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## IRRI Limited Proceedings Series

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# Foreword

The IRRI-Japan collaborative research project, functioning through a donation by the Japanese government, started in 1989. Under this project, research to improve the productivity of the rice plant and establish environment-friendly technology in the tropics has been carried out until now through four phases. Each stage was set up to last five years. Plant breeders and agronomists have been seconded from Japan, and research has been done according to the following phases (listing dates, subjects, and participating scientists by research area).

## Phase I (December 1984 to November 1989)

“The development of material-saving technology in rice cultivation”

Genetics and breeding: Drs. Tsuguhumi Ogawa and Ryoichi Ikeda

Agronomy and plant physiology: Dr. Genshichi Wada

## Phase II (December 1989 to November 1994)

“The development of stabilization technology for rice double cropping in the tropics”

Genetics and breeding: Drs. Ryoichi Ikeda and Tokio Imbe

Agronomy and plant physiology: Dr. Minoru Yamauchi

## Phase III (December 1994 to November 1999)

“Stabilization of rice culture under water stress in the tropics using a broader spectrum of genetic resources”  
Genetics and breeding: Drs. Tokio Imbe and Hiroshi Kato

Agronomy and plant physiology: Dr. Motohiko Kondo

## Phase IV (October 1999 to September 2004)

“Physio-genetic study on yield determination and ecological adaptability for sustainable rice culture”

Genetics and breeding: Dr. Yoshimichi Fukuta

Agronomy and soil science: Dr. Takuhito Nozoe

The results have already been explained in several hundred publications. Based on these results, a workshop was held to discuss these results and knowledge for future policy at JIRCAS on 24-25 September 2002. In the presence of staff members who were involved in research planning at JIRCAS, IRRI, and MAFF (Ministry of Agriculture, Forestry, and Fisheries), scientists from each stage, and collaborators, all the researchers presented their results. This Proceedings summarizes the research results from each phase.

# **Breeding and genetics**

# Research on resistance to bacterial blight in rice

T. Ogawa, T. Yamamoto, S. Taura, N. Endo, H. Kaku, R. Ikeda, G.S. Khush, and T.-W. Mew

This project on resistance to rice bacterial blight (BB) has concentrated on the breeding of international differentials, gene analysis using races and differentials of Japan and IRRI, and the specific reaction between rice cultivars and races of BB pathogen in various countries.

The genes for BB resistance identified in each country were reported on the basis of analysis using local bacterial isolates. These genes could not be compared directly with each other. Therefore, it was necessary to reanalyze key cultivars using a uniform set of races and to compare the results of previous studies in each country to have a common base for defining the relationships between the groups of BB pathogens and the resistance of rice cultivars. Japanese and IRRI differentials were analyzed using Japanese and Philippine BB isolates. Four resistance genes were identified.

To develop near-isogenic lines (NILs) having each BB resistance gene, we used an inoculation test with 7 Japanese races and 13 Philippine ones to select recurrent parents: IR24 (indica), Milyang 23 (indica-japonica), and Toyonishiki (japonica). NILs were developed to establish a set of international differentials with a monogenic base of the pathogen with resistance to BB and to supply reliable materials to breeding programs for resistant cultivars. An initial set of NILs (36 lines) with diverse genes for resistance was developed in 1987 and seeds distributed to scientists of various countries. From then on, systematic research using these materials as a common base was carried out internationally.

We collected more than 500 rice leaves affected by BB from 1982 to 1988 and examined variation in the pathogenicity of these isolates at the isolation greenhouse in TARC. The isolates were grouped into 28 races on the basis of their pathogenicity to Japanese and Philippine differentials. The distribution of races was quite specific to each country.

To identify new sources of resistance to BB, we inoculated about 30,000 rice cultivars using six BB races of the Philippines. The reaction pattern mostly corresponded to those of rice cultivars with known BB resistance genes (*Xa3*, *Xa4*, *xa5*, *Xa10*, *Xa4 + Xa10*, *xa5 + Xa7*, *Xa4 + xa5*, *xa5 + xa13*, and *Xa14*). We continued inoculation tests on rice cultivars (about 1,000 per country) stored at IRRI to examine the gene distribution in each country. Results showed that the distribution of each gene appeared to be geographic. In addition, the morphological characters of cultivars in each group suggested that the differentiation of BB resistance genes was probably linked with the differentiation of rice cultivars into distinct ecotypes.

To increase the genetic resources for resistance to BB, we carried out a study to induce mutants with resistance to BB by chemical mutagen. We found two resistant mutant lines (XM5 and XM6).  $M_3$  progenies derived from  $M_2$  plants in both mutant lines were tested for segregation for resistance; the results showed that these mutant lines have single recessive genes for BB races. From an allelic test between XM5 or XM6 and known recessive genes (*xa5*, *xa8*, and *xa13*), we concluded that the two recessive genes are new ones, and designated the genes as *xa19* and *xa20*.

This project has developed the first NILs for rice diseases and insects and established a common basis of research on resistance to BB disease by exchanging materials among rice-growing countries.

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the major diseases of rice in the rice-growing countries of Asia. Yield losses in severely infected fields ranged from 20% to 30% (Ou 1985) and data showed that yield loss could reach about 80% (Singh et al 1977). Reliance is considerably high on the use of resistant cultivars for controlling the disease because of the ineffectiveness of bactericidal agents.

Since the pathogenic specialization in the causal bacterium of BB was first reported in Japan by Kuhara et al (1958) and Kusaba et al (1958), several reports have been published on the variability of pathogenicity in the bacterium and of the resistance.

Mostly Japanese researchers and IRRI (International Rice Research Institute, Philippines) scientists carried out recent research on BB. Many researchers (Ezuka and Horino 1974, Reddy and Ou 1976, Sato et al 1976, Choi et al 1976, 1977) mentioned the existence of the specialization of races. Nishimura (1961) began genetic studies on the resistance of rice to BB. Four major genes (*Xa1*, *Xa2*, *Xaw*, and *Xakg*) were identified in Japan (Sakaguchi 1967, Ezuka et al 1974, Ogawa et al 1978). Seven new loci for resistance (*Xa4*, *xa5*, *Xa6*, *Xa7*, *xa8*, *xa9*, and *Xa10*) were identified by IRRI scientists (Petpisit et al 1977, Olufowote et al 1977, Librojo et al 1976, Sidhu et al 1978, Sidhu and Khush 1978, Singh et al 1983, Yoshimura et al 1983).

Since the differential cultivars and bacterial races used at both sites (Japan and Philippines) were different, the two groups of scientists had difficulty in distinguishing the resistance gene. To control the disease, it is important to have a common base to define the relationship between the virulence of the groups of the BB pathogens and the resistance of rice cultivars to the races. Thus, it is desirable at this stage to compare and analyze the results of the studies conducted in Japan and at IRRI.

Dr. N.C. Brady, former IRRI director general, proposed collaborative research on the development of BB-resistant isogenic lines between Japan and IRRI in 1978. As a result, TARC (Tropical Agricultural Research Center), of the MAFF (Ministry of Agriculture, Fisheries, and Forestry), Japan, sent one plant pathologist to IRRI for collaborative research during a few months in 1979, 1980, and 1981. However, the collaborative research was limited to the area of plant pathology. The first workshop of IRRI-MAFF on research collaboration was in Tsukuba, Japan, in September 1981. During the workshop, it was agreed that basic studies on the control of pests and diseases, particularly bacterial blight, would be one of the appropriate collaborative research subjects, which should begin immediately. It was further decided that both IRRI and TARC would coordinate the implementation of the collaborative research. After the first IRRI-MAFF workshop, a meeting on the BB project was held at TARC in February 1982, in which Japanese scientists/administrators participated. In this meeting, the participants discussed the plans on BB collaborative research proposed by TARC. Then, they agreed that four main subjects should be studied in BB collaborative research. They also decided

that a TARC plant breeder should be assigned to IRRI on a long-term basis and a TARC plant pathologist should visit regularly for a short time to participate in the BB program. As a follow-up to the discussion held in September 1981, a planning workshop was held at IRRI in March 1982 between MAFF and IRRI scientists/administrators. Subsequently, four proposed studies presented by MAFF were agreed upon. In April 1982, one plant breeder and one plant pathologist joined TARC, started a preliminary experiment at TARC, and then joined IRRI to conduct the collaborative program in September 1982.

In 1984, IRRI and the government of Japan agreed to initiate collaborative efforts on selective aspects of low-input rice cultivation, in recognition of the need to develop low-input technology for irrigated paddy rice in the tropics and semitropics. The collaborative program mentioned above for research on resistance to BB was included as a component of this Low-Input Technology Research Project.

This paper deals only with developing the international differentials because of a limitation in space. For those interested in learning more about the overall achievements of the project, please see the published references.

## Breeding of near-isogenic lines

Near-isogenic lines (NILs) with diverse genes for resistance to major diseases and insects can be powerful tools in identifying races and biotypes of diseases and insects. In rice, no NILs can be used among the countries because of some difficulties in exchanging materials among rice-growing countries.

To establish a common basis of research on resistance to BB, we began developing NILs with diverse genes for resistance to BB disease. As a result, we have developed an initial set of NILs, although a few isolates avirulent to recurrent plants were identified during the development of the NILs.

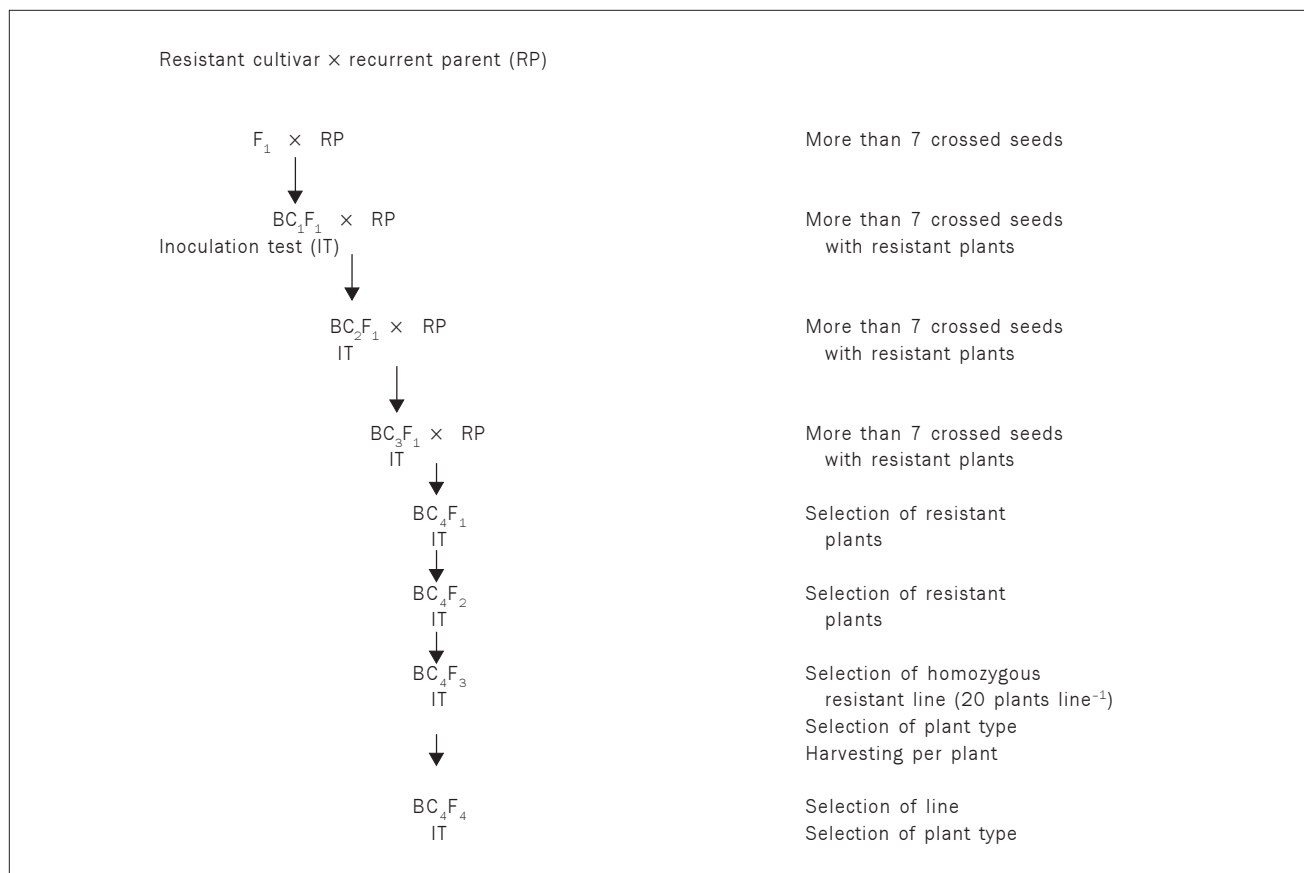
## Materials and methods

The initial plan for developing NILs with diverse genes for resistance to BB was to breed the BC<sub>4</sub>F<sub>4</sub> lines with each monogenic basis for the resistance.

The resistant donors for developing NILs are as follows: Kogyoku for *Xa1* and *Xa12*, Te-tep for *Xa2*, Chugoku 45 for *Xa3*, Java 14 for *Xa3*, IR8 for *Xa11*, IR20 for *Xa4*, IR1545-339 for *xa5*, DV85 for *Xa7*, Zenith for *Xa3* (*Xa6*), Sateng for *Xa3* (*xa9*), Cas 209 for *Xa10*, and PI231129 for *xa8*.

The recurrent parents for developing NILs are as follows: IR24 for indica genetic background, Toyonishiki for japonica genetic background, and Milyang 23 for indica-japonica hybrid genetic background. The sources of original seeds for these materials were already described.

The basic procedure is shown in Figures 1–5. All crossing work for developing NILs was done continuously at IRRI. After the original crossing between recurrent parents



**Fig. 1. Procedural steps in breeding for lines carrying dominant gene for Philippine races.**

and each resistant donor, the first backcrosses to  $F_1$  hybrids were made, and the selection of plants carrying a dominant resistance gene was started by inoculation using Philippine isolates. For the selection of plants carrying a recessive gene,  $F_2$  progenies of backcrossed hybrids were inoculated with Philippine isolates. From the second backcross, at least seven plants of each line were crossed with each recurrent parent to get a progeny carrying each resistance gene with more than a 99% possibility. This process was repeated until the  $BC_4F_2$  generation of each breeding line carrying resistance genes.

On the other hand, progenies between recurrent parents and resistant donors for developing NILs were inoculated mostly from the  $BC_3F_1$  advanced generation with Japanese isolates at TARC because the rice-cropping season is once per year in Japan. To select backcrossed progenies, more than seven crossed seeds were sent to TARC.

With this process, backcrossed progenies carrying each resistance gene were advanced until the  $BC_4F_2$  generation. The  $BC_4F_2$  plants showing resistance to BB isolates and having an introduced recessive gene, such as *xa5* in IR1545-339, were considered to be homozygous for the recessive gene. On the other hand, the homozygosity of breeding lines having the introduced dominant gene was tested in the  $F_3$  lines. From the  $BC_4F_3$ , each line was inoculated with suitable BB isolates and was compared with the recurrent par-

ent. Uniformity for plant type was also considered within each line.

