the reaction of this variety it is assumed that it is dependent on the horizontal resistance from be reactions of Zenith (Pi-z, Pi-a) and Aichi-Asahi. The replacement of Fukunishiki is needed for identifying the Pi-z gene.

(7) K 1 (Pi-ta): always showed typical susceptible reactions to all isolates excepting race 305. The reaction showed severe symptoms by both the spray and injection methods. To date no isolate showed an ambiguous reaction to K 1.

(8) Pi Nº 4 (Pi-ta^2): showed a typical resistance reaction to all the isolates which obtained from the main upland varieties no symptoms appears on the inoculated leaf, however, shows M reaction with several pG type lesions to Race 303 which was isolated from a lowland variety IRGA 408 in the state of Santa Catarina. The Pi-ta^2 major R. gene of Pi Nº 4 is inherited from the philippines variety

Tadukan.

(9) Toride 1 (Pi-z^t): showed a typical resistant reaction. No symptom and no hypersensitive reaction appears in all isolates obtained from all locations to date.
The Pi-z^t major R. gene of this variety is inherited from the Indian variety TKM 1.

(10) K 60 (Pi-k^p): always showed a typical susceptible reaction with pG type lesions. To date only one of all the isolates showed a resistant reaction (no symptom). K 60 shows the clear reaction symptom as well as that of Aichi-Asahi.

(11) BL 1 (Pi-b): showed a typical resistance reaction to all the isolates obtained from the main upland varieties. However, shows M reaction with several pG type lesions to Race 305 which isolated several lowland varieties cultivated in the state of Santa Catarina. BL 1 is the one of the usuful variety to identify the isolates obtained from main lowland varieties in Brazil.

(12) K 59 (Pi-t): showed clearly two different reactions: one is the typical susceptible reaction with pG type lesions, another is the resistant reaction with b or bg type lesions.
Sometimes there were lesions which resemble one of hypersensitive reaction.

2) Individual reaction of international differentials to Brazilian isolates.

(1) Raminad Str 3: no susceptible reaction appears in all the isolates to date. There was sometimes a hypersensitive and resistant reaction with b or pg type lesions.

(2) Zenith (Pi-z, Pi-a): Zenith is well knownto have Pi-z and Pi-a resistance genes. Aichi-Asahi (Pi-a) shows susceptible reaction to all isolates. According to the reaction of Fukunishiki (Pi-z), the reaction of Zenith will also be affected. However, some isolates did not show a susceptible reaction to Fukunishiki whereas in Zenith. They produced typical susceptible symptoms. It is assumed that Fukunishiki has relatively high level of horizontal resistance to mask symptom formation in Fukunishiki.

(3) NP-125: showed susceptible reaction to most races. It serves to be a different for typical susceptible reaction with pG type lesions.

(4) Usen (Pi-a): showed susceptible reaction to most races. It serves as a typical susceptible reaction with pG type lesions as does NP-125.

(5) Dular (Pi-k): sometimes showed a typical hypersensitive reaction. But this appeared to be typical susceptible symptoms with pG lesions to several isolates. However, the hypersensitive reaction showed very severe reaction sometimes covering whole leaves. This

is not susceptible symptom of this variety. Dular showed the typical susceptible reaction with pG lesions when matched races existed.

(6) Kanto 51 (Pi-k) showed typical susceptible reaction but the number of lesions are less than in Aichi-Asahi, Sha-tiao-tsao or Caloro. Sometimes resistant symptoms were found with b or bg type lesions.

(7) Sha-tiao-tsao ($Pi\text{-}k^s$): always showed the typical susceptible reaction. The number of susceptible lesions is almost the same as in Aichi-Asahi. These varieties showed susceptible symptoms to all the isolates.

(8) Caloro ($Pi\text{-}k^s$): always showed the typical susceptible reaction with pG lesions as does Sha-tiao-tsao or Aichi-Asahi. Reactions between Caloro and Shin 2 are sometimes different.

IV- THE REACTION OF BRAZILIAN BLAST FUNGUS ISOLATES OBTAINED FROM THE MAIN UPLAND VARIETIES OF FIVE STATES TO KIYOSAWA's (JAPANESE) AND INTERNATIONAL DIFFERENTIAL VARIETIES

1) Introduction

Brazil is the biggest upland producer in the world. The upland rice area covers more than 4.2 million ha and produced about 4.5 million tons of rice in 1980. The major upland rice cultivated area is covered by Cerrados (Fig. 1).

From the east-central to the south-east, upland cultivations is predominant and is the typical transition to develop the Cerrados region for pasture crop in the future.

Fig. 2 shows the main upland cultivated area and the main varieties in each state in Brazil.

At present IAC 47 and IAC 25 are the most common varieties grown in this country and were released by the Institute of Agriculture of Campinas (IAC), São Paulo in the year 1971 and 1974, respectively. The breakdown of the varietal resistance of these two varieties has recently been reported and is increasing rapidly with the expansion of the cultivated area.

Unfortunately there are now no improved varieties with resistance to blast that can replace IAC 47 and IAC 25.

The Centro Nacional de Pesquisa de Arroz e Feijão (CNPAF) is improving the rice varieties by incorporating blast resistance and is testing it in several key locations in Brazil.

However, as mentioned at the biggining of this report, there is little information about the race frequency and constitution, and resistance gene of rice varieties in Brazil.

Following literatures about race identification are available AMARAL (1980) identified 16 physiological races from 28 collections in São Paulo according to the reaction of international differentials in the state of São Paulo. YUNOKI and IGARASHI (1980) started the race differentiation using Japanese and international differentials. They classified into 10 international races from 45 isolates in the state of Paranã; after that 10 international races were classified into 12 races based on the reaction to the 12 Japanese differential varieties. It is concluded that further studies on the choice of differential varieties and the race identifying are needed in the future.

This experiment will be carried out to identify the race constitution and frequency at each locations in the multi-location trials. It may provide information on the resistance source as well as on race constitution.

A total of 218 isolates from 17 locations in 5 states were differentiated to the race based on the reaction patterns of pathogenicity to the international and Kiyosawa's differentials.

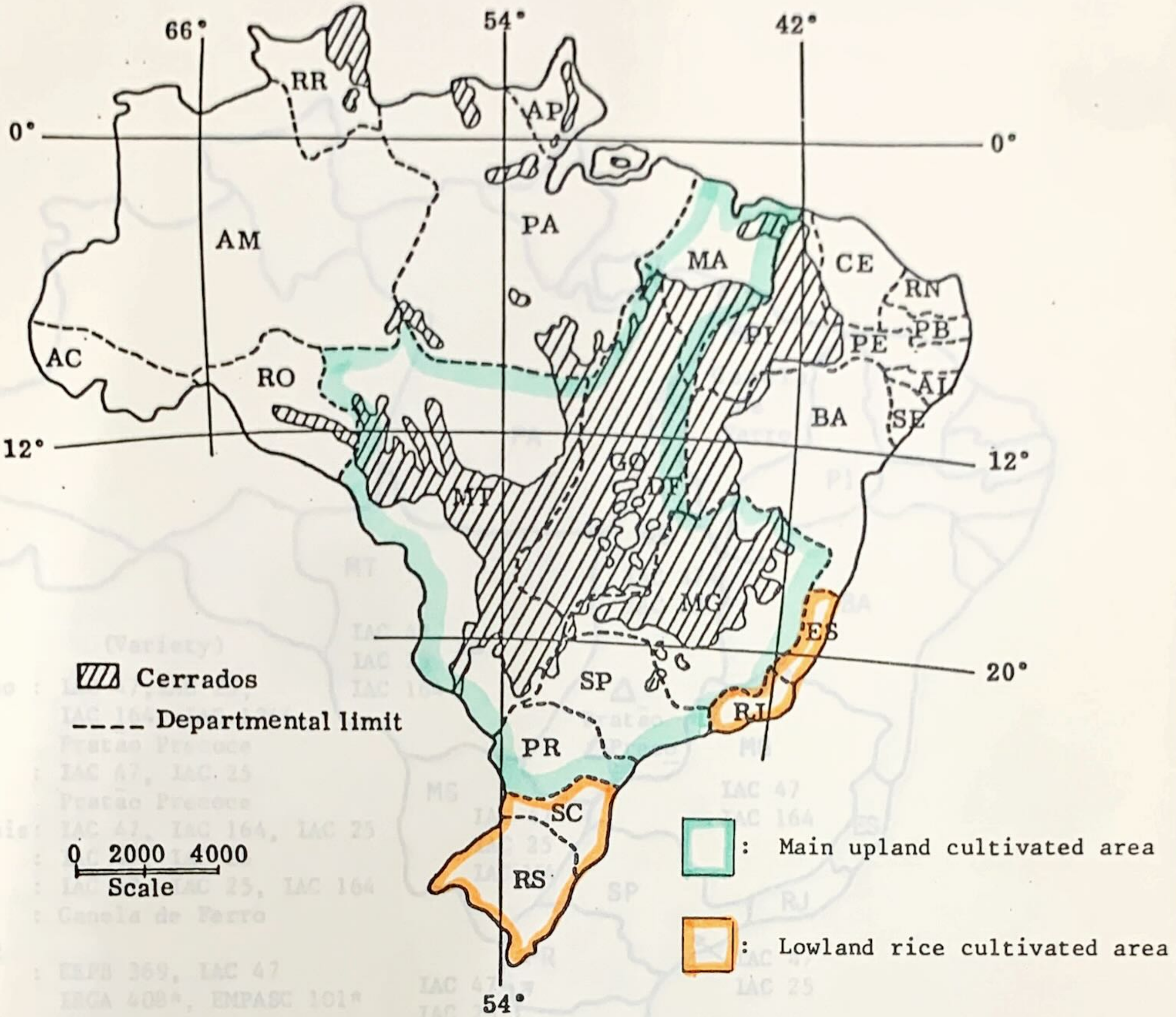
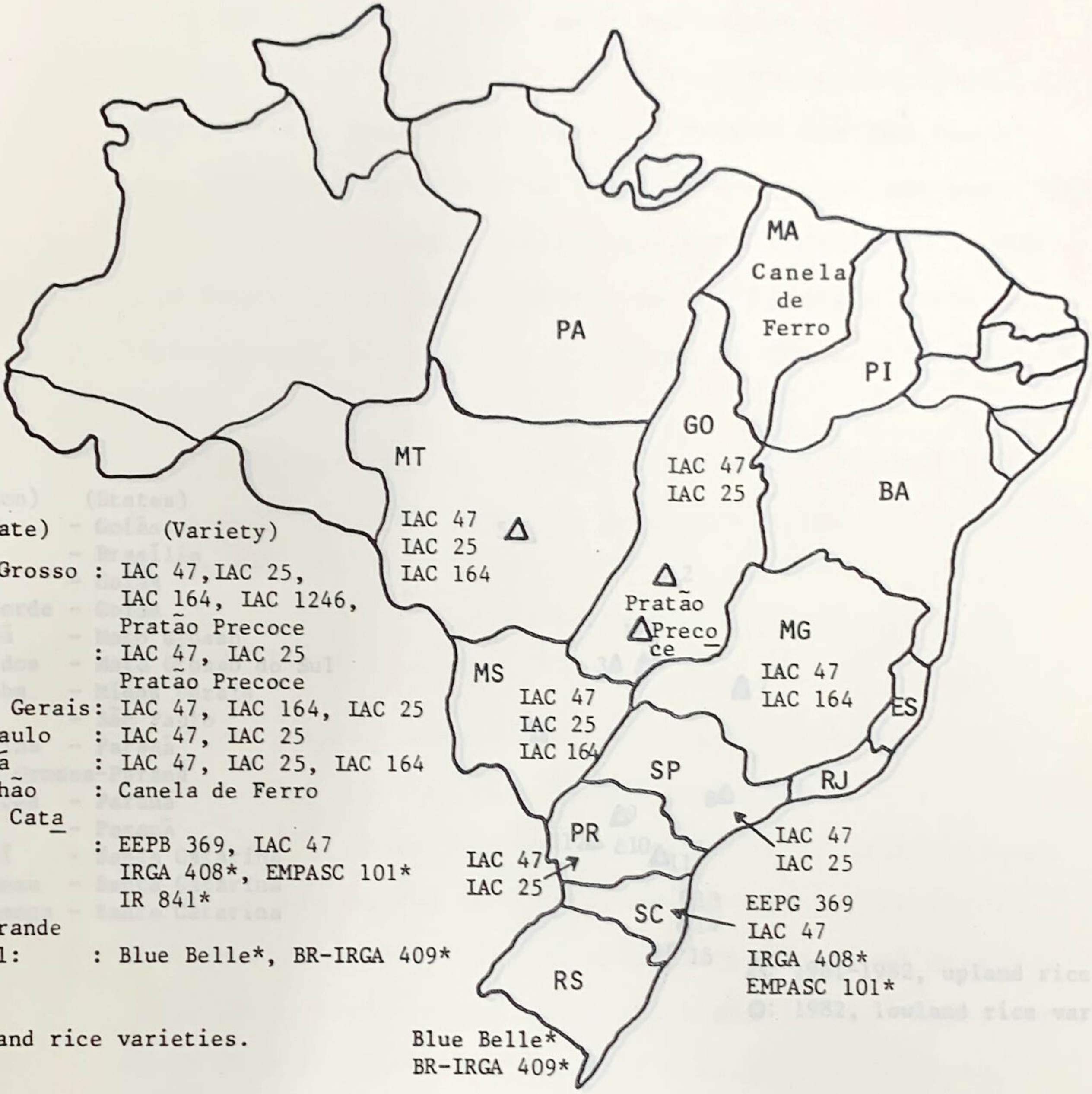


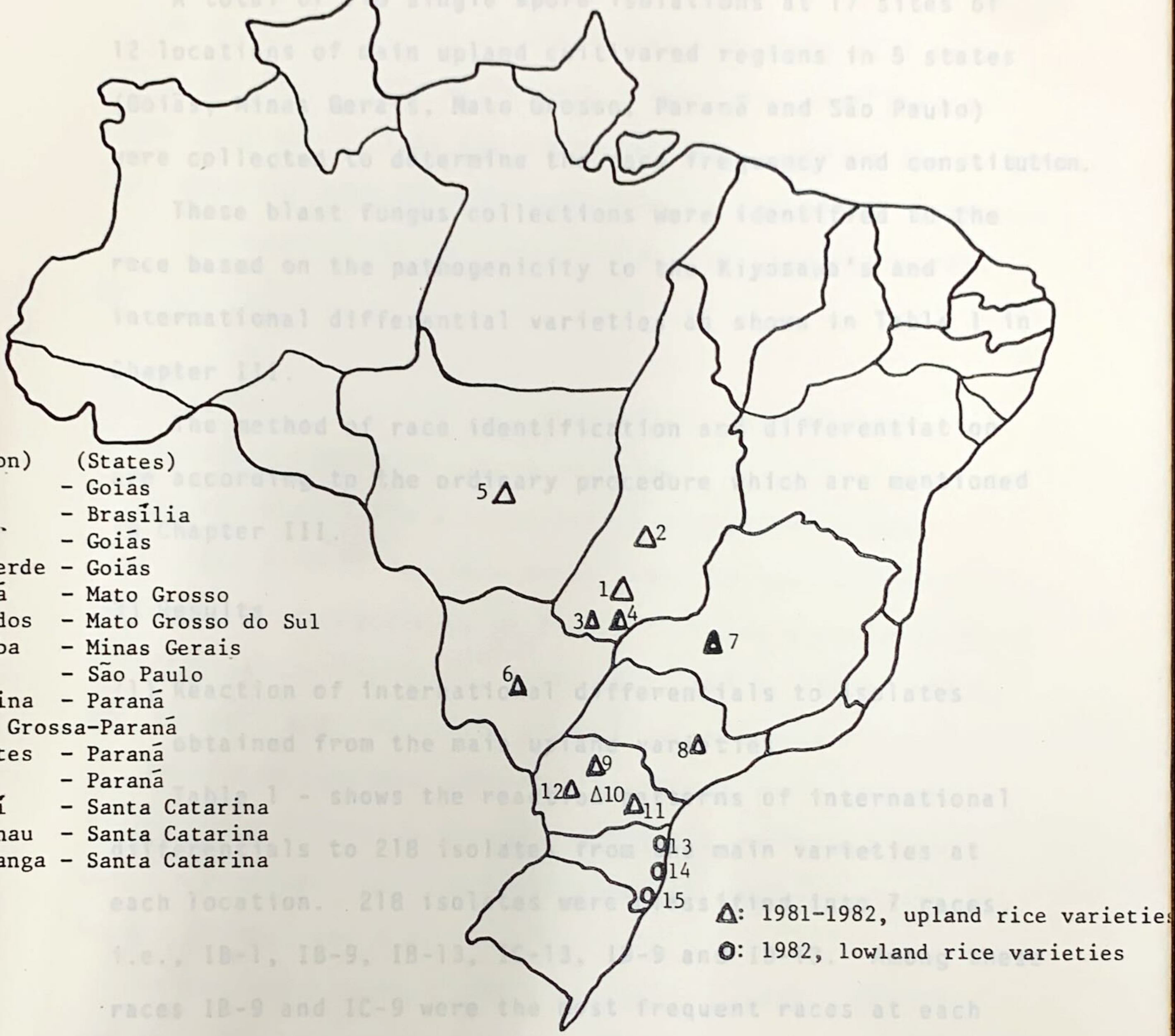
Fig. 1. Main upland rice cultivated area and present distribution of "Cerrados" in Brazil.



(State)	(Variety)
1.Mato Grosso	IAC 47, IAC 25, IAC 164, IAC 1246, Pratão Precoce
2.Goiás	IAC 47, IAC 25 Pratão Precoce
3.Minas Gerais	IAC 47, IAC 164, IAC 25
4.São Paulo	IAC 47, IAC 25
5.Paraná	IAC 47, IAC 25, IAC 164
6.Maranhão	Canela de Ferro
7.Santa Catarina	EEPB 369, IAC 47 IRGA 408*, EMPASC 101* IR 841*
8.Rio Grande do Sul:	Blue Belle*, BR-IRGA 409*

*: lowland rice varieties.

Fig. 2. Main upland cultivated areas and main varieties in each state in Brazil.



(Location)	(States)
1.CNPAF	- Goiás
2.CPAC	- Brasília
3.Jataí	- Goiás
4.Rio Verde	- Goiás
5.Cuiabá	- Mato Grosso
6.Dourados	- Mato Grosso do Sul
7.Uberaba	- Minas Gerais
8.IAC	- São Paulo
9.Londrina	- Paraná
10.Ponta Grossa	-Paraná
11.Morretes	- Paraná
12.Irati	- Paraná
13.Itajaí	- Santa Catarina
14.Blumenau	- Santa Catarina
15.Urussanga	- Santa Catarina

Δ: 1981-1982, upland rice varietie

O: 1982, lowland rice varieties

Fig. 3. Locations of blast fungus collections in Brazil.

2) Material and Method

Fig. 3 shows the locations of blast fungus collections in Brazil.

A total of 218 single spore isolations at 17 sites of 12 locations of main upland cultivared regions in 5 states (Goiãs, Minas Gerais, Mato Grosso, Paranã and São Paulo) were collected to determine the race frequency and constitution.

These blast fungus collections were identified to the race based on the pathogenicity to the Kiyosawa's and international differential varieties as shown in Table 1 in Chapter III.

The method of race identification and differentiation are according to the ordinary procedure which are mentioned in Chapter III.

3) Results

(1) Reaction of international differentials to isolates obtained from the main upland varieties.

Table 1 - shows the reaction patterns of international differentials to 218 isolates from the main varieties at each location. 218 isolates were classified into 7 races, i.e., IB-1, IB-9, IB-13, IC-13, ID-9 and ID-13. Among these races IB-9 and IC-9 were the most frequent races at each location.

Raminad Str. 3 always showed a resistant reaction but sometimes showed a hypersensitive reaction; on the other hand, Shao-tiao-tsao and Caloro showed susceptible reactions with typical pG type lesions to all the isolates. Among these varieties, Dular sometimes showed a very severe hypersensitive reaction to some isolates.

Details of reaction of individual varieties will be mentioned later. The difference between the most frequent races IB-9 and IC-9 is the pathogenicity to Zenith (Pi-z, Pi-a). This suggests that the gene constitution for blast resistance is to be expected to be controlled by the Pi-z resistance gene.

(2) Reaction of Kiyosawa's differentials to isolates obtained from main upland varieties.

As shown in Table 2 these international races were classified into 20 Japanese races based on the reaction patterns of pathogenicity to the 12 Kiyosawa's (Japanese) differentials. Specially, Race 157.5, 147.5, 146.5, 107.5, 107.1, 106.5 and 106.1 were predominant everywhere.

Among the Japanese differentials Aichi Asahi (Pi-a), and Fujisaka 5 (Pi-i), showed susceptible reactions to all the isolates and only one isolate did not show pathogenicity to K 1 (Pi-ta) and K 60 (Pi-k^p).

On the other hand, Pi Nº 4 (Pi-ta^2), Toride 1 (Pi-z^t) and BL 1 (Pi-b) showed resistant reactions to all the isolates. From these reaction patterns, predominant races may able to be divided into some groups based on pathogenicity to Japanese differentials as follows:

Fukunishiki type (Pi-z) - Race 157.5, 147.5, 146.5
Tsuyuake type (Pi-k^m) - Race 137.1, 127.1
Kusabue type (Pi-k) - Race 117.5, 117.1
Shin 2 type (Pi-k^s) - Race 107.5
K 60 type (Pi-k^p) - Race 107.1

This suggests that the constitutions of the resistance gene in Brazilian varieties are mainly controlled by Pi-z, Pi-k^s, Pi-k^p, Pi-a, Pi-i, Pi-t and not identified genes.

This result suggests that the constitution and frequency of the blast races varies according to location and variety.

Further study using more isolates is needed to identify the race constitution and frequency.

(3) Comparison of isolates from the Philippines and Japan

Table 3 shows the number of isolates attacking each of Kiyosawa's differential varieties collected from the Philippines and Japan.

218 isolates obtained from 7 locations in 5 states in Brazil were compared with isolates from the Philippines and Japan. Isolates between countries show quite different reaction patterns. It suggests that the constitution and frequency of the blast fungus varies among the countries.

The predominant races are quite different from those of the Philippine, Japan and Brazil. To date, isolates able to attack to Pi Nº 4, Toride 1 and BL 1 are not yet identified in Brazil.

4) Discussion

A total of 218 single spore isolations of rice blast fungus obtained from main upland varieties at 17 locations in 5 states was differentiated to international and Japanese races based on the pathogenicity to international and Kiyosawa's differentials.

From the results of race identification with international differentials, only 7 international races were identified. This is less than the number here before "identified". IB-9 and IC-9 are predominant every where (TANAKA, 1981a).

From international races, they were classified into 20 Japanese (Kiyosawa's) races. This suggests that a set of Kiyosawa's differentials is more isogenic to the blast resistance gene and more sensitive in reaction to the isolations.

To date, one of the international differentials Raminad Str. 3, always shows resistant reaction to all isolates and the reaction of Kiyosawa's differentials. Pi Nº 4, Toride 1 and BL 1 shows a typical resistant reaction to all isolates which have the Pi-ta^2, Pi-z^t and Pi-b single resistance gene, respectively. Table 4 shows the resistance genes which were identified in different varieties of mainly of <u>indica</u> type.

The resistance gene of Pi-ta^2 in Pi Nº 4 and Pi-z^t in Toride 1 are inherited from Tadukan (Pi-ta, Pi-ta^2, Pi-k) of the Philippines, and from TKM 1 (Pi-z^t) of India, respectively.

If these parent varieties of Toride 1 and Tadukan have a high level of horizontal resistance they will be the contributors of the resistance source to blast in Brazil.

The results of race differentiation in Brazilian isolates suggest the possibility of the introduction of vertical resistance genes from the varieties given in Table 4. Some of varieties are promising gene sources not only for vertical (gene-specific) resistance but also some of them have a high level of horizontal or broad spectrum resistance.

Te-tep and Dawn are well known varieties which have broad spectrum resistance (IRRI). This suggests that one of the resistance genes of both varieties the Pi-k^h resistance gene may provide an important function on blast resistance.

The results of race differentiation provide not only race frequency and constitution but also provide the possibility of introduction of resistance gene sources under the genetic basis.

On the other hand, some of local land varieties showed the Toride 1 type reaction; this will be mentioned in a later chapter. There is also the possibility of introduction of resistance background from local Brazilian land varieties.

In Chapter V and VIII there will be discussion about the screening of the resistance gene of local varieties with six differential races which have been obtained from Brazilian isolates.

In terms of the differential varieties, the establishment of Brazilian differential varieties is urgently needed as well as Brazilian differential races.

At first, some of the international and Kiyosawa's differentials may have the same kind of resistance gene, for example, Shao-tiao-tsao, Caloro and Shin 2 have Pi-k^{s} gene.

The reaction of some differential varieties is duplicated. Some of them should be eliminated in order to select one variety only.

About the Pi-z gene, Fukunishiki, one of the Japanese differentials may have a high level of horizontal resistance, which sometimes prevents the reaction to a pathogenic race. Another substitute variety with susceptible reaction to Pi-z gene should be looked for. In chapter VI and VII there will be discussion about the differential ability and the level of horizontal resistance of the differentials.

The conclusion will be discussed in a later chapter and further study is needed for the selection and establishment of differential varieties in Brazil.

TABLE 1. Reaction of International differential varieties to isolates obtained from main varieties in 5 states.

Variety	REACTION							
	1	2	3	4	5	6	7	
Raminad Str. 3	-	-	-	-	-	-	-	
Zenith	S	S	-	S	-	-	-	
NP 125	S	S	S	S	S	-	-	
Usen	S	S	S	S	S	S	S	
Dular	S	-	-	-	-	-	-	
Kanto 51	S	S	S	-	-	S	-	
Sha-Tiao-Tsao	S	S	S	S	S	S	S	
Caloro	S	S	S	S	S	S	S	
International Race	IB-1	IB-9	IC-9	IB-13	IC-13	ID-9	ID-13	
Number of isolates	6	98	97	8	5	3	1	(218)

S: Susceptible reaction

-: Resistant reaction

TABLE 2. Reaction of Kiyosawa's differential varieties to isolates obtained from main varieties in 5 states.

Differential Variety	Resistance Gene	Code Nº	REACTION							
			1	2	3	4	5	6	7	
Shin 2	Pi-k^s	1	S	-	S	S	-	S	S	74
Aichi-Asahi	Pi-a	2	S	S	S	S	S	S	S	76
Fujisaka 5	Pi-i	4	S	S	S	S	S	S	S	76
Kusabue	Pi-k	10	S	S	S	S	S	-	-	25
Tsuyuake	Pi-k^m	20	S	S	-	-	-	-	-	2
Fukunishiki	Pi-z	40	S	S	S	S	S	S	S	76
K 1	Pi-ta	100	S	S	S	S	S	S	S	76
Pi Nº 4	Pi-ta^2	200	-	-	-	-	-	-	-	0
Toride 1	Pi-z^t	400	-	-	-	-	-	-	-	0
K 60	Pi-k^p	.1	-	S	S	S	S	S	S	76
BL 1	Pi-b	.2	-	-	-	-	-	-	-	0
K 59	Pi-t	.4	S	S	S	-	S	S	-	74
Japanese race			117.5	176.5	157.5	157.1	156.5	147.5	147.1	
Number of isolates			1	1	21	1	1	50	1	(76)

S: Susceptible reaction

-: Resistant reaction

TABLE 2. Continued.