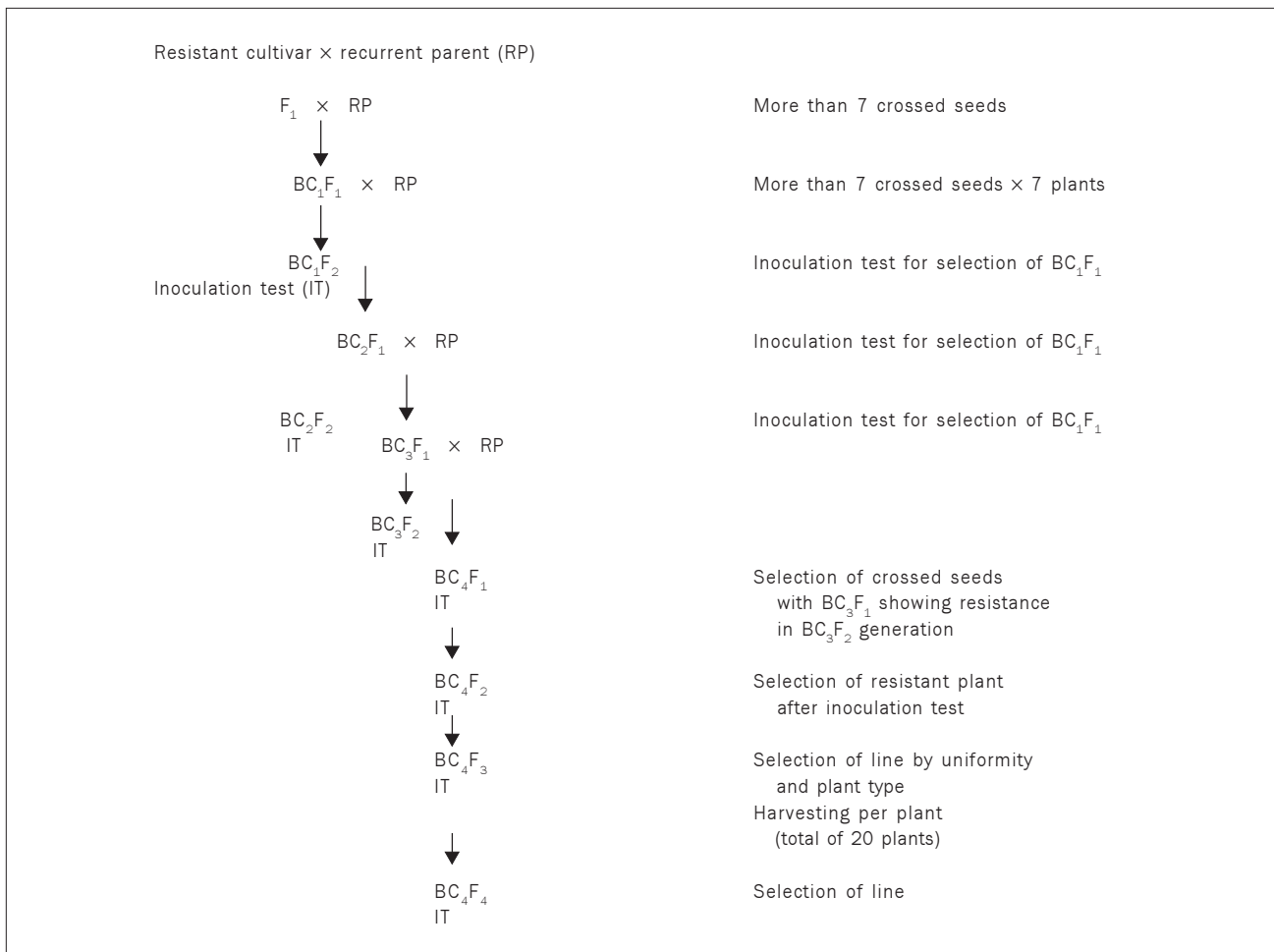
Thus, in the  $BC_4F_4$  generation, or in a more advanced generation, each breeding line was designated by the name IR-BB as a NIL. A number is assigned to each gene with resistance to BB. The recurrent parent has a background from three varieties: “0” represents IR24, “1” represents Toyonishiki, and “2” represents Milyang 23. For example, the NIL carrying the *Xa3* gene and backcrossed by Toyonishiki is designated as IR-BB 103. The number “3” represents the *Xa3* gene.

## Results and discussion

### Developing NILs carrying the *Xa3* gene

Chugoku 45 and Java 14 were used as resistant donors for developing near-isogenic lines carrying the *Xa3* gene. Moreover, Zenith (originally identified as *Xa6*) and Sateng (originally identified as *xa9*) were also used as resistant donors for developing NILs. During our studies, *Xa6* in Zenith and *xa9* in Sateng were revealed to be identical to *Xa3* in Chugoku 45 and Java 14. Therefore, Zenith and Sateng also serve as donors of NILs carrying the *Xa3* gene.

Initial crossing between recurrent parents (female) and cultivars harboring the *Xa3* gene was done from December 1982 to March 1983, and the first backcross between these



**Fig. 2. Procedural steps in breeding for lines carrying recessive gene for Philippine races.**

F<sub>1</sub> hybrids and IR24 was completed during April to May 1983. About 12 BC<sub>1</sub>F<sub>1</sub> progenies of every cross-combination were planted with 20 cm between plants and every plant was inoculated with Philippine race 1 (PXO61).

The inoculation test for all BC<sub>1</sub>F<sub>1</sub> lines indicated that several plants of every line were resistant to PXO61 (1). That is, it was confirmed that Sateng also carried a dominant gene for the Philippine isolate as mentioned in the genetic study in an earlier section. More than seven plants in several BC<sub>2</sub>F<sub>1</sub> progenies were backcrossed with IR24 during December 1983 to January 1984. Every plant of the BC<sub>2</sub>F<sub>1</sub> lines was also inoculated with PXO61 (1).

After the backcross to each plant of the BC<sub>2</sub>F<sub>1</sub> lines, our results of genetic studies indicated that plants carrying the *Xa3* gene could be selected by Philippine isolates. Therefore, after that, we selected plants of backcrossed progenies by using only Philippine isolates. The backcrossed seeds of BC<sub>2</sub>F<sub>1</sub> plants were sent to TARC and BC<sub>3</sub>F<sub>1</sub> plants were inoculated with T7174 (IA) and T7133 (IIIA) to remove the plants carrying *Xa1* and *Xa12* in Java 14 and IR20.

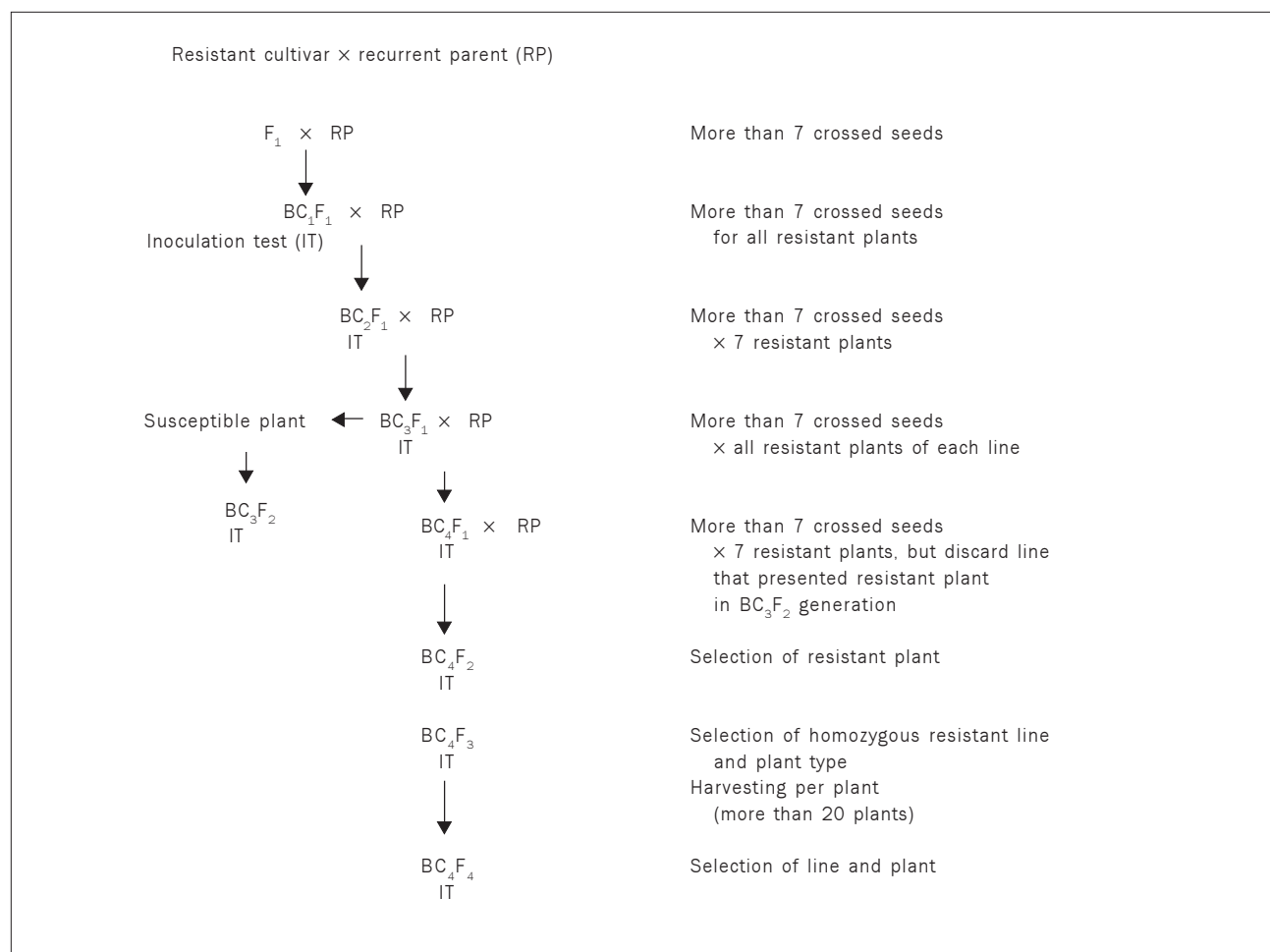
The fourth backcrossing to plants in selected BC<sub>3</sub>F<sub>1</sub> lines was done during June to July 1984. Before the backcrossing, all plants were inoculated with PXO61 (1). The

few BC<sub>4</sub>F<sub>1</sub> lines derived from the resistant BC<sub>3</sub>F<sub>1</sub> plants were planted and inoculated with four Philippine isolates. About five lines of the BC<sub>4</sub>F<sub>2</sub> generation derived from resistant BC<sub>4</sub>F<sub>1</sub> plants were planted and inoculated with four Philippine isolates at IRRI. The seeds of the BC<sub>4</sub>F<sub>2</sub> seeds were sent to TARC and BC<sub>4</sub>F<sub>2</sub> plants from the seeds were inoculated with one or two Japanese isolates. From the BC<sub>4</sub>F<sub>2</sub> generation, the plant types of each line were also compared with those of the recurrent parents.

More than 20 plants in the BC<sub>4</sub>F<sub>2</sub> generation with shorter lesion length than the resistant plants were harvested. Each ten BC<sub>4</sub>F<sub>3</sub> lines of different cross-combinations were planted and inoculated with four Philippine races. From the inoculation test, homozygous lines for resistance were selected and harvested. After the BC<sub>4</sub>F<sub>4</sub> generation, selection for plant type was again repeated and compared with that of the recurrent parents. Each line of the BC<sub>4</sub>F<sub>4</sub> generation was also planted and inoculated at TARC.

Finally, we designated line names of the NILs mostly in the BC<sub>4</sub>F<sub>5</sub>. In the advanced generations after the designation of each NIL, plant types of the breeding lines were compared with those of the recurrent parents for selection of a better line.





**Fig. 3. Procedural steps in breeding for lines carrying Xa7 of DV85 (xa5, Xa7) for Philippine races.**

The resistant plants are all heterozygous genotypes for the resistance gene *Xa3*, that is, the data show the degree of resistance under the heterozygous genotype carrying the *Xa3* gene. Recurrent parents Milyang 23 and IR24 showed typical susceptibility of Japanese and Philippine BB isolates, whereas Toyonishiki showed moderate susceptibility in our preliminary experiment. In the  $BC_4F_1$  generation, the heterozygous plants (R+) with a Toyonishiki background showed short lesion length for four Philippine isolates. On the other hand, the heterozygous plants (R+) with a Milyang 23 or IR24 background showed much longer lesions than those with a Toyonishiki background. Most of the lesion lengths in the resistant plants (R+) were longer than those of the susceptible plants (++) with a Toyonishiki background. However, the lesion length of the resistant plants (R+) with a Milyang 23 or IR24 background was clearly shorter than that of the susceptible plants (++) . Therefore, these data indicate that the lesion length of resistant plants carrying *Xa3* for BB is variable according to the genetic background.

The inoculation results with these developed NILs indicated that NILs carrying *Xa3* showed a slightly shorter lesion length with a Toyonishiki background than those with a Milyang 23 or IR24 background. However, the dif-

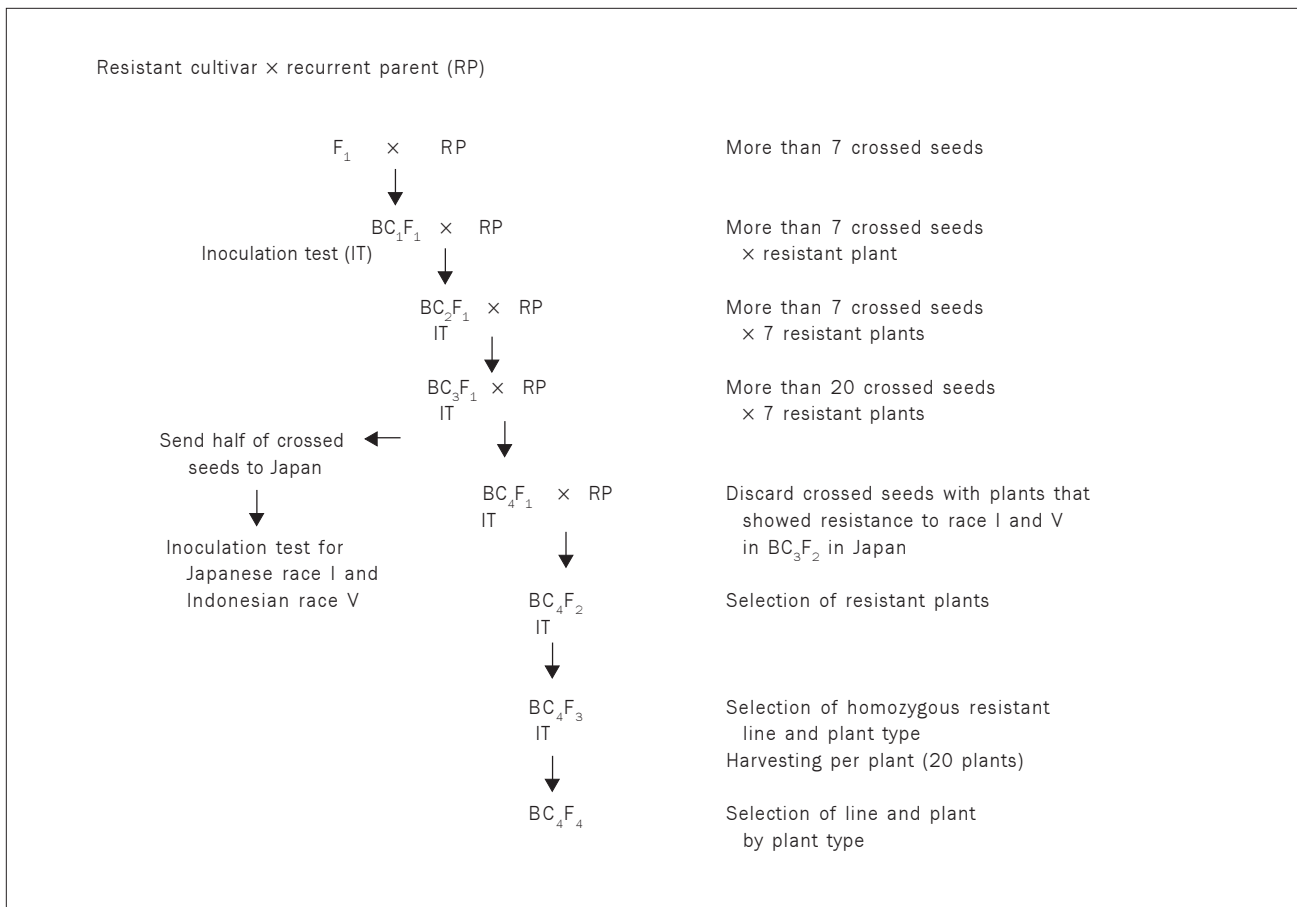
ference in lesion length between different backgrounds was less in homozygous resistant plants than in the heterozygous resistant plants mentioned above.