Differential Variety	Resistance gene	Code Nº	REACTION							
			8	9	10	11	12	13	14	(TOTAL)
Shin 2	Pi-k^s	1	-	-	S	S	S	S	S	13
Aichi-Asahi	Pi-a	2	S	S	S	S	S	S	S	29
Fujisaka 5	Pi-i	4	S	S	S	S	S	S	S	29
Kusabue	Pi-k	10	-	-	S	S	-	-	S	20
Tsuyuake	Pi-k^m	20	-	-	S	S	S	S	-	7
Fukunishiki	Pi-z	40	S	S	-	-	-	-	-	16
K 1	Pi-ta	100	S	S	S	S	S	S	S	29
Pi Nº 4	Pi-ta^2	200	-	-	-	-	-	-	-	0
Toride 1	Pi-z^t	400	-	-	-	-	-	-	-	0
K 60	Pi-k^p	.1	S	S	S	S	S	S	S	29
BL 1	Pi-b	.2	-	-	-	-	-	-	-	0
K 59	Pi-t	.4	S	-	S	-	S	-	S	23
Japanese Race			146.5	146.1	137.5	137.1	127.5	127.1	117.5	(29)
Number of isolates			15	1	1	2	1	3	6	

TABLE 2. Continued.

Differential Variety	Resistance Gene	Code Nº	REACTION						
			15	16	17	18	19	20	(Sub Total)
Shin 2	Pi-k^{s}	1	S	S	S	-	-	S	14
Aichi-Asahi	Pi-a	2	S	S	S	S	S	S	113
Fujisaka 5	Pi-i	4	S	S	S	S	S	S	113
Kusabue	Pi-k	10	S	-	-	-	-	-	1
Tsuyuake	Pi-k^{m}	20	-	-	-	-	-	-	0
Fukunishiki	Pi-z	40	-	-	-	-	-	-	0
K 1	Pi-ta	100	S	S	S	S	S	-	112
Pi Nº 4	Pi-ta^{2}	200	-	-	-	-	-	-	0
Toride 1	Pi-z^{t}	400	-	-	-	-	-	-	0
K 60	Pi-k^{p}	.1	S	S	S	S	S	-	112
BL 1	Pi-b	.2	-	-	-	-	-	-	0
K 59	Pi-t	.4	-	S	-	S	-	-	52
Japanese Race			117.1	107.5	107.1	106.5	106.1	7.0	
Number of isolates			1	30	42	22	17	1	(113)

S: Susceptible reaction

-: Resistant reaction

TABLE 3. The number of isolations attacking each of Kiyosawa's differential varieties from isolates collected in 1977 and 1980 in the Philippines, 1975 to 1980 in Japan and in 1980/81 in Brazil.

Differential	Resistance	Philippines*		Japan		Brazil	
Variety	Gene	1977	1978	1975	1980	1980	1981
Shin 2	Pi-k^{s}	52	57	1282		161	
Aichi Asahi	Pi-a	65	68	678		218	
Fujisaka 5	Pi-i	9	10*	163		218	
Kusabue	Pi-k	5	4	731		46	
Tsuyuake	Pi-k^{m}	6	4	712		9	
Fikunishiki	Pi-z	0	11	97		92	
K 1	Pi-ta	8	19*	72		217	
Pi Nº 4	Pi-ta^{2}	1	14	0		0	
Toride 1	Pi-z^{t}	32	41	43		0	
K 60	Pi-k^{p}	6	4	745		217	
BL 1	Pi-b	10	33*	22		0	
K 59	Pi-t	58	50*	0		149	
Total number of isolates tested		65	70	1284		218	

*: In these cases, the total number of isolates were 69, as one isolate was removed for its number reaction.

**: Data from Kiyosawa et al. 1980.

TABLE 4. Resistance genes identified in different varieties.

COUNTRY	VARIETY	Nº OF GENE	RESISTANCE GENE
Philippines	Tadukan	⩾ 3	Pi-ta, Pi-ta^2, Pi-k
India	HR-22	2	Pi-k^h
	CO 25		Pi-z^t, Pi-a
	TKM 1		Pi-z^t
	Charnack	⩾ 3	Pi-k
	CO 4		Pi-z^t
	Modan		Pi-k
	ADT 10		Pi-z^t
Pakistan	Dular		Pi-k
	Pusur	3	Pi-k^p, Pi-a
Viet Nam	Te-tep	⩾ 3	Pi-k^h
Malaysia	Morak Sepilai		Pi-z^t
	Kontor		Pi-z^t
	Milek huning	3	Pi-b
Thailand	Leuang Tawng 77-12-5		Pi-z^t
	Chao Leuang		Pi-z^t
Indonesia	Chao Leuang II		Pi-z^t
	Tjina		Pi-b
	Tjahaja		Pi-b
	Bengawan		Pi-b, Pi-t
U.S.A.	Zenith	2	Pi-z, Pi-i, Pi-a
	Caloro		Pi-k^s
	Lacrosse		Pi-k^s
	Blue Bonnet		Pi-a
	Dawn		Pi-k^h, Pi-i, Pi-a
	Saturn		Pi-z
U.S.S.R.	Russian nº 33		Pi-k

V. The reaction of the blast fungus isolates obtained from the main lowland varieties and breeding materials in the state of Santa Catarina.

1) Introduction

The breakdown of varietal resistance of the main varieties such as IRGA 408, BR-IRGA 409, IRGA 410 and EMPASC 103 are recently reported from several locations in the state of Santa Catarina.

Main lowland cultivars, IRGA 408, BR-IRGA 409 and IRGA 410 were released from IRGA/Rio Grande do Sul during the years 1978 to 1980 and EMPASC 103 was released from EMPASC/Santa Catarina in 1980 as a recommended variety for high digree of resistance.

The breadkdown of these varieties suggests that the main varietal resistance is controlled by the vertical (gene-specific, major gene) resistance. Differentiation of the major R. genes in these varieties is important besides studying the constitution and frequency of blast fungus races.

Twenty-seven blast fungus isolates were established from main cultivars grown ten different locations in the state of Santa Catarina.

The blast fungus isolates were identified and differentiated into races according to the reaction patterns of the Japanese and Kiyosawa's differential varieties.

The results showed quite different reaction pattern from the blast fungus isolates collected from the main upland varieties such as IAC 47, IAC 25 et al. from five state in Brazil, Goiás, Mato Grosso, Paraná, São Paulo and Minas Gerais.

Many upland varieties cultivated in the above five states possibly carry Zenith type (Pi-z, Pi-a) major resistance gene (Tanaka 1981a). On the other hand, the results of race differentiation of isolates collected from the state of Santa Catarina showed that the major resistance apparing to be controlled by Pi-ta^2 (Tadukan type) Pi-b (BL 1 type) and Pi-k^h (Dawn type), major R. gene.

The matching race to Pi-ta^2 and Pi-b was not found in the blast collections obtained from major upland varieties of all five states.

It is necessary to know the frequency and constitution of prevalent races to estimate the major R. genes. The results presented this chapter, show the frequency and constitution of prevalent races obtained from the main lowland varieties in the state of Santa Catarina. Also, from the results of the estimation of major R. genes by the artificial inoculation method using a set of six Brazilian differential races, it can be concluded that the resistance in the main lowland varieties in the state of Santa Catarina appears to be controlled by a few major R. genes as follows: Pi-ta^2, Pi-b and Pi-k^h.

2) Material and Method

Origin of blast fungus isolates obtains from the state of

Santa Catarina is shown in Table 1.

A total of 27 blast fungus isolates were obtained 8 varieties at 10 different locations of main lowland cultivating areas in the state of Santa Catarina.

Method for race identification and evaluation of major R. gene using six differential races are according to the ordinary procedure which are mentioned in chapter III.

TABLE 1. The origin of blast fungus isolates obtained from the state of Santa Catarina. (April, 1982).

Nº of Isolate	Most variety	Location	Isolate from
1	IRGA 410	Nova Veneza-1	neck
2	IRGA 410	Nova Veneza-1	neck
3	IRGA 410	Nova Veneza-2	neck
4	IRGA 410	Nova Veneza-2	leaf
5	IRGA 410	Urussanga	neck
6	IRGA 410	Urussanga	leaf
7	IRGA 410	Blumenau	leaf
8	IRGA 410	Blumenau	neck
9	IRGA 408	Nova Veneza-1	neck
10	IRGA 408	Nova Veneza-1	neck
11	IRGA 408	Urussanga	leaf
12	IRGA 408	Urussanga	neck
13	EMPASC 101	Urussanga	leaf
14	EMPASC 101	Urussanga	leaf
15	EMPASC 101	Urussanga	leaf
16	EMPASC 103	Urussanga	neck
17	EMPASC 103	Urussanga-4	neck
18	EMPASC 103	Urussanga-4	leaf
19	EMPASC 103	Urussanga-4	leaf
20	IR 9202-21-1	EMPASC/Itajaī	leaf
21	IR 9202-21-1	EMPASC/Itajaī	leaf
22	IR 6441-JN6B	EMPASC/Itajaī	leaf
23	IR 6441-JN6B	EMPASC/Itajaī	leaf
24	IRGA 409	Urussanga-4	leaf
25	IRGA 409	Urussanga-4	neck
26	EMPASC 102	Urussanga	leaf
27	EMPASC 102	Blumenau	leaf

3) Results

(1) Race identification with a set of Kiyosawa's differential varieties.

Table 1 shows that the origin of blast fungus isolates collected from the state of Santa Catarina. A total of 27 isolates were obtained from 8 varieties at 10 different locations of main lowland cultivating areas. Based on the analysis of physiologic races, the breakdown of varietal resistance in main varieties in Santa Catarina was shown to be due to the vertical (major R. gene, gene specific) gene effect.

Based on the results of race identification with a set of Kiyosawa's differentials, only two main blast races were identified from 27 isolates (Table 2).

Fourteen differential varieties which can differentiate 12 of 13 major R. genes, have already been identified in Japan.

The variety which can differentiate the Pi-k^h major R. gene was not used in this experiment. It showed very clear reaction between races and the differential varieties.

It can be seen from the Table 2 that both the 2 races showed typical susceptible reaction to 4 varieties, Shin 2, Aichi Asahi, Fujisaka 5 and K 60, which carry Pi-k^s, Pi-a, Pi-i and Pi-k^p major R. genes, respectively.

Race 303 (Code nº 307.1) can match to K 1 (Pi-ta) with S reaction and Pi Nº 4 (Pi-ta^2) with M reaction. On the other hand, Race 305 (Code nº 7.3) showed R^h (no symptom) reaction and can match to BL 1 (Pi-b) with M reaction.

Both races did not show any remarkable symptom on the rest of the differential varieties.

The frequency of Race 303 and Race 305 were 14.8% and 85.2%, respectively.

Race 303 (307.1) was isolated from IRGA 408 and one of the breeding materials IR 920-2-21-1, the characteristics of this isolate is able to match to Pi-ta^2 major R. gene (Tadukan-type).

On the other hand, Race 305 (7.3) is able to match to Pi-b major R. gene which was isolated from IRGA 410, BR-IRGA 409, EMPASC 101, EMPASC 102, EMPASC 103 and one of the breeding materials from IRRI, IR 6441.

The isolates of IRGA 410 were collected from four different locations in the state of Santa Catarina and isolated from different portions of rice plant, necks and leaves. The race frequency did not show any difference between locations. There was no difference among 27 isolates obtained from different portion of rice plant. Since only 2 races were identified, it showed that the race constitution in the state of Santa Catarina is more simple than the isolates obtained from the main upland varieties in five states. The results further suggest that the resistance gene diversity seems to be more simple than in the upland varieties.

(2) Estimation of major R. genes by the artificial inoculation method using six different pathogenic races.

To date, the races which can attack to major R. genes Pi-ta^2 and Pi-b were not obtained from the blast fungus collections of upland varieties from the five state.

From these results, a set of six differential races of blast fungus was obtained, which can identify the six major R. genes as follows: Pi-k, Pi-z, Pi-k^m, Pi-ta^2, Pi-b and Pi-k^p.

Since three of 13 major R. genes, Pi-a, Pi-i, and Pi-k^s are supposed to be inadequate donars of resistance to blast, the existing set of six differential races are quite sufficient to identify some of the major R. gene or genes in Brazilian materials.

Table 3 shows the reaction patterns of Brazilian lowland varieties and breeding materials to six different pathogenic races by the spray inoculation method in the greenhouse.

Blue Belle and IAC 435 showed almost same reaction patterns. Blue Belle is well known lowland variety which carrys Pi-a (Aichi-Asahi type) major R. gene.

According to the similarity of reaction pattern of above two varieties, IAC 435 probably have Pi-a major R. gene.

Only EMPASC 100 showed remarkable difference in reaction pattern as compare to than that of other varieties. It showed the susceptible reaction to 5 out of 6 races. The race 303 (307.1) isolated from a variety IRGA 410 did not infect EMPASC 100.

IAC 899 showed S reaction to Race 39 and Race 162 and ambiguous reaction to race 305 (7.3). It suggests that the resistance of IAC 899 seems to be an upland type rather than that of lowland variety.

Rest of the varieties can be classify into following four categories.

1) S reaction to Race 305 (7.3): BR-IRGA 409, IRGA 410, EMPASC 100, 102, 103, IR 22, IR 24, IR 841, IR 6441-JN-6B, P 895-B4-4-1T BG 90-2

2) S reaction to Race 303(307.1): IRGA 408, IR 9202-21-1

3) S reaction to Race 240 (37.4): Dawn, Lebonnet, RS 16-516-1-16-1T-1

4) R^h reaction to all the 6 races: CICA 8, CICA 9

First category, Race 305 can match to Pi-b major R. gene specifically. These varieties possibly have Pi-b major R. gene. Only IR 841 showed Carreon type hypersensitive reaction with numerous small lesions.

Second Category, IRGA 408 and IR 9202-21-2 seems to control the main resistance by Pi-ta^2 (Tadukan type) major R. gene.

Dawn, Lebonnet and RS 16-516-1-16-1T-1 showed S reaction by the inoculation of Race 240 (37.4). These two varieties are known to carry Pi-k^h major R. gene. It is necessary to confirm whether Race 240 (37.4) can match to Pi-k^h major R. gene or not using adequate differential variety.

From these results, these three varieties probably have the same type of major R. gene in third category.

CICA 8 and CICA 9 did not show any remarkable reaction to all the 6 races. It suggests that these two varieties have different major R. gene beside Pi-b, Pi-ta^2 or Pi-k^h.

4) Discussion

The breakdown of blast resistance varieties with vertical (major R. gene, gene specific) resistance gene alone leads breeders and pathologists to re-evaluate its horizontal (non-gene specific) resistance breeding. So far, we realized that breeding for horizontal resistance is important as well as vertical resistance to obtain higher and more stable yield. However, to evaluate the horizontal resistance, it is necessary to exclude the effect of major R. resistance. On the other hand, to evaluate the major R. gene it is necessary to know the relation between races and resistance gene constitution of each variety.

The progress in genetic studies on blast resistance has greatly influenced the field of plant pathology. The differential system of blast fungus races established by many researchers has been widely used in Japan.

It is difficult to compare the genetic studies with different varieties from different gene sources and races in different countries. However, according to the results which I obtained to date, many of Brazilian upland rice varieties belong to the intermediate (javanica) type instead of indica type. As many of upland varieties in Brazil are supposed to belong to the intermediate type, it may be possible to identify the common major R. gene or genes between Japanese and Brazilian varieties by using a set of the Japanese differential varieties.

About the lowland rice varieties cultivated at the southern part of Brazil, in the state of Santa Catarina and Rio Grande do Sul, many varieties are introduced from IRRI and CIAT.

It indicates that these varieties and pedigrees are supposed to be controlled by vertical resistance gene or genes which are introduced from indica sources. Some varieties from IRRI were selected for blast resistance by vertical resistance before re-evaluating the horizontal resistance. For example, one of the variety Tongil has been subjected to the "breakdown" of varietal resistance caused by its major R. genes Pi-a, b and t.

It is necessary to identify the major R. gene in a cultivar to avoid the breakdown and further resistance breeding in Brazil.

As mentioned in the last report (Tanaka, 1981a), the resistance in local varieties such as IAC 47, IAC 25, IAC 164, IAC 165 seems to be controlled by Zenith type resistance. On the other hand, the results from the race differentiation of isolates from main lowland varieties of IRGA, EMPASC, Santa Catarina, showed that these varieties appears to control the main resistance by Pi-ta^2 (Tadukan type) and Pi-b (BL 1 type) major R. genes.

The reason for the breakdown of varietal resistance of main cultivars at the state of Santa Catarina is presumbly due to the increase in number of pathogenic races which are able to attack to Pi-ta^2 and Pi-b major R. genes.

The varietal resistance of main lowland varieties in the state of Santa Catarina is probably controlled by vertical resistance genes and based on breeding for specific gene resistance.

These results should be confirmed by F_2, F_3 progeny test (genetic analysis).

Fig. 1 shows the pedigrees of main varieties in the state of Santa Catarina.

It shows more clear relationships between varieties. Many of the main varieties, EMPASC 101, 102, 103, BR-IRGA 409 and IRGA 410 have been bred from the crosses with IRRI materials and same parent lines. The sib lines of IRRI materials such as IR 930-2, IR 930-53 and IR 930-80 may carry same vertical resistance gene. EMPASC 101, 102, 103, BR-IGA 409 and IRGA 410 seems to carry Pi-b major R. gene according to the results of race identification.

It suggests that these IRRI breeding materials probably have same Pi-b major R. gene.

And also the estimation of major R. gene by the artificial inoculation method using six different pathogenic races showed that these above mentioned five varieties probably have Pi-b major R. gene. It is possible that may not have major R. gnes such as Pi-ta^2, z, k, ta, k^m, k^p and t.

On the other hand, IRGA 408 is supposed to have Pi-ta^2 major R. gene according to the both results of race identification of isolates and inoculation method with six different pathogenic races.

IRGA 408 was derived from the cross between IR 8 and IR 12-178-2-3, the major R. gene Pi-ta^2 probably has inherited from IR 8.

One of the breeding materials, P 805-B4-4-1T showed the same reaction pattern. It shows that this carries the same major R. gene. Lebonnet is one of the resistant variety in the state of Santa Catarina. The variety Lebonnet was derived from as cross of Blue Belle (Pi-a) and (Belle Patna x Dawn). Dawn is known to carry Pi-a, i and k^h major R. genes. It is known that Te-tep carrys Pi-k^h and other genes.

According to the results of the estimation of major R. gene by the inoculation method, it showed R reaction to Race 303 (307.1), and R^h reaction to the rest of races. Te-tep showed same reaction pattern, indicating thereby that Lebonnet and Te-tep may carry Pi-k^h major R. gene inherited from American variety Dawn. On the other hand, one of the breeding materials, RS 16-516-1-16-15-1 was bred from the cross with Dawn and IRGA 407.

RS 16-516-1-16-1T-1 probably carries Pi-b major R. gene which has inherited from IRGA 407 according to the reaction patterns with six different pathogenic races. However, it showed R reactions to Race 39 (147.5) and Race 303 (307.1) indicating that this material may carry other gene or genes inherited from Dawn.

These results should be confirmed by genetical analysis.

EMPASC 100 showed susceptible reaction under the blast nursery condition at EMPASC (1980)(Table 4). This has been bred by a cross between IAC 435 and Taichung 24. Only EMPASC 100 showed quite different reaction pattern from others it exhibited S reaction to Race 11, 39 and 162. It suggests that the resistance gene of EMPASC 100 was inherited from Taichung 24 and may carry Pi-a major R. gene as in the case of Blue Belle.

From the results of the estimation of major R. gene of main lowland varieties and these pedigrees showed the typical vertical resistance. Their resistance may consist in a few major R. genes such as Pi-ta^2 and Pi-b.

CICA 8, CICA 9, Dawn and Lebonnet showed R^h reaction to all the six races. The resistance genes of Dawn have been analysed by Kiyosawa (1974), it consists of three known genes, Pi-a, Pi-i and Pi-k, and additional unknown gene (SS) which was not allelic to Pi-z, Pi-z^t and Pi-ta. Finally, Kiyosawa (1978) designated above additional unknown gene to Pi-k^h.

One of the well known variety of resistance gene donar, Tetep also has been identified as possessing Pi-k^h gene.

These results suggest that above four varieties probably have Pi-k^h major R. gene.

IRRI varieties, IR 22, IR 24 and IR 841 showed S reaction to only Race 305 (Code nº 7.3), suggesting that these varieties may carry Pi-b major R. gene as well as EMPASC cultivars.

As these six differential races were selected from the reaction patterns to the Kiyosawa's and Japanese differential varieties, they may possess the differential ability to identify the thirteen major R. genes indicated in Table 2. However, the results of genetic analysis were carried out in Japan using Japanese differentials and races. The resistance gene constitution and component are likely to be different in <u>indica</u>, <u>javanica</u> and <u>japonica</u> varieties.

It is necessary to confirm these results by further genetic analysis using adequate gene marker varieties and differential pathogenic races.

To date the breakdown of IRGA 408, BR-IRGA 409 and 410, and EMPASC 101, 102 and 103 have been reported at many locations at Santa Catarina. However, the varieties which carry same resistance gene constitution such as IR 22, IR 24, IR 841, IR 6441, IR 9292, and RS 16-516 will be subjected to severe epidemic of blast within a few years.

It is urgently needed to introduce other resistance gene sources instead of Pi-ta^2 (Tadukan type) and Pi-b (BL 1 type) major R. genes. Also, necessary to evaluate the horizontal resistance by artificial inoculation method using the adequate pathogenic races.

Multi-line consist in wide diversity of resistance genes and gene accumulation of major R. genes are supposed to be the important strategies both in lowland and upland rice breeding in future.

However the knowledge on the relation between races and major R. genes are essential.

(Fig. 2 shows the distribution of main race of blast fungus at each state in Brazil. 1981-1982).

TABLE 2. Reaction patterns of Kiyosawa's differentials to blast isolates from the state of Santa Catarina.

Nº	Variety	Resistance Gene	Code Nº	REACTION 303	REACTION 305
1	Shin 2	Pi-k^{s}	1	S	S
2	Aichi Asahi	Pi-a	2	S	S
3	Fujisaka 5	Pi-i	4	S	S
4	Kusabue	Pi-k	10	R^{h}	R^{h}
5	Tsuyuake	Pi-k^{m}	20	R^{h}	R^{h}
6	Fukunishiki	Pi-z	40	R^{h}	R^{h}
7	K 1	Pi-ta	100	S	R^{h}
8	Pi Nº 4	Pi-ta^{2}	200	M	R^{h}
9	Toride 1	Pi-z^{t}	300	R^{h}	R^{h}
10	K 60	Pi-k^{p}	0.1	S	S
11	BL 1	Pi-b	0.2	R^{h}	M
12	K 59	Pi-t	0.4	R^{h}	R^{h}
13	Kanto 51	Pi-k	10	R^{h}	R^{h}
14	Zenith	Pi-a, Pi-z	(42)	R^{h}	R^{h}
Code Nº (Japanese race)				307.1	7.3
Nº of isolates				9,10,11,12	1,2,3,5,6 7,8,13,14,15,16 17,18,19,20,21,22 23,24,25,26,27
TOTAL				(4)	(23)

TABLE 3. The reaction of Brazilian lowland varieties and breeding materials to six different pathogenic races by the artificial inoculation method at the greenhouse.

Race Code Nº	11	39	162	240	303	305	Major R. Gene Estimated
Nº Variety	157.5	147.5	117.5	37.4	307.1	7.3	
1-BR IRGA 409	R^h	R^h	R^h	R^h	R^h	S	Pi-b
2-IRGA 410	R^h	R^h	R^h	R^h	R^h	S	Pi-b
3-IRGA 408	R^h	R^h	R^h	R^h	M	S	Pi-ta^2
4-CICA 8	R^h	R^h	R^h	R^h	R^h	R^h	
5-CICA 9	R^h	R^h	R^h	R^h	R^h	R^h	
6-DAWN	R^h	R^h	R^h	S	R^h	R^h	Pi-a, i, k^h
7-EMPASC 100	SS	SS	SS	SS	R^h	S	
8-EMPASC 101	R^h	R^h	R^h	R^h	R^h	S	Pi-b
9-EMPASC 102	R^h	R^h	R^h	R^h	R^h	S	Pi-b
10-EMPASC 103	R^h	R^h	R^h	R^h	R^h	M	Pi-b
11-IAC 899	R^h	S	S	R^h	R^h	S	Pi-b
12-IR 22	R^h	R^h	R^h	R^h	R^h	M	Pi-b
13-IR 24	R^h	R^h	R^h	R^h	R	M	Pi-b
14-IR 841	R^h	R^h	R^h*	R^h	R^h	M	Pi-b
15-IR 6441-JN-6B	R^h	MR	R^h	R^h	R^h	M	Pi-b
16-IR 9202-21-1	R^h	R	R^h	R^h	M	MS	Pi-ta^2
17-LEBONNET	R^h	R^h	R^h	S	R	R^h	(Pi-k^h)
18-BLUE BELLE	SS	SS	SS	S	S	SS	Pi-a
19-P 805-B4-4-1T	R^h	R	R^h	R^h	M	R^h	Pi-ta^2
20-RS 16-516-1-16-1T-1	R^h	R	R^h	S	R	S	(Pi-b)
21-BG 90-2	R^h	R	R^h	R^h	R^h	S	
22-IAC 435	S	S	S	S	S	M	Pi-a
23-ZENITH	SS	SS	M	R^h	R^h	R^h	Pi-z, Pi-a

* Hypersensitive reaction, Carreon type.

TABLE 4. The reaction of main lowland cultivars to blast (*Pyricularia oryzae* Cav.) under the blast nursery condition at EMPASC, Itajaī, Santa Catarina (EMPASC, Dec. 1979).

	Cultivar	REP. I	REP. II	Average	Classification
1	EMPASC 102[a]	2.0	1.0	1.5	Resistant
2	P 798-B4-4-1T	2.0	1.0	1.5	
3	P 791-B4-14	2.0	1.0	1.5	
4	CICA 9	2.0	1.0	1.5	
5	IRGA 408	2.0	3.0	2.5	
6	EMPASC 101[b]	2.0	3.0	2.5	
7	BR IRGA 409	3.0	2.0	2.5	
8	IR 841[c]	4.0	2.0	3.0	
9	IAC 2092	3.0	4.0	3.5	Moderate Resistant
10	Dawn	4.0	3.0	3.5	
11	Lebonnet	3.0	5.0	4.0	
12	CICA 7	4.0	5.0	4.5	
13	Batatais Longo	5.0	7.0	6.0	
14	EMPASC 100[d]	7.0	7.0	7.0	Susceptible
15	IAC 435	8.0	9.0	8.5	
16	Blue Belle	9.0	8.0	8.5	

[a] (P738-137-4-1)

[b] (P780-55-1-1)

[c] (IR 841-63-5-L-9-33)

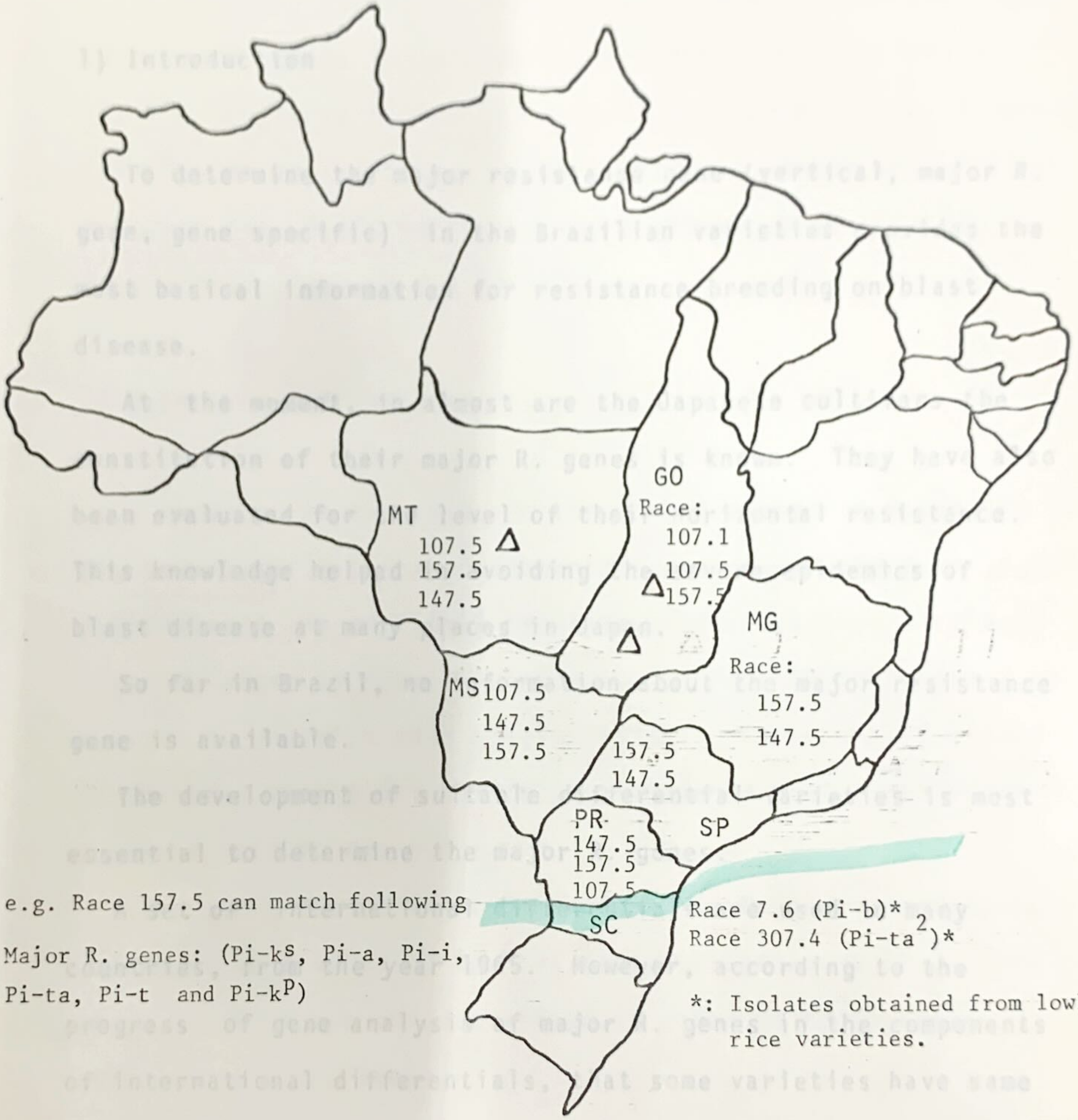
[d] (P 69-205)

TABLE 5. Pedigrees of main cultivars from the State of Santa Catarina, (lowland rice).

	(Variety or line)
IR 930-80 IR 532-E-208	EMPASC 101
IAC 435 Taichung 24	EMPASC 100
IR 579-160 IR 930-53	EMPASC 102 (Pi-b)
IR 930-53 IR 665-31-2	IRGA 410 (Pi-b)
IR 665-31-2 IR 930-2	BR-IRGA 409 (Pi-b)
IR 930-2 IR 665-31-5-8	EMPASC 103 (Pi-b)
IR 8 IR 12-178-2-3	IRGA 408 (Pi-ta^2)
Blue Belle (Pi-a) Belle Patna Dawn (Pi-a,i,k^h)	LEBONNET
Dawn (Pi-a,i,k^h) BR IRGA 407	RS 16-516-1-16-1T-1
Pérola Matão Jaguari Yora	IAC 435

( ): estimation of major R. gene by the artificial inoculation method.

* : according to the result of the evaluation of resistance gene. (Kiyosawa, 1982).



e.g. Race 157.5 can match following Major R. genes: (Pi-k^s, Pi-a, Pi-i, Pi-ta, Pi-t and Pi-k^p)

Race 7.6 (Pi-b)*
Race 307.4 (Pi-ta^2)*

*: Isolates obtained from lowland rice varieties.

Fig. 2. The distribution of main race of blast fungus at each State in Brazil - (1981-1982).

VI. The comparison of differential ability among the Japanese differential varieties and cultivars to Brazilian blast fungus isolates

1) Introduction

To determine the major resistance gene (vertical, major R. gene, gene specific) in the Brazilian varieties provides the most basical information for resistance breeding on blast disease.

At the moment, in almost are the Japanese cultivars the constitution of their major R. genes is known. They have also been evaluated for the level of their horizontal resistance. This knowledge helped in avoiding the severe epidemics of blast disease at many places in Japan.

So far in Brazil, no information about the major resistance gene is available.

The development of suitable differential varieties is most essential to determine the major R. genes.

A set of international differentials are used in many countries, from the year 1965. However, according to the progress of gene analysis of major R. genes in the components of international differentials, that some varieties have same major R. gene. It is necessary to establish a set of Brazilian differentials which are able to differentiate the major R. gene in Brazilian varieties, however Japanese or Kiyosawa's differentials may be tentatively usuful to differentiate rice blast races in

Brazil. In this experiment, several varieties which carry same major R. gene were tested to know the differential ability of major R. genes and their level of horizontal resistance.

15 from 69 varieties were selected to differentiate the 13 major R. genes as follows: Aichi Asahi (Pi-a), Shin 2 (Pi-k^s), Fujisaka 5 (Pi-i), Kanto 51, Kusabue (Pi-k), Tsuyuake (Pi-k^m), Pi Nº 1 (Pi-ta), Pi Nº 4 (Pi-ta^2), Fukunishiki (Pi-z), Toride 1 (Pi-z^t), K 59 (Pi-t), BL 1 (Pi-b), K 60 (Pi-k^p), Zenith (Pi-z, a) and K 3 (Pi-k^h).

2) Materials and Methods

A total of 71 Japanese and introduced varieties including the Kiyosawa's and Japanese differential varieties were used in this experiment. 46 out of 71 varieties are known with single major R. genes as shown in Table 1, and rest of them have 2 to 4 major R. genes in each single variety. These materials were distributed from Aichi Agricultural Experimental Station, Inahashi, Aichi, Japan.

Some varieties of them, Homarenishiki, Akibare, Toyonishiki and Koganehare supposed to have different other major R. gene or genes which are not identified in Japan using Japanese differentials and races.

A set of six different pathogenic races (temporary Brazilian differential races) which is mentioned in chapter III is used for the estimation of major R. gene in each variety in this experiment.

Methods used in this experiment is in accordence with the ordinary procedures mentioned in chapter III.

The evaluation of rating of blast epidemic at the blast nursery is mentioned in chapter VII.

Table 1. Representative varieties carring different single major R. gene.

Resistance gene	Variety
Pi-a:	Aichi Asahi* Kinmaze Shyuhō Homarenishiki Akibare Toyonishiki
Pi-i:	Fujisaka 5* Todorokiwase Inabawase Yoneshiro
Pi-k^{s}:	Shin 2* Sha-tiao-tsao Caloro
Pi-k:	Mangetsu-mochi Kusabue** Kanto 51* Tastumi-mochi Kanto 52 Kanto 53
Pi-k^{m}:	Tsuyuake* Inakei 140 Inakei 159 Nuaki
Pi-k^{p}:	K 60**
Pi-ta:	Pi nº 1* Pi nº 2 Yashiromochi K 1**
Pi-ta^{2}:	Pi nº 4* Yamahikari Fukuhonami
Pi-z:	Fukunishiki* Fukuhikari Ōu 244
Pi-z^{t}:	Toride 1* TYB TYC
Pi-t:	K 59**
Pi-b:	BL 1 BL 7
Pi-k^{h}:	K 3**

* Japanese differential varieties

** Kiyosawās differential varieties

3) Results

Table 2 shows that the reaction patterns of Japanese varieties with known major R. genes to six different pathogenic races by the artificial inoculation method in the greenhouse and the evaluation of degree of blast under the blast nursery condition.

The six different pathogenic races used in this experiment were differentiared by a set of Kiyosawa's differential varieties. It is based on the 12 out of 13 major R. genes which have been identified in Japan using Japanese differential varieties and races.

Till now, the matching race to the major R. gene Pi-z^t (Toride type) was not found in the blast fungus collections from both upland and lowland varieties. Also, one differential variety which can differentiate the Pi-k^h major R. gene is not available.

The results of reaction to six different pathogenic races were mentioned at each major R. gene as follow:

(Pi-a)

Homarenishiki, Akibare and Toyonishiki showed more resistant reactions to all the six races than in other varieties, and they showed MR-R reaction under the blast nursery condition.

It suggests that these three varieties possibly have other unidentified major R. gene according to the reaction patterns.

Aichi Asahi, Kinmaze and Fujiminori showed S reaction to all of six races whereas Yamabiko and Shyuhō showed MS and M reaction to Race 11 and 39, respectively.

Former three varieties possible de not have other major R. gene beside Pi-a major R. gene and later two varieties, the level of resistance M-S will be altered according to the circumstance.

Under the blast nursery condition, Aichi Asahi showed the most severe reaction than others. It showed that Aichi Asahi may possess more lower horizontal resistance than others.

(Pi-i)

Fujisaka 5 and Ishikarishiroke showed S reaction to all the six races. According to the reaction patterns, Todoroki-wase, Yoneshiro and Koganehare showed MR-R reaction to majority of the races. It suggests that they are supposed to have different major R. genes beside Pi-i or have considerably high level of horizontal resistance. From the results of evaluation at the blast nursery, they showed M-S reaction.

(Pi-k)

Mangetsu-mochi, Kanto 51, Kanto 52 and Kanto 53 showed almost same reaction patterns to all the six races. They are supposed to have same major R. gene Pi-k and the same level of horizontal resistance according to the reaction patterns to six races and the evaluation under the blast nursery condition. Kanto 51 is the most convenient variety to differentiate the

Pi-k major R. gene from the result of the level of horizontal resistance. On the other hand, Kusabue showed R, R and M reaction to the race 11(157.5), race 162(117.5) and race 240(37.4), respectively.

It suggests that Kusabue may possess other unidentified major R. gene beside Pi-k or may have relatively high level of horizontal resistance to Brazilian pathogenic races.

From the results of the evaluation in the blast nursery, Kusabue showed relatively high level of varietal resistance than others.

($Pi\text{-}k^s$)

All the three varieties, Shin 2, Sha-tiao-tsao and Caloro showed SS reaction to six races as well as in the blast nursery. It did not show any difference in reaction pattern between three varieties.

($Pi\text{-}k^m$)

Tsuyuake showed SS reaction and other three varieties showed MS reaction to Race 240(37.4) which was isolated from one of the breeding material SR 2041-50-1 introduced from Korea.

The race which can attack to major R. genes Pi-b, $Pi\text{-}ta^2$, $Pi\text{-}z^t$ and $Pi\text{-}k^m$ was not identified under the blast nursery condition at CNPAF/EMBRAPA, Goiânia.

However the evaluation at the blast nursery showed that disease index 1.3 - 3,3 (R-MR). It suggests that the population of matching race to $Pi\text{-}k^m$ major R. gene or the other factor probably effected the reaction.

($Pi\text{-}k^p$)

K 60 showed clear SS reaction to Race 11, 39, 162 and 240 and showed SS reaction at the blast nursery.

(Pi-ta)

Four varieties showed almost same reaction patterns to all the six races. It showed clear R^h reaction to Races 240 and 305.

It showed that these varieties have only Pi-ta major R. gene matching to Brazilian races.

($Pi\text{-}ta^2$)

Three varieties showed M reaction to Race 303(307.1). It showed that Race 303 can attack to $Pi\text{-}ta^2$ major R. gene however, it is possible that Race 303 has another pathogenicity to other major R. gene.

They showed typical R^h reaction to the rest of the 5 races.

As virulent race which can match to $Pi\text{-}ta^2$ major R. gene was not present in the blast nursery, they did not show any symptom.

(Pi-t)

K 59 showed clear SS reaction to five races beside R^h reaction to race 240(37.4). In blast nursery it showed SS reaction.

(Pi-z)

The differential variety which can differentiate Pi-z major R. gene is very important to identify the major R. gene in Brazilian upland varieties and breeding materials.

Three representative varieties which can differentiate Pi-z major R. gene were used in this experiment.

They showed S and M reactions to Race 11(157.5) and 39(147.5), respectively. Both races are supposed to be virulent to these varieites.

However they showed MR-R (disease index 2.8 - 3.5) at the blast nursery. It suggest that these three varieties have same major R. gene to match to Brazilian races. However they may have relatively high level of horizontal resistance.

(Pi-b)

From this blast nursery, matching race to Pi-b major R. gene was not identified BL 1 and BL 7 showed S reaction to Race 305(7.3) by the spray method. BL 1 and BL 7 have same major R. gene to match to Brazilian races.

(Pi-z, Pi-a)

Zenith is one of the international differential variety in which is known both Pi-a and Pi-z gene are identified.

Zenith showed SS reaction to Race 11(157.5) and Race 39(147.5) and showed SS reaction under the blast nursery condition.

($Pi\text{-}z^t$)

Three varieties are known - which possess $Pi\text{-}z^t$ major R. gene, Toride 1, TYB and TYC showed R^h reaction to all the six races.

Till now, it was not possible to obtain the race which can match to $Pi\text{-}z^t$ major R. gene.

TABLE 2. The reaction of Japanese varieties with known major R. gene to six different pathogenic races by the artificial inoculation method at the greenhouse and the evaluation under the blast nursery condition: Pi-a, Pi-i major R. genes.

Resistance Gene	Variety	Race Code Nº 11 157.5	39 147.5	162 117.5	240 37.4	303 307.1	305 7.3	Evaluation at the Blast Nursery	
(Pi-a)	Aichi Asahi	SS	SS	SS	S	SS	SS	8.0	SS
	Yamabiko	MS	SS	S	S	S	S	6.5	SS
	Kimmaze	S	SS	SS	S	S	S	6.0	SS
	Shyūho	M	SS	S	S	SS	S	6.3	SS
	Homarenishike**	R	MR	M	M	M	M	3.0	MR
	Akibare**	MR	MR	M	R	S	S	2.5	R
	Fujiminori	SS	SS	S	S	S	S	6.0	SS
	Toyonishiki**	MR	MR	M	R	M	M	2.8	R
(Pi-i)	Todoroki-ware**	S	MR	R	MR	MR	MR	4.5	M
	Fujisaka 5	SS	SS	SS	S	SS	SS	7.8	SS
	Inaba-wase	SS	S	M	MR	SS	MS	5.3	S
	Yoneshiro**	MR	MR	MR	MR	R	R	5.0	S
	Hourei	R	-	S	MR	MR	MR	6.8	SS
	Koganehare**	R	MR	R	R	R	R	5.8	S
	Ishikari-Shiroke	SS	SS	SS	S	SS	S	7.5	SS

The races which can be attacked to major R. genes, Pi-b, Pi-ta^2, Pi-z^t were not existed in the blast nursery.

* The variety is supposed to have unidentified major R. gene or genes.

TABLE 2. Continued. Pi-k, Pi-k^s, Pi-k^m and Pi-k^p major R. genes.

Resistance Gene	Race Code nº / Variety	11	39	162	240	303	305	Evaluation at the Blast Nursery	
		157.5	147.5	117.5	37.4	307.1	7.3		
(Pi-k)	Tatsumi-mochi	MS	MR	MS	MS	R^h	R^h	5.8	S
	Mangetsu-mochi	S	R	S	S	R^h	R^h	6.3	SS
	Kusabue**	R	R	M	M	R^h	R^h	5.2	S
	Kanto 51	SS	MR	SS	SS	R^h	R^h	6.3	SS
	Kanto 52	SS	MR	S	SS	R^h	R^h	6.0	SS
	Kanto 53	SS	MR	SS	SS	R^h	R^h	5.8	S
(Pi-k^s)	Shin 2	SS	SS	SS	SS	SS	SS	8.0	SS
	Sha-tiao-tsao	SS	SS	SS	SS	SS	SS	9.0	SS
	Caloro	SS	SS	SS	SS	SS	SS	9.0	SS
(Pi-k^m)	Tsuyuake	R^h	R^h	R^h	SS	R^h	R^h	2.5*	R
	Inakei 140	R^h	R^h	R^h	MS	R^h	R^h	2.5*	R
	Inakei 159	R^h	R^h	R^h	MS	R^h	R^h	3.3*	MR
	Nuaki	R^h	R^h	R^h	MS	R^h	R^h	1.3*	R
(Pi-k^p)	K 60	SS	SS	SS	SS	R^h	R^h	8.8	SS

*: The races which can be attacked to major R. genes Pi-b, Pi-ta^2, and Pi-z^t were not existed in the blast nursery.

**: The variety is supposed to have unidentified major R. gene or genes.

TABLE 2. Continued. Pi-ta, Pi-ta^2, Pi-t, Pi-z and Pi-b major R. genes.

Resistance	Race Code nº	11	39	162	240	303	305	Evaluation at the	
Gene	Variety	157.5	147.5	117.5	37.4	307.1	7.3	Blast	Nursery
(Pi-ta)	Yashiro-mochi	SS	S	S	R^h	SS	R^h	8.0	SS
	Pi Nº 1	SS	SS	SS	R^h	SS	R^h	8.0	SS
	Pi Nº 2	SS	SS	SS	R^h	S	R^h	8.3	SS
	K 1	SS	SS	SS	R^h	SS	R^h	6.8	SS
(Pi-ta^2)	Pi Nº 4	R^h	R^h	R^h	R^h	M	R^h	0*	R^h
	Yamahikari	R^h	R^h	R^h	R^h	M	R^h	0*	R^h
	Fukuhonami	R^h	R^h	R^h	R^h	M	R^h	0*	R^h
(Pi-t)	K 59	SS	SS	SS	R^h	SS	SS	9.0	SS
(Pi-z)	Fukunishiki	S	M	R	R^h	R^h	R^h	2.8	R
	Fukuhikari	S	M	R	R^h	R^h	R^h	3.5	MR
	Oû 244	S	M	R	R^h	R^h	R^h	3.5	MR
(Pi-b)	BL 1	R^h	R^h	R^h	R^h	R^h	S^h	0*	R^h
	BL 7	R^h	R^h	R^h	R^h	R^h	S^h	0*	R^h
(Pi-z, Pi-a)	Zenith	SS	SS	R	R^h	R^h	R^h	7.8	SS

*: The races which can be attacked to major R. genes, Pi-b, Pi-ta^2 and Pi-z^t were not existed in the blast nursery.

TABLE 2. Continued. (+), k^h and z^t major R. genes.

Major R. Gene	Race Code nº / Variety	11 157.5	39 147.5	162 117.5	240 37.4	303 307.1	305 7.3	Evaluation at the Blast	Nursery
(+)	Yamaji-wase	MR	R	R	R^h	R	R^h	2.8	R
	Nihon-bare	MR	MR	R^h	R^h	R^h	R^h	4.3	M
	Rikuto-Norin 4	R^h	MR	R	R	R	R	6.3	SS
	Rikuto-Norin 12**	R	R	R^h	R	S	R	7.8	SS
	Koshi-Riku A	R	R	R	R^h	R	R	2.5	R
	Koshi-Riku B	R	MR	R	R^h	R	R	2.3	R
(Pi-k^h)	K 3	R^h	R^h	R^h	S	S	R^h	1.0*	R^h
(?)	K 50	S	SS	S	S	SS	R^h	7.5	SS
(Pi-z^t)	Toride 1	R^h	R^h	R^h	R^h	R^h	R^h	0*	R^h
	TYB	R^h	R^h	R^h	R^h	R^h	R^h	0*	R^h
	TYC	R^h	R^h	R^h	R^h	R^h	R^h	0*	R^h
(Pi-ta^2,ta,a)	Tadukan	R^h	MR	MR	R^h	MS	R^h	0*	R^h
(Pi-a,i,k^h)	Dawn	R^h	R^h	R^h	R^h	R^h	R^h	0*	R^h
(Pi-k^h +α)	Te-tep	R^h	R^h	R^h	R^h	R^h	R^h	0*	R^h

*: The races which can be attacked to major R. genes, Pi-b, Pi-ta^2, Pi-z^t and Pi-k^h were not existed in the blast nursery.

**: The variety is supposed to have unidentified major R. gene or genes.

TABLE 2. Continued. The varieties which have two or more major R. genes.

Resistance Gene	Race Code nº / Variety	11	39	162	240	303	305	Evaluation at the Blast	Nursery
		157.5	147.5	117.5	37.4	307.1	7.3		
(Pi-a, Pi-i)	Chyubu 26	R^h	R^h	R^h	R^h	R^h	R^h	2.5	R
	Chyubu 35	R^h	R^h	R^h	R^h	R^h	R^h	1.8	R
(Pi-a, Pi-k^m)	BR Nº 1	R^h	R^h	R^h	M	R^h	R^h	1.0*	R^h
	Minehikari	R^h	R^h	R^h	M	R^h	R^h	1.0*	R^h
	Chyubu 36	R^h	R^h	R^h	M	R^h	R	0.3*	R^h
	Hokushitamai	R	R^h	MR	MR	SS	MR	6.0	SS
(Pi-i, Pi-k)	Inakei 115**	R^h	R^h	R^h	R^h	MR	R^h	1.0	R^h
(Pi-a, Pi-k)	Toto	S	S	S	R^h	R^h	R^h	7.0	SS
(Pi-a, Pi-ta^2)	Reiho	R^h	R^h	R^h	R^h	M	R^h	0.5*	R^h
(Pi-a,Pi-z)	Zenith	SS	SS	R	R^h	R^h	R^h	7.8	SS
(Pi-a,Pi-k,Pi-z)	Akiyutaka	MR	M	R^h	R^h	R^h	R^h	2.3	R
(Pi-a,b,i,k)	Hamaasahi	R^h	R^h	R^h	R^h	R^h	R^h	0*	R^h

*: The races which can be attacked to major R. genes, Pi-b, Pi-ta^2 and Pi-z^t were not existed in the blast nursery.

**: The variety is supposed to have unidentified major R. gene or genes.

4) Discussion

The most important step to identify the major R. gene is to establish a set of differential varieties and races in Brazil.

It is necessary to collect as many different pathogenic races as possible in order to be able to differentiate the single specific resistance gene by genetic analysis. Differential races should have a clear remarkable reaction against each differential variety.

On the other hand, differential varieties should have following characteristics:

1) Each differential variety should have only simple resistance gene that can differentiate different P. oryzae isolates.
2) It should exhibit definite and stable reaction to the Japanese isolates.
3) The differential variety should possess low degree of horizontal resistance for avoiding the overlapping effects on symptoms.
4) Multiplication of seeds of differentials should be easy under different climatic conditions.
5) The varieties with better combining ability should be selected as differentials.

Japanese differentials such as a set of Kiyosawa's are essential for race identification. However according to the latest literature Kiyosawa and Ikehashi (1981), Japanese, the Kiyosawa's and Ikehashi's differential sets were not suitable for differenting philippines isolates, although the Kiyosawa's differentials are suitable for differentiating Japanese isolates and have single gene for blast resistance.

However, the Kiyosawa's differentials may be tentatively useful to differentiate rice blast races in a country which does not have suitable differential varieties.

Particularly, in countries where indica type varieties are prevalent differential varieties have to be composed of indica type.

An approach toward this is to find such a particular indica type variety that is susceptible to the fungus strains in the world, and to introduce the identified genes from Japanese varieties to the particular variety through backorossing.

On the other hand, a set of international differentials are used to differentiate the international races: however the gene constitution of these, and some of varieties is not yet identified.

Therefore international differentials are useful only for brief screening of isolates.

From this aspect, the comparison of differential ability of Japanese varieties including several candidate varieties for differentials was carried out.

From the reuslts of the comparison of the varieties by the inoculation tests using six different pathogenic races and at the blast nursery, a set of recommendable differential varieties are determined as shown in Table 3.

The determination of the suitable set of differential varieties should wait the result of genetic analysis in local varieties.

A set of differential varieties as shown in Table 3 is sufficient to identify the race in Brazil at the moment. However, as will be mentioned in later Chapter, the adequate differential races to distinguish the difference of main upland varieties such as IAC 47 and IAC 25 are needed.

It is necessary to look for more suitable differential races to identify the unidentified major R. genes beside above mentioned 13 major R. genes.

Threre are many aspects at each stage to determine the suitable differential varieties and races should be identified by genetic analysis.

TABLE 3. A set of Japanese (Kiyosawa's) differential varieties to identify the Brazilian blast fungus strains.

Nº	Variety	Resistance Gene	Code Nº
1	Shin 2	Pi-k^{s}	1
2	Aichi-Asahi	Pi-a	2
3	Fujisaka 5	Pi-i	4
4	Kanto 51	Pi-k	10
5	Tsuyuake	Pi-k^{m}	20
6	Fukunishiki	Pi-z	40
7	K 1	Pi-ta	100
8	Pi Nº 4	Pi-ta^{2}	200
9	Toride 1	Pi-z^{t}	400
10	K 60	Pi-k^{p}	.1
11	BL 1	Pi-b	.2
12	K 59	Pi-t	.4
13	K 3	Pi-k^{h}	1,000
14	Kusabue	Pi-k	For Reference
15	Zenith	Pi-z, Pi-a	
16	K 2	Pi-k^{p}, Pi-a	

VII. Evaluation of Horizontal resistance of Japanese and Brazilian Rice varieties under the blast nursery condition

1) Introduction

Seventy Japanese varieties with known level of horizontal resistance and major R. gene in Japan and fifty Brazilian main varieties were tested for the level of horizontal resistance (field, non-gene specific) in the blast nursery CNPAF/EMBRAPA, Goiânia.

Later few were selected for evaluating their resistance by artificial inoculation method in the greenhouse using a set of six differential races which are mentioned in chapter III. Ten out of seventy Japanese entries have been bred to incorporate the high level of horizontal resistance at the Aichi prefectue Agricultural Experiment Station, Inahashi, Japan.

The degree of epidemics was estimated by a set of Japanese, Kiyosawa's and international differential varieties which are planted in the blast nursery.

The frequency and constitution of blast races were evaluated based on the identification of isolates using Kiyosawa's differentials. In the blast nursery, there were races which are able to attack to following major R. genes: Pi-a, Pi-k^s, Pi-k, Pi-i, Pi-t, Pi-k^p, Pi-z and Pi-ta.

The race analysis of forty-five isolates obtained from the blast nursery, showed the following race constitution. 177.5 (2.2%), 157.5 (84.4%), 157.1 (2.2%), 156.1 (2.2%), 147.5 (2.2%), 117.5 (2.2%) and 117.1 (2.2%). They are indicated by code number in accordance with the Kiyosawa's differentials as mentioned in chapter III.