#### **Developing NILs carrying the Xa4 gene**

IR20, an IRRI differential, was used as a resistant donor for developing NILs carrying the *Xa4* gene.

The initial crossing between the three recurrent parents and IR20 was done in January 1983, and the first backcross in April to May 1983. The second backcrosses to the  $BC_1F_1$  progenies were done in August 1983. At the same time, the  $BC_1F_1$  plants were inoculated with four Philippine races. The  $BC_2F_1$  progenies obtained by backcross between IR24 and resistant  $BC_1F_1$  plants were also inoculated with four Philippine races and were then backcrossed again with each recurrent parent.  $BC_1F_2$  progenies and  $BC_3F_1$  progenies were also inoculated with Japanese isolates at TARC to remove the *Xa1* gene from backcrossed progenies. From the  $F_2$  analysis of Kogyoku/IR20, IR20 was identified as carrying the same gene(s) of Kogyoku in addition to *Xa4*.

After the inoculation of  $BC_3F_1$  progenies at IRRI, the last backcross was made to them from December 1983 to February 1984. The  $BC_4F_1$  progenies were inoculated with



**Fig. 4. Procedural steps in breeding for lines carrying *Xa4* of IR20 (*Xa1*, *Xa4*, *Xa12*) for Philippine races.**

four Philippine races to select resistant plants carrying *Xa4*. The data indicate that the *Xa4* gene in IR20 has a different degree of resistance to Philippine races 1 and 4. The degree of resistance to race 1 was slightly variable between different backgrounds, and variable to race 4 by different backgrounds. Moreover, *Xa4* might convey resistance to races 2 and 3 at a very minor level.

After the selection of resistant  $BC_4F_1$  plants, the generation of each progeny was advanced by self-pollination. The  $BC_4F_2$ ,  $BC_4F_3$ , and  $BC_4F_4$  progenies were inoculated with Philippine races at IRRRI and with Japanese races at TARC. At the same time, the plant type of these progenies was compared with the plant type of each recurrent parent. In  $BC_4F_3$  progenies, homozygous lines carrying the *Xa4* gene could not be identified. Afterward, 5 to 0 plants of a fixed line in the resistance gene were selected based on the similarity of plant type with each recurrent parent.

In  $BC_4F_5$  lines carrying *Xa4*, we finally selected NILs per each recurrent parent and designated them as IR-BB4 (IR24 background), IR-BB 104 (Toyonishiki background), and IR-BB204 (Milyang 23 background), respectively. The *Xa4* gene conveys relatively stable resistance to race 1, but variable resistance in different backgrounds to race 4. Moreover, the reaction to races 2 and 3 of NILs did not show complete susceptibility in the lines with an IR24 or

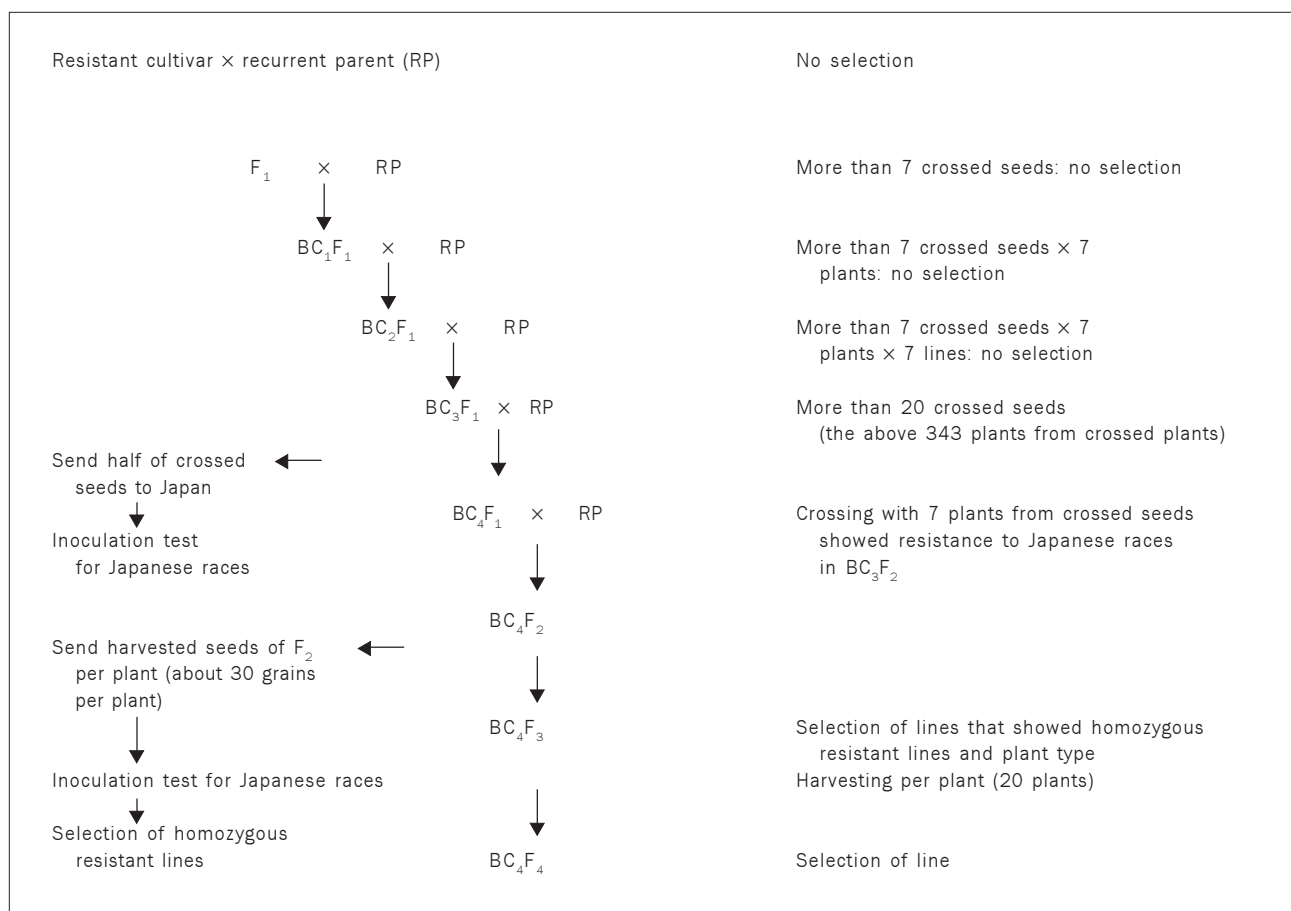
Toyonishiki background. Therefore, the *Xa4* gene might express pleiotropy on resistance to BB isolates.

#### Developing NILs carrying the *xa5* gene

IR1545-339, an IRRRI differential, was used as a resistant donor for developing NILs carrying the *xa5* gene. An IRRRI-bred line, IR1545-339, was developed from the cross between IR24 and DZ192. Therefore, the *xa5* gene in IR1545-339 was derived from that of DZ192.

The initial cross between recurrent parents and IR1545-339 was made during January to February 1983, and then the first backcrosses between recurrent parents and  $F_1$  hybrids were made during April to May 1983. More than seven plants of  $BC_1F_1$  progenies per different recurrent parent were backcrossed again. At the same time, plants of  $BC_1F_2$  progenies were inoculated with four Philippine races, and then  $BC_1F_1$  plants carrying the *xa5* gene were specified. The genetic study of IR1545-339 for resistance confirmed that IR1545-339 had only one recessive gene for Japanese isolates and it was identical to *xa5*. Therefore, the progenies of the backcrossing program were inoculated and selected only with Philippine races and the process was repeated from the  $BC_1F_1$  to  $BC_4F_2$  generation.

Since *xa5* is a recessive gene, we can get the homozygous genotype for the resistance in  $BC_4F_2$  progenies, that is,



**Fig. 5. Procedural steps in breeding for lines carrying gene with resistance to Japanese race only.**

if a BC<sub>4</sub>F<sub>2</sub> progeny shows resistance, it is considered a homozygous genotype for the gene. Thus, we have developed NILs from the generation by inoculation test and comparison of plant type with that of each recurrent parent. From BC<sub>4</sub>F<sub>3</sub> lines, the inoculation test with Japanese races and comparison of plant type with each recurrent parent were also done at TARC. As a result, IR-BB5 (IR24 background), IR-BB 105 (Toyonishiki background), and IR-BB 205 (Milyang 23 background) were developed in 1987. After the designation of NILs, we still continued selecting better lines among NILs.

The lesion length of NILs for four Philippine isolates showed that the *xa5* gene expressed a longer lesion length in a Toyonishiki background than in an IR24 background, although parental Toyonishiki had a shorter lesion length than that of parental IR24. Moreover, the *xa5* gene was confirmed to convey resistance to race 4.

#### Developing NILs carrying the *Xa7* gene

An IRRI differential, DV85, was used as a resistant donor for developing NILs carrying *Xa7*. DV85 was identified to have one recessive gene, *xa5*, and one dominant gene, *Xa7* (Sidhu et al 1978). Actually, no cultivar with the *Xa7* gene only has been found yet.

The initial crosses between recurrent parents and DV85 were made from December 1982 to January 1983. Then, the first backcrosses to the F<sub>1</sub> hybrids were completed in April 1983. The BC<sub>1</sub>F<sub>1</sub> progenies were inoculated with four Philippine races and the plants resistant to Philippine races 2 and 3 were backcrossed with recurrent parents from July to August 1983. The third backcrosses to the BC<sub>2</sub>F<sub>1</sub> progenies were made using more than seven plants per each recurrent parent. The BC<sub>2</sub>F<sub>2</sub> progenies were inoculated with four Philippine races. Moreover, to remove the recessive gene, *xa5*, or to separate the recessive gene, *xa5*, and the dominant gene, *Xa7*, the BC<sub>2</sub>F<sub>3</sub> progenies were inoculated on 17 plants per line with four Philippine races and then the carrying gene(s) of each BC<sub>2</sub>F<sub>1</sub> and BC<sub>3</sub>F<sub>1</sub> progeny were identified by the results.

At that time, Saha (1984) reported that DV85 had two dominant genes and one recessive gene. Therefore, we had to confirm this result, though our inoculation results with the backcrossed progenies showed that DV85 had one dominant gene, not more than one dominant gene. Thus, we advanced the backcross program only to IR24 backcrossed progenies, and then, after the confirmation of the resistance gene in DV85, we continued backcrossing to Toyonishiki and Milyang 23 progenies.

As a result, the last backcrosses were made with IR24 in February 1985, and with Toyonishiki or Milyang 23 in July 1986. The BC<sub>4</sub>F<sub>1</sub> and BC<sub>4</sub>F<sub>2</sub> progenies were inoculated with four Philippine races. In the BC<sub>4</sub>F<sub>3</sub> progenies, homozygous lines for resistance were selected. Afterward, NILs with a homozygous genotype were compared with each recurrent parent based on uniformity and plant type. Now, NILs were advanced to the BC<sub>4</sub>F<sub>7</sub> generation with an IR24 background and to BC<sub>4</sub>F<sub>4</sub> with a Toyonishiki or Milyang 23 background. We designated NILs as IR-BB 7 (IR24 background), IR-BB 107 (Toyonishiki background), and IR-BB 207 (Milyang 23 background).

The data showed that the *Xa7* gene conveyed high resistance to Philippine races 1, 2, and 3, but was susceptible to race 4. However, the resistance to race 1 was slightly lower than that to races 2 and 3.

### Developing NILs carrying the *xa8* gene

PI231129 has been identified as carrying the *xa8* gene for resistance by Sidhu et al (1978). No other cultivars were identified to have this *xa8* gene. However, PI231129 is not included in IRRI differentials. Thus, PI231129 was used as a resistant donor for developing NILs carrying the *xa8* gene.

The initial crosses between recurrent parents and PI231129 were made in January 1983, and then the first backcrosses to F<sub>1</sub> hybrids were carried out from May to July 1983. The second backcrosses to BC<sub>1</sub>F<sub>1</sub> progenies were made using more than seven plants per recurrent parent from August to November 1983. The BC<sub>2</sub>F<sub>2</sub> progenies were inoculated with four Philippine races for specifying the BC<sub>2</sub>F<sub>2</sub> plants carrying the recessive gene *xa8*. The third backcrosses to BC<sub>2</sub>F<sub>1</sub> progenies were made from March to April 1984.

The BC<sub>3</sub>F<sub>2</sub> progenies were also inoculated with four Philippine races. However, there were some difficulties in specifying plants carrying the resistance gene. Therefore, every BC<sub>3</sub>F<sub>1</sub> plant was crossed with PI231129 and recurrent parents to confirm which plants have the resistance gene of PI231129. The crosses were made from February to April 1986. Afterward, the inoculation test with four Philippine races was carried out for the progenies of IR24/PI231129//3\*IR24//PI231129 and the BC<sub>4</sub>F<sub>1</sub> progenies carrying the resistance gene were specified by the test.

In BC<sub>4</sub>F<sub>2</sub> progenies, more than 20 plants having moderate resistance to Philippine races 1, 2, and 3 were selected per recurrent parent. From the BC<sub>4</sub>F<sub>3</sub> lines, every plant was inoculated with four Philippine races and the uniformity of the reaction was considered. At the same time, the plant type of each line was compared with that of each recurrent parent.

During the season of NILs evaluation, PI231129 did not head because it has high photosensitivity. The lesion length of selected NILs for four Philippine races was longer, although it was shorter than the lesion length of each recurrent parent. Moreover, lesion development of the NILs did not stop after inoculation. The resistance conveyed by the *xa8* gene might be different from that of the other genes identified for resistance to BB.

### Developing NILs carrying the *Xa10* gene

An IRRI differential, Cas 209, was used as a resistant donor for developing NILs carrying the *Xa10* gene. At that time, only Cas 209 was identified as having *Xa10* for BB. *Xa10* conveys resistance to race 2 only and Cas 209 shows susceptibility to all Japanese races. Therefore, the breeding of NILs carrying *Xa10* has been done only at IRRI in the early generations, but, in the later generations, breeding lines were planted at TARC for comparison of plant type with that of the recurrent parents.

The initial crosses between recurrent parents and Cas 209 were made from December 1982 to January 1983, and the first backcrosses between F<sub>1</sub> hybrids and recurrent parents were completed from April to May 1983. The BC<sub>1</sub>F<sub>1</sub> progenies were inoculated with PXO86 (2) for selecting resistant plants, and then crossed with recurrent parents (second backcrosses) from July to September 1983. This procedure was repeated until the fourth backcrosses with recurrent parents.

The BC<sub>4</sub>F<sub>2</sub> progenies were inoculated with four Philippine races to confirm the reaction of the plants carrying *Xa10*. The results of inoculation show that plants carrying *Xa10* have a similar degree of resistance to race 2 among different backgrounds, that is, every heterozygous (resistant) plant with a different background had a short lesion length for race 2. At the same time, susceptible plants with a different background showed a lesion length similar to that of each recurrent parent.

After the BC<sub>4</sub>F<sub>2</sub> progenies, the developed plants were compared with the plant type of each recurrent parent at both IRRI and TARC. The NILs did not show a difference in lesion length for race 2 among each background, that is, the lesion lengths were very short. Therefore, the expression of *Xa10* for resistance appears not to be affected by genetic background.