From Brazilian varieties and breeding materials, Catalão, 3 Meses Branco and IRAT 13, showed relatively high level of horizontal resistance both in the blast nursery and the artificial inoculation method. Two varieties, Tiririca and Três Marias did not show any symptom both in the blast nursery and the artificial inoculation tests with six different races; they maybe have Toride type or unidentified major R. gene. It should be confirmed by genetic analysis.

From the results of the evaluation of resistance of Japanese varieties, Rikuto-Norin 4 and Rikuto-Norin 12 showed very susceptible reaction both under the blast nursery condition and the artificial inoculation method.

On the other hand, Koganenishiki (+), Nihon-bare (+), Yamaji-Wase (+), Homarenishiki (a), Toyonishiki (a) and Akibare (a) showed that they maybe have the high level of horizontal resistance or other different major R. genes. It should be confirmed by genetic analysis. Rikû 132 did not showed any symptom in this experiment it possibly has other unidentified major R. gene.

2) Materials and Methods

A total of seventy varieties with well known level of horizontal resistance and major R. gene in Japan and fifty

Brazilian varieties were used.

The frequency and constitution of pathogenic races are observed by a set of Kiyosawa's, Japanese and international differential varieties.

Each variety was planted in the upland blast nursery, 15 m x 1.2 m, at 12 cm between rows and there were about 50 seeds per variety.

1.5 kg of N. P. K., Zn (5:30:15, + 0,3%) of fertilizer and 1.0 kg of ammonium sulphate were applied before sowing.

Standard Varieties: To know the level of epidemics, three varieties Aichi Asahi (Pi-a), Norin 22 (+) and Fujisaka 5 (Pi-i) were used as standard check varieties. The checks as well as Kiyosawa's and international differentials were planted every 50 entries.

Spreader Varieties: To expose to as many pathogenic races as possible under the blast nursery condition, spreader varieties were mixed with many variety which carry different major R. genes as follows; same amount of Japanese, Kiyosawa's international differentials. Koshihikari (Pi-a), Kusabue (Pi-k), Aichi Asahi (Pi-a), Norin 22 (+) Tsuyuake (Pi-k^{m}) and 20 Brazilian upland varieties.

Observation: According to the criterion of the blast nursery test, observations and recording were made on 19, 25 Feb, 01, 05 and 10 March. The criterion of observation is according to the standard evaluation system for rice, IRRI as follows.

Scale	General observation
1	None to small brown specks of pinthread size
2	Larger brown specks
3	Small, roundish, to slightly elongated, necrotic gray spot, about 1-2 mm in diameter with a brown margin
4	A typical blast lesion: elliptical, 1-2 cm long, usually confined to the area of the two main veins, less than 2% of leaf area infected with typical blast lesions.
5	Less than 10% of leaf area infected with typical blast lesions
6	Average of about 25% of leaf area infected with typical blast lesions.
7	Average of about 50% of leaf area infected with typical blast lesions
8	Average of about 75% of leaf area infected with typical blast lesions.
9	About 100% of leaf area infected

To determine the level of horizontal resistance at each variety, the number of susceptible lesions are counted in accordance with the daily progress of development of blast. First recording was started 19 days after sowing in the blast nursery, five times on 19/Feb., 25/Feb., 1/Mar. and 10/Mar.

The infection rates, which is one of the indicators of the level of horizontal resistance were obtaind by the following equation.

$$r = \frac{2.3}{t_2 - t_1} \times \log \frac{x_2}{x_1}$$

r: regression coefficient of $\log_e x$ on time. (Infection rate)

X_1, X_2: number of susceptible lesions at the date t_1 and t_2, respectively.

3) Results

1) Race frequency and constitution of blast fungus in the blast nursery, (March, 1982).

Forty-five blast fungus isolates were obtained from the infected plants in the blast nursery. Isolates were collected from infected lesions at ramdom including small and large lesions.

The result of race differentiation according to the reaction patterns of Kiyosawa's differential varieties, shows the race constitution in the blast nursery as follows: 177.5 (2.2%), 157.5 (84.4%), 157.1 (2.2%), 156.1 (2.2%), 147.5 (2.2%), 117.5 (2.2%) and 117.1 (2.2%). The code number were indicated by the Kioysawa's differentials, which are mentioned in chapter III.

First symptom appeared on the leaves in the spreader on 16, Feb. 16 days after sowing and after 2 days the symptoms appeared on the some of entry varieties especially on Aichi Asahi and Fujisaka 5.

The main races were 157.5 and 147.5 (total 86.6% of all the isolates). It showed that in the blast nursery, that the races can attack the following resistance genes, Pi-k^s, a, i, k, ta, k^p, t and z according to the reaction patterns of Kiyosawa's differentials.

However, the race which can match to Pi-ta^2, b, z^t and k^m could not be identified under this blast nursery condition.

One out of thirteen resistance genes, (Pi-k^h gene) was not identified because a differential variety which can match Pi-k^h major R. gene was not available.

(2) The level of horizontal resistance under the blast nursery condition.

The level of horizontal resistance was evaluated by the daily progress in number of susceptible lesions per plant.

The varieties which carry the same major R. gene or genes were evaluated to eliminate the overlapping effect of major R. gene. Under this blast nursery conditions, the races which can match to following major R. genes, Pi-k^s, Pi-a, Pi-i, Pi-k, Pi-z, Pi-t, Pi-k^p and Pi-t were found.

Major R. gene (+): (Fig. 1-1)

The daily progess in number of susceptible lesions are shown in Fig. 1-1.

Most of the varieties do not have difinite major R. gene (indicated as +) in Japan by using Japanese differential races. The symptoms of these entries first appeared 18 days after sowing.

After 33 days, 5.0~6.0 leaf stage, Norin 8, Rikutō Norin 12 and Rikutō Norin 4 showed typical susceptible reaction, especially Rikuto Norin 12 was completely dead 38 days after sowing. In addition, Norin 6, and Akishino-mochi showed severe reaction under the blast nursery condition.

On the other hand namely Koganenishiki, Nihonbare, Yamajiwase and Ginga showed the high level of varietal resistance.

Rikū 132 did not show any symptom even hypersensitive reaction, it suggests that Rikū 132 possibly have another major R. gene or genes which could not be identified in Japan using Japanese differential races. If the varieties Koshi-Riku A and Koshi-Riku B do not carry any major R. gene or genes, they possess the high level of horizontal resistance.

Pi-a: (Fig. 1-2)

Aichi Asahi showed typical susceptible reaction under the blast nursery condition as well as Zenith (Pi-a, z). Fujiminori, Shyūho and Kinmaze showed susceptible reaction, however the daily progress of susceptible lesions was slower and smaller than those of Aichi Asahi and Zenith. On the other hand, Homarenishiki, Akibare and Toyonishiki showed resistant reaction. It showed that these varieties probably carry another, specific resistance gene beside Pi-a major R. gene.

Pi-i: (Fig. 1-3)

The varieties which have Pi-i major R. gene can classify into two categories, m and s. Fujisaka 5, one of Japanese and Kiyosawa's differentials always showed susceptible reaction, which can classify into later category. Other five varieties showed relatively high level of varietal resistance.Yoneshiro, Koganebare and Todoroki-wase showed the high level of horizontal resistance according to the daily progress of susceptible lesions.

Pi-k: (Fig. 1-4)

Kanto 51, 52 and 53 showed almost same reaction pattern, however according to the result of replication of Kanto 51 showed a little bit higher resistance than others, it may be affected by the nutrition level or depend on the density of distribution of pathogen under the blast nursery condition. Mangetsu-mochi, Tatsumi-mochi showed moderatly resistant reaction than others. For example Kusabue showed the relatively high resistant reaction in four replications. Kusabue is one of the Kiyosawa's differentials, which is a well known variety carring Pi-k major R. gene. It suggests that Kusabue probably carry another major R. gene. It should be confirmed by genetic analysis.

Reishiko is also well known variety and carries Pi-k major R. gene. It has used for resistance breeding in Japan before. However, it showed moderate resistant reaction and is supposed to carry another major R. gene.

Pi-ta: (Fig. 1-5)

Four varieties, Pi Nº 4, Pi Nº 2, Yashiro-mochi and K 1 showed almost the same level of resistance and reaction patterns according to the daily progress of epidemics.

It did not show much difference in the level of horizontal resistance among four varieties. It suggests that they may not carry another resistance gene beside Pi-ta major R. gene.

Pi-z:

The race which can match to Pi-z major R. gene exists under the blast nursery condition according to the race identification.

The susceptible lesions appeared on the leaves of three varieties, Fukunishiki, Fukuhikari and Oû 244. However, they showed relatively high resistant reaction. It suggests that these three varieties are supposed have a high level of horizontal resistance.

Pi-k^{m}:

Tsuyuake and other three varieties showed a few susceptible lesions. However, the analysis of the races using isolates from them as well as forty-five isolates from the blast nursery showed that the race which can attack to Pi-k^{m} specific gene were absent under this blast nursery condition. Six isolates obtained from Tsuyuake and Inakei 159 differentiated the races of 157.5 (4) and 147.5 (2). Tsuyuake showed typical susceptible symptoms with the race which can attack to Pi-k^{m} major R. gene in artificial inoculation tests under the glass house condition.

Other genes; Pi-ta^{2}, b, z^{t}

The varieties which control their resistance by major R. genes such as Pi-ta^{2}, (Pi Nº 4, Yamahikari, Fukuhonami), Pi-b (BL 1, BL 7), Pi-z^{t} (Toride 1, TYB, TYC) and Hokkai 188 (?) did not show any symptom because of the absence of matching races in the blast nursery.

Brazilian varieties: (Fig. 1-6, 7)

Results are shows in Table 2.

Two varieties, Tiririca and Três Marias out of fifty Brazilian entries did not show any symptom. It shows that these two varieties probably carry other major R. gene besides Pi-a, k, i, z, k^{p}, ta and k^{s}.

Três Meses Branco, Catalão, and CNAx 104 showed high level of varietal resistance than others. SR 2041-50-1 showed typical hypersensitive reaction, suggesting thereby that it probably carry another major R. gene.

30 varieties were classified into the category of moderate resistance including IAC 47, IAC 25, IRAT 13 and other

commercial varieties. 19 varieties were classified into s category of the level of horizontal resistance according to the daily progress of symptoms and r-coefficient values.

In the m category, IRAT 13, Pratão Precoce and some other varieties are well known which possess the high leve of horizontal resistance under normal cultivating condition.

The lesion type on the varieties of m categories was quite different. They showed numerous small brown lesions instead of large, susceptible or acute lesions. Many of the varieties in s category showed large acute lesions as in Japanese susceptible varieties.

(3) Estimation of the effect of accumlation of major R. genes into a single cultivar.

According to the reaction patterns, the races which can match to 7 of 13 major R. genes Pi-k^s, Pi-a, Pi-i, Pi-k, Pi-ta, Pi-z and Pi-k^p were existed under this blast nursery condition.

Some of entries have been bred to accumulate two or more major R. gene, which were used for evaluation of the effect of gene accumulation. The gene components were as follows, a + k^m, a + i, a + k, i + k, a + ta^2, a + k + z and a + b + i + k.

From this combination, the races which can match to Pi-k^m, Pi-ta^2 and Pi-b were eliminated from evaluation.

The varieties which carry the major R. gene matching to pathogenic races under this blast nursery condition are as follows:

Major R. gene	Variety	Degree of Horizontal Resistance
a, i	Chyubu 26, Chyubu 35	r
i, k	Inakei 115	r
a, k, z	Akiyutaka	r
a, b, i, k	Hama asahi	r
a, z	Zenith	SS
a, k	Toto	S
a, k^m	Hokushitamai	M

The varieties which have non-matching gene, such as Kongo (BR 1) , Minehikari and Chyubu 36 (Pi-a, k^m) showed resistance reaction, however Hokushitamai (Pi-a, k^m) showed the moderate resistant reaction.

Reiho (Pi-a, ta^2) did not show any lesion even hypersensitive reaction. Akiyutaka (Pi-a, k, z) showed resistant reaction (Fig.1-4).

According to the results, if these varieties have not any other specific resistance gene, the effect of accumulation of specific resistance gene into one cultivar shows significant result under this blast nursery condition.

However, Zenith is well known variety which has Pi-a and Pi-z major R. gnes, it showed susceptible reaction as well as Toto. It is necessary to compare with of the reaction of Zenith to other varieties which carry same major R. gene components.

4) Discussion

The level of horizontal resistance is commonly evaluated underthe blast nursery condition in Japan besides the artificial inoculation method in the greenhouse.

Horizontal resistance is considered as a quantitative characters which is inherited by polygenes or minor genes, it can be measured either by the daily progress of number of susceptible lesions or disease leaf area.

To evaluate the level of horizontal resistance, it is necessary to exclude the effect of vertical resistance gene (major R. gene).

In other words, it should be measured under the presence of virulent races matching are vertical resistance genes.

It is necessary to know the relation between virulent races and resistance gene constitution of each variety.

The level of horizontal resistance was evaluated in the varieties which carry matching major R. gene or genes under matching virulent races.

Based on the results of race identification, matching virulent races to the following major R. genes were found under the blast nursery condition at CNPAF/EMBRAPA, Goiãnia: Pi-a, Pi-k^s, Pi-i, Pi-k, Pi-t, Pi-z, Pi-k^p and Pi-ta. Therefore under the blast nursery condition, it could not be possible to estimate the level of horizontal resistance of the varieties which carry other major R. genes such as Pi-ta^2, Pi-b, Pi-k^m and Pi-z^t.

The prevalent race in the blast nursery was race 157.5 (84.4%), which can match to Pi-z and Pi-k major R. genes mainly.

As most of upland varieties are supposed to carry Pi-z major R. gene, the level of horizontal resistance of major entries was evaluated under the blast nursery condition.

(1) The evaluation of the level of horizontal resistance of Japanese varieties with known major R. gene.

Three varieties Ginga, Honarenishiki and Norin 22 were known varieties, are supposed to have unidentified gene in Japan. They were classified into r category of the level of horizontal resistance.

These three varieties showed stable high level of horizontal resistance in the blast nursery test. It suggests that this reaction is supposed to due to the horizontal resistance.

Rikuto Norin 4 and Rikuto Norin 12 showed quite susceptible reaction. These varieties are believed to possess a high level of horizontal resistance and have been trying to introduce the stability of horizontal resistance to Japanese lowland rice varieties. However, these two varieties seems to carry certain specific resistance gene or genes according to the reaction under the blast nursery condition. The leaf stage of initiation of blast epidemics may or may be not be affected by the appearance of the gene action of horizontal resistance. For example, a variety Zenith is an useful resistance gene sources to incorporate the major R. gene (Pi-z) to Japanese varieties. However Zenith is quite susceptible both in the blast nursery and in the artificial inoculation test. If it is possible to determine the gene specificity in Rikuto Norin 4 and Rikuto Norin 12 under different pathogenic races condition in Brazil, it provides very important information to the rice breeding program in Japan.

It further shows that not only the frequency and constitution of blast fungus races but also resistance gene components are supposed to be quite different from Japanese varieties.

Rikū 132 did not show any symptom even hypersensitive reaction. It showed that Rikū 132 probably have certain specific resistance gene which is not identified in Japan using a set of Japanese differential varieties and races.

The varieties Rikū 132, Homarenishiki (Pi-a), Akibare (Pi-a), Toyonishiki (Pi-a), Hokkai 188 (unidentified) as low as others showed r reaction of the level of horizontal resistance. It is necessary to confirm whether have certain major R. gene or not by genetic analysis using adequate gene marker varieties and the specific races.

(2) The evaluation of the level of horizontal resistance of the varieties from Aichi Prefectural Experimental Station, Inahashi, Japan.

Ten varieties, Chyubu 32, Chyubu 22, Inakei 153, Inakei 154, 70 GR, Koshi-Riku A, Koshi-Riku B, Rikuto Norin 4, Rikuto Norin 12 and Chyubu mochi 37 have been bred for horizontal resistance avoiding major R. gene in Japan.

Seven out of ten varieties were classified into r level of horizontal resistance, beside Chyubu mochi 37 was m and Rikuto Norin 4 and Rikuto Norin 12 were s level.

Chyubu 26 and Chyubu 35 (Pi-a, i), Inakei 115 (Pi-i, k) have been bred combining horizontal resistance with two major R. genes.

These varieties showed high level of horizontal resistance even though appeared typical susceptible lesions under the presence of matching races to them. The daily progress of number of susceptible lesions seemed to be prolonged by their varietal resistance. If this resistance is stable under different conditions , it is possible to state that the resistance is horizontal.

Chyubu 26 and Chyubu 36 have both Pi-a and Pi-i major R. genes. Both major R. genes probably are not adequate resistant gene donars of major R. gene because of the frequency of the races which can match to both major R. genes. However Chyubu 26 and 36 considerably high level of horizontal resistant reaction. If they do not carry another major R. gene or genes. They can be considered to have enough level of horizontal resistance. There are many aspects at each stage which should be confirmed by genetic analysis.

(3) Difference in the level of horizontal resistance between Kanto 51 and Kusabue.

Kanto 51 is one of the international differentials and Kusabue is one of the Kiyosawa's differentials. Both Kanto 51 and Kusabue possess the ability to identify the Pi-k major R. gene as differential variety.

According to many investigators in Japan the level of horizontal resistance of Kanto 51 is higher than that of Kusabue. But, the results obtained under the blast nursery condition at CNPAF/EMBRAPA, Goiânia, Brazil, Kusabue showed more resistant reaction than that of Kanto 51. The difference in reaction between Kusabue and Kanto 51 has been reported in the Annual Report 1981.

It further suggests that the variety Kusabue probably have another major R. gene which could not be identified in Japan using a set of Japanese differentials and races. These results are interesting in showing that certain resistance gene may function differently under different conditions.

F_2 progenies of crosses between above two varieties and gene marker varieties were obtained and the genetic analysis is expected to reveal these questions.

(4) Evaluation of the level of horizontal resistance of Brazilian varieties and breeding materials.

As shown in Table 2, the test varieties were classified into 5 categories, majority of them were categorized into m and s of the level of horizontal resistance.

However five varieties, Tiririca, Três Marias, SR 2041-50-1, Catalão, CNAx 104 and 3 Meses Branco showed exceptional reaction than other entry varieties.

According to the results of artificial inoculation method using six different pathogenic races, SR 2041-50-1, CNAx 104 (IAC 47 x SR 2041-50-1) and Catalão showed susceptible reaction by the inoculation of matching race to Pi-k^m major R. gene. These three varieties are supposed to have Pi-k^m (Tsuyake type) major R. gene. Tiririca probaly has Pi-b major R. gene as in the case of some IRGA and EMPASC lines of the Santa Catarina and Rio Grande do Sul States.

As Três Marias showed R^h reaction to all the six races, it further suggests that this variety carry Pi-z^t (Toride type) or inidentified major R. gene. The level of horizontal resistance of above 5 varieties should be evaluated in the presence of certain matching virulent races.

When the entries showed r reaction of varietal resistance under blast nursery condition, it is essential to confirm whether major R. gene is present or not. Otherwise, we may select the major R. gene variety instead of horizontal resistance.

Experimentally , IRAT 13, Amarelão, Pratão Precoce and Dourado Precoce are known varieties which possess the relatively high level of horizontal resistance under natural cultivated condition. And also IRAT 13 is one of the promising resistance donar varieties. However, according to the results of evaluation of the level of horizontal resistance these varieties were classified into m category under the blast nursery condition.

The screening of varietal resistance under the blast nursery condition is the most easiest method, however it is necessary not only to know the race frequency and constitution but also to relate the degree of epidemics between the blast nursery and natural conditions.

The epidemics in blast nursery are seems to more severe than under natural cultivated condition, because of dense plant population, high level of nitrogen fertilizer and abnormal spore concentration from spreaders.

The considering this aspect the varieties classified into m category possibly have relatively high level of horizontal resistance.

It is necessary to compare their horizontal resistance results obtained from the blast nursery with the artificial inoculation tests using adequate races in the greenhouse.

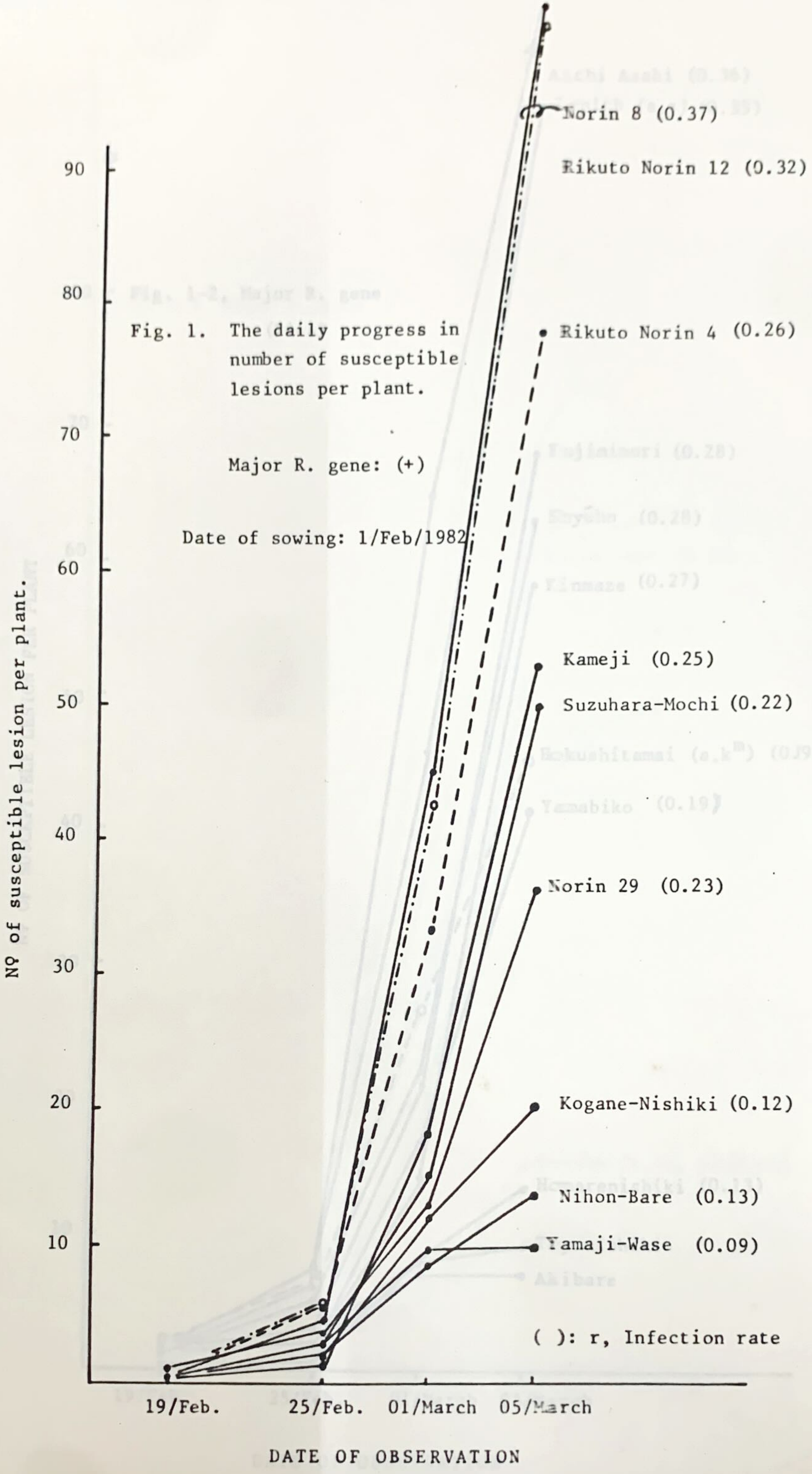


Fig. 1. The daily progress in number of susceptible lesions per plant.