### Developing NILs carrying the *Xa11* gene

An IRRI differential, IR8 (susceptible check), was used as a resistant donor for developing NILs carrying *Xa11*. *Xa11* was identified originally in RP9-3 (Ogawa and Yamamoto 1986), and then in this study, Elwee and IR8 were also identified as carrying the *Xa11* gene. *Xa11* conveys resistance to some Japanese races but not to any Philippine races. Therefore, crossing work for developing NILs has been done only at IRRI, but selection for plants carrying *Xa11* was done at TARC.

Initial crossing between recurrent parents and IR8 was done in January 1983. The first backcrossing to F<sub>1</sub> hybrids was carried out from April to June 1983 and the second backcrossing to BC<sub>1</sub>F<sub>1</sub> progenies was done using about seven plants per recurrent parent during August to November 1983. BC<sub>1</sub>F<sub>2</sub> progenies for the Toyonishiki or Milyang 23 backcross program were inoculated with Japanese races at TARC and BC<sub>1</sub>F<sub>1</sub> plants carrying *Xa11* were selected according to the results.

Inoculation can be done once a year in the field at TARC. Therefore, we continued crossing the necessary num-

ber of combinations with recurrent parents to involve *Xa11* in the backcross progenies. As a result, crossing of 50 BC<sub>2</sub>F<sub>1</sub> plants with IR24, 59 BC<sub>2</sub>F<sub>1</sub> plants with Toyonishiki, and 58 BC<sub>2</sub>F<sub>1</sub> plants with Milyang 23 was done from March to May 1984. Furthermore, crossing of 102 BC<sub>3</sub>F<sub>1</sub> plants with IR24, 62 BC<sub>3</sub>F<sub>1</sub> plants with Toyonishiki, and 62 BC<sub>3</sub>F<sub>1</sub> plants with Milyang 23 was completed during November 1984.

The fourth backcrossed seeds were sown at TARC in 1985, and the plants were inoculated with Japanese races IB and IIIA. The BC<sub>4</sub>F<sub>1</sub> plants showing resistance to the two races were harvested and then the BC<sub>4</sub>F<sub>2</sub> progenies were planted at TARC in 1986. Twenty lines per recurrent parent were evaluated based on resistance to Japanese races, plant type, and grain shape. Five lines per recurrent parent were selected from plant type and grain shape and then, within the selected lines, plants resistant to Japanese races were harvested.

At TARC in 1987, the BC<sub>4</sub>F<sub>3</sub> lines were further evaluated based on resistance and plant type. As a result, few lines per recurrent parent having homozygous resistance were selected. The selected lines were evaluated based on plant type at IRRI in 1988. Finally, each line per recurrent parent was selected and designated as IR-BB 11 (IR24 background), IR-BB 111 (Toyonishiki background), and IR-BB 201 (Milyang 23 background).

#### **Developing NILs carrying the *Xa1* and *Xa12* gene**

A Japanese differential, Kogyoku, was used as a resistant donor for developing NILs carrying *Xa1* and *Xa12*.

First, we repeatedly backcrossed and inoculated plants to get backcrossed progenies resistant to Japanese race IA and an Indonesian isolate, Xo7306, because the genes *Xa1* and *Xa12* could not be separated from each other due to the very close linkage between them.

Initial crosses between recurrent parents and Kogyoku were made from December 1982 to February 1983, and the first backcrosses were made in April 1983. The progenies of BC<sub>1</sub>F<sub>1</sub> plants (BC<sub>1</sub>F<sub>2</sub>) were inoculated with a Japanese isolate and an Indonesian isolate in the isolation greenhouse at TARC, where the BC<sub>1</sub>F<sub>1</sub> plants carrying a resistance gene were identified. Backcrosses with recurrent parents were repeated more than three times without selection by inoculation of a Japanese and Indonesian isolate due to space limitations of the isolation greenhouse at TARC.

The second backcrossing was done from July to August 1983, the third from December 1983 to February 1984, and the fourth in October 1984. All BC<sub>4</sub>F<sub>1</sub> progenies were sent to TARC. The BC<sub>4</sub>F<sub>1</sub> progenies taken from 191 cross-combinations were inoculated with a Japanese isolate, T7174, and an Indonesian isolate, Xo7306, at the isolation greenhouse of TARC in 1985. After selection of plants resistant to the two isolates, more than 2,000 plants of the BC<sub>4</sub>F<sub>2</sub> progenies per recurrent parent were inoculated with the two isolates.

Several plants appeared to show a different reaction to the two isolates, but no plant was susceptible to an isolate in the progenies. Therefore, we decided first to develop ho-

mozygous lines resistant to the two isolates, and at the same time we continue looking for plants showing resistance to only one isolate. Thus, we selected homozygous resistant lines with resistance to two races in BC<sub>4</sub>F<sub>3</sub> progenies at TARC in 1987; these lines were designated as IR-BB 1 (IR24 background), IR-BB 101 (Toyonishiki background), and IR-BB 201 (Milyang 23 background).

We still need to continue to segregate plants with two kinds of resistance: resistant to only the Japanese isolate and resistant to only the Indonesian isolate. It is possible that the two genes, *Xa1* and *Xa12*, are located on the same locus.

#### **Developing NILs carrying the *Xa2* gene**

A Japanese differential, Te-tep, was used as a resistant donor for developing NILs carrying *Xa2*. However, Te-tep has been identified as carrying *Xa1* and *Xa2* and it might carry one more gene (such as *Xa12*) with resistance to Indonesian isolate Xo7306 (Sakaguchi 1967, Ogawa et al 1978). Furthermore, a recent inoculation test showed that Te-tep was resistant to Indonesian isolate Xo7435 (IV) while other Japanese differentials were susceptible to it. Therefore, we initially developed NILs carrying all resistance genes in Te-tep, and later on tried to separate each resistance gene in Te-tep.

Crosses between recurrent parents and Te-tep were made in January 1983 and then the first backcrosses to F<sub>1</sub> hybrids were completed from April to May 1983. The BC<sub>1</sub>F<sub>2</sub> progenies were inoculated with four isolates, T7174 (IA), T7147 (II), Xo7435 (IV), and Xo7306 (V), at the isolation greenhouse of TARC. The selected BC<sub>1</sub>F<sub>1</sub> plants from the above results were backcrossed with recurrent parents from August to November 1983. The third backcrosses were from January to February 1984 and the fourth backcrosses from February to March 1985. The fourth backcrossed seeds (BC<sub>4</sub>F<sub>1</sub>) were sent to TARC in 1985 and selection was then made to identify resistant plants in backcrossed progenies by inoculation with the four isolates.

More than 2,000 plants (BC<sub>4</sub>F<sub>2</sub> progenies) were inoculated per recurrent parent in 1986 and BC<sub>4</sub>F<sub>3</sub> progenies were evaluated in 1987. We selected homozygous lines resistant to Japanese isolates T7174 (IA) and T7147 (II) and Indonesian isolate Xo7306, and homozygous lines resistant to Indonesian isolate Xo7435 (Table 78) because there were still some difficulties in separating lines resistant to either T7147 or Xo7306.

In addition to the relationship between *Xa1* and *Xa12*, it is possible that the resistance genes in Te-tep are each located on the same locus, though we need to continue to segregate plants with one resistance gene.

Thus, we finished developing a basic set of NILs for research on resistance to rice bacterial blight. The seeds have already been distributed to scientists of various countries and biotechnologists in the U.S. and Japan, and reports on which lines were used have been published.

The reactions of the development of NILs for the Japanese and Philippine races of BB pathogen are shown in Table 1. It should be noted that only two Myanmar isolates



**Table 1. Reaction<sup>a</sup> of near-isogenic lines (NILs) to Japanese and Philippine races of BB pathogen (corrected according to Ogawa et al 1991).**

NILs	Japanese race <sup>b</sup>						Philippine race					
	IA	IB	II	IIIA	IIIB	IV	1	2	3	4	5	6
IR-BB 1	HR	HR	M	S	S	S	S	S	S	S	S	S
IR-BB 2	HR	HR	HR	S	S	S	S	S	S	S	S	S
IR-BB 3	RB	RB	RB	RB	RB	S	RB	RB	RB	RB	RB	S
IR-BB 4	R	R	R	R	R	R	R	S	S	M	R	S
IR-BB 5	R	R	R	R	R	R	R	R	R	M	R	S
IR-BB 7	HR	HR	HR	HR	HR	HR	R	HR	HR	S	HR	S
IR-BB 8	R	HR	HR	HR	R	R	R	R	R	M	R	M
IR-BB 10	S	S	S	S	S	S	S	HR	S	S	HR	S
IR-BB 11	S	R	R	R	S	S	S	S	S	S	S	S

<sup>a</sup>Reaction at booting stage, HR = highly resistant, RB = resistant with browning margin, R = resistant, M = moderately susceptible to moderately resistant, S = susceptible.

<sup>b</sup>Standard isolates for each race are as follows: T7174 for race IA, T7156 for race IB, T7147 for race II, T7133 for race IIIA, Q6803 for race IIIB, H75373 for race IV, PX061 for race 1, PX086 for race 2, PX079 for race 3, PX071 for race 4, PX0112 for race 5, PX0 for race 6. The reaction to Japanese race V (H75304) is not included in this table because of the low virulence.

among more than 500 isolates of our collection from Asian countries (which we will describe later) were not virulent to the recurrent parents, while they were virulent to a few BB differentials. In addition, one Japanese isolate was not virulent to IR24 and Milyang 23, while it was virulent to Toyonishiki (Yamamoto and Ogawa 1990).

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## Notes

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# Developing rice blast differential lines and evaluating partial resistance for the breeding of durable rice varieties in the tropics

H. Kato, T. Imbe, H. Tsunematsu, and Y. Fukuta

Breeding of rice varieties with durable resistance to blast is the most logical and environment-friendly approach. Major gene and partial resistance are known. Information about the virulence of rice blast isolates is important for genetic studies and the breeding of resistant varieties. We have tested the pathogenicity of a few hundred blast isolates by inoculating them to Japanese differential varieties and IRRI near-isogenic lines (NILs) having known resistance genes. Based on the results of inoculation, standard blast isolates are selected. However, the virulence of each resistance gene was not clear. Extra genes seemed to exist in the differentials or NILs.

Varieties with a single but different resistance gene are ideal as differentials for pathogenicity tests. We developed a set of lines with single but different blast resistance genes as monogenic lines. Thirteen genes for blast resistance were incorporated. Monogenic lines with nine genes were also developed.

The reaction patterns of almost all of the donors and differential varieties could be explained by the reaction patterns of the monogenic lines or combination of lines. However, resistant reactions of some donors were higher than expected. This higher resistance can be ascribed to either complementary gene action or the presence of additional resistance genes besides known ones.

Partial resistance to blast has not been clearly distinguished from major gene resistance in the tropics. Several major genes for resistance to blast were identified recently in IR varieties at IRRI and the reactions of these genes to Philippine blast isolates were studied. Consequently, suitable blast isolates could be selected to overcome the effect of these major genes for evaluating partial resistance to blast under field conditions.

Seventy-two varieties and breeding lines were inoculated with three blast isolates during two seasons. Serious blast infection was induced by partial shading, continuous irrigation, and the application of a large amount of fertilizer. Disease damage was evaluated using the Standard Evaluation System for rice blast.

Partial resistance was clearly distinguished from major gene resistance and the differences between moderate levels of resistance conferred by major genes and partial resistance were clarified. Among the materials used, IR64 showed a high level of partial resistance. Partial resistance was moderate in IR60 and IR36 and low in IR50 and CO39. These results were consistent with the results of sequential planting done at IRRI earlier. Partial resistance levels of several Japanese varieties at IRRI are also consistent with their evaluations in Japan. Significant positive correlations observed among partial resistance to the three isolates indicate that partial resistance is horizontal in the tropics. A distinction between major gene and partial resistance to blast is necessary for identifying donors for developing durably resistant varieties.

Rice blast, caused by *Pyricularia oryzae* Cav., is the most important and potentially damaging disease of rice (*Oryza sativa* L.). Breeding of rice varieties with durable resistance to this disease is the most logical and environment-friendly approach. Major gene and partial resistance to diseases are known in rice and other crops. In major gene resistance, there is incompatibility between the resistance gene in the host and the avirulence gene in the pathogen. Partial resistance reduces the extent of pathogen reproduction within the context of a compatible interaction. Several strategies to develop durable resistance to blast are possible. The pyramiding of major genes for resistance, the accumulation of genes for partial resistance, the combination of major and partial resistance genes, and the use of multiline varieties composed of isogenic lines with major resistance genes are some feasible approaches.

### Pathogenicity of IRRI blast isolates

Information about the virulence of rice blast isolates is important for genetic studies and the breeding of resistant varieties. A set of international differential varieties for rice blast established by Atkins et al (1967) has been used in various parts of the world. However, since genetic analysis of the resistance genes of the varieties is lacking, differentiation of races based on their avirulence could not be carried out. Two sets of differential varieties (Yamada et al 1976, Kiyosawa 1984) have been used in Japan to identify races of the blast fungus. Each differential variety with one resistance gene effective against Japanese races was selected based on genetic analysis. Shin 2, one of the differentials, carries *Pik-s*, which is not effective against Japanese isolates but is effective against a Philippine blast isolate (Kiyosawa 1969a). *Pish* was also identified in it by using a Japanese isolate (Imbe and Matsumoto 1985). It is not effective against most of the Japanese isolates. The presence of *Pik-s* and *Pish* in the differentials did not seem to be important in Japan. These sets enabled us to differentiate blast races based on their avirulence in Japan.

Blast resistance genes in most of the indica varieties, including IRRI-bred IR cultivars, have not been identified because of their complexities. Among Japanese varieties, 14 alleles on 8 loci have been identified. Except for *Pia*, *Pii*, *Pik-s*, and *Pish*, the other 10 alleles have been introduced into Japanese varieties from indica donors. Therefore, those genes may also exist in indica varieties.

We have tested the pathogenicity of a few hundred blast isolates by inoculating them to Japanese differential varieties and IRRI near-isogenic lines (NILs) having known resistance genes. These blast isolates had been collected from all over the Philippines or were found in the IRRI blast nursery. They were classified into around 30 different pathotypes according to their pathogenicities. The pathogenicities of the blast isolates are usually used for the analysis of resistance genes of cultivars. Based on the results of inoculation, the standard blast isolates are selected (Table 1).

The pathogenicity of these isolates had been analyzed by using Japanese differentials and IRRI NILs. *Pia*, *Pib*, *Pit*, *Pi19(t)*, and *Pik-s* were resistant to a few isolates. *Pish* seemed to be moderately resistant to many isolates. *Pii* and *Pi3(t)* showed reaction patterns similar to each other. *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, and *Pil* showed a lower number of moderate reactions and their patterns were also similar to one another. *Piz*, *Piz-5*, and *Piz-t* showed moderate reactions against many isolates and their reaction patterns are also similar to one another. *Pita*, *Pita-2*, and *Pi20* showed fewer moderate reactions. According to these results, the virulence of each resistance gene was not clear. Extra genes seemed to exist in the differentials or NILs.

### Developing monogenic lines for blast resistance

Varieties with a single but different resistance gene are ideal as differentials for pathogenicity tests of a pathogen (Flor 1956). A set of differential lines with a single known resistance gene was needed to identify the virulence of rice blast isolates. We therefore developed a set of lines with single but different blast resistance genes as monogenic lines. A set of monogenic lines could be suitable for use as differentials for rice blast because of their superior ability for race differentiation. The monogenic lines can also be used for genetic analysis of genes for blast resistance.

*Pi19(t)* was identified in the Japanese variety Aichi Asahi (Hayashi et al 1998). This gene could not be incorporated into the monogenic lines at IRRI as it was not effective against any of the isolates at IRRI. Therefore, selection of *Pi19(t)* and confirmation of the existence of *Pia*, *Pik-s*, and *Pish* in the lines were carried out at the National Agricultural Research Center (NARC) in Japan through the Japan-IRRI shuttle research project.

To develop a line with a single blast resistance gene, we applied the backcross breeding method. Varieties and lines with known genes for resistance were used as donors. Lijiangxintuanheigu (LTH) was used as a recurrent parent in the backcrossing. LTH, which is a japonica variety from Yunnan Province in China, is highly susceptible to rice blast. No isolate incompatible with LTH was found in China (Ling et al 1995) and all of the blast isolates evaluated at IRRI so far were also compatible with the variety. No major resistance gene for rice blast has been identified in LTH. All of the materials were planted under long-day treatment to extend the growing period because most of the lines probably carry the photosensitivity inherited from LTH. A 30-minute interruption of the dark period was performed as a long-day treatment from 21 days after seeding (DAS) to 70–75 DAS.

To confirm the existence of a single resistance gene, BC<sub>1</sub>F<sub>2</sub> populations and their selected progenies were inoculated with suitable isolates. A maximum of 20 pregerminated seeds of a line was used for the inoculations. Inoculation was performed at the 4.0- to 4.9-leaf seedling stage by using the spraying method (Inukai et al 1994, Hayashi et al 1998).

**Table 1. Reaction patterns of differential varieties to Philippine blast isolates.<sup>a</sup>**

Group	Isolate	Differential variety or donor of resistance gene																		
		Caloro	K 59	Aichi Asahi	Shin 2	BL1	Fujisaka	C104PKT	Kanto 51	K 3	Tsuyuake	K 60	C101 LAC	Fukumishiki	C101A51	Toride 1	Yashitomochi	Reiho	IR 24	
		Piks (?)	Pit (Piks)	Pia PI19(t)	Piks, Pish	Pib, Pish	Pij, Piks	Pi3 (Pia)	Pik	Pikh	Pikm	Pikp	Pi1 (Pia)	Piz, Pish	Piz-5 (Pia)	Piz-t (Pish)	Pita (?)	Pita-2 (Pia)	Pi20 Pib, Piks	
XXIXb	B90002	S	S	R	M	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
XXIXb	C92349	S	S	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
IXb	BN111	S	S	S	M	M	R	M	R	R	R	R	R	R	R	M	S	R	R	R
XIVa	BN209	S	M	S	M	R	S	S	R	R	R	R	R	R	M	R	R	R	R	R
XXIb	JMB840610	S	S	S	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R
Ive	V86010	R	S	S	M	R	S	S	R	R	R	R	R	R	M	R	S	R	R	R
XXVIII	V850196	R	R	S	R	-	R	R	R	R	R	R	R	R	M	M	R	R	R	R
II	IK81-3	M	S	S	S	R	S	S	R	R	R	R	R	R	M	M	M	R	R	S
XX	IK81-25	R	S	S	M	M	R	R	S	S	R	R	S	R	M	M	R	R	R	S
V	P06-6	S	S	S	M	M	R	M	R	R	R	R	R	R	R	M	S	S	S	S
Ib	Ca89	S	S	S	S	M	S	S	R	R	R	R	R	R	M	M	S	S	S	S
IIIb	V850256	S	S	S	M	M	S	S	R	R	R	R	R	R	M	R	M	R	R	S
XVIIb	M36-1-310-1	-	S	S	R	R	R	R	R	S	R	R	M	R	R	R	S	R	R	-
IIIa	M64-1-39-1	S	S	S	S	M	S	S	S	S	S	M	M	R	R	R	M	R	R	S
IIIa	M39-1-38-1	S	S	S	S	M	M	M	S	S	S	M	M	M	R	R	M	R	R	S
XIVa	M101-129-1	-	S	S	M	R	S	S	R	R	R	R	R	S	R	R	R	R	R	-

<sup>a</sup>R = resistant, S = susceptible, M = moderately resistant.



Each seedling was examined 5 to 7 days after inoculation using a modified classification based on a 0–5 scale (Mackill and Bonman 1992). All the isolates of *Pyricularia grisea* used at IRRI were either maintained as stock cultures in the Entomology and Plant Pathology Division of IRRI or were isolated from the blast nursery of IRRI. All the isolates used at NARC were maintained at the Rice Pathology Laboratory in NARC.

Only one resistance gene was transferred from a donor harboring more than one resistance gene. As an example, Aichi Asahi, one of the Japanese differential varieties, carries two blast resistance genes, *Pia* (Yamasaki and Kiyosawa 1966) and *Pi19(t)* (Hayashi et al 1998). Aichi Asahi was used as a donor for the two genes. Aichi Asahi was crossed and backcrossed with LTH. Twenty-four BC<sub>1</sub>F<sub>1</sub> plants were self-pollinated. The 24 BC<sub>1</sub>F<sub>2</sub> families were inoculated with Ina72 (*Av-a* and *Av-19+*) and CHNOS58-3-1 (*Av-a+* and *Av-19*) at NARC.

To select the monogenic line with *Pia*, a family that showed segregation for resistance to Ina72 and a susceptible reaction to CHNOS58-3-1 was selected. This family was expected to be heterozygous for *Pia* but lacked *Pi19(t)*. The same BC<sub>1</sub>F<sub>2</sub> families were inoculated with B90002 (*Av-a* and *Av-19+*) and C923-49 (*Av-a* and *Av-19+*) at IRRI. The segregation pattern of *Pia* in these lines was identical at both NARC and IRRI. Nine resistant plants in the selected BC<sub>1</sub>F<sub>2</sub> family were self-pollinated. Among the nine BC<sub>1</sub>F<sub>3</sub> lines, two lines were homozygous-resistant to B90002. Thus, these two lines were homozygous-resistant to *Pia* but did not carry *Pi19(t)*. From the two lines, seven resistant plants were selected and self-pollinated. All of these BC<sub>1</sub>F<sub>4</sub> lines were homozygous-resistant to B90002. These BC<sub>1</sub>F<sub>4</sub> lines were also inoculated with TH68-140 (*Av-a* and *Av-19+*) at NARC. All of the plants in each line were resistant to TH68-140. The homozygosity of *Pia* was thus confirmed. These lines were self-pollinated until they were morphologically uniform.

To obtain a monogenic line with *Pi19(t)*, three BC<sub>1</sub>F<sub>2</sub> families that showed segregation for resistance to CHNOS58-3-1 but a susceptible reaction to Ina72 were selected at NARC. These families, which were expected to be heterozygous for *Pi19(t)* but lacked *Pia*, were inoculated with B90002 and C923-49 at IRRI. All the plants in these families were susceptible to both isolates. Eight plants of the selected families were self-pollinated and BC<sub>1</sub>F<sub>3</sub> lines were obtained. Ten BC<sub>1</sub>F<sub>4</sub> lines were derived from each BC<sub>1</sub>F<sub>3</sub> line. A total of 80 BC<sub>1</sub>F<sub>4</sub> lines were tested with CHNOS58-3-1 at NARC. Ten lines were found to be homozygous-resistant. These lines were homozygous for *Pi19(t)* but did not have *Pia*. From the 10 BC<sub>1</sub>F<sub>4</sub> lines, 22 plants were randomly selected and self-pollinated. These 22 BC<sub>1</sub>F<sub>5</sub> lines were inoculated with CHNOS58-3-1 and TH68-140 at NARC. All the plants of these lines were resistant to CHNOS58-3-1. Twenty-one lines showed a susceptible reaction to TH68-140 and only one line was moderately resistant to this isolate. Two lines that were resistant to CHNOS58-3-1 but susceptible to TH68-

**Table 2. Monogenic lines with different genes for blast resistance.**

Line	Resistance gene	Donor	Generation
IRBLa-A	<i>Pia</i>	Aichi Asahi	BC <sub>1</sub> F <sub>10</sub>
IRBLa-C	<i>Pia</i>	C039	BC <sub>2</sub> F <sub>10</sub>
IRBLi-F5	<i>Pii</i>	Fujisaka 5	BC <sub>1</sub> F <sub>10</sub>
IRBLks-F5	<i>Pik-s</i>	Fujisaka 5	BC <sub>1</sub> F <sub>10</sub>
IRBLks-S	<i>Pik-s</i>	Shin 2	BC <sub>1</sub> F <sub>10</sub>
IRBLk-Ka	<i>Pik</i>	Kanto 51	BC <sub>1</sub> F <sub>9</sub>
IRBLkm-Ts	<i>Pik-m</i>	Tsuyuake	BC <sub>1</sub> F <sub>6</sub>
IRBLkp-K60	<i>Pik-p</i>	K60	BC <sub>1</sub> F <sub>8</sub>
IRBLkh-K3	<i>Pik-h</i>	K3	BC <sub>1</sub> F <sub>8</sub>
IRBLz-Fu	<i>Piz</i>	Fukunishiki	BC <sub>1</sub> F <sub>10</sub>
IRBLz5-CA	<i>Piz-5(= Pi2(t))</i>	C101A51	BC <sub>3</sub> F <sub>8</sub>
IRBLzt-T	<i>Piz-t</i>	Toride 1	BC <sub>1</sub> F <sub>10</sub>
IRBLta-K1	<i>Pita(= Pi4(t))</i>	K1	BC <sub>1</sub> F <sub>8</sub>
IRBLta-CT2	<i>Pita</i>	C105TTP2L9	BC <sub>3</sub> F <sub>8</sub>
IRBLta-CP1	<i>Pita</i>	C101PKT	BC <sub>5</sub> F <sub>6</sub>
IRBLta2-Pi	<i>Pita-2</i>	Pi No. 4	BC <sub>1</sub> F <sub>5</sub>
IRBLta2-Re	<i>Pita-2</i>	Reiho	BC <sub>1</sub> F <sub>6</sub>
IRBLb-B	<i>Pib</i>	BL1	BC <sub>1</sub> F <sub>8</sub>
IRBLsh-S	<i>Pish</i>	Shin 2	BC <sub>1</sub> F <sub>10</sub>
IRBLsh-B	<i>Pish</i>	BL1	BC <sub>1</sub> F <sub>8</sub>
IRBL1-CL	<i>Pi1</i>	C101LAC	BC <sub>3</sub> F <sub>8</sub>
IRBL3-CP4	<i>Pi3</i>	C104PKT	BC <sub>3</sub> F <sub>8</sub>
IRBL5-M	<i>Pi5(t)</i>	RIL249 <sup>a</sup>	BC <sub>3</sub> F <sub>8</sub>
IRBL7-M	<i>Pi7(t)</i>	RIL29 <sup>a</sup>	BC <sub>3</sub> F <sub>8</sub>
IRBL9-W	<i>Pi9(t)</i>	WHD-IS-75-1-127	BC <sub>3</sub> F <sub>8</sub>
IRBL11-Zh	<i>Pi11(t)</i>	Zhaiyeqing 8	BC <sub>2</sub> F <sub>8</sub>
IRBL12-M	<i>Pi12(t)</i>	RIL10 <sup>a</sup>	BC <sub>2</sub> F <sub>8</sub>
IRBL19-A	<i>Pi19(t)</i>	Aichi Asahi	BC <sub>1</sub> F <sub>10</sub>
IRBL20-IR24	<i>Pi20</i>	ARL24 <sup>b</sup>	BC <sub>1</sub> F <sub>6</sub>

<sup>a</sup>Recombinant inbred lines from a cross of C039 and Moroberekan (Wang et al 1994).

<sup>b</sup>Recombinant inbred line from a cross of Asominori and IR24 (Tsunematsu et al 1996).

140 were selected. These lines were self-pollinated until they were morphologically uniform.

To develop other lines, inoculation and selection with suitable isolates were carried out in the same way as for Aichi Asahi. Some of the lines were also backcrossed to eliminate unnecessary genes. From the 25 resistant donors, a total of 29 lines with single genes were developed (Table 2). Monogenic homozygous lines for 23 known resistance genes were obtained. These lines were designated as IRBL, followed by the resistance gene and an abbreviation for the resistant donor. For example, IRBLa-A is the monogenic line with *Pia* developed from Aichi Asahi.

Fourteen genes for blast resistance—*Pia*, *Pii*, *Pik* (Yamasaki and Kiyosawa 1966), *Pita* (Kiyosawa 1966), *Pita-2* (Kiyosawa 1967a), *Piz* (Kiyosawa 1967b), *Pik-s* (Kiyosawa 1969a), *Pik-p* (Kiyosawa 1969b), *Pik-h* (Kiyosawa and Murty 1969), *Piz-t* (Yokoo and Kiyosawa 1970), *Pib* and *Pit* (Kiyosawa 1972), *Pik-m* (Kiyosawa 1978), and *Pish* (Imbe and Matsumoto 1985)—were identified in Japan. Monogenic lines with all of these genes except for *Pit* were developed. The monogenic line with *Pit* is under development. In accordance with the naming and symbolization of blast resistance genes, new blast resistance genes are designated

as *Pi* followed by a numeral. *Pi1* to *Pi20* have been designated (Kinoshita 1998). Monogenic lines with nine of the genes—*Pi1*, *Pi3*, *Pi5(t)*, *Pi7(t)*, *Pi9(t)*, *Pi11(t)*, *Pi12(t)*, *Pi19(t)*, and *Pi20*—were developed. Mackill and Bonman (1992) developed NILs of CO39 with *Pi1(t)*, *Pi2(t)*, *Pi3(t)*, *Pi4-a(t)*, and *Pi4-b(t)*. Inukai et al (1994) investigated the relationship between the resistance genes in the NILs of CO39 and the Japanese differentials. It was found that *Pi1* and *Pi3* were new genes. *Pi2(t)* was renamed as *Piz-5* because it was allelic to *Piz*. *Pi4-a(t)* was found to be identical to *Pita*. Wang et al (1994) identified and mapped *Pi5(t)* and *Pi7(t)* using recombinant inbred lines derived from the cross between CO39 and Moroberekan. Hayashi et al (1998) identified *Pi19(t)* in Aichi Asahi and Imbe et al (1997) reported *Pi20* in IR24. Although *Pi9(t)*, *Pi11(t)*, and *Pi12(t)* are not registered by the committee on rice gene symbolization of the Rice Genetics Cooperative, monogenic lines with these resistance genes were developed. Brar and Khush (1997) reported *Pi9(t)* in an introgression line of *Oryza minuta*. Zhu et al (1993) identified *Piz-h* in Zhaiyeqing 8 and it was renamed as *Pi11(t)* (Kinoshita et al 1994). Inukai et al (1996) reported *Pi12(t)* in the recombinant inbred line. The remaining resistance genes identified recently have not yet been incorporated into the monogenic lines. Monogenic resistant lines with only the major resistance genes were developed and genes conferring partial resistance were not considered in the development of monogenic lines.

The monogenic lines, their donors, and some of the differential varieties were inoculated with 12 representative blast isolates at IRRI (Table 3). Both IRBLsh-S from Shin 2 and IRBLsh-B from BL1 for *Pish* showed a moderately resistant reaction to the isolates used and the lines displayed a broad spectrum of resistance to the Philippine isolates. Other blast isolates tested so far also showed the same reactions to the gene. This result is contrary to that obtained with Japanese blast isolates; most of these were compatible to *Pish*. It is thus considered that *Pish* could be useful as a source of durable resistance to blast in the Philippines. IRBL19-A, which carries *Pi19(t)* from Aichi Asahi, showed a susceptible reaction to all of the isolates. So far, we have not found any Philippine blast isolates that are incompatible to *Pi19(t)*. *Pi19(t)* was identified in Aichi Asahi and other Japanese differentials against a blast isolate from Yunnan Province, China (Hayashi et al 1998). It was allelic or closely linked to the *Pita* locus.