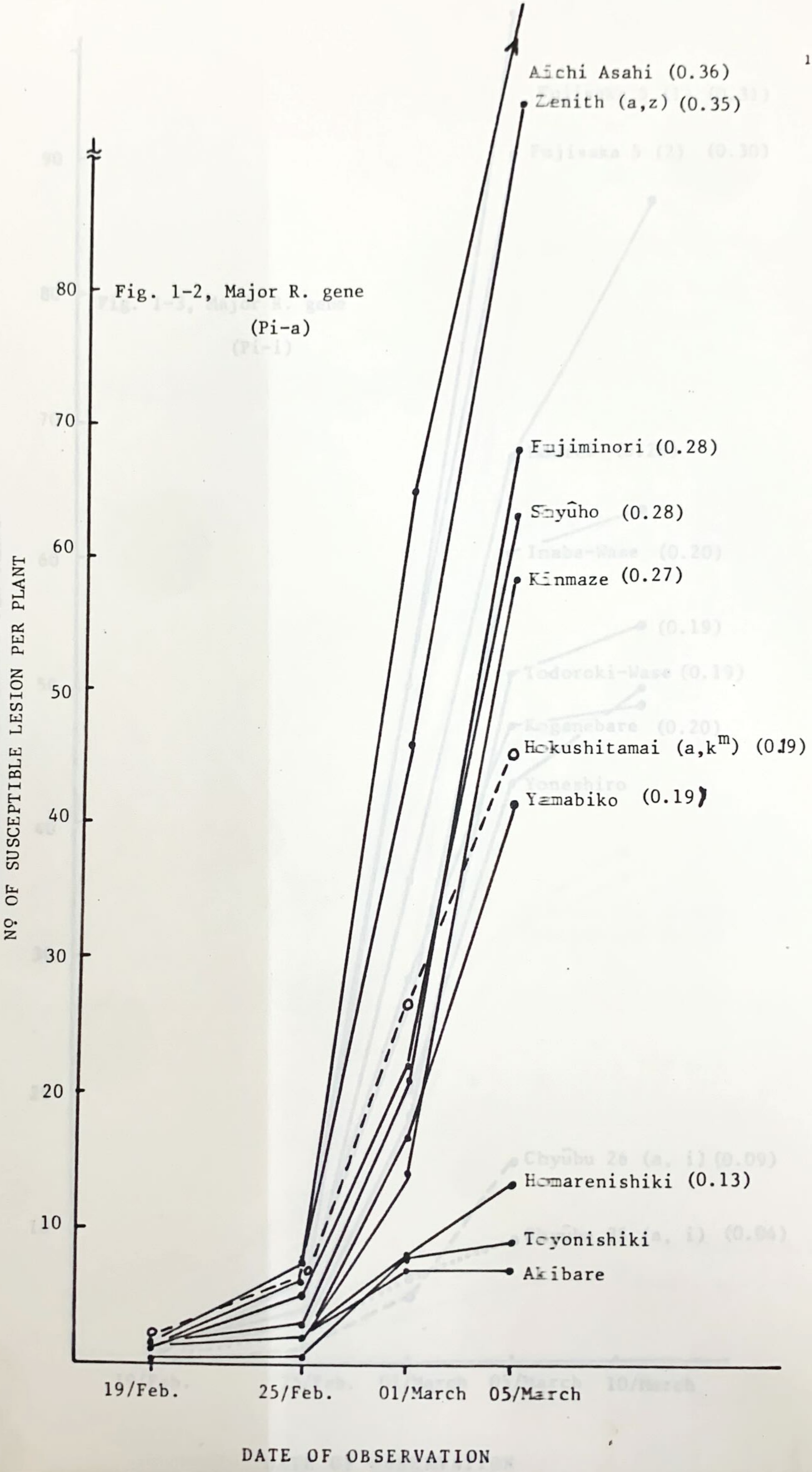


Fig. 1-2, Major R. gene (Pi-a)

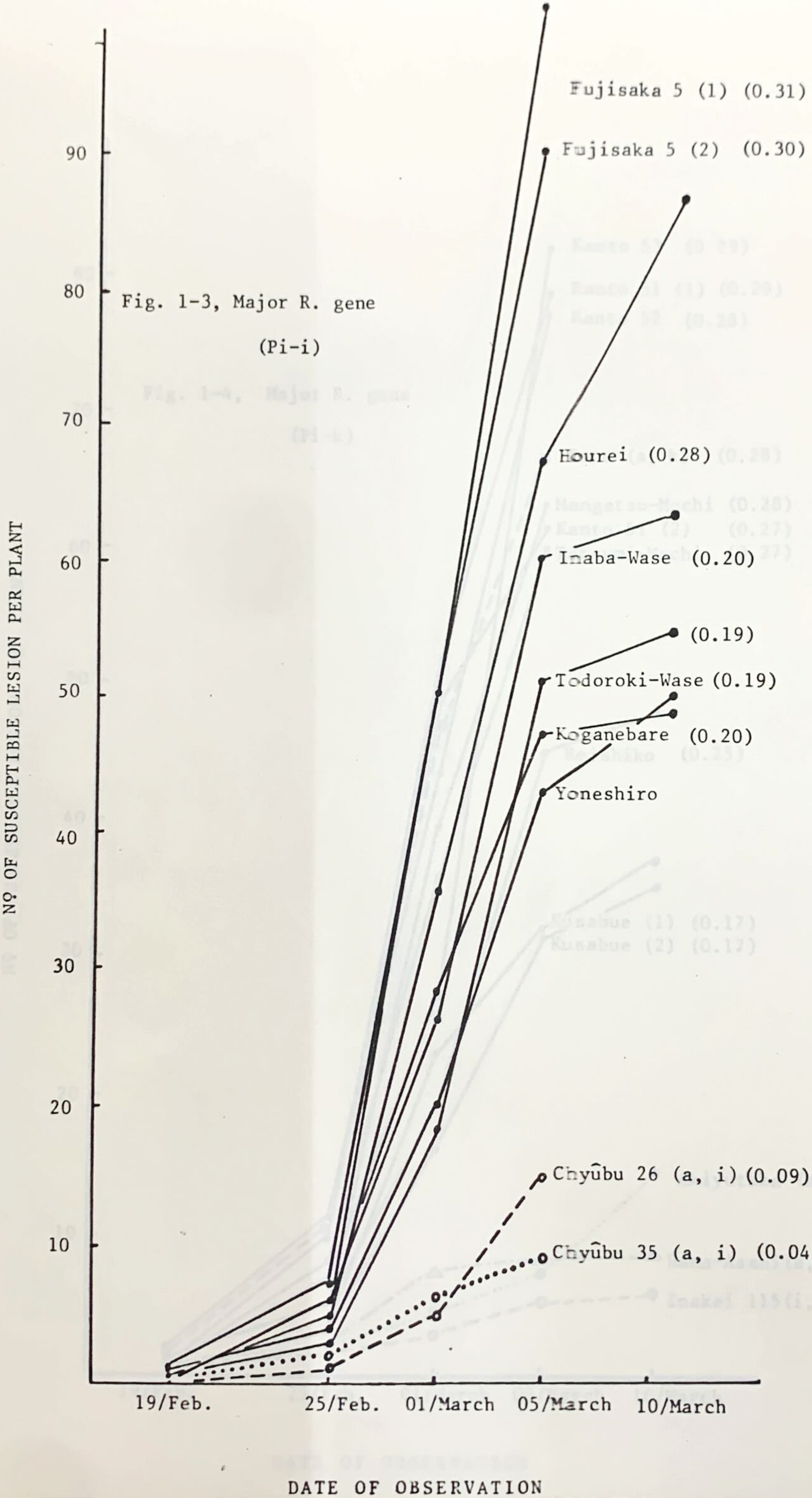


Fig. 1-3, Major R. gene

(Pi-i)

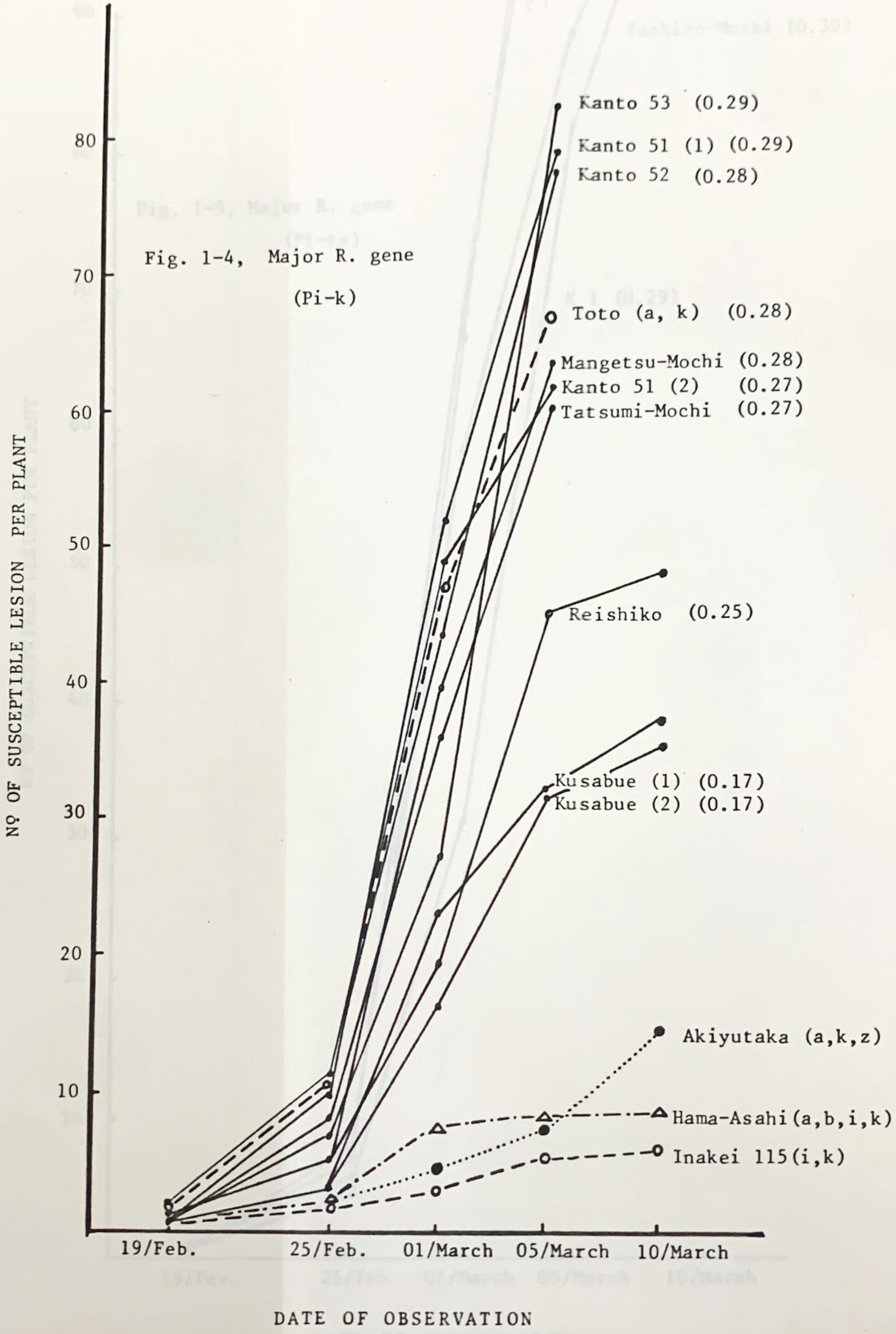


Fig. 1-4, Major R. gene

(Pi-k)

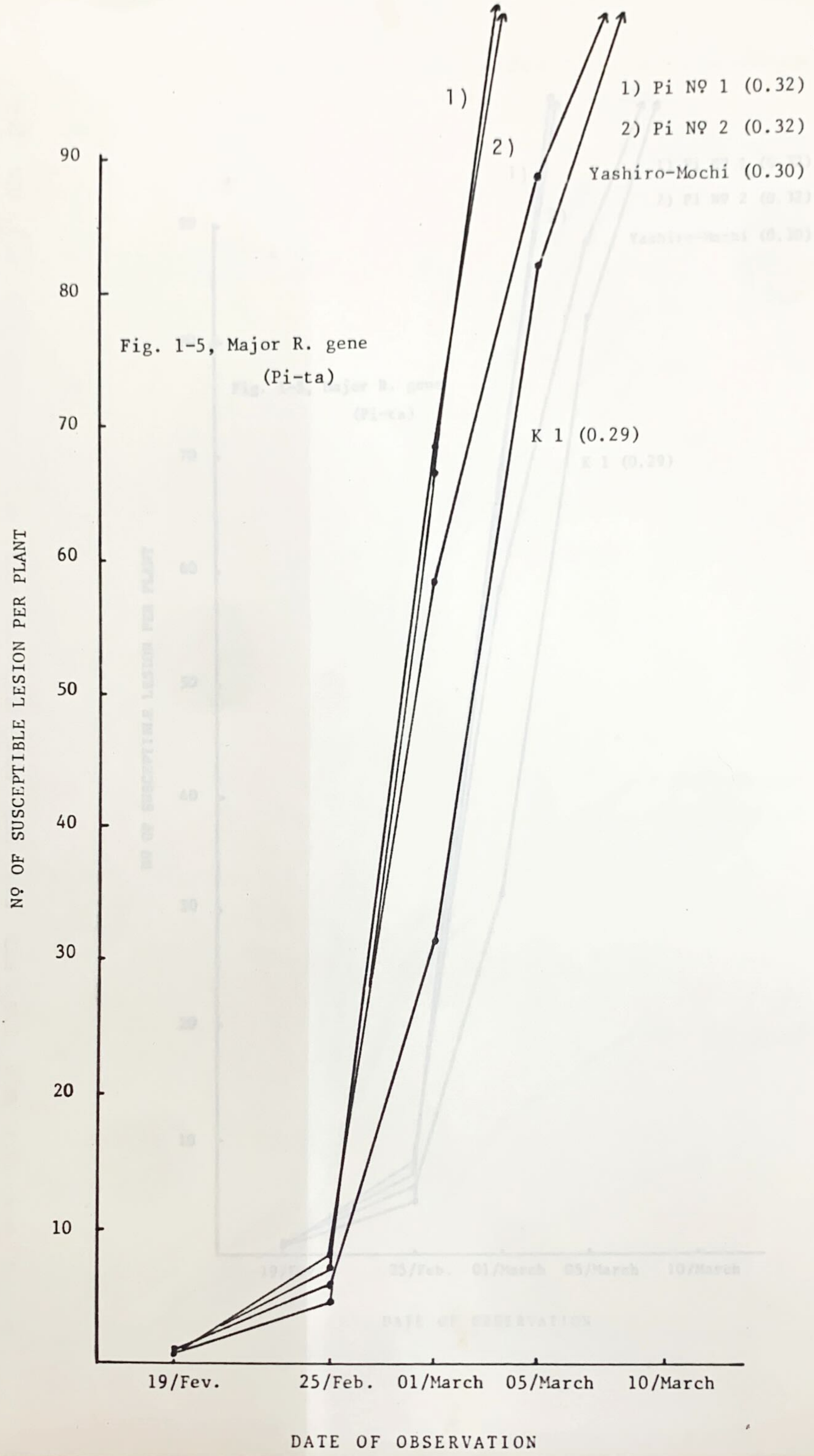


Fig. 1-5, Major R. gene (Pi-ta)

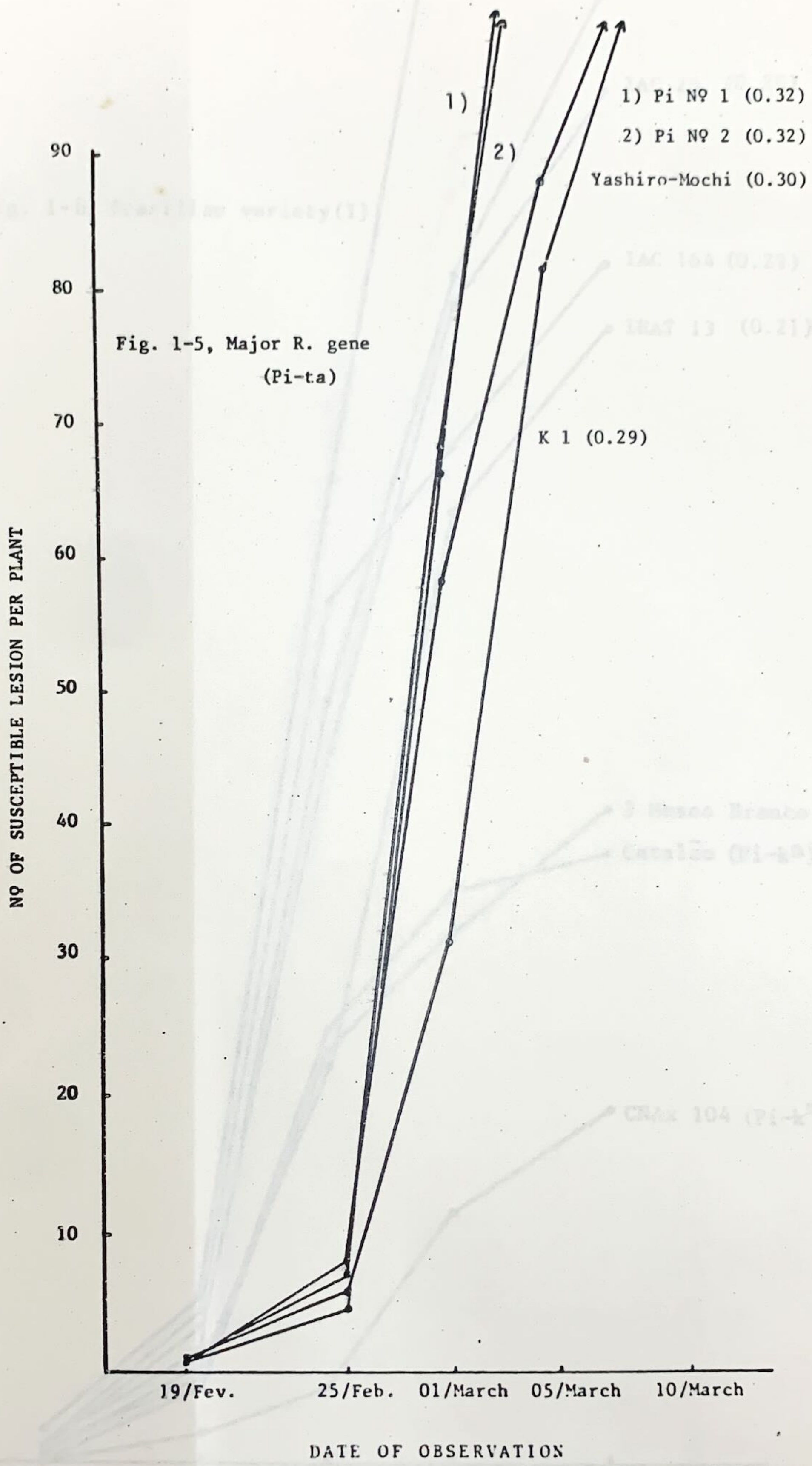


Fig. 1-5, Major R. gene (Pi-ta)

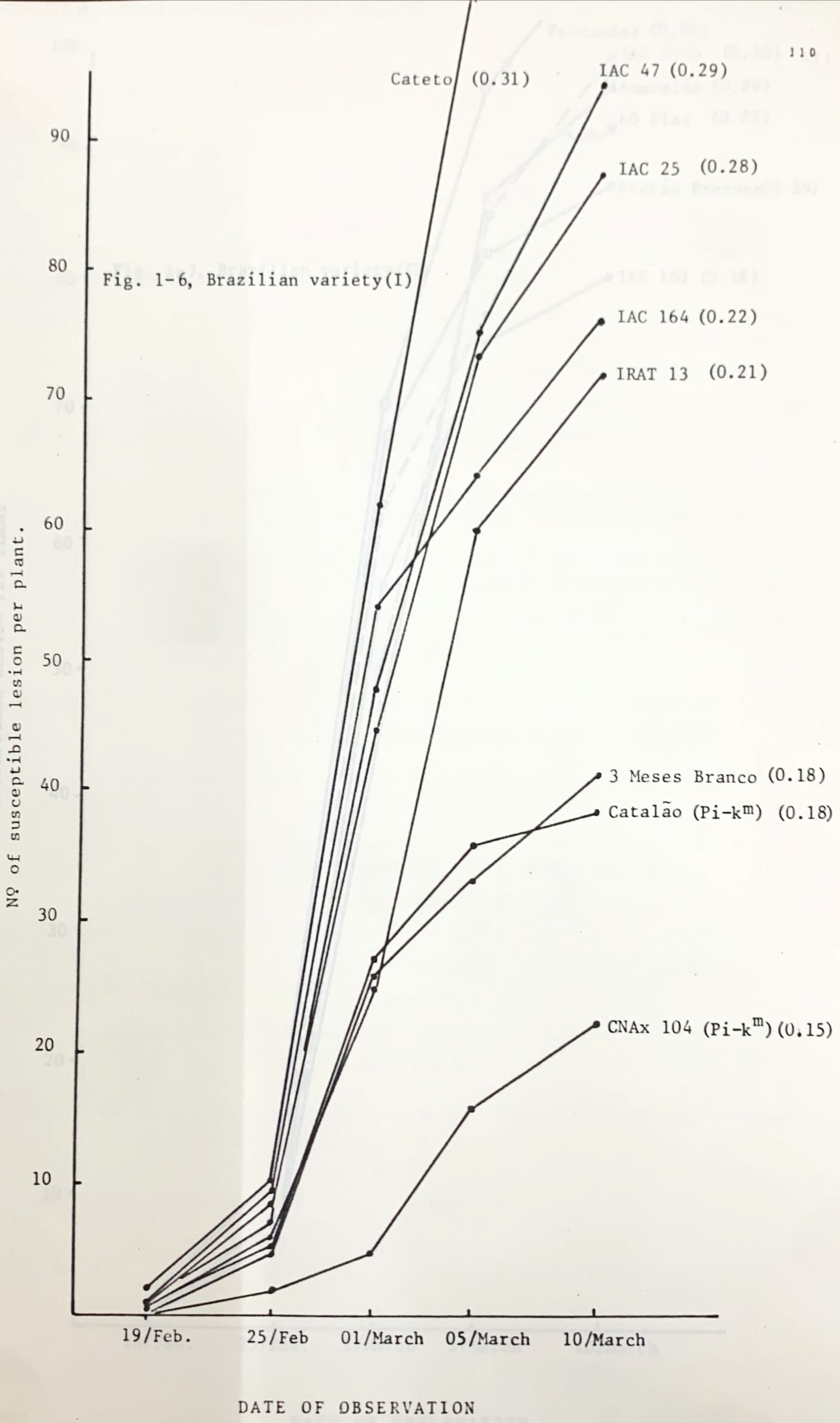


Fig. 1-6, Brazilian variety(I)

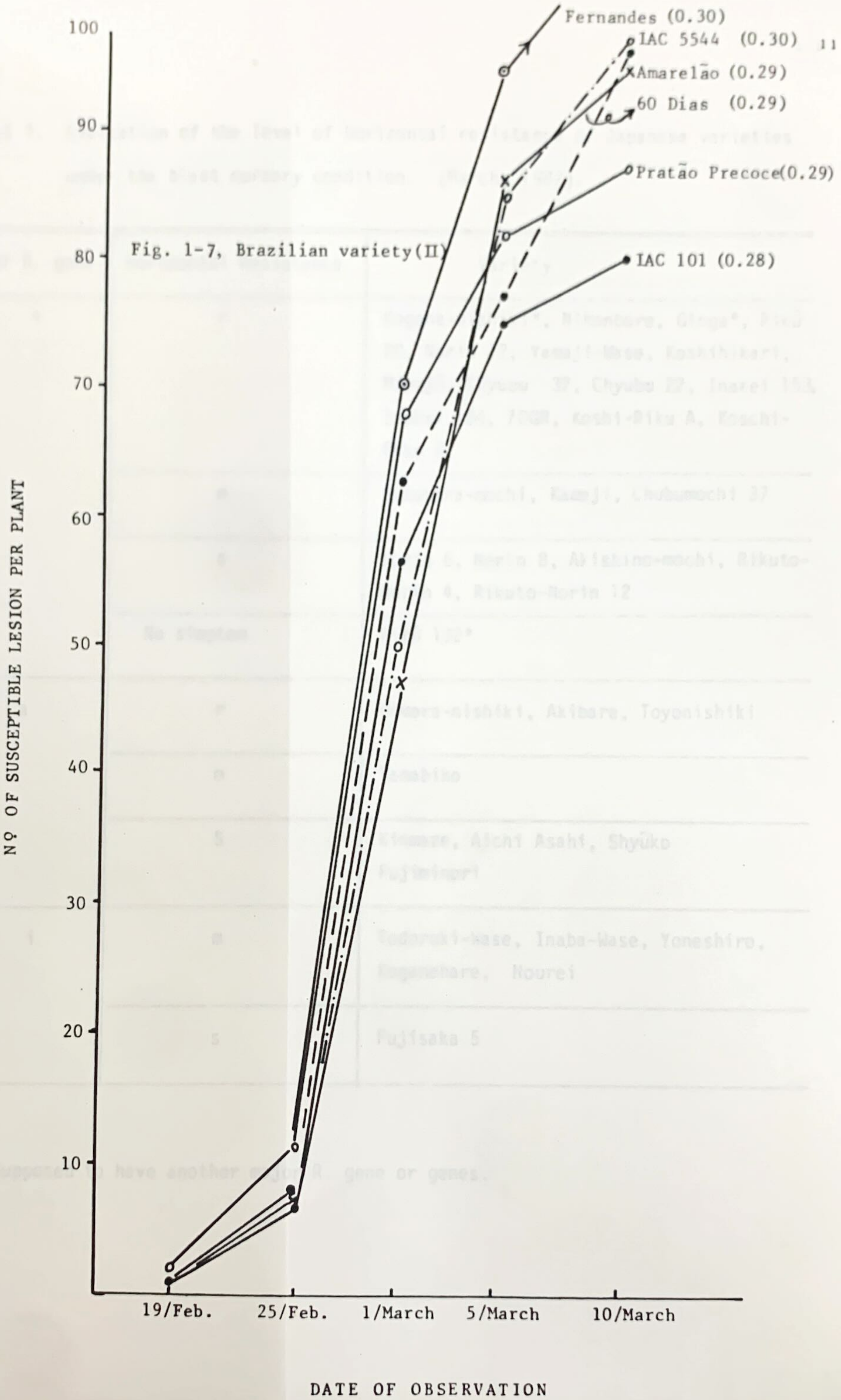


Fig. 1-7, Brazilian variety(II)

TABLE 1. Evaluation of the level of horizontal resistance of Japanese varieties under the blast nursery condition. (March, 1982).

Major R. gene	Horizontal Resistance	Variety
+	r	Kogane-nishiki*, Nihonbore, Ginga*, Rikū 20, Norin 22, Yamaji-Wase, Koshihikari, Miyojô, Chyubu 32, Chyubu 22, Inakei 153, Inakei 154, 70GR, Koshi-Riku A, Koschi-Riku B
	m	Suzuhara-mochi, Kameji, Chubumochi 37
	s	Norin 6, Norin 8, Akishino-mochi, Rikuto-Norin 4, Rikuto-Norin 12
	No simptom	Rikū 132*
a	r	Homore-nishiki, Akibare, Toyonishiki
	m	Yamabiko
	S	Kinmaze, Aichi Asahi, Shyūko Fujiminori
i	m	Todoroki-Wase, Inaba-Wase, Yoneshiro, Koganehare, Nourei
	s	Fujisaka 5

*: Supposed to have another major R. gene or genes.

TABLE 1. Continued.

Major R. gene	Horizontal Resistance	Variety
k	m	Kusabue, Tatsumi-mochi, Reishiko
	s	Mangetsu-mochi, Kanto 51, Kanto 52, Kanto 53
ta	s	Yoshiro-mochi, Pi nº 1, Pi nº 2
z	r	Fukunishiki, Fukuhikari, Oȗ 244
k^m	r	Tsuyuake, Inakei 140, Inakei 159, Nuaki

TABLE 1. Continued.

Major R. gene	Horizontal Resistance	Variety
a, k^m	r	Kongo, Minehikari, Chyubu 36
	m	Hokushitamai
a, i	r	Chyubu 26, Chyubu 35
a, k	S	Toto
i, k	r	Inakei 115
a, ta^2	No Sympton	Reiho
a, k, z	r	Akiyutaka
a, b, i, k	r	Hama-asahi
ta^2	No Symptom	Pi nº 4, Yamahikari, Fukuhonami
b	No Symptom	BL 1, BL 7
z^t	No Symptom	TYB, TYC, Toride 1
Not identified	r	Hokkai 188
	m	Futaba

TABLE 2. Evaluation of the level of the horizontal resistance of Brazilian varieties under the blast nursery condition. (March 1982).

The level of Horizontal Resistance	Variety
No symptom	Tiririca, 3 Marias
rr	SR 2041-50-1
r	Catalão, 3 Meses Branco, CNAx 104
m	IAC 47, IAC 25, IAC 164, Pratão Precoce, IAC 165, IAC 5544, Amarelão, IRAT 13, IAC 1246, Pérola, Matão. Dourado Precoce, IAC 1311, EEPG 369, EEPG 269, Agulha, Zebu, 64 Dias, IAC 101, Triste Tristão, Lageado, Chatão, Tomba Morro, Pela Mão, 60 Dias, Catetão Precoce, Quebra Cacho, Arroz de Revenda, Beira Campo, CGA 90 e IAC 46.
s	Cateto, Fernandes, Bico Ganga, Iguape Redondo, Blue Belle, Pratão, Batatais, Come Cru, Japonês, IAC 435, Toro, Montanha Liso, Sagrimão, Fortuna, Pindaré, Maçarico, Pingo D'Água, 90 Dias e 100 Dias.

VIII. Estimation of major R. gene in Brazilian rice varieties and breeding materials by the artificial inoculation method using six different pathogenic races.

1) Introduction

A set of six different pathogenic races was obtained from the blast fungus collections as mentioned in the Chapter III and IV.

A set of six Brazilian different pathogenic races can be used to identify the folllowing six of thirteen major known R. genes, Pi-k, Pi-z, Pi-k^m, Pi-ta^2, Pi-b and Pi-k^p, in Japanese varieties. They have been identified in Japan using Japanese differentials and races by Kiyosawa et al.

Since three of thirteen major R. genes Pi-a, Pi-i and Pi-k^s are supposed to be inadequate donars of resistance to blast, the existing set of six differential races are quite sufficient to identify the some of the major R. gene or genes in Brazilian varieties and breeding materials.

However there are possibly many unidintified major R. genes in Brazilian varieties. It is necessary to determine a suitable differential varieties from Brazilian materials.

A set of six different pathogenic races were selected in accordance with the reaction patterns of the Kiyosawa's differentials.

Japanese differentials such as a set of Kiyosawa's are essential for race identification to date; however according to the latest literature (Kiyosawa and Ikeashi, 1981), Japanese, the Kiyosawa"s and Ikehashi's differentials sets were not suitable for differentiating Philippines isolates, although the Kiyosawa's differential varieties are suitable for differentiating Japanese isolates and have single gene for blast resistance.