IRBLk-Ka, IRBLkp-K60, IRBLkh-K3, IRBLkm-Ts, IRBL1-CL, and IRBL7-M showed almost the same reaction pattern to all the isolates used. Multiple alleles at the *Pik* locus (*Pik*, *Pik-p*, *Pik-h*, and *Pik-m*, except for *Pik-s*), *Pi1* and *Pi7(t)*, could not be differentiated using these isolates. Other isolates not listed in the table also showed the same reactions to the genes, and we have no isolate to distinguish these genes from each other at IRRI. Till distinguishable isolates are identified, these genes are designated *Pik\** in this report. The *Pi1* gene was closely linked to the *Pik* locus (Inukai et al 1994), and *Pi7(t)* was found to be allelic or

closely linked to *Pi1* (Wang et al 1994, Inukai et al 1996). Therefore, the origin of these genes was presumed to be the same.

IRBLi-F5 and IRBL3-CP4 showed almost the same reaction pattern to the isolates used. The isolates could not differentiate between *Pii* and *Pi3*. The *Pii* and *Pi3* genes were closely linked to each other (Inukai et al 1994). Therefore, the two genes should also be treated as one. The *Pi5(t)* gene showed a tendency similar to that of *Pii* and *Pi3* in terms of reaction to the isolates (Wang et al 1994, Inukai et al 1996). However, the line with *Pi5(t)* showed a higher resistance level than those with *Pii* and *Pi3*. We have to confirm the resistance pattern of the *Pi5(t)* gene by inoculating the NILs with *Pi5(t)* because we could not know whether the line possesses extra genes for a moderate reaction or whether *Pi5(t)* itself showed higher resistance. IRBL9-W, with *Pi9(t)* from WHD-IS-75-1-127, showed a resistant reaction to almost all the isolates used.

The reaction patterns of almost all of the donors and differential varieties could be explained on the basis of the reaction patterns of the monogenic lines or combination of the lines. However, resistant reactions of some of the donors were higher than expected. For example, BL1 has *Pib* (Yokoo et al 1978) and *Pish* (Imbe and Matsumoto 1985). It was used as a donor for these genes. IRBLb-B for *Pib* and IRBLsh-B for *Pish* were derived from BL1. The resistance pattern of BL1 was different from that expected from the combined resistance of *Pib* and *Pish*. BL1 was resistant to the isolates PO6-6, Ca89, BN111, M36-1-3-10-1, and V850196, whereas IRBLsh-B was moderately resistant to these isolates and IRBLb-B was susceptible, presumably because of complementation of *Pib* and *Pish*, or the presence of other genes in BL1.

RIL249 is one of the recombinant inbred lines with *Pi5(t)* derived from a cross between CO39 and Moroberekan (Wang et al 1994). RIL249 may also have inherited *Pia* from CO39. RIL249 was resistant to the isolates B90002 and C923-49, to which IRBL5-M was susceptible. IRBLA-C was resistant to these isolates. From the reaction patterns of RIL249, IRBL5-M, and IRBLA-C, we conclude that RIL249 has *Pia*. Resistant or moderately resistant reactions of RIL249 to the isolates BN209, M36-1-3-10-1, and M101-1-2-9-1 could not be explained by the combined reaction of *Pia* and *Pi5(t)*. The higher resistance of RIL249 can be ascribed to either complementation of *Pia* and *Pi5(t)* or to the presence of an additional gene or genes for resistance besides *Pia* and *Pi5(t)*.

Zhaiyeqing 8 is the donor of *Pi11(t)* in IRBL11-Zh (Zhu et al 1993). This donor showed a resistant or moderately resistant reaction to the isolates PO6-6, IK81-25, V850196, and M64-1-3-9-1, which were compatible with IRBL11-Zh. These results indicated that Zhaiyeqing 8 might harbor a resistance gene or genes in addition to *Pi11(t)*.

The monogenic lines we developed could be useful for studying the pathogenicity of blast isolates and this information could contribute to the elucidation of genetic as-

**Table 3. Reactions of monogenic lines, their donors, and differentials to selected Philippine blast isolates.**

Line/ variety	Resistance gene	Reaction to isolate <sup>a</sup>											
		1	2	3	4	5	6	7	8	9	10	11	12
IRBLa-A	<i>Pia</i>	S	S	S	S	S	S	R	R	S	S	S	S
IRBL19-A	<i>Pi19(t)</i>	S	S	S	S	S	S	S	S	S	S	S	MS
Aichi Asahi	<i>Pia, Pi19(t)</i>	S	S	S	S	S	S	R	R	S	S	S	S
IRBLa-C	<i>Pia</i>	S	S	S	S	S	S	R-M	R	S	S	S	S
CO39	<i>Pia</i>	S	S	S	S	S	S	R	R	S	S	S	MS
IRBLi-F5	<i>Pii</i>	M	S	R-M	S	R	S	S	S	R	S	S	S
IRBLks-F5	<i>Pik-s</i>	S	S	S	S	S	S	S	S	R	R	S	S
Fujisaka 5	<i>Pii, Pik-s</i>	R-M	S	R	S	R	S	MS	S	R	R	S	M-S
IRBLks-S	<i>Pik-s</i>	M	S	S	S	S	S	MS-S	S	R	R	S	S
IRBLsh-S	<i>Pish</i>	R-M	M-MS	R-M	R-M	R	R-M	R-M	M	M	R-M	R-M	M-MS
Shin 2	<i>Pik-s, Pish</i>	M	M	M	R-M	R	R	M	M-MS	R	R	M-MS	M
IRBLk-Ka	<i>Pik</i>	R	R	S	R	R	S	R	R	R	R	S	R
IRBLkp-K60	<i>Pik-p</i>	R	R	S	R	R	S	R	R	R	R	S	R
IRBLkh-K3	<i>Pik-h</i>	R	R	S	R	R	S	R	R	R	R	S	R
IRBLkm-Ts	<i>Pik-m</i>	R	R	S	R	R	S	R	R	R	R	S	R
Kanto 51	<i>Pik</i>	R	R	S	R	R	S	R	R	R	R	S	R
IRBLz-Fu	<i>Piz</i>	R-M	M	M	R-M	S	R	R-M	M	MS	M	M	MS
Fukunishiki	<i>Piz, Pish</i>	R	M	R	R	R	R	R	R	R	R-M	R-M	R
IRBLz5-CA	<i>Piz-5</i>	R-M	R-M	R-M	R-M	R	R	R-M	R	R-M	R-M	R-M	S
C101A51	<i>Piz-5 (Pia)</i>	R	R	M	R-M	R	R	R	R	R	R	R	M-MS
IRBLzt-T	<i>Piz-t</i>	S	S	R-M	S	S	S	R	R	S	R	R	S
Toride 1	<i>Piz-t (Pish)</i>	M	M	R	M	M	R-M	R	R	M	R	R	M
IRBLta-K1	<i>Pita</i>	S	S	R-M	M-S	S	S	R	S	R	S	R	S
IRBLta-CT2	<i>Pita</i>	S	S	MS-S	S	S	S	R	S	R	S	R	S
IRBLta-CP1	<i>Pita</i>	S	S	R	S	S	S	M	S	R	S	R-M	S
Yashiromochi	<i>Pita</i>	S	S	R	MS	S	R-M	R	S	R	S	R	S
IRBLta2-Pi	<i>Pita-2</i>	S	S	R	R	R	R	R	S	R	R	R	R
IRBLta2-Re	<i>Pita-2</i>	S	S	R	R	R	R	R	S	R	R	R	R
Reiho	<i>Pita-2, Pi a</i>	S	S	R	R	R	R	R	R	R	R	R	R
IRBLb-B	<i>Pib</i>	S	S	S	R	S	S	R	R	S	R-M	S	R
IRBLsh-B	<i>Pish</i>	M	M-S	M	M	M	M	M	S	M	R-M	MS	MS
BL1	<i>Pib, Pish</i>	R	R	R-M	R	R	R	R	R	R	R	M	R
IRBL1-CL	<i>Pi1</i>	R	R	S	R-M	R	S	R	R	R	R	S	R
C101LAC	<i>Pi1 (Pia)</i>	R	R	S	R	R	S	R	R	R	R	S	R
IRBL3-CP4	<i>Pi3</i>	R	S	R	S	R	S	S	S	R	S	S	MS
C104PKT	<i>Pi3 (Pia)</i>	R	S	M	S	R	MS-S	R	R	R	S	S	M-MS
IRBL5-M	<i>Pi5(t)</i>	S	S	R	S	R	S	M-MS	MS-S	R	MS-S	S	MS
RIL 249	<i>Pi5(t)</i>	MS-S	MS-S	R	R	R	M	R	R	R	MS	S	R
IRBL7-M	<i>Pi7(t)</i>	R	R	S	R	R	S	R	R	R	R	S	R
RIL 29	<i>Pi7(t)</i>	R	R	S	R	R	MS	R	R	R	R	S	R
IRBL9-W	<i>Pi9(t)</i>	R	R	M	R	R	R	R	R	R	R-M	R	R
WHD-IS-75-1-127	<i>Pi9(t)</i>	R	R	R-M	R	R	R	R	R	R-M	R	R-M	R
IRBL11-Zh	<i>Pi11(t)</i>	S	S	S	R	S	S	R-M	R	S	M	S	R
Zhaiyeqing 8	<i>Pi11(t)</i>	M	MS-S	R	R	MS	M-MS	R	R	R	R	R	R
IRBL12-M	<i>Pi12(t)</i>	S	S	S	R	S	S	R-M	R	S	MS-S	S	R
RIL 10	<i>Pi12(t)</i>	S	S	S	R	S	MS-S	R	R	M-MS	M	S	R
IRBL20-IR24	<i>Pi20</i>	S	S	S	S	R	R	R	R	S	R	S	S
ARL 20	<i>Pi20</i>	S	S	S	S	R	R	R	R	S	R	S	S

<sup>a</sup>1 = P06-6, 2 = Ca89, 3 = IK81-25, 4 = BN209, 5 = BN111, 6 = M36-1-3-10-1, 7 = B90002, 8 = C923-49, 9 = V850196, 10 = V86010, 11 = M64-1-3-9-1, 12 = M101-1-2-9-1.  
Reactions: R = resistant, M = moderately resistant, MS = moderately susceptible, S = susceptible.

pects of rice blast. A set of NILs is the most suitable material for race differentiation. We have begun developing NILs with single resistance genes. However, it may take time to establish them. A set of monogenic lines could be suitable for use as differentials for rice blast until a set of NILs is completed. These monogenic lines will be distributed as

candidates for blast differentials to scientists working on rice blast in collaboration with the International Network for Genetic Evaluation of Rice (INGER). Global applicability of the monogenic lines as differentials for blast will be evaluated through international collaboration.



## Studies on partial resistance to rice blast in the tropics

Partial resistance to blast in the tropics is not well studied for two reasons. First, most of the studies concerning partial resistance were carried out in Japan and published in Japanese journals (Yunoki et al 1970, Asaga 1981, Shindo and Asaga 1989, Higashi 1995, Kiyosawa and Ando 1997, Naito and Yaegashi 1997). Second, partial resistance of the variety must be evaluated under the conditions where the type and number of major genes for resistance are known. Until recently, information on the presence of major genes in tropical varieties was lacking. For example, the number of major resistance genes in IR36 was not known. In addition, the interaction of natural or purified blast isolates with major genes for resistance (pathogenicity) was not known in the tropics until recently. This information is necessary to overcome the effect of major resistance genes for evaluating partial resistance.

Ikehashi and Kiyosawa (1981) studied partial resistance of Japanese rice varieties against blast isolates from the tropics. They introduced Philippine blast isolates to Japan and studied the partial resistance of Japanese varieties against those isolates in the field. However, they could not eliminate the effect of *Pish*, which was not known at that time. *Pish* was reported by Imbe and Matsumoto (1985) and it is widely distributed among Japanese varieties. It showed moderate resistance against all blast isolates from the Philippines (Imbe et al 2000, Tsunematsu et al 2000). Bonman and Bandong (1989) studied partial resistance in the tropics. They equated moderately resistant reactions with partial resistance. However, moderately resistant reactions are not always equivalent to partial resistance (Imbe et al 2000). Wang et al (1994) also tried to identify partial resistance in the tropics. They used Moroberakan and CO39 recombinant inbred lines. Unfortunately, they could not obtain the blast isolates virulent against Moroberakan, which is supposed to possess several major resistance genes. Moreover, they used blast isolate PO6-6 to distinguish between plants with major genes for resistance (those with no disease symptoms) and partial resistance (those with moderately resistant reactions). However, the isolate they used showed moderately resistant reactions on varieties with *Pii*, *Pi3*, *Piz*, *Piz-5*, and *Pish* (Imbe et al 2000, Tsunematsu et al 2000). These moderately resistant reactions are due to major genes for resistance and the reactions are specific between PO6-6 and the major genes. As a result, Imbe et al (2000) and Tsunematsu et al (2000) could not clearly distinguish partial resistance from major gene resistance. Studies on durable blast resistance in a sequential planting experiment with 2- or 4-week seeding intervals were conducted at IRRI (IRRI 1994). Partial resistance could not be clearly distinguished from major gene resistance because information about blast population dynamics was lacking.

Imbe et al (2000) and Ebron et al (2004) recently identified major genes for resistance in IR varieties. At the same time, the pathogenicities of Philippine blast isolates were

determined against known major resistance genes (Imbe et al 2000, Tsunematsu et al 2000). These important breakthroughs enabled us to carry out our study successfully. By selecting suitable blast isolates, we could eliminate the effect of major resistance genes and evaluate the partial resistance of rice varieties. International differential lines with single resistance genes for rice blast were also developed (Tsunematsu et al 2000). We could monitor the pathogenicity of blast isolates in the field by using these lines. By using this information and materials, we tried to identify the partial resistance of IR varieties in the tropics.

## Materials and methods

### Inoculation and disease evaluation

The sporulation of blast and production of lesions on plants are necessary conditions for the evaluation of partial resistance because partial resistance reduces the extent of pathogen reproduction. We prepared the plots by surrounding them with 2-m-high plastic fences to prevent the migration of blast spores from other fields and natural populations (Fig. 1). These fences were also effective in diminishing wind velocity in the plots. A less windy condition favors the multiplication of blast spores. We planted spreader rows of susceptible varieties that were inoculated with blast isolates to encourage the multiplication and spread of disease to the test rows. One blast isolate was inoculated to each spreader row, which was the first row in a plot. Inoculation was done in the evening at the fourth-leaf stage by spraying 240 mL m<sup>-2</sup> spore suspension, adjusted to approximately 1 × 10<sup>5</sup> spores mL<sup>-1</sup>. During inoculation, plastic barriers between rows were installed to prevent the escape of blast spores to other rows. Inoculated rows were covered with plastic sheets until the next morning to maintain moisture on the leaves.

Two experiments were conducted at IRRI, Los Baños, Laguna, Philippines. Experiment I was conducted from January to March 1999, when the average temperatures were relatively low. Experiment II was conducted from October to December 1999. To reduce sunlight and keep high hu-

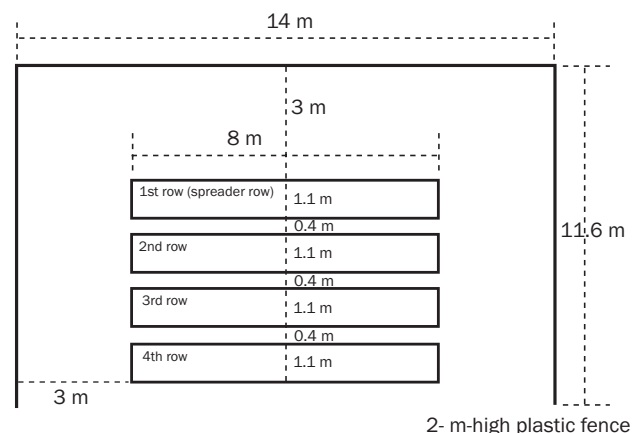


Fig. 1. Plot design of experiment I.