However, the Kiyosawa's differentials may be tentatively useful to differentiate rice blast races in a country which does not have suitable differential varieties.

The main purpose of this experiment is not only to estimate the major R. gene in Brazilian varieties but also to find the suitable differential varieties which have single major R. gene.

2) Material and Method

To date, totally 72 Brazilian upland varieties and breeding materials were used in this experiment as shown in Table 3.

Method and procedure is discribed in Chapter III.

Races which were used for this experiment are identified and differentiated according to the reaction of Kiyosawa's differentials. Table 1 shows the origin and characteristics of six different pathogenic races used in this experiment.

3) Results

The reaction patterns using six different pathogenic races

TABLE 1. Reaction patterns of six different pathogenic races to the Kiyosawa's differential varieties.

Nº	Variety	Resistance gene	Code Nº	RACE					
				11	39	162	240	303	305
1	Shin 2	Pi-k^s	1	S	S	S	S	S	S
2	Aichi Asahi	Pi-a	2	S	S	S	S	S	S
3	Fujisaka 5	Pi-i	4	S	S	S	S	S	S
4	Kusabue	Pi-k	10	S	R	S	S	R^h	R^h
5	Tsuyuake	Pi-k^m	20	R^h	R^h	R^h	S	R^h	R^h
6	Fukunishiki	Pi-z	40	M	MR	R^h	R^h	R^h	R^h
7	K 1	Pi-ta	100	S	S	S	R^h	S	R^h
8	Pi Nº 4	Pi-ta^2	200	R^h	R^h	R^h	R^h	M	R^h
9	Toride 1	Pi-z^t	400	R^h	R^h	R^h	R^h	R^h	R^h
10	Zenith	Pi-a,Pi-z		S	S	R^h	R^h	R^h	R^h
11	Kanto 51	Pi-k	10	S	R	S	S	R^h	R^h
12	K 60	Pi-k^p	0.1	S	S	S	R^h	S	S
13	BL 1	Pi-b	0.2	R^h	R^h	R^h	R^h	R^h	M
14	K 59	Pi-t	0.4	S	S	S	S	R^h	R^h
	CODE Nº			157.5	147.5	117.5	37.4	307.1	7.3
	ORIGIN	Variety		IAC 25	IAC 25	IAC 47	SR2041-50-1	IRGA 408	BR IRGA 409
		Location		CNPAF GOIÂNIA GOIÁS	CNPAF GOIÂNIA GOIÁS	JATAÍ GOIÁS	CNPAF GOIÂNIA GOIÁS	URUSSANGA SANTA CA- TARINA	URUSSANGA SANTA CA- TARINA

showed the quite different result form that of the lowland varieties of the state of Santa Catarina as shown at the Chapter IV and Table 2.

Many of lowland varieties and breeding materials showed typical susceptible reaction to race 303 and 305. Therefore, these varieties possibly control their resistance by Pi-ta^2, Pi-b or Pi-k^h major R. genes. On the other hand, as shown in Table 3, many of upland varieties and breeding materials showed R^h reaction to Race 303 and 305. It suggests that the major R. gene constitution is quite different between lowland and upland varieties.

(1) IAC lines

IAC 25 and IAC 47 shows almost same reaction patterns to all the six races.

IAC 165, IAC 5544 and IAC 1246 showed R^h-R, MR-R and R-MS reaction patterns to Race 11, 39 and 162, respectively.

IAC 46 showed MR reaction to Race 240 as same as IAC 1311.

The reaction patterns showed the similarity of these varieties. IAC 435 showed quite different reaction from above varieties, IAC 435 showed S reaction to five races except Race 303 as R^h reaction.

Fig. 1 shows the pedigree history of IAC upland varieties.

The parent varieties, Cateto, Perola, Dourado Precoce, Pratão and Jaguari showed MR-S reaction to Race 11 and 39 and showed the similarity of reaction patterns in some varieties.

Pratão and Jaguari showed R^h and R reaction to race 162, respectively. Dourado Precoce showed MR reaction to Race 303 and 305.

From this reaction patterns with six races, the gene diversity of IAC lines supposed to be relatively narrow as well as that of lowland varieties.

(2) IRAT lines

IRAT 13, IRAT 112 and IRAT 146 were used in this experiment. All of varieties have been bred to incorporate the high level of horizontal resistance, however IRAT 112 and IRA 136 showed the gene specificity to 4 and 2 races, respectively.

IRAT 13 showed typical hypersensitive reaction with numerous number of brown spot, however showed R-R^h reaction to all of six races.

(3) Varieties without any symptom or hypersensitive reaction

Três Marias, EEPG 269, Raminad Str. 3, Carreon, CO 25, 3 Meses Branco and TKM 1 showed R^h-R reaction to all the six races. CO 25 and TKM 1 were known which have Pi-z^t major R. gene.

It suggests that these varieties supposed to have Pi-z^t (Toride type) or unidentified resistance genes.

(4) Breeding lines at CNPAF/EMBRAPA

CNAx 104 is one of the breeding lines, of CNPAF/EMBRAPA which has been bred from the cross of IAC 47 and SR 2041-50-1 as high horizontal resistance variety. Both CNAx 104 and SR 2041-50-1 showed S reaction to Race 240. It suggests that both varieties supposed to have Pi-k^m (Tsuyuake-type) major R. gene.

SR 2041-50-1 is one of the introduced breeding materials from Korea.

(5) Local land varieties

Amarelão, Catetão Precoce, Fernandes, Iguape Redondo, Quebra Cacho and Zebu, showed R^h reaction to three races, 240, 303 and 305. The reaction patterns are quite different from that of lowland varieties. Majority of them showed R-S reaction to Race 11.39 and 162. It suggests that the gene diversity on resistance is quite different from lowland varieties.

(6) Others

Tiririca, BG 90-2 and Santa Catarina showed S reaction to only Race 305. These varieties showed R^h reaction under the blast nursery condition at CNPAF/EMBRAPA, Goiânia 1981 and 1982.

Carreon showed R-MR ambiguous reaction to race 305 and Carreon showed hypersensitive reaction with small slender spots to all the six races. Lebonnet and Dawn showed S reaction to race 240.

Both varieties are known which carry Pi-k^h major R. gene. It suggests that race 240 maybe able to match to Pi-k^h major R. gene as well as Pi-k^m.

From these results, according to the difference of the pathogenicity of three races, some varieties can be classified into three categories by S reaction as shown in Table 4.

The frequency of varieties which can match by three races 240, 303 and 305 is lower than that of lowland varieties. A some of these varieties, IET 1444, Santa Catarina and Japanese are lowland varieties, majority of upland varieties showed resistant reaction to above three pathogenic races.

It can be seen from these results that many of the upland varieties and breeding materials showed MR-MS, ambiguous reaction. These results further suggest that in many varieties that the resistance may be controlled not only by single major R. gene but also possess a high level of horizontal resistance.

4) Discussion

Three major R. genes Pi-a, Pi-i and Pi-k are mainly contributing to the resistance of japonica rice varieties in Japan.

The geographical distribution of major R. genes shows the means of introduction of resistant gene sources.

It is very important to know the pedigrees and their origin of local land varieties.

It gives enough informations to identify the major R. genes. Fig. 1 shows that the pedigree history of IAC upland varieties and lines.

Some of them are javanica type, it suggests that these varieties maybe introduced from Indo-China countries at 15th century through some latin American countries. It is necessary to know the pedigree of these local varieties.

The reaction patterns of upland varieties and breeding materials were different from those of the lowland varieties from the state of Santa Catarina when inoculated with six races.

Race identification and the estimation of major R. genes of main lowland varieties revealed that the major R. genes contributing to lowland varieties are supposed to be Pi-ta^2 (Tadukan type), Pi-b (BL 1 type) and Pi-k^h (Dawn type) as mentioned in Chapter IV.

The upland varieties and breeding materials derived part of their resistance from Pi-z (Fukunishiki-type) or Pi-k (Kusabue or Kanto 51 type). Many varieties showed ambiguous reaction such as MR-MS, which could be due to combined reaction of major R. genes.

It further suggests that they may have either unidentified gene or combined major R. genes.

Race 11 showed the pathogenicity to Pi-z (Fukunishiki), Pi-k (Kanto 51) and Zenith (Pi-a, Pi-z) in accordance with a set of Kiyosawa's differentials.

The race analysis of the blast fungus isolates and reaction patterns of IAC 25 and IAC 47 showed existence of Pi-z (Fukunishiki) and K 60 (Pi-k^p) as shown in Table 5.

From these results it can be concluded that IAC 25 may possess Pi-z major R. gene. However, the reaction patterns to Race 11 and Race 39 showed MR (intermediate) reaction and M reaction to Race 162 showing there by the same reaction patterns of IAC 47.

The cultivars IAC 47 and IAC 25 possibly have common major R. gene beside Pi-z and Pi-k^p genes or unidentified major R. gene or genes.

It is necessary to use another race to identify the difference of major R. gene or genes between IAC 25 and 47.

It will be revealed by genetic analysis.

IAC 165 and IAC 1246 showed R and R^h reaction to race 11 and Race 39, respectively. However, IAC 165 and IAC 1246 showed R and MS reaction to race 11 and Race 39, respectively.

Difference in the resistance between IAC 165 and IAC 1246 is the reaction to race 162.

The difference of Race 39 and Race 162 is only pathogenicity to Fukunishiki (Pi-z) major R. gene.

From these results, Pi-z major R. gene maybe important in function with their resistance, however it suggests the existence of another unidentified major R. gene in both varieties.

Perola, Matão, Fernandes and Pratão Precoce showed similar results.

It is necessary to use another race to identify the difference of major R. gene in function and also genetic analysis is needed.

Generally, from these results, the gene constitution of resistance of upland varieties and breeding materials appears to be more complicated than those of lowland materials in the state of Santa Catarina.

It further suggests that these upland varieties possibly possess high level of horizontal resistance than those of lowland varieties.

EEPG 369, T 1, Catalão, CNAx 104 and SR 2041-50-1 showed S reaction to only Race 240 beside showed R^h reaction to rest of five races.

It suggests that these varieties maybe carry Pi-k^m major R. gene. Since race 240 can attack Dawn (Pi-k^h) it is necessary to identify the difference of pathogenicity of race to Pi-k^m and Pi-k^h major R. gene.

Both major R. genes showed quite clear resistance reaction to five races according to the reaction patterns.

Another major R. gene sources, CO 25 and TKM 1 are one of parent of Toride line, which were known carrying Pi-z^t major R. gene. Both varieties showed R^h reaction to all the six races. As Três Marias, EEPG-269, Raminad Str. 3, Carreon and Três Meses Branco showed R^h-R reaction to all the six races, they are supposed to have Pi-z^t gene or unidentified major R. gene or genes.

One of the breeding lines at CNPAF/EMBRAPA, CNAx 104 and one of parent varieties SR 2041-50-1 showed S reaction to Race 240. It suggests that these two varieties supposed to have Pi-k^m or Pi-k^h major R. gene. CNAx 104 has shown R^h reaction under the blast nursery condition CNPAF/EMBRAPA, Goiânia through 1980 to 1982.

It suggests that SR 2041-50-1 probably has major R. gene, there are many aspects at each stage to be revealed by further genetic analysis.

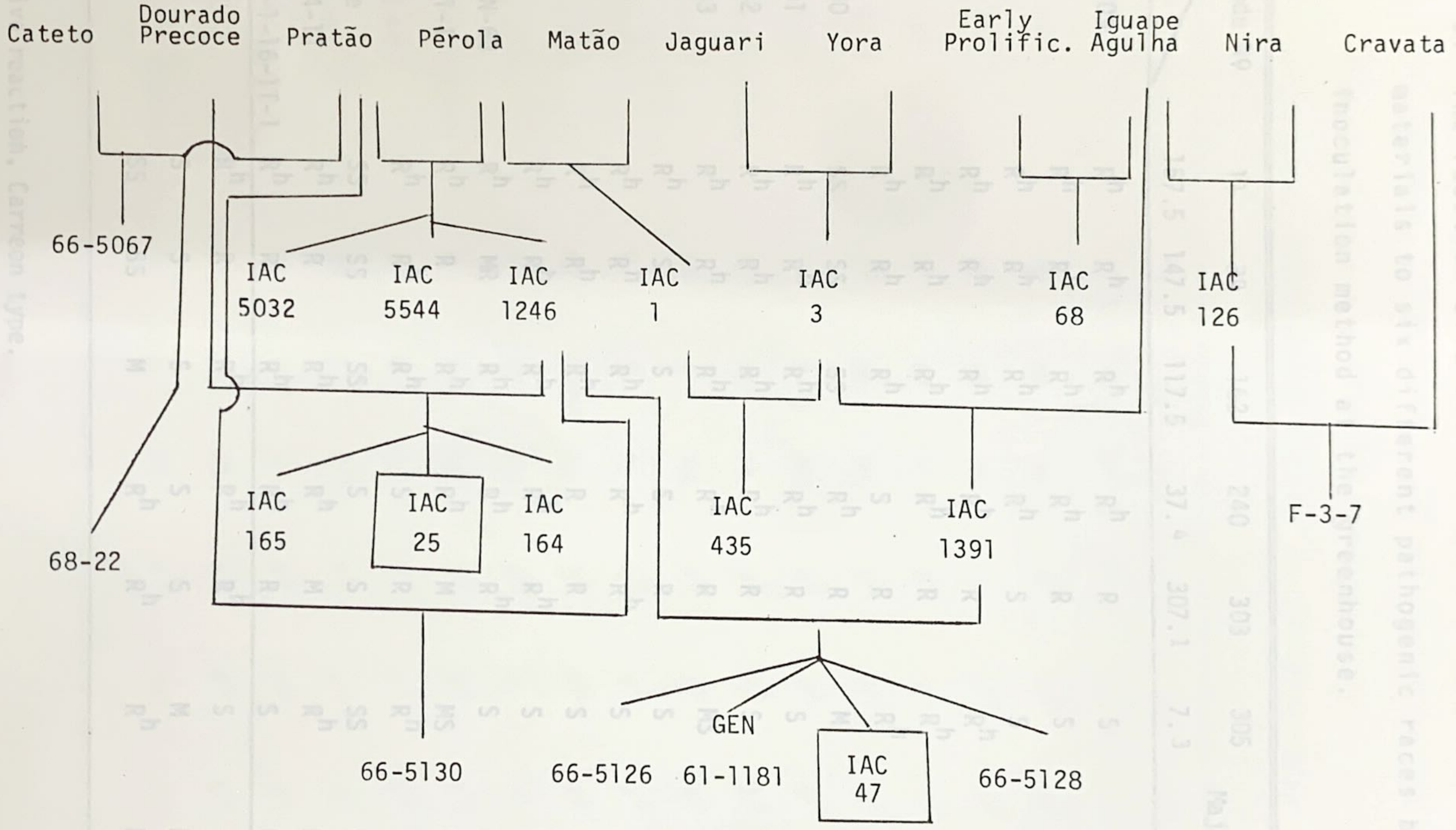


FIG. 1. Pedigree history of IAC upland varieties.

TABLE 2. The reaction of Brazilian lowland varieties and breeding materials to six different pathogenic races by the artificial inoculation method at the greenhouse.

Race Code Nº	11	39	162	240	303	305	Major R. Gene Estimated
Nº Variety	157.5	147.5	117.5	37.4	307.1	7.3	
1- BR IRGA 409	R^h	R^h	R^h	R^h	R	S	Pi-b
2- IRGA 410	R^h	R^h	R^h	R^h	R	S	Pi-b
3- IRGA 408	R^h	R^h	R^h	R^h	S	S	Pi-ta^2
4- CICA 8	R^h	R^h	R^h	R^h	R	R^h	
5- CICA 9	R^h	R^h	R^h	R^h	R	R^h	
6- Dawn	R^h	R^h	R^h	S	R	R^h	Pi-a, i, k^h
7- EMPASC 100	SS	SS	SS	R^h	R	M	
8- EMPASC 101	R^h	R^h	R^h	R^h	R	S	Pi-b
9- EMPASC 102	R^h	R^h	R^h	R^h	R	S	Pi-b
10- EMPASC 103	R^h	R^h	R^h	R^h	R	MS	Pi-b
11- IAC 899	R^h	S	S	S	R	S	Pi-b
12- IR 22	R^h	R^h	R^h	R^h	R^h	S	Pi-b
13- IR 24	R^h	R^h	R^h	R	R	S	Pi-b
14- IR 841	R^h	R^h	R^h	R^h	R^h	S	Pi-b
15- IR 6441-JN-6B	R^h	MR	R^h	R^h	R^h	S	Pi-b
16- IR 9202-21-1	R^h	R	R^h	R^h	M	MS	Pi-ta^2
17- Lebonnet	R^h	R^h	R^h	S	R	R^h	(Pi-k^h)
18- Blue Belle	SS	SS	SS	S	S	SS	Pi-a
19- P 805-B4-4-1T	R^h	R	R^h	R^h	M	R^h	Pi-ta^2
20- RS 16-516-1-16-1T-1	R^h	R	R^h	R^h	R	S	Pi-b
21- BG 90-2	R^h	R	R^h	R^h	R^h	S	
22- IAC 435	S	S	S	S	S	M	Pi-a
23- Zenith	SS	SS	M	R^h	R^h	R^h	Pi-z, Pi-a

* Hypersensitive reaction, Carreon type.

TABLE 3. The reaction of Brazilian upland varieties and breeding materials to six different pathogenic races by the artificial inoculation method at the greenhouse.

Nº	Variety / Race Code Nº	11 157.5	39 147.5	162 117.5	240 37.4	303 307.1	305 7.3	Major R. Gene Estimated
1	IAC 25	MR	MR	MS	R^h	R^h	R^h	Pi-z
2	IAC 47	MR	MR	MS	R^h	R^h	R^h	Pi-z
3	IAC 165	R	MR	R	R^h	R^h	R^h	Pi-z
4	IAC 5544	R^h	MR	MR	R^h	R^h	R^h	
5	IAC 1246	R^h	R	MS	R^h	R^h	R^h	
6	Pratão Precoce	R	MR	MR	R^h	R^h	R^h	
7	Cateto	MR	S	MS	R^h	R^h	R^h	Pi-z
8	Fernandes	R	S	M	R^h	R^h	R^h	
9	Amarelão	MR	M-MR	S	R^h	R^h	R^h	
10	Bico Ganga	R	MR	MS	R^h	M	R^h	
11	Iguape Redondo	R	R	MS	R^h	R^h	R^h	
12	IRAT 13	R	R	R^h	R^h	R^h	R^h	
13	Pratão	MR	S	R^h	R^h	R^h	R^h	Pi-z
14	Pérola	MR	S	S	R^h	R^h	R^h	
15	Matão	R	MR	MS	R^h	R^h	R^h	
16	Dourado Precoce	MR	S	MS	R^h	MR	MR	

TABLE 3. Continued.

Nº	Race Code Nº / Variety	11	39	162	240	303	305	Major R. Gene Estimated
		157.5	147.5	117.5	37.4	307.1	7.3	
17	60 Dias	R^h	MR	R^h	MR	R^h	R^h	
18	Catetão Precoce	R	M	R	R	R^h	R^h	
19	3 Meses Branco	R^h	R^h	R	R	R^h	R^h	
20	Zebu	R	M	S	R	R^h	R^h	
21	EEA 407	S	S	R^h	R	R^h	R^h	Pi-z
22	IRAT 146	MS	MR	R^h	R	R^h	R^h	Pi-z
23	Jaguari	SS	S	R	R	R^h	R^h	Pi-z
24	3 Marias	R^h	R^h	R^h	R	R^h	R^h	Toride 1 (Pi-z^t)type
25	CNAx 104**	R^h	R^h	R^h	S	R^h	R^h	Pi-k^m
26	SR 2041-50-1*	R^h	R^h	R^h	SS	R^h	R^h	Pi-k^m
27	Tiririca	R^h	R^h	R^h	MR	R^h	S	Pi-b
28	Carreon	R^h	R^h	R^h	R	R^h	MR	Pi-b
29	TOX 502	MR	R	R	M	R^h	MR	
30	IRAT 112	MR	S	SS	SS	R^h	R	
31	IAC 435	S	S	S	SS	R^h	S	
32	Tadukan	R^h	MR	MR	R	MS	R^h	Pi-ta^2, Pi-ta
33	Zenith***	SS	SS	R	R	R^h	R^h	Pi-z, Pi-a
34	BG 90-2	R^h	R	R^h	R	R^h	S	
35	IAC 435	S	S	S	S	S	M	Pi-a
36	Lebonnet	R^h	R^h	R^h	S	R	R	Pi-k^h

*: Breeding material from Korea.

**: IAC 47 x SR 2041-50-1.

***: International Differential variety.

TABLE 3. Continued.

Nº Variety / Race Code Nº	11	39	162	240	303	305	Major R. Gene Estimated
	157.5	147.5	117.5	37.4	307.1	7.3	
37 EEPG 269	R	R	R	R^h	R^h	R^h	
38 EEPG 369	R^h	R	R	S	R^h	R^h	Pi-k^m
39 T 1	R^h	R^h	R	S	R^h	R^h	Pi-k^m
40 Catalão	R^h	R^h	R	S	R	R^h	Pi-k^m
41 Come Cru	R	S	S	S	R^h	S	
42 64 Dias	R^h	R-MR	R-MR	S	M-S	R^h	
43 Agulha	R^h	R-MR	R-MR	S	R^h	R^h	Pi-k^m
44 Batatais	R^h	S	MR	S	R^h	R^h	
45 90 Dias	R	MR	MR	-	R^h	R^h	
46 100 Dias	R^h	M	MR	S	R^h	R^h	
47 IAC 46	R	MR	R	MR	R^h	R	
48 IET 1444	R^h	R	R	S	R^h	S	
49 Nanicão	M-MS	M	MR	S	R^h	MR-R	
50 IRAT 146	MR	SS	R^h	R	R^h	R^h	
51 CGA 90	S	S	S	S	R^h	R^h	
52 Santa Catarina	R^h	R^h	R^h	R	M	S	Pi-b
53 EEA 407	S	S	R^h	R^h	R^h	S	
54 Brejeiro	MR	M	S	MR	R^h	MR	
55 Arroz de Revenda	MR	R	S	R	R^h	R^h	
56 Quebra Cacho	R	MR	R	R^h	R^h	R^h	

TABLE 3. Continued.

Race Code Nº	11	39	162	240	303	305	Major R. Gene
Nº Variety	157.5	147.5	117.5	37.4	307.1	7.3	Estimated
57 Beira Campo	R	M	S	S	R^h	R	
58 Palha Murcha	MR	SS	S	S	R^h	R	
59 Diwani	R^h	R^h	R	R^h	R^h	MR	
60 Raminad Str. 3**	R^h	R^h	R	R^h	R^h	R^h	
61 Dawn	R^h	R^h	R^h	S	R^h	R^h	
62 64 Dias Itumbiara	MR	S	R	MR	R	M	
63 IAC 1311	R	R-MR	S	MR	R^h	R	
64 Japonês	S	S	S	S	MR	S	
65 CO 25	R^h	R^h	R^h	R^h	R^h	R^h	Pi-z^t
66 TKM 1	R^h	R^h	R^h	R^h	R^h	R^h	Pi-z^t
67 NP-125***	S	S	S	S	R^h	R	
68 Sha-tiao-tsao***	SS	SS	SS	S	SS	SS	Pi-k^s
69 Dular***	R	R^h	R	R^h	R^h	R^h	Pi-k
70 SR 2041-50-1	R^h	R^h	R^h	S	R^h	R^h	Pi-k^m
71 BG 90-2	R^h	R^h	R^h	R	R^h	S	
72 CNAx 104	R^h	R^h	R^h	S	R^h	R^h	Pi-k^m

***: International Differential variety.

TABLE 4. Classified into 3 categories by S reaction in accordance with the difference of the pathogenicity of three races.

S reaction to	Variety
Race 240 (37.4)	EEPG 369, T 1, Catalão, Come Cru, 64 Dias, Agulha, Batatais, 100 Dias, IET 1444**, Nanicão, CGA 90, Beira Campo, Palha Murcha, Japanese**
Race 303 (307.1)	64 Dias, Sha-tiao-Tsao*
Race 305 (7.3)	Come Cru, IET 1444**, Santa Catarina**, EEA 407, Japanese**

( ): Code number in accordance with the reaction by Kiyosawa's differential varieties

* : International differential variety

**: Lowland variety

IX. Gene analysis of blast resistance in Brazilian varieties and breeding materials

1) Introduction

Resistance to plant disease is described in functional and genetic terms. In functional term resistance may be specific or effective against some biotypes.

Van Der Plank (1963) introduced the terms Vertical and Horizontal to describe the differences in function. A variety with race specific resistance has vertical or perpendicular resistance where as a variety with non-specific resistance has Horizontal resistance.

The genetic term that describe resistance is based on the mode of inheritance. Oligogenic and polygenic are used to designate Vertical and Horizontal resistance. Oligogenic is determined by one or a few genes where as polygenic resistance is determined by many genes of individual small effect. Polygenic resistance is also called general resistance, tolerance and race non-specific resistance. It gives resistance to a wide spectrum of pathogenic races.

Sasaki (1923) reported investigations on the inheritance of resistance to blast in Japan. Resistance versus susceptibility was controlled by genes and behaved as a Mendelian charactor.

The genetic study of disease resistance was complicated by the discovery of physiologic races present in various pathogenic organisms.

The gene hypothesis was submitted by Flor (1955, 1959) working on flax rust fungus cited by Takahashi (1963).

Sasaki (1922, 1923) suggested the possibility of at least two physiologic races of Pyricularia oryzae. Goto and his collaborators in 1954 detected a certain number of races different in their pathogenic reaction with the genotype of host plant.

Gene analysis of blast resistance was begun by Sasaki (1922).

Till now, thirteen genes for resistance were found in Japan. Among the thirteen resistance genes, some of them are multiple allele. Pi-k gene was studied by Yamasaki and Kiyisawa (1966), Pi-k^s (Kiyosawa 1969a), Pi-k^p (Kiyosawa 1969b), Pi-k^h (Kiyosawa and Murty 1969), Pi-k^m on the Pi-k locus and Pi-ta (Kiyosawa 1966, 1969b), Pi-ta^2 (Kiyosawa 1967b) on the Pi-ta locus, and Pi-z and Pi-z^t are on the Pi-z locus.

The allelic genes on the same locus are different in their reaction to fungus races with each other.

Pi-a, Pi-i and Pi-k^s major R. genes were found commonly in japonica varieties in Japan.

Both Pi-a and Pi-k^s major R. genes are not adequate resistance gene donnars in Japan because the fungus races matching Pi-a and Pi-k^s genes are widely distributed throughout Japan.

Pi-b gene was introduced from some Indonesian varieties to incorporate the major R. gene source.

Table 1 shows the major R. genes identified in foreign rice varieties by Japanese researchers (Kiyosawa 1976 et al.).

2) Materials and Methods

A. Material: Table 1 shows that the crosses of upland varieties and breeding materials for genetic analysis on blast resistance.

Fig. 1. shows the pedigrees of IAC upland varieties.

The crosses of these materials were initiated from Dec. 1981. Number F_1 and F_2 seeds obtained to date are shown in Table 1.

From the corsses made using 35 main upland varieties and breeding materials and some of Kiyosawa's differentials the major R. gene will be identified by genetic analysis.