**Table 4. SES leaf blast scoring data for isolate BN111.**

Entry no.	Variety or breeding line	Scoring date					
		10 Feb	16 Feb	19 Feb	23 Feb <sup>a</sup>	26 Feb	2 Mar
Check	IR50	5	7	7	8	9	9
PR-1	IR5	5	5	6	6	6	6
Check	IR50	6	7	7	8	9	9
PR-2	IR8	2	4	4	5	5	5
Check	IR50	6	7	8	8	9	9
PR-3	IR20	6	6	7	7	7	8
Check	IR50	6	7	8	8	8	9

<sup>a</sup>The data on this day were used in this row. 0 = no lesions observed; 1 = small brown specks of pin-point size or larger brown specks; 2 = small roundish to slightly elongated, necrotic gray spots about 1–2 mm in diameter with a distinct brown margin. Lesions are mostly found on the lower leaves; 3 = lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves; 4 = typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area; 5 = typical blast lesions infecting 4–10% of the leaf area; 6 = typical blast lesions infecting 11–25% of the leaf area; 7 = typical blast lesions infecting 26–50% of the leaf area; 8 = typical blast lesions infecting 51–75% of the leaf area and many leaves dead; 9 = more than 75% of the leaf area affected.

midity in the plots, double-layered cheesecloth was installed at 2-m height above the plots. Sunlight penetration rates were 57.8% and 32.4% in experiments I and II, respectively. Seedlings were grown on raised beds like upland paddy fields. Plants were irrigated by using steel pipes with holes several times a day. The cheesecloth was made wet from 0800 to 0900 but we made sure blast spores were not washed off the leaves. Disease intensity was evaluated using the Standard Evaluation System (SES, IRRI 1996) for rice (Table 4).

### Experiment I

Seventy varieties and breeding lines were used in experiment I (Table 5), 34 of which are IRRI or Philippine Seed Board-released varieties. Twenty-four were differential lines with single genes for blast resistance (Tsunematsu et al 2000). By using these lines, we could confirm the pathogenicity of the inoculated blast isolates in the plots. CO39 is a blast-susceptible indica variety. IR49830-1-7-2-2 is an elite line suitable for rainfed lowland conditions. Ten japonica varieties and lines were also included. Each plot was inoculated with blast isolates PO6-6 and BN111 on 3 February 1999. Both of these isolates were collected in the Philippines and maintained at IRRI. The major genes for resistance of the materials used and the expected major resistance gene reactions are shown in Table 5 (Imbe et al 2000, Ebron et al 2004).

Each plot had four rows. All 70 varieties and breeding lines were sown with check variety IR50 alternately (Fig. 2). IR50 is susceptible to blast (Bonman and Mackill 1988). In five long horizontal rows, we seeded a mixture of IR52 and CO39 seeds. One hundred seeds were sown in a 30-cm slot about 3 cm deep. The seeding of the first row (spreader row) was done on 15 January 1999. The second to fourth rows were sown on 25 January. A total of 240 kg N ha<sup>-1</sup> was applied in four split doses at 10-d intervals starting from the day of seeding. An additional 80 kg of ammonium sulfate ha<sup>-1</sup> was applied to the second to fourth rows on 24 Febru-

ary. Leaf blast disease was evaluated twice a week from 10 February to 9 March using the SES. Blast-damaged areas on the third leaf of six plants of each variety were measured. Sampling was done on the first row on 16 February and on the second to fourth rows on 1 March.

### Experiment II

Fifty varieties and lines were used in experiment II (Table 5). IR64 and US-2 were additional entries. IR64 has *Pita*, *Pi20*, *Piz-t*, *Pib*, and *Pik-s* major genes for resistance. US-2 is a japonica susceptible line with no major resistance gene. Blast isolates PO6-6 and M36-1-3-10-1 (M36) were used for inoculating each plot. M36 was collected from the Philippines and stored in the Entomology and Plant Pathology Division at IRRI.

Each plot had four rows. All 50 varieties and breeding lines were sown with check variety IR50 (Fig. 3). The row ratio of IR50 to test entries was 1 to 2 in this experiment. We also seeded IR50 in five long horizontal rows. One hundred seeds were sown in a 30-cm seeding slot about 3 cm deep, the same as in experiment I. Seeding of the first row (spreader row) was done on 27 October 1999. The second to fourth rows were sown on 3 November. A total of 320 kg N ha<sup>-1</sup> was applied in four split doses at 10-d intervals starting from the day of seeding. Leaf blast disease was evaluated twice a week using the SES, from 6 to 20 December. The damaged area on the third leaf of six plants of each variety was measured. Sampling was done on the first row on 15 December.

## Results

### Experiment I

The spreader rows were inoculated on 3 February 1999. The first lesions were observed 5 d after inoculation or on 8 February. Six days later, or on 14 February, lesions were observed on the second to fourth rows, where artificial inoculation was not done. Lesions were observed on the same days in both plots inoculated with different blast isolates.

**Table 5. Partial resistance of IR varieties and monogenic lines.<sup>a</sup>**

Variety or breeding line	Estimated major resistance gene	Feb 1999		Dec 1999		Av	Ranking of partial resistance
		P06-6	BN111 <sup>b</sup>	P06-6 <sup>b</sup>	M36 <sup>b</sup>		
IR5	ta,b	1.75	1.76	1.40	0.87	1.45	6
IR8	20,b,k-s	1.50	<b>R</b>	1.40	<b>R</b>	1.45	5
IR20	b,k-s	0.00	-0.05	-	-	-0.03	41
IR22	20,b,k-s	0.25	<b>R</b>	-	-	0.25	36
IR24	20,b,k-s,a	1.38	<b>R</b>	0.76	<b>R</b>	1.07	11
IR26	20,b,k-s	2.00	<b>R</b>	1.63	<b>R</b>	1.82	1
IR29	z-t,b,k-s	1.50	0.86	-0.05	-0.01	0.57	27
IR30	b,k-s	-0.38	-0.83	0.07	0.33	-0.20	44
IR32	ta,b	0.25	0.08	-	-	0.16	38
IR34	z-t,b,k-s,a,?	0.75	0.21	0.82	0.37	0.54	28
IR36	ta,z-t,b,k-s,?	0.63	0.21	0.24	0.08	0.29	35
IR38	ta,b	0.50	-0.18	-	-	0.16	39
IR40	ta,b	0.63	0.60	-	-	0.61	25
IR42	ta,b	0.75	1.24	0.65	0.33	0.74	21
IR43	20,b	1.63	<b>R</b>	1.75	<b>R</b>	1.69	2
IR44	ta,b	0.00	0.60	-	-	0.30	34
IR46	ta,20,b,k-s,?	0.88	<b>R</b>	0.99	<b>R</b>	0.93	15
IR48	ta,20,b	0.88	<b>R</b>	-	-	0.88	16
IR50	ta,b	-0.13	-1.22	0.01	0.05	-0.32	46
IR52	ta,b	0.38	0.99	-	-	0.68	23
IR54	ta,b,?	<b>M</b>	<b>M</b>	-	-	-	-
IR56	ta,k*	<b>R</b>	<b>R</b>	-	-	-	-
IR58	ta,b	1.50	0.99	0.47	0.02	0.74	20
IR60	ta,z-t,b,k-s,?	0.50	0.99	0.07	0.27	0.46	31
IR62	ta,b	0.63	0.47	0.59	0.21	0.47	30
IR64	20,ta,z-t,b,k-s,?	-	-	1.25	<b>R</b>	1.25	7
IR70	ta,k*	<b>R</b>	<b>R</b>	-	-	-	-
IR72	ta,b	0.75	-0.44	0.70	0.40	0.35	33
IR74	20,k*,b,a	<b>R</b>	<b>R</b>	-	-	-	-
PSBRc 1	k*	<b>R</b>	<b>R</b>	-	-	-	-
PSBRc 2	20,b,k-s,a,z-t,?	<b>R</b>	<b>R</b>	-	-	-	-
PSBRc 4	ta,b,?	0.50	<b>M</b>	1.17	0.90	0.86	17
PSBRc 10	20,ta,i,b	<b>M</b>	<b>R</b>	-	-	-	-
PSBRc 18	20,ta,i,b	<b>M</b>	<b>R</b>	-	-	-	-
PSBRc 20	20,ta,i,b	<b>M</b>	<b>R</b>	-	-	-	-
IRBLa-A	a	0.63	1.50	0.13	0.78	0.76	18
IRBLa-C	a	-0.5	-0.31	-0.45	0.24	-0.26	45
IRBLi-F5	i	<b>M</b>	<b>M</b>	<b>M</b>	<b>M</b>	-	-
IRBLks-F5	k-s	1.00	0.60	0.24	0.15	0.50	29
IRBLks-S	k-s,?	<b>M</b>	<b>M</b>	<b>M</b>	0.71	0.71	22
IRBLk-ka	k	<b>R</b>	<b>R</b>	<b>R</b>	0.75	0.75	19
IRBLkp-K60	k-p	<b>R</b>	<b>R</b>	<b>R</b>	1.25	1.25	8
IRBLkh-K3	k-h	<b>R</b>	<b>R</b>	<b>R</b>	1.13	1.13	9
IRBLz-Fu	z	<b>M</b>	<b>M</b>	<b>M</b>	<b>M</b>	-	-
IRBLz5-CA	z-5	<b>M</b>	<b>R</b>	<b>M</b>	<b>M</b>	-	-
IRBLzt-T	z-t	-0.38	-0.31	0.18	0.27	-0.06	42
IRBLta-K1	ta	1.75	2.04	1.28	1.13	1.55	4
IRBLta-CT2	ta	-1.00	-1.35	-0.57	0.33	-0.65	49
IRBLb-B	b	-0.25	0.86	0.99	0.81	0.60	26
IRBLt-K59	+	0.63	1.37	1.05	0.71	0.94	14
IRBLsh-S	sh	<b>M</b>	<b>M</b>	<b>M</b>	<b>M</b>	-	-
IRBLsh-B	sh	<b>M</b>	<b>M</b>	<b>M</b>	<b>M</b>	-	-
IRBL1-CL	1	<b>R</b>	<b>R</b>	<b>R</b>	0.97	0.97	13
IRBL3-CP4	3	<b>M</b>	<b>M</b>	<b>M</b>	<b>M</b>	-	-
IRBL5-M	5(t)	<b>R</b>	<b>R</b>	<b>R</b>	<b>M</b>	-	-
IRBL7-M	7(t)	<b>R</b>	<b>R</b>	<b>R</b>	0.08	0.08	40
IRBL9-W	9(t)	<b>R</b>	<b>R</b>	<b>R</b>	<b>R</b>	-	-
IRBL12-M	12(t)	-1.00	0.08	-0.63	-0.17	-0.43	47
IRBL19-A	19(t)	0.75	0.99	0.30	0.43	0.62	24
CO39	a	-1.5	-2.5	-0.28	-0.08	-1.09	50

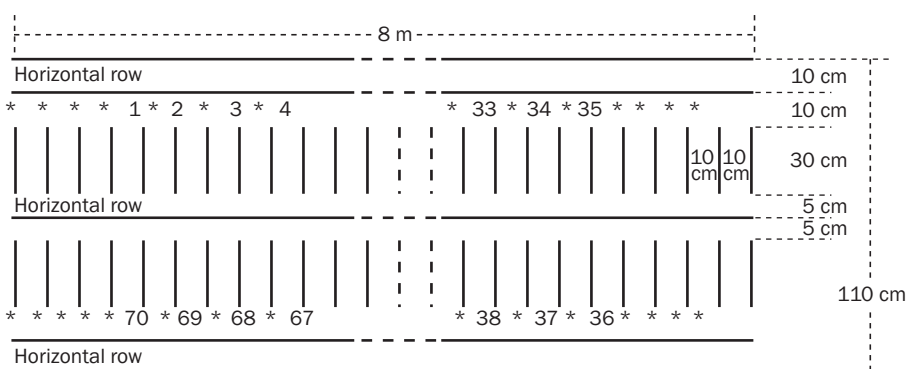
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**Table 5 continued.**

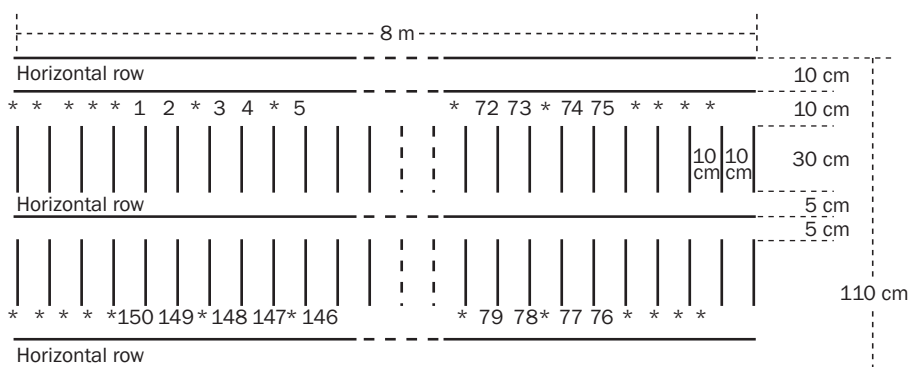
Variety or breeding line	Estimated major resistance gene	Feb 1999		Dec 1999		Av	Ranking of partial resistance
		P06-6	BN111 <sup>b</sup>	P06-6 <sup>b</sup>	M36 <sup>b</sup>		
IR49830-7-1-2-2	k-s,b	0.00	-0.05	0.59	0.46	0.25	37
LTH	+	0.25	0.73	0.36	0.46	0.45	32
US-2	+	-	-	-1.15	0.08	-0.53	48
Aichi Asahi	a,19(t)	0.00	-0.31	0.01	0.02	-0.70	43
Fujisaka 5	i,k-s	<b>M</b>	<b>M</b>	-	-	-	-
Kanto 51	k	<b>R</b>	<b>R</b>	-	-	-	-
Tsuyuake	k-m,sh	<b>R</b>	<b>R</b>	-	-	-	-
Yashiromochi	ta,?	0.88	<b>M</b>	1.34	<b>M</b>	1.11	10
Reiho	ta-2,a	1.13	<b>R</b>	0.99	<b>R</b>	1.06	12
K59	t,k-s	1.13	2.28	1.57	1.28	1.57	3
Norin 6	k	<b>R</b>	<b>R</b>	-	-	-	-
Zenith	z,a	<b>M</b>	<b>M</b>	-	-	-	-
LSD (5%)		1.06					

<sup>a</sup>R = resistant, M = moderately resistant. - = evaluation not done.

<sup>b</sup>Levels of partial resistance were transformed to equivalent values for P06-6 in February by the regression formulas.



**Fig. 2. Row pattern used in the blast nursery of experiment I. \* = check variety IR50.**



**Fig. 3. Row pattern used in the blast nursery of experiment II. \* = check variety IR50.**

The period from conidia attachment to the formation of lesions was about 6 days in this experiment. Kato and Kozaka (1974) reported that at 25–32 °C, the number of conidia per lesion was highest on the third day after the appearance of lesions. The first peak of sporulation was estimated on 10 February and the second one on 16 February. The clearest differences in blast damage between susceptible check IR50 and the test materials were observed from late February to early March. We analyzed the data from that period. The cycles of blast sporulation were considered to be third to fourth generations at that time. Most of the seedlings of IR50 died by early March.

A part of the SES-based leaf blast scores are shown in Table 4. The control and the test materials got blast damage gradually. We chose the scores of 23 February for blast damage estimation of each entry in this row. Resistance level was calculated as the difference between the blast scores of each variety and the adjacent IR50 check. For example, the resistance level of IR5 was 2, which was calculated as follows:  $(8 + 8)/2 - 6 = 2$ . We measured blast damage based on damaged leaf area using the SES. The correlation coefficient between the percent blast-damaged leaf area actually measured and SES scores was 0.811 and highly significant. We used 578 pairs of data for this calculation.