Table 2 shows that the crosses of some Japanese varieties. They probably have different major R. gene which may not have been identified in Japan using Japanese differentials and races. These varieties were selected from the experiments in chapter VI and VII.

Two varieties Aichi Asahi (Pi-a) and Toride 1 (Pi-z^t) were used for the resistance gene marker with the cross to the above mentioned varieties and breeding materials.

Aichi Asahi is one of the most susceptible variety among the Kiyosawa's differentials, and susceptible to all the blast fungus isolates which have been collected from main rice varieties both upland and lowland from the rice growing regions in Brazil.

On the other hand, Toride 1 (Pi-z^t) is resistance to all the races as mentioned above.

As the sterility in F_2 and F_3 progenies will affect the ratio of segregation of major R. gene, it is necessary to cross with other gene marker varieties such as Caloro (Pi-k^s), instead of Aichi Asahi to avoid the sterility of hybrids.

Table 3 shows the crosses of lowland varieties and breeding materials for genetic analysis and Fig. 2 shows the pedigrees of main cultivars.

Many of them are introduced from IRRI and CIAT, therefore they are suppose to bilong to <u>indica-</u> type instead of <u>Javanica</u>-type.

The materials from IRRI and CIAT were crossed with Caloro (Pi-k^s) and Toride 1 (Pi-z^t) as the gene marker varieties, which are susceptible and resistant to all the races, respectively.

B. Race: Six different pathogenic races were usde for genetic analysis in this experiment. The details of these six races was described in the section of material and methods in chapter III.

C.Methods: 1) F_2 or F_3 seeds were planted in plastic trays, 28 x 16 x 12cm in the greenhouse to avoid contamination.

Each plant was planted in 3cm rows and there were 20 seedlings per row. On both sides of F_2 or F_3 plants, parent varieties were planted to check the reaction, there were 20 ~ 30 seedlings per row.

A minimum of 100 to 300 F_2 and F_3 seeds of each cross were planted to avoid the confusion of ratio of segregation. Also F_1 seeds were planted to determine the resistance gene constitution in F. hybrids.

Before sowing, all the parents, F_1, F_2 and F_3 seeds were treated with Benlate (to avoid seed transmissible disease).

2) Inoculation was done by the spray method, the details of procedure such as multiplication of culture, incubation and inoculation are mentioned in chapter III.

TABLE 1. Crosses for genetic analysis on rice blast. (I) upland variety. (August, 1982).

Nº	CROSS	GENETIC ANALYSIS	Nº OF F_1 SEED OBTAINED	F_2 SEED
1	Kusabue x Kanto 51	Kusabue	6	
2	Kusabue x Aichi Asahi		36	20000
3	Kanto 51 x Aichi Asahi	Kanto 51	1	2000
4	Pi Nº 4 x Aichi Asahi	Pi Nº 4		
5	Toride 1 x Aichi Asahi	Toride 1	1	
6	Pi Nº 4 x Toride 1		40	6000
7	IAC 47 x Aichi Asahi	IAC 47	20	
8	IAC 25 x Aichi Asahi	IAC 25	42	
9	IAC 25 x IAC 47		31	5000
10	IAC 47 x Toride 1		18	5000
11	IAC 25 x Toride 1		88	5000
12	Pratão x Pērola	Pratão,Pērola	6	
13	IAC 1246 x Dourado Precoce	IAC 1246	10	
14	IAC 1246 x IAC 1391			
15	IRAT 13 x Aichi Asahi	IRAT 13	13	
16	IRAT 13 x Toride 1		20	10000
17	IRAT 13 x IAC 25		2	3000
18	IRAT 13 x IAC 47		15	5000
19	Matão x Pērola		30	5000
20	Matão x Aichi Asahi	Matão	19	
21	Pērola x Aichi Asahi	Pērola	20	
22	Pratão x Aichi Asahi		16	
23	Pratão x Matão	Pratão	15	5000
24	Pratão Precoce x Aichi Asahi	Pratão Precoce	13	8000
25	Pratão Precoce x Toride 1			
26	Dourado Precoce x Aichi Asahi	Dourado Precoce	2	10000
27	3 Marias x IAC 47	3 Marias	5	1500
28	3 Marias x Toride 1		29	
29	3 Marias x Aichi Asahi		2	
30	Tiririca x IAC 47	Tiririca	21	
31	Tiririca x Toride 1			

TABLE 1. Continued.

Nº	CROSS	GENETIC ANALYSIS	Nº OF F_1 SEED OBTAINED	F_2 SEED
32	Tiririca x Aichi Asahi			
33	CNAx 104 x IAC 47	CNAx 104	10	
34	CNAx 104 x Aichi Asahi		20	
35	CNAx 104 x Toride 1		10	
36	SR 2041-50-1 x IAC 47	SR 2041	28	
37	SR 2041-50-1 x Aichi Asahi		17	
38	SR 2041-50-1 x Toride 1		28	
39	Carreon x IAC 47	Carreon	2	2000
40	Carreon x Aichi Asahi		20	
41	Carreon x Toride 1		5	
42	TOx 502 x Aichi Asahi	TOx 502		
43	TOx 502 x Toride 1			
44	TOx 502 x Catetão		2	2000
45	TOx 502 x IAC 47			
46	BG 90-2 x Aichi Asahi	BG 90-2		
47	BG 90-2 x Toride 1			
48	BG 90-2 x IRAT 13		2	1200
49	BG 90-2 x IAC 47			
50	IRAT 112 x IAC 47	IRAT 112	2	1500
51	Jaguary x Aichi Asahi	Jaguary		
52	Jaguary x Toride 1			
53	Amarelão x Aichi Asahi	Amarelão		
54	Amarelão x Toride 1			
55	Amarelão x IAC 47		1	2000
56	Pratão x Tsuyake	Tsuyuake	2	3000
57	R. Norin 4 x IRAT 13	R.Norin 4	5	5000
58	R. Norin 4 x Kusabue		8	10000
59	Amarelão x IRAT 13			1200
60	Fujisaka 5 x IRAT 13	Fujisaka 5	1	2000
61	Kanto 51 x K 59	K 59	1	5000
62	Toride 1 x Aichi Asahi	Toride 1	1	
63	Carreon x Fujisaka 5	Carreon	2	
64	Toride 1 x IAC 47//IAC 47	IAC 47	5	
65	Toride 1 x IAC 25//IAC 25	IAC 25	1	

TABLE 2. Crosses for genetic analysis of some Japanese varieties in which maybe have different resistance gene which are not identified in Japan. (June, 1982).

	Variety	Major R. gene	Gene Marker Variety
1	Homare-nishiki	Pi-a	x Aichi Asahi
2			x Toride 1
3	Akibare	Pi-a	x Aichi Asahi
4			x Toride 1
5	Toyonishiki	Pi-a	x Aichi Asahi
6			x Toride 1
7	R. Norin 4	+	x Aichi Asahi
8			x Toride 1
9	R. Norin 12	+	x Aichi Asahi
10			x Toride 1
11	Rikū 132	+	x Aichi Asahi
12			x Toride 1
13	Kogane-nishiki	+	x Aichi Asahi
14			x Toride 1
15	Ginga	?	x Aichi Asahi
16			x Toride 1
17	Nihon-bare	+	x Aichi Asahi
18			x Toride 1
19	Yamaji-wase	+	x Aichi Asahi
20			x Toride 1

TABLE 3. Crosses for genetic analysis on blast (II): Lowland variety.

Nº	Cross		Genetic analysis
1	BR-IRGA 409	x Caloro	IRGA 409
2		x Toride 1	
3	BR-IRGA 410	x Caloro	IRGA 410
4		x Toride 1	
5	CICA 8	x Caloro	CICA 8
6		x Toride 1	
7	CICA 9	x Caloro	CICA 9
8		x Toride 1	
9	EMPASC 100	x Caloro	EMPASC 100
10		x Toride 1	
11	EMPASC 101	x Caloro	EMPASC 101
12		x Toride 1	
13	EMPASC 102	x Caloro	EMPASC 102
14		x Toride 1	
15	EMPASC 103	x Caloro	EMPASC 103
16		x Toride 1	
17	IAC 899	x Caloro	IAC 899
18		x Toride 1	
19	IR 22	x Caloro	IR 22
20		x Toride 1	
21	IR 24	x Caloro	IR 24
22		x Toride 1	
23	IR 841	x Caloro	IR 841
24		x Toride 1	
25	IR 6441	x Caloro	IR 6441
26		x Toride 1	
27	IR 9202	x Caloro	IR 9202
28		x Toride 1	
29	IRGA 408	x Caloro	IRGA 408
30		x Toride 1	
31	Lebonnet	x Caloro	Lebonnet
32		x Toride 1	

Caloro and Toride 1 are used for gene marker varieties, carry P-k^{s} and Pi-z^{t} major R. gene, respectively.

Chapter X STRATEGY OF FUTURE BREEDING FOR BLAST RESISTANCE

As mentioned at the introduction in this report, to overcome the difficulties caused by the breakdown of vertical resistance, various methods for breeding have been reported by many researches, as follows:

1) Utilization of horizontal resitance (gene-non-specific) alone.

2) The combination of horizontal resistance and vertical resistence.

3) Mixed cultivation of varieties with different vertical resistance genes (multiline).

4) Rotation of varieties with different vertical resistance genes.

5) Accumulation of many vertical resistence genes in a single variety (pyramidation of resistance gene).

1) Utilization of horizontal resistance (gene non-specific) alone.

Vertical resistance (major R gene, gene specific) shows a specifc interaction between host and parasite, in other words resistance gene and blast fungus race. This is one of the reasons of breakdown of varietal resistance (vertifolia effect). On the other hand, horizontal resistance does not show the gene-specific interection in general. However according to the progress of genetic analysis of resistance genes revealed that some horizontal resistance varieties showed the gene specificity to the fungus races.

One of representative variety Te-tep was believed to possess non-specific and broad spectrum resistance to blast reces according to the results of the international evaluation trials. However, Kiyosawa (1974) found that Te-tep carrys pi-k^h major R. gene and more than three major R. genes supposed to control the resistance of it. The race which can attack to Te-tep also found at some locations(IRRI 1971). It suggests that the resistance of Te-tep maybe a function of the effect of the accumulation of major R. genes.

Rikuto Norin 4 and Rikuto Norin 12 are well known varieties with hign level of horizontal resistance in Japan, therefore these varieties were used for the breeding materials to introduce their high level of horizontal resistance to Japanese lowland cultivars.

However, due to the results of the blast nursery observation and by the spray method with six different pathogenic Brazilian races, they showed the gene-specific reaction. It showed that these varieties appears to have specific resistance gene (major R. gene) which operate against to Brazilian pathogenic races. In spite of gene specificity of major R. gene, Toriyama (1975) reported that the level of horizontal resistance differ for different races. It suggests that even though the horizontal resistance is controlled by polygenes, it exhibited certain degree of specificity to the different pathogenic races.

This will be further examined by careful observations using more pathogenic races and sensitive differential varieties.

Generally, vertical resistance breeding (major R. gene) is relatively easy. However, the breeding for horizontal resistance which is controlled by polygenic resistance is more difficult than that of vertical resistance. The former conflicts with yield, quality and other characteristics. Moreover, the effect of horizontal resistance itself is usually lower than that of vertical resistance. It is desirable to develop varieties with a high level of polygenic resistance.

In the main upland cultivated regions in Brazil, the drought stress (veranico) is the most important factor which effect the epidemics. It is well known that blast epidemics are promoted after drought stress. Under such circumstance, vertical resistance (major R. gene) is more effective than horizontal resistance.

As horizontal resistance itself is usually lower than vertical resistance, its fluctuation due to the enviromental factors and conditions is greater than vertical resistance. It has been mentioned in the introduciton of this report, that the breakdown of varietal resistance makes breeders and pathologists reconsider and reevaluate horozontal resistance and vertical resistance breeding. Though screening from a blast nursery and from multilocation trials are one of the easiest selection methods of varietal resistance if the knowledge of the genetic base of the variety is lacking, breakdown of varietal resistance inevitably occurs as a natural phenomenon.

So far, we realized that horizontal resistance breeding is important as well vertical resistance to obtain higher and more stable yields. However, to evaluate horizontal resistance it is necessary to exclude the effect of vertical resistance. On the other hand, to evaluate vertical resistance it is necessary to know the relatioship between races and resistance gene constitution of varieties.

These basic studies require a long term project for the success of resistance breeding in Brazil.

One of my major subjects is to identify the major R. gene in a local variety for further resistance breeding work in Brazil. From a series of experiments of the race differentiation of isolates from main upland varieties including IAC 47, IAC 25, IAC 164, IAC 165, it is concluded that local varieties seems to control the part of their resistance by pi-z major R. gene (Fukunishiki type). On the other hand, the results from the race differentiation of isolates from main lowland varieties of IRGA, EMPASC, Santa Catarina, showed that these varieties supposed to control the main resistance by pi-ta^2 (Tadukan type) and pi-b (BL 1 type) major R. genes.

Though these results should be confirmed by F_2, F_3 progeny test (genetic analysis), we can initiate the evaluation of horizontal resistance of these varieties and breeding materials using above blast fungus races. At first, it is necessary to find the adequate donar parents with high level of horizontal resistance from Brazilian varieties and breeding materials.

2) The combination of horizontal resistance and vertical resistance

Table 1 showed the results of breakdown of varietal resistance incorporated from major R. gene sources.

It has happened due to the lack of horizontal resistance during the screening of varietal resistance. Van der Plank (1963) call this phenomenon "Vertifolia effect" from the name of Potato variety.

It is not easy to evaluate the effect of combination of horizontal and vertical resistance. Because, it is necessary to use the virulent race to major R. gene which is required to be incorporated in the variety with horizontal resistance.

It is not easy to find the matching race by mutation. In this case, the mutants have to be use in glasshouse to prevent its escape to open field. It is not a pratical method under improperly equipped conditions.

Toriyama (1980) showed that is possible for combining horizontal and vertical resistance. Rice varieties Ishikari and Matsumae were selected as possessing high level of horizontal resistance under the presence of the virulent race matching pi-k major R. gene at Hokkaido Agricultural Experimental Station, Japan.

The other method is an indirect evaluation method developed by Asaga and Higashi (1973). Fig. 1 shows the procedure of selection method combined with horizontal and vertical resistence.

In the Cross of Toride 1 and Yamabiko, Toride 1 is well known variety possessing pi-z^t major R. gene introduced from an Indian variety TKM 1. Yamabiko has only pi-a major R. gene, however it has considerably high level of horizontal resistance.

They attempt to incorporate the horizontal resistance of Yamabiko with the pi-z^t major R. gene of Toride 1.

In F_2 progeny: the plants segregated to ratio 3:1 as R. and S, respectively, according to the manner of single dominant gene.

At F_2 progeny, susceptible rice seedling will be eliminated. From F_3 progeny, the homogeneous pedigrees with no-symptom or susceptible will be eliminate as shown (x), the pedigrees in mixture of resistance and susceptible individuals were tested for horizontal resistance by the ordinary inoculation method in the greenhouse or in the field.

From these results, they succeeded in developing varieties combining the major R. gene pi-z^t from Toride 1 with the horizontal resistance which is as high as Yamabiko.

This constitutes one of the successful attempts to combine both horizontal and vertical resistance in a single variety.

As it is not necessary to use specific races, practical use at the experimental station without special equipment.

3) Mixed cultivation of varieties with different vertical resistance gene (multi-line).

Multiline, multilineal variety or multi-mixture cultivation are reported from many researchers (Borlaug 1959. Browning and Frey 1969, Okabe 1967. Van der Plank 1963, Kiyosawa 1975a).

Isogenic line is the concept of the variety which has same characteristics such as plant height, panicle type, quality and maturity beside carry different specific resistance gene.

Multiline is already in practical use in several countries for the control of rusts of wheat, oat and sunflower.

Table 2 showed the unpblished data (Shindo) cited from Ezuka (1978), which demonstrated that the mixture culture of three rice varieties with different major R. gene gave a remarkably lower blast occurance than the single culture of each variety. Borloug (1959) developed the near-isogenic line of wheat variety

Yaqui 59 to produce the multi-line composite Yaqui 59.

It showed the possibility of multi-line variety in rice and blast resistance. However, it is not easy to develop a series of near isogenic line to serve as a multiline.

On the other hand, it is necessary to forcast the frequency and constitution of pathogenic race to concern how to mix the component variety according to the different major R. gene.

As multilene is the mixture of the varieties which carry different major R. gene, the progress of epidemics will be espected to be slower than that of the pure genotype.

However, there is a possibility of appearance of so-called "super-race", which is matching all of the major R. genes in the multiline. If the matching super race to all the major R. genes appears multiline method will not beeffective.

Kiyosawa et al. (1975) suggested that such a possibility may be avoided if the stabilizing selection existed in the rice varieties - blast fungus race relationship.

And also, Borloug (1959) demonstrated the evidence for the relationship between wheat rust fungus, which have maintained balance for long time. He provided evidence that super-race will not appear.

Further study on stabilizing selection and the capacity of survival of super race will be needed.

4) Rotation of varieties with different vertical resistence genes.

The basic knowledge on vertical resistance genes (major R. genes) is essential to carry out this method. It requirs a system to provide the seeds by the replacement of one variety before, or just after, the vertical resistance of one or the other is broken down.

A siries of varieties with different major R. genes is essential. If a useful level of horizontal resistance is combined with vertical resistance in each variety, the longevity of each variety will be extended.

However, it is not easy to provide the large amount of seed for large scale of cultivation.

The frequency and constitution of pathogenic races and the situation of epidemics each cultivated region should be observed very carefully, and should establish the well organized system between the forcasting of epidemics and multiplication of seeds for replacement of the variety with different major R. gene.

In a small scale rice cultivation, this method will be one of the most effective weapons to blast.

5) Accumulation of many vertical resistance geneS into a single variety (Pyramidation of resistance genes).

To date thirteen major R. genes were identified by Kiyosawa et al. in Japan. This method is to develop a super variety against a super race of blast.

It is relatively easy to accumulate the certain major R. gene into a single variety. However, it is not easy how to identify the resistance genes accumulated in the hybrids. It is necessary to have a set of specific races to identify the gene accumulation at each stage of hybridzation.

Ezuka (1977) mentioned that no fungus isolate is available now in Japan distinguish the plant possessing four resistance genes, pi-a, pi-k, pi-i and pi-z from the plant possessing these genes, pi-a, pi-k and pi-z. He also suggested that the possibility that the super-race may be produced by the cultivation of super-variety. Kiyosawa et al. (1975) pointed out the importance of stabilizing selection also in this case.

According to the results of race idenfification, all races obtained from blast collections in Brazil till now can atack pi-a and pi-i. It suggests that these two major R. gene are not adequate resistant donars to Brazilian varieties.

The effect of gene accumulation for blast resistance was mentioned in Chapter VI. The effect of gene accumulation depends upon the major R. gene combination. For example, Chyubu 26 and Chyubu 35 have pi-a and pi-i genes. They showed high varietal resistance both in artificial inoculation tests using 6 races and in the blast nursery, even though the combination of major R. genes pi-a and pi-i supposed to have less effect. On the other hand, Zenith (pi-z, pi-a) and Toto (pi-a, pi-k) showed susceptible reation to virulent race under the blast nursery condition. These results should be confirmed by genetic analysis. And also the effect of horizontal resistance by the gene accumulation should be confirmed using more pathogenic races and sensitive gene marker varieties.

TABLE. 1- SITUATION OF BREAKDOWN OF VARIETAL RESISTANCE OF COMMERCIAL VARIETIES IN CORPORATED WITH RESISTANCE GENES FROM FOREIGN VARIETIES IN JAPAN. (Watanabe, 1980)

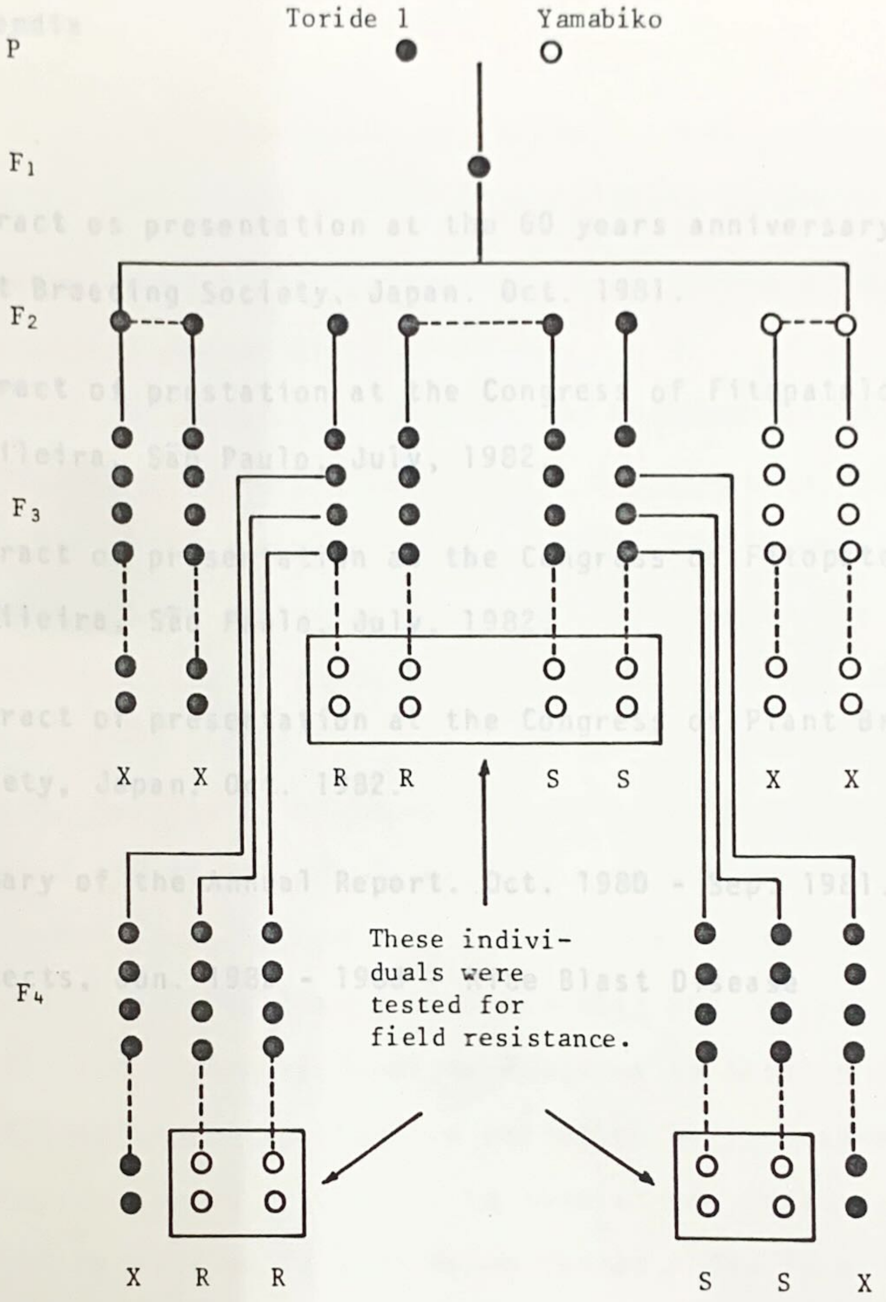
Variety	Major R. gene	Variety of resistance gene Source	Year released	Year of epidemics started	Period to epidemics started after released (years)	Locations
Pi Nº 5		Tadukan	(1959)	1962	3	Hiroshima
Kusabue	pi-k	Reishiko	1961	1963	2	Ibaraki
Kusabue	pi-k	Reishiko	1961	1963	2	Tochigi
Kusabue	pi-k	Reishiko	1960	1964	4	Fukushima
Kusabue	pi-k	Reishiko	1961	1964	3	Yamanashi
Kusabue	pi-k	Reishiko	1962	1964	2	Toyama
Kusabue	pi-k	Reishiko	1961	1963	2	Saitama
Kusabue	pi-k	Reishiko	1963	1965	2	Gunma
Kusabue	pi-k	Reishiko	1963	1964	1	Aichi
Kusabue	pi-k	Reishiko	1961	1967	6	Kanagawa
Yukara	pi-k, pi-a	Reishiko	1962	1964	2	Hokkaido
Teine	pi-k, pi-a	Reishiko	1962	1964	2	Hokkaido
Ugonishiki	pi-k	Toto	1963	1965	2	Akita
Tachihonami	pi-k	Reishiko	1966	1968	2	Yamagata
Minehikari	pi-a, pi-k^m	Kitashitamai	1965	1968	3	Aichi
Shimokita	pi-ta, pi-a	Tadukan	1962	1969	7	Aomori
Shimokita	pi-ta, pi-a	Tadukan	(1967)	1969	2	Yamagata
Fukunishiki	pi-z	Zenith	1964	1969	5	Fukushima
Yamatenishiki	pi-z	Zenith	1976	1979	1	Yamagata

The year of epidemics are according to the report from each Prefectural Agriculture Experimental Station.

YT/lmd

TABLE 2 . Effect of mixture cultivation of varieties with different true (major R. gene) resistance on the blast occurence (Shindo, unpublished).

Expt. Nº	Variety	True resistance genotype	Nº of S-type lesion per hill	Index to panicle infection	Yield kg per 3.3 m^2
I.	Single culture				
	Fukei 69	Pi-k	12.25	8.68	1.58
	Tohoku 108	Pi-a, Pi-i	25.75	23.78	1.58
	Toyonishiki	Pi-a	7.25	4.08	1.95
	Average		15.08	12.18	1.70
	Mixed culture of 3 varieties		2.25	1.68	1.98
II.	Single culture				
	Bikei 91	Pi-k	0	0.03	1.83
	Miyoshi	Pi-a, Pi-i	3.25	6.18	1.75
	Norin 41	Pi-a	15.50	23.10	1.75
	Average		6.25	9.97	1.80
	Mixed culture of 3 varieties		1.75	3.45	1.83



○ : Individual infected (without Pi-z^1)

● : Individual remained healthy (with Pi-z^1)

X : Line discarded

R : Resistant line } in the sense of field resistance

S : Susceptible line } in the sense of field resistance

FIG. 1. Procedure for evaluation of field resistance of the progenies from the cross between Toride 1 and Yamabiko (Asaga & Higashi, 1973).

XI Appendix

1. Abstract os presentation at the 60 years anniversary of Plant Breeding Society, Japan. Oct. 1981.
2. Abstract of prestation at the Congress of Fitopatologia Brasileira, São Paulo, July, 1982.
3. Abstract of presentation at the Congress of Fitopatologia Brasileira, São Paulo, July, 1982.
4. Abstract of presentation at the Congress of Plant Breeding Society, Japan. Oct. 1982.
5. Summary of the Annual Report. Oct. 1980 - Sep. 1981.
6. Projects, Jun. 1982 - 1983 - Rice Blast Disease

Appendix - 1

TANAKA, Y. - (CNPAF/EMBRAPA/IICA, Goiânia, Goiás, Brazil).

Breeding for resistance to rice blast disease in Brazil.

1. The reaction of Brazilian isolates to Kiyosawa's and international differential varieties.

Brazil is the biggest upland rice producer in the world. The upland rice area covers more than 4.2 million ha, and produced abaut 4.5 million tons of rice in 1979. IAC 47 and IAC 25 are the most common varieties grown in this country and were released by the Institute of Agriculture of Campinas (IAC), São Paulo in the year 1971 and 1974, respectively. The breakdown of the varietal resistance of these two varieties has recently been reported and is increasing rapidly with the expansion of the cultivated area. Unfortunately there are now no improved varieties with resistance to blast that can replace IAC 47 and IAC 25. The Centro Nacional de Pesquisa de Arroz e Feijão (CNPAF) is improving the rice varieties by incorporating blast resistance and is testing it in several key locations in Brazil. Drought resistance is also being tested. The rice blast fungus was collected from main upland varieties at 17 sites in different states of Brazil (Goiás, Minas Gerais, Mato Grosso, Paraná and São Paulo). The races of rice blast disease are then differentiated by using Kiyosawa's and international differential varieties. According to the reaction to international differentials IB-9, IC-9 and IB-13 are the predominant races at each location in Brazil. But by using Kiyosawa's differentials more races can be identified. Table 1 shows the difference in reaction pattern of isolates to Kiyosawa's differentials from some countries.

Further study is needed to obtain suitable differentials which have singly different genes that may be found in Brazil.

Table 1. - The number of isolates attacking each of Kiyosawa's differential varieties from isolates collected in 1977 and 1980 in the Philippines, 1975 to 1980 in Japan and in 1980/81 in Brazil.

Differential variety	Resistance gene	Philippines**		Japan**	Brazil
		1977	1978	1975 1980	1980 1981
Shin 2	Pi - K^{s}	52	57	1982	112
Aichi-Asahi	Pi - a	65	68	678	164
Fujisaka 5	Pi - i	9	10*	163	164
Kusabue	Pi - k	5	4	731	23
Tsuyuake	Pi - k^{m}	6	4	712	3
Fikunishiki	Pi - z	0	11	97	49
K 1	Pi - ta	8	19*	72	163
Pi 4	Pi - ta^{2}	1	14	0	0
Toride 1	Pi - z^{t}	32	41	43	0
K 60	Pi - k^{p}	6	4	745	163
BL 1	Pi - b	10	33*	22	0
K 59	Pi - t	58	50*	0	106
Total number of isolates tested		65	70	1284	164

* : In these cases, the total number of isolates was 69, as one isolate was removed because of its unclear reaction.

**: Data from Kiyosawa et al. 1980.

Appendix 2

REAÇÕES DE ISOLADOS BRASILEIROS DE *Pyricularia oryzae* EM CULTIVARES DE ARROZ SA SÉRIE DIFERENCIAL INTERNACIONAL E DA SÉRIE DE KIYOSAWA. Y. Tanaka (CNPAF/EMBRAPA/IICA, Cx. P. 179, 74000 Goiânia/ GO). The reaction of Brazilian isolates to Kiyosawa's and international differential varieties.

As cultivares de arroz IAC 47 e IAC 25 são as mais plantadas no Brasil para condições de sequeiro. A suscetibilidade destas cultivares à brusone tem sido constantemente observada e a incidência da doença tem aumentado com a expansão da área cultivada. Não existe no momento variedades melhoradas que possam substituí-las. O CNPAF está promovendo melhoramento de cultivares de arroz através da incorporação de resistência à brusone e testando-as em vários locais do Brasil.

Isolados de *P. oryzae* foram coletados em 17 locais do Brasil, abrangendo Goiás, Minas Gerais, Mato Grosso, Paraná e São Paulo. As raças do fungo foram diferenciadas utilizando-se as cultivares componentes da Série Diferencial Internacional e da Série de Kiyosawa. De acordo com a reação na Série Internacional constatou-se que as raças IB-9, IB-13 e IC-9 são predominantes em todos os locais amostrados. No entanto, usando a Série de Kiyosawa um maior número de raças pode ser identificado.

Este trabalho mostrou também que as raças predominantes no

Brasil são diferentes daquelas predominantes no Japão e nas Filipinas, em relação à Série de Kiyosawa.

Sugere-se que se desenvolva uma Série de Diferenciais Brasileiras para futuros estudos de raças fisiológicas do fungo.

Appendix 3

COMPARAÇÃO ENTRE CULTIVARES JAPONESAS DE ARROZ QUANTO À CAPACIDADE DE DIFERENCIAÇÃO DE RAÇAS DE *Pyricularia oryzae* ISOLADAS NO BRASIL. Y. Tanaka (CNPAF/EMBRAPA/IICA, Cx. P. 179, 74000 Goiânia/GO). The comparison of differential ability among the Japanese differential varieties to Brazilian blast isolates.

O melhoramento para resistência à brusone requer um estudo básico para caracterizar a presença de genes resistentes nas cultivares brasileiras de arroz. Esta informação já é conhecida para quese todas as cultivares japonesas, e é útil para evitar quebra de resistência desencadeando uma epidemia.

Este estudo envolve a determinação de uma série de diferenciais brasileiras cada uma delas possuindo um gene de resistência característico. A Série Diferencial Internacional tem sido utilizada em muitos países, porém tem sido mostrado que algumas cultivares da série possuem o mesmo gene de resistência.

As cultivares diferenciais Japonesas ou de Kiyosawa podem ser utilizadas para determinação de raças, enquanto não se dispõem de diferenciais brasileiras.

Neste trabalho comparou-se cultivares de arroz que possuem o mesmo gene de resistência para se determinar quais as que tem maior capacidade para diferenciar raças de *P. oryzae* coletadas no Brasil. Dentre as 48 cultivares testadas, 13 foram selecionadas e são listadas a seguir juntamente com o gene diferenciador que possuem: Aichi Asahi (pi-a), Shin 2 (pi-k^s) , Fujisaka 5 (pi-i), Kanto 51 (pi-k), Tsuyuake (pi-k^m), Pi Nº 1 (pi-ta), Pi Nº 4 (pi-ta^2), Fukunishiki (pi-z), Toride 1 (pi-z^t), K 59 (pi-t), BL 1 (pi-b), K 60 (pi-k^P) e Zenith (pi-z, a).

Appendix 4

TANAKA, Y., (CNPAF/EMBRAPA/IICA, Goiânia, Goiás, Brazil). Breeding for resistance to rice blast disease in Brazil. II. Estimation of major R genes in Brazilian varieties and breeding materials by artificial inoculation method using six different pathogenic races.

The race frequency and constitution of blast fungus isolates obtained from the main upland varieties in five states of Brazil have been reported last year. During this year, severe epidemic of blast have occured in the state of Santa Catarina. Several varieties such as EMPASC 101, 103, IRGA 408 and BR-IRGA 409 which were released from EMPASC and IRGA a few years ago as highly resistant varieties were severely attacked by blast. The race identification revealed that the breakdown of varietal resistance of these varieties were caused by major R genes. Differences in reaction pattern were observed between the isolates collected from Santa Catarina and five other states (Goiás, Minas Gerais, Mato Grosso, São Paulo and Paraná) of Brazil. Many of the upland varieties which are cultivated in the later five states probably have mainly the Zenith type major R gene. On the other hand, the reaction pattern exhibited by the Kiyosawa's differentials to the isolates of Santa Catarina indicated that the varietal resistance in these varieties in controlled by pi-ta^2 and pi-b genes. Till now, the matching races to the major R. gene pi-ta^2 and pi-b were not found in the blast fungus collections from upland varieties at the above five states. From these results, a set of six races of blast fungus was obtained which can identifity the following six major R genes pi-k, pi-z, pi-k^m, pi-ta^2, pi-b and pi-k^p. The reaction pattern to six races was distinctly different between upland and lowland varieties (Table 1). These results further showed that the upland varieties have been improved by the crosses with local land varieties mainly *javanica* - type. On the other hand, main lowland varieties and breeding materials were introduced from *indica* - type improved at IRRI and CIAT. The genetic analysis of resistant genes of the rice cultivars including the pedigrees of them is urgently needed.

Table 1. The reaction of Brazilian varieties and breeding materials to six different pathogenic races in the greenhouse.

Nº	Variety	Race Code Nº*	11 157.5	39 147.5	162 117.5	240 37.5	303 307.5	305 7.6	Major R Gene Estimated
	(Upland variety)								
1	IAC 25		MR	MR	MS	R^h	R^h	R^h	pi-z
2	IAC 47		MR	MR	MS	R^h	R^h	R^h	
3	IAC 165		R	MR	R	R^h	R^h	R^h	pi-z
4	IAC 5544		R^h	MR	MR	R^h	R^h	R^h	
5	IAC 1246		R^h	R	MS	R^h	R^h	R^h	
6	Pratão Precoce		R	MR	MR	R^h	R^h	R^h	
7	Cateto		MR	S	MS	R^h	R^h	R^h	
8	Pratão		MR	S	R^h	R^h	R^h	R^h	pi-z
9	Pérola		MR	S	S	R^h	R^h	R^h	
10	Matão		R	MR	MS	R^h	R^h	R^h	
11	Dourado Precoce		MR	S	MS	R^h	MR	MR	
12	Jaguari		SS	S	R	R^h	R^h	R^h	pi-z
13	SR 2041-50-1**		R^h	R^h	R^h	SS	R^h	R^h	Pi-k^m
14	CNAx 104 ***		R^h	R^h	R^h	S	R^h	R^h	Pi-k^m
15	Tiririca ****		R^h	R^h	R^h	MR	R^h	S	Pi-b
	(Lowland variety)								
16	BR-IRGA 409		R^h	R^h	R^h	R^h	R^h	S	pi-b
17	IRGA 410		R^h	R^h	R^h	R^h	R^h	S	Pi-b
18	IRGA 408		R^h	R^h	R^h	R^h	S	R	pi-ta^2
19	EMPASC 100		SS	SS	SS	R^h	R^h	M	
20	EMPASC 101		R^h	R^h	R^h	R^h	R^h	S	pi-b
21	EMPASC 102		R^h	R^h	R^h	R^h	R^h	S	pi-b
22	EMPASC 103		R^h	R^h	R^h	R^h	R^h	MS	pi-b
23	IR 6441-JN-6B		R^h	MR	R^h	R^h	R^h	S	pi-b
24	IR 9202-21-1		R^h	R	R^h	R^h	M	S	
25	Blue Belle		SS	SS	SS	S	S	SS	pi-a
25	Dawn		R^h	R^h	R^h	R^h	R^h	R^h	pi-a, i, k^h
26	Lebonnet		R^h	R^h	R^h	R^h	R^h	R^h	K^h
27	IR 22		R^h	R^h	R^h	R^h	R^h	M	(pi-b)
28	IR 24		R^h	R^h	R^h	R^h	R^h	M	(pi-b)
29	IR 841		R^h	R^h	R^h	R^h	R^h	M	(pi-b)
30	CICA 8		R^h	R^h	R^h	R^h	R^h	R^h	

* : Code number, in accordance with Kiyosawa's differentials.

** : Breeding material from Korea.

*** : IAC 47 x SR 2041-50-1

****: Lowland variety

Appendix 5

SUMMARY of the Annual Report 1980-1981

This study was carried out with the main purpose of accumulating basic and genetic data for future resistance breeding programs. Results obtained in this study are as follows:

Chapter III - Establishment of the method to identify the Brazilian isolates according to international and Japanese (Kiyosawa's) diffential varieties.

1. Tried to establish the material and method for race identification and differentiation of Brazilian isolates. Japanese (Kiyosawa's) and international differential varieties were used for this study.

2. From the result of the comparison between spray and injection methods, to differentiate the races to be screened by the spray method as a convenience. If there appears to be an ambiguous reaction or if weak pathogenic races are used confirmation of resistance reaction may be established by the injection method.

3. Kiyosawa's set of criteria for race differentiation was used. At times the IRRI set of criteria was also used.

Chapter IV - Trials of observation and identification of prevalent race at CNPAF.

1. From single spore isolations obtained from the blast nursery at CNPAF, Japanese and international differential varieties were identified to collect the different pathogenic races for the establishment of the Brazilian differential races.

2. International races IB-9 and IC-9 were predominant at CNPAF. From these international races were classified into more races based on the reaction to Japanese differentials.

3. Some of the isolates from this collection may be able to be used for the Brasilian differential races.

Chapter V - Proposed investigation of race constitution and frequency of rice blast at the sites for multi-location trials in Brazil.

1. This experiment were carried out to determine the race constitution and frequency at each site for multi-location trials to establish resistance on a genetical basis.

2. A total of 76 isolates at 5 sites from IAC 47 and 4 sites from IAC 25 were differentiated. Race constitution and frequency of the isolates obtained from IAC 47 and IAC 25 were quite different. This suggests that the resistance

gene components in the 2 varieties are different.

3. This result suggests that one of the reasons for the breakdown of varietal resistance of IAC 47 and IAC 25 is the expansion of the pathogenic race to attack the pi-k^s and the pi-z vertical (specific) resistance genes, respectively.

4. Part of the resistance of the IAC 47 and IAC 25 is considered to be controlled by the Pi-k^s and the Pi-z resistance genes, respectively. IAC 47 may be one of Fukunishiki (pi-z) type variety.

5. The collections from IAC 47 and IAC 25 in the Cuiabā region will be identified in a further experiment.

Chapter VI - The reaction of Brazilian isolates to Japanese (Kiyosawa's) and international differential varieties.

1. A total of 218 isolates obtained from many different varieties at 17 locations in 5 states were differentiated with 12 Japanese and 8 international differential varieties.

2. From the result of race identification, only 7 international races were identified. This is less than the number here before identified. IB-9 and IC-9 are predominant everywhere. They are classified into 20 Japanese races. This suggests that a set of Japanese differentials is more

isogenic with the blast resistance gene and more sensitive in reaction to the isolations. Therefore international differentials are useful only for brief screening of isolations.

3. Brazilian predominant races may be able to be divided into some groups based on pathogenicity to Kiyosawa's differentials as follows:

Fukunishiki	type (pi-z) -	Race 157.5, 147.5, 146.5
Tsuyuake	type (pi-k^m)-	Race 137.1, 127.1
Kusabue	type (pi-k) -	Race 117.5, 117.1
Shin 2	type (pi-k^s)-	Race 107.5
K 60	type (pi-k^p)-	Race 107.1

This suggests that the constitutions of the resistance gene in Brazilian varieties are mainly controlled by pi-z, pi-k^s, pi-k^p, pi-a, pi-i, pi-t and possibly other as yet unidentified genes.

4. To date, no isolate is obtained to match to Raminad Str. 3, Pi Nº 4 (pi-ta^2), Toride 1 (pi-z^t) and BL 1 (pi-b). The resistance genes of pi-ta^2 in Pi Nº 4 and pi-z^t in Toride 1 are inherited from Tadukan (pi-ta^2, pi-ta, pi-a) of the Philippines, and from TKM 1 (pi-z^t) of India, respectively. This suggests the possibility of the introduction of vertical resistance genes resistance sources.

5. Race constitutions and frequencies are quite different among the countries, the Philippines, Japan and Brazil.

6. Kanto 51 is one of the Japanese and international differentials, and Kusabue is one of the Kiyosawa's differentials. Both varieties have the ability to identify the pi-k resistance gene but with quite different reaction patterns. From this experiment the presence of the pi-k^p gene in Kanto 51 is assumed. Whether Kanto 51 has the pi-k^p gene or not needs to be confirmed by genetic analysis of crosses between Kanto 51, Kusabue and other marker varieties. This results is a very interesting point in that it suggests the inability to identify in Japan with Japanese differential races. The gene pi-k^s which was found in Shin 2 with a Philippine strain is an example.

7. These are many aspects at each stage of this study which need identification by genetic analysis.

Chapter VII - Trials to Establish the Brazilian Differential Races and varieties

1. A total of 127 local land varieties will be screening by the six different pathogenic races. Some of the varieties which showed typical resistant and susceptible reaction in preliminary experiments may be able to be used for one of

the differential varieties.

Further experimentation will be done.

Chapter VIII - Genetics of Blast Resistance in Local Varieties.

1. Identification of the resistance gene in local varieties with 18 main cultivars and 19 Japanese different pathogenic races. Study will be continued.

Chapter IV - Evaluation of Horizontal Resistance to Blast in Hain Local Varieties.

1. To evaluate horizontal resistance in the greenhouse conditions with artificial inoculation method. The result will be compared with the experiments under field conditions. Study will be continued.

Appendix 6

PROJECTS, 1982 - RICE BLAST DISEASE

1) Basic study of Breeding for Resistance to Blast Disease in Brazil.

   1. The reaction of Brazilian isolates to kiyosawa's and international differential varieties.
   2. The comparisons of differential ability among the Japanese diffenrential varieties to Brazilian blast strains.
   3. The effect of accumulation of major resistance gene into one cultivar using Japanese varieties for resistance to blast.
   4. Survey of blast race, identification, differentiation and frequency of the prevalent race in the main upland and lowland cultivating area.
   5. Establishment of the Brazilian differential varieties.
   6. Establishment of the Brazilian differential races.
   7. Genetic analysis of the resistance gene of the main upland varieties including IAC 47, IAC 25 and breeding materials.
   8. Genetic analysis of the resistance gene of the main lowland varieties including IRGA and EMPASC materials.
   9. The new aproach to control the blast such as mixed varieties: mixture cultivation of varieties with different major R. genes.

XII. Summary

This study was carried out with the main purpose of accumulating basic and genetic data for future resistance breeding programs in Brazil.

Results obtained in this study are as follows:

Chapter III- Material and Method

1. Methods including single spore isolation, incubation, inoculation, identification and differentiation of blast fungus isolates are summarized in order to ordinary producedure, which are used in this study. The Material and Method which used in this study is modified by the resultsof trials in last report.
2. Kiyosawa's set of differentials and criteria for race differentiation were used from the resultsof trials in last report.
3. A set of six different races, which are used for estimation of major R. genes are summarized with reaction patterns to a set of Kiyosawa's differential varieties. A set of six different pathogenic races are used as temporary Brazilian differential races through this study.
4. The characteristics on reaction of components of the Kiyosawa's and international differential varieties are summarized for race identification.

Chapter IV - The reaction of Brazilian blast fungus isolates obtained from the main upland varieties of five states to Kiyosawa's (Japanese) and international

differential varieties.

1. A total of 218 isolates obtained from main upland varieties at 17 locations in 5 states were differentiated with 12 Japanese and 8 international differential varieties.
2. From the result of race identification, only 7 international races were identified. IB-9 and IC-9 are prodominant in 5 states. They further can classified into 20 races according to the reaction patterns of Kiyosawa's differentials. This suggests that a set of Kiyosawa's differentials is more isogenic with the blast resistance gene and more sensitive in reaction to the blast fungus isolates. Therefore a set of international differentials is usuful only for brief screening of isolations.
3. Brazilian predominant races may be able to be divided into some groups based on pathogenicity to Kiyosawa's differentials as follows:

Fukunishiki type (Pi-z) - Race 157.5, 147.5, 146.5
Tsuyuake type (Pi-k^m)- Race 137.1, 127.1
Kusabue type (Pi-k) - Race 117.5, 117.1
Shin 2 type (Pi-k^s)- Race 107.5
K 60 type (Pi-k^p)- Race 107.1

This suggests that the constitutions of the resistance gene in Brazilian upland varieties are mainly controlled by Pi-z, Pi-k^s, Pi-k^p, Pi-a, Pi-i, Pi-t and possibly other as yet unidentified genes.

4. To date from these blast collections collected from main upland varieties, no isolate is obtained to match to Raminad Str. 3, Pi Nº 4 (Pi-ta^{2}), Toride 1 (Pi-z^{t}) and BL 1 (Pi-b). The resistance genes of Pi-ta^{2} in Pi Nº 4 and Pi-z^{t} in Toride 1 are inherited from Tadukan (Pi-ta^{2}, Pi-ta, Pi-a) of the Philippines, and from TKM 1 (Pi-z^{t}) of India, respectively. This suggests that the possibility of the introduction of major resistance genes resistance sources.
5. Race constitutions and frequencies are quite different among the countries, the Philippines, Japan and Brazil. It provides much informations about relation between races and varieties among different countries.
6. There are many aspects at each stage of this study which need identification by genetic analysis.

Chapter V. The reaction of the isolates obtained from the main lowland rice varieties and breeding materials from the state of Santa Catarina.

1. A total of 27 isolates obtained from 8 main lowland rice varieties from 10 different locations were identified to the race according to the reaction patterns of the Kiyosawa's differentials.

   They were classified into two races, which are Race 303 (Code nº 307.1) and Race 305 (Code nº 7.3).

   Since only two races were identified, it shows that the race constitution of blast fungus in the state of Santa Catarina is more simple than the isolates obtained from the main upland varieties from five states, which are mentioned in Chapter IV. It further suggests that the gene diversity on blast resistance supposed to be more simple than that of upland varieties.

2. From the results of race identification, it suggests that the reason of breakdown of the varietal resistance of main cultivars in the state of Santa Catarina is due to the expansion in number of virulent races which can attack to Pi-ta^2 and Pi-b major R. genes.
3. The major R. genes of the main lowland varieties and these pedigrees were estimated by the artificial inoculation method using six different pathogenic races. From these results of the evaluation, it suggests that the constitutions of the resistance genes in lowland varieties in Santa Catarina are mainly controlled by a few major R. genes such as Pi-ta^2, Pi-b and Pi-k^h. It concluded that the gene diversity on blast resistance supposed to be narrow than that of upland varieties.
4. It further suggests that the main lowland varieties and their pedigrees have been selected under vertical resistance gene at CIAT and IRRI before re-evaluated the horizontal resistant breeding.
5. Genetic analysis of main lowland varieties and their pedigrees as well as to estimate the level of horizontal resistance are needed.

Chapter VI. The comparison of differential ability among the Japanese differential varieties and cultivars to Brazilian blast fungus isolates.

1. A total of 48 Japanese varieties with known gene constitution on blast resistance were tested the differential ability of major R. genes by the artificial inoculation method using six Brazilian differential races.
2. Based on the differential reactions of 48 varieties obtained from NIAS and Aichi prefectural Experimental Station, Inahashi Japan, the following fifteen differential varieties were selected to identify thirteen major R. genes in Brazil; Shin 2 (Pi-k^{s}), Aichi Asahi (Pi-a), Fujisaka 5 (Pi-i), Kusabue and Kanto 51 (Pi-k), Tsuyuake (Pi-k^{m}), Fukunishiki (Pi-z), K 1 (Pi-ta), Pi Nº 4 (Pi-ta^{2}), Toride 1 (Pi-z^{t}), Zenith (Pi-z, Pi-a), K 60 (Pi-k^{p}), K 59 (Pi-t), BL 1 (Pi-b) and K 3 (Pi-k^{h}). Brazilian varieties will be included in accordance with the results of genetic analysis.
3. As many of upland varieties in Brazil are supposed to belong the intermediate type (*javanica* type) it maybe able to identify the common major R. gene or genes between Japanese and Brazilian varieties by using a set of the Japanese differential varieties.

Chapter VII. Evaluation of horizontal resistance of Japanese and Brazilian rice varieties under the blast nursery condition.

1. A total of 70 varieties with known level of horizontal resistance and major R. gene in Japan and 50 Brazilian varieties and breeding materials were estimated the level of horizontal resistance under the blast nursery condition at CNPAF/EMBRAPA, Goiânia.
2. Ten of 70 Japanese entries have been bred to incorporate with the high level of horizontal resistance at the Aichi Prefectural Agricultural Experimental Station, Inahashi, Japan.
3. The part of entry varieties were evaluated their varietal resistance by the artificial inoculation method by using a set of six Brazilian differential races.
4. Nine of 13 vertical resistance genes were attacked under the blast nursery condition.

   No race has been identified which has been able to match to Pi-b, Pi-ta^2, Pi-z^t and consequently no major R. gene or genes have been identified. Therefore under this blast nursery condition, it could not evaluate the level of horizontal resistance of the varieties which carry Pi-b, Pi-ta^2 and Pi-z^t major R. genes.
5. Results obtained from the blast nursery test, showed a typical vertical resistant reaction in several varieties including Ginga, Homarenishiki, Norin 22, Três Marias, Tiririca, Catalão and CNAx 104. And also Rikuto Norin 4

and Rikuto Norin 12 showed quite susceptible reaction.

From the result of artificial inoculation method, it suggests that SR 2041-50-1, CNAx 104 and Catalão supposed have Pi-k^m major R. gene. Três Marias and Tiririca supposed to have Pi-z^t and Pi-b major R. genes, respectively.

It is necessary to determine the level of horizontal resistance in Brazilian materials and to identify which major R. gene or genes materials have.

6. As mentioned in the last report, Kusabue supposed have another major R. gene beside Pi-k.

   According to many investigators in Japan, the level of horizontal R. of Kanto 51 is higher than that of Kusabue, however Kusabue showed more resistant reaction than that of Kanto 51 under the blast nursery condition at CNPAF/ EMBRAPA through 1981 and 1982. This result supports that the variety Kusabue probably has another major R. gene beside Pi-k.

   These results are interesting in showing that certain resistance gene may function differently under different condition.

   F_2 progenies of crosses between above two varieties and gene marker varieties were obtained to date. Genetic analysis is expected to reveal these questions.

7. Majority of Brazilian entry varieties were classified into M and S category of the level of horizontal resistance. From the result obtained under the blast nursery condition, it could not compare the horizontal resistance among the

Brazilian materials.

It should be confirmed the level of horizontal resistance by the artificial inoculation method using the Brazilian differential races.

Chapter VIII. Estimation of major R. genes in Brazilian rice varieties and breeding materials by the artificial inoculation method using six different pathogenic races.

1. A total of 72 main upland varieties and breeding materials were used for the estimation of major R. genes by the artificial inoculation method using a set of six different pathogenic races.
2. The reaction patterns with six different races showed the quite different results between upland and lowland varieties.

   From these observations, it concluded that the constitution of major R. genes is quite different between upland and lowland varieties in Brazil.
3. The constitution of the resistance gene in upland varieties and breeding materials are mainly controlled by Pi-z, Pi-k^s, Pi-k^p, Pi-a, Pi-t, Pi-i and possibly other as yet unidentified genes. On the other hand, many of lowland varieties and breeding materials in the state of Santa Catarina are mainly controlled by Pi-ta^2, Pi-b, Pi-k^h and unidentified genes.
4. From the reaction patterns to a set of differential races, some of upland varieties and breeding materials

showed that they probably have the high level of horizontal resistance.

It should be confirmed by further careful observation.

Chapter IX. Gene analysis of blast resistance in Brazilian varieties and breeding materials.

1. The crosses of Brazilian varieties and gene markers for genetic analysis on blast resistance gene are initiated from December 1981.

   F_1 hybrids of 60 cominations and F_2 progenies of 30 combinations of the Brazilian varieties to Japanese gene marker varieties were obtained for genetic analysis. And also more than 30 cominations of F_2 are being obtained.

2. Aichi Asahi (Pi-a , susceptible to all the races) and Toride 1 (Pi-z^t, resistance to all the races) are used for the gene marker varieties.

   The sterility at F_2 progenies did not occur among the crosses between Brazilian and Japanese gene marker varieties. It shows that many of the Brazilian materials are intermediate - javanica type varieties. Utilizing these crosses, the major R. gene of main Brazilian materials such as IAC 47, IAC 25, IAC 164, Matão, Pērola and other will be determined by the genetic analysis.

3. Crossing program including the 20 main cultivars and their pedigrees from the state of Santa Catarina for genetic analysis has been initiated. To avoid the sterility problems at the F_2 progenies of crosses of

indica materials, Caloro (Pi-k^s: susceptible to all the race) is used as the gene marker variety instead of Aichi Asahi.

Chapter X. Strategy of future breeding for blast resistance

The strategies of future breeding in relation to blast resistance are discussed in this chapter.

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