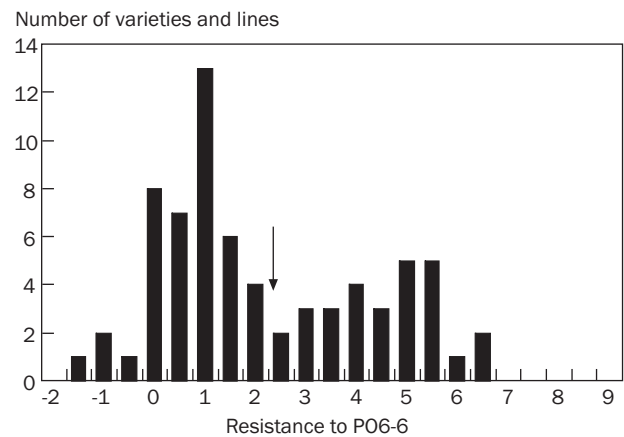
The distribution of resistance to isolate PO6-6 is shown in Figure 4. The lowest score was -1.5 (CO39) and the highest was 6.4 (PSBRc10). We sorted out partial resistance from major gene resistance. According to our results, all the entries distributed on the right-hand side of the arrow had incompatible major resistance genes for PO6-6. Among them, the lowest resistance level was 2.1 (Norin 6 with incompatible gene *Pik*). There were no known blast-incompatible major resistance genes in all the other varieties and lines on the left-hand side of the arrow. The highest score (2.0) was seen in IR26 and the average was 0.54.

The lowest score for isolate BN111 was -1.4 (CO39) and the highest was 7.9 (IR74). The distribution of major gene and partial resistance did not overlap (data not shown). All the entries on the right-hand side of the distribution had incompatible major resistance genes for BN111. The lowest resistance level among them was 3.3 (IRBLks-S with unknown gene). There were no known blast-incompatible major resistance genes in all the other varieties and lines. Their average score was 1.41. K59 had the highest score (3.3).

The pathogenicities of blast isolates used were monitored throughout the period by the evaluation of IRBL lines with single genes for resistance. They were consistent through the evaluation (data not shown).

## Experiment II

The spreader rows were inoculated on 12 November 1999. The first lesions were observed on 16 November or 4 d after inoculation. Second-generation lesions were observed on 23 November on the second to fourth rows. The time course of blast infection was almost the same as that in experiment I. However, growth of seedlings in the spreader rows was not



**Fig. 4. Distribution of resistance to rice blast isolate PO6-6, February 1999.**

good and blast infection in all the rows was less than that in experiment I. Differences in blast damage based on SES scores were most distinct between susceptible check variety IR50 and the test entries observed on 10 and 20 December. At that time, blast sporulation cycles were considered at the fourth to sixth generations.

The correlation coefficient between the percent blast-damaged leaf area actually measured and SES scores was 0.678 and highly significant. We used the data from 73 varieties and lines for this calculation. The lowest score for isolate PO6-6 was -2.2 (US-2) and the highest was 6.9 (IRBL3-CP4). The distribution of major gene resistance and partial resistance overlapped slightly (data not shown). The lowest score for major gene resistance was 1.3 (IRBL7-M with *Pi7(t)*). Among varieties and lines without incompatible resistance genes, 3.3 (IR43) was the highest score and their average was 1.04. The lowest score for isolate M36 was -0.6 (IRBL12-M) and the highest was 8.3 (IRBL5-M). Major gene and partial resistance overlapped slightly (data not shown). The lowest score of major gene resistance was 3.8 (IR8 with *Pi20*). Among varieties and lines without incompatible resistance genes, 4.56 (K59) was the highest score and their average was 1.66.

The pathogenicities of blast isolates used were monitored throughout the period by the evaluation of IRBL lines. They were consistent through the evaluation (data not shown).

## Discussion

Partial resistance to rice blast is not well studied in the tropics because the pathogenicity of blast isolates in the tropics was not well known until recently. Without sufficient knowledge about pathogenicity, it is impossible to distinguish between partial resistance and major gene resistance. In this study, moderate levels of resistance governed by major genes showed a higher resistance level than partial resistance, even though a limited level of overlapping was observed. Wang et al (1994) used blast isolate PO6-6 to distinguish major



gene resistance from partial resistance. The results of our study indicated that their supposed partial resistance based on a moderate level of resistance was different from partial resistance. In addition, the isolate they used showed moderately resistant reactions against *Pii*, *Pi3*, *Piz*, *Piz-5*, and *Pish* (Imbe et al 2000, Tsunematsu et al 2000). Those moderate reactions were in fact due to major genes and the resistance of these genes to the blast isolates was specific. Thus, ours is the first report to clearly distinguish partial resistance from major gene resistance in the tropics.

The highest SES score of partial resistance observed in this study was 4.56 of K59 against M36. Almost all the varieties with blast-compatible reactions died in experiment I, even though they showed the highest level of partial resistance. On the other hand, plants with major gene resistance had almost no blast damage. However, under field conditions, even a low level of partial resistance is expected to be more effective than in this study. In a commercial field when a partially resistant variety is cultivated, the density of blast spores is much less than in this experiment because there is no surrounding susceptible variety such as IR50 as in this experiment. Newly introduced major genes for resistance were easily broken down within several years of intensive cultivation in blast-prone areas in Japan (Fujita 1997) and Korea (Chang 1994). Partial resistance is considered effective and indispensable for reducing the use of fungicides for blast control in Japan. To evaluate partial resistance of each variety or breeding line, major gene resistance is often considered as an obstacle.

Partial resistance, also known as horizontal resistance, is expressed against all compatible blast isolates (Yunoki et al 1970, Asaga 1981). A positive and highly significant relationship was observed between partial resistance to blast isolates PO6-6 and BN111 in a February 1999 experiment ( $r = 0.780^{**}$ ). For this calculation, we selected 32 varieties or lines that are compatible to both isolates. In the rest that have major genes for resistance, there was no significant correlation between the resistance to PO6-6 and BN111 ( $r = 0.277$ ). This is because major genes confer vertical resistance. In this study, partial resistance was horizontal as elaborated by Asaga (1981) in Japan. Our results are different from those of Ou (1979), who reported that partial resistance of Japanese varieties was vertical. His results were obtained without knowledge about the existence of the major gene *Pish* in Japanese varieties.

Correlation coefficients among partial resistance to different blast isolates and seasons are shown in Table 5. For this calculation, we selected 24 varieties or lines that are compatible to three isolates. Among the three blast isolates over two seasons, we always observed significant positive correlations. These results strongly indicate that partial resistance is also horizontal in the tropics. The correlation coefficients were relatively high in the same season ( $0.79^{**}$  and  $0.78^{**}$ ). These correlation coefficients were even higher than that of the same isolate for different seasons, which was  $0.68^{**}$ . These results suggest that partial resistance may be slightly modified by seasonal and varietal factors.

**Table 6. Correlation coefficients among partial resistance to different blast isolates over two seasons.**

Date	Isolate	Feb 99			Dec 99		
		BN111	PO6-6	M36	BN111	PO6-6	M36
Feb	PO6-6	0.79**	0.68**	0.43*			
Feb	BN111		0.69**	0.66**			
Dec	PO6-6			0.78**			

There were significant positive correlations between partial resistance for different seasons and isolates, but there were large differences among their averages. By using regression equations, we transformed the partial resistance to M36, BN111, and PO6-6 (December 1999 experiment) to the equivalent of the partial resistance to PO6-6 in the February 1999 experiment (Table 6).

Durable blast resistance was studied at IRRI in sequential planting (IRRI 1994). Even though information about blast population dynamics was lacking, IR64 was reported to have lower disease severity in all 15 sequential crop cycles. Intermediate levels of infection on IR36 and IR60 were maintained throughout the whole duration of planting. CO39 and IR50 had higher disease severity. These observations were quite consistent with the results of this study. Among the 50 varieties and lines studied, IR64 ranked 7th in level of partial resistance, and IR60 and IR36 were 31st and 35th, respectively. IR50 and CO39 were 46th and were the lowest. Aichi Asahi is known to have very low partial resistance in Japan and so does US-2 (Hayashi, personal communication). Yashiromochi is also ranked “low” and Reiho is ranked “slightly low” for partial resistance. The ranking of Yashiromochi and Reiho for partial resistance in Japan is not completely consistent with the results of this study. However, the difference is small. We conclude that the results of the present study are quite consistent with partial resistance evaluations done in Japan in the past.

Wang et al (1994) reported that more than 80% diseased leaf area of CO39 was observed in the Philippines. Bonman and Bandong (1989) reported slower disease progress in the Philippines than in Korea. Induction of serious blast infection is not always possible in the Philippines. In experiment I, a very serious blast infection was induced and most of the susceptible plants died within 1 mo after inoculation. This success could be attributed to the special shading and irrigation method used and to the application of a large amount of fertilizer. On the other hand, blast infection in experiment II was not so severe. There are three possible explanations for this. First, the temperature was higher during experiment II than during experiment I. February is one of the coolest months in Laguna, Philippines. Cool weather is favorable for blast sporulation (Kato and Kozaka 1974). Second, the shading in experiment II was too strong to induce good blast infection. The light penetration in experiment II was almost half that in experiment I. Third, seedling growth in the spreader rows was not so

good and blast sporulation was affected by smaller leaf development of the spreader rows.

Disease score was recorded following the SES and the percentage of damaged leaf area. The correlation coefficients were high and significant. However, many points were distributed far from the regression equations (scatter diagram not shown). This could be the result of a too small sampling size of the damaged leaf area. We took six plants and measured the leaf area affected of the third leaf of each plant only. These measurements required much labor even with a small number of plants. In the case of the SES evaluation, we evaluated around 50–80 plants per variety at one glance. This evaluation was quite easy. Moreover, SES evaluation results were quite reliable, even though assessments were done on different days.

### Strategy for breeding rice varieties with durable resistance to blast

Newly introduced major genes for blast resistance were easily broken down within several years of intensive cultivation in blast-prone areas such as those in Japan (Yamada 1965) and Korea (Chang 1994). We consider that this phenomenon is inevitable when using accumulations or pyramidings of major genes. To avoid this risk, it is important to evaluate the partial resistance of varieties. To evaluate them in each country, the following steps are needed. Our study has shown that this can be done.

1. Collecting and stocking as many as a few hundred indigenous blast isolates.
2. Evaluating their pathogenicities by using monogenic lines.
3. Reconstructing monogenic lines in each country. As shown in this report, *Pish* and *Pik-s* in Japanese differential varieties are not very effective in Japan, but they are incompatible in the Philippines. Our monogenic lines may have new unknown genes in them against blast races in a newly introduced area. If they do, reconstructing of monogenic lines is necessary.
4. Studying the major genes in major varieties by using cross combinations among monogenic lines and major varieties (Toriyama et al 1983).
5. Evaluating partial resistance of major varieties and breeding lines.

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## Notes

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# Genetic analysis of blast resistance genes in elite indica-type rice

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Seven resistance genes, *Pia*, *Pib*, *Pik-s*, *Pi-zt*, *Pi20*, *Pita*, and one other *Pik* allele, were identified in International Rice Research Institute (IRRI)-bred rice (*Oryza sativa* L.) varieties by genetic analyses based on the differential system using isolates of the blast fungus *Pyricularia grisea* (Cooke) Sacc. from the Philippines. Segregation analysis using BC<sub>1</sub>F<sub>2</sub> populations derived from crosses of these varieties with a susceptible indica-type line, CO39, and allelism tests using F<sub>2</sub> hybrid populations from crosses of IRRI varieties with differential varieties having known resistance genes was carried out with nine varieties (IR34, IR24, IR36, IR60, PSBRc1, IR74, IR56, IR70, and IR64) and six varieties (IR34, IR36, IR60, IR74, IR46, and IR64), respectively. Selected blast isolates with known avirulence were used in these analyses, and the genotype in each variety was identified. Among the genes identified, two, *Pib* and one *Pik* allele, were detected in almost all the IRRI varieties used. New genes, which were not estimated based on the reaction patterns of blast isolates due to the masking effect of *Pita*, *Pik-h*, and *Pi20* in a previous analysis, were also identified. We could confirm the genotypes of resistance genes in IRRI-bred lines and demonstrate the efficiencies of genetic analysis for blast resistance genes based on the differential system according to the gene-for-gene theory.

In rice (*Oryza sativa* L.), genetic analysis for blast has been governed based on the differential system according to the gene-for-gene theory between the host and the fungus, *Pyricularia grisea* (Cooke) Sacc. (Kiyosawa 1972, Silue et al 1992). Genetic studies performed in this context led to the identification of many blast resistance genes. In the tropics, genetic studies on blast resistance in indica-type varieties were limited. The extremely changeable virulence of the blast fungus (Ou 1985, Bonman et al 1986) and the presence of several resistance genes in indica-type cultivars (Mackill et al 1985, Yu et al 1987) make genetic studies of blast resistance genes difficult.

The limitation in genetic studies of blast resistance could also be attributed to the lack of a differential system for blast resistance genes in the tropical zone. Yamada et al (1976) and Kiyosawa (1984) selected 12 japonica-type differential varieties for blast resistance, and these were used to develop the differential system. These differential varieties were not well adapted for the blast isolates in the tropical zone because these have an additional gene or genes, *Pish* or other, which masked the target gene's reaction (Noda et al 1999). Yanoria et al (2000) tried to clarify the pathogenicities of blast isolates from the Philippines using Japanese differential varieties (Yamada et al 1976, Kiyosawa

1984). Among these blast isolates, several with distinct pathogenicities were selected and studied in detail by Tsunematsu et al (2000) using a set of monogenic lines, which have a single resistance gene in each genetic background. Using this new system, Ebron et al (submitted) could estimate the genotypes of 42 IRRI-bred rice varieties, and found a total of seven resistance genes, *Pi20*, *Pita*, *Pik-h*, *Pib*, *Pik-s*, *Piz-t*, and *Pii* or *Pi3(t)*, included in them. The number of resistance genes varied from one to four among the IRRI varieties, and these were classified into seven variety groups (VG) based mainly on the presence of *Pi20*, *Pita*, and one *Pik* allele. In the pathogenicity analysis for blast isolates from the Philippines, *Pik-s* could be distinguished from the other four *Pik* alleles, *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*, but available isolates have not yet been found to classify these four alleles (Tsunematsu et al 2000, Yanoria et al 2000). However, Ebron et al (submitted) estimated that the allele might be *Pik-h* based on the pedigree research and genotypic information of the parental lines. Among these genes estimated, two, *Pib* and one *Pik* allele (*Pik-s* or *Pik<sup>+</sup>*), were common in almost all IRRI varieties. Because of the masking effect of *Pi20*, *Pita*, and *Pik* alleles, the presence of the other genes might not be expected in these varieties.

Toriyama et al (1986) demonstrated that the estimation

of genes with resistance to blast in rice varieties by using segregation analysis with the hybrid population derived from a cross between resistant and susceptible varieties was effective. Genetic analysis based on gene segregation including the allelism test with a differential variety is necessary to clarify in detail genotypes in each variety, which were estimated by Ebron et al (n.d.). The present study was therefore undertaken to confirm the genes previously estimated or masked by reaction pattern analysis based on the differential system.

## Materials and methods

### Segregation analysis using a hybrid population derived from the cross between an IRR1 variety and a susceptible variety

Six IRR1 varieties, IR34 (VG 1b), IR36 and IR60 (VG 3), IR74 (VG 5), and IR46 and IR64 (VG 7a), previously classified by patterns of reaction to blast isolates from the Philippines (Ebron et al, submitted), were backcrossed with a susceptible indica-type variety, CO39, as the recurrent parent.

The BC<sub>1</sub>F<sub>2</sub> populations, consisting of 53 to 140 lines derived from different cross combinations, were inoculated with selected blast isolates avirulent to the expected genes. Following the procedure of Toriyama et al (1986), the number of genes conferring resistance in a variety was estimated from the segregation of BC<sub>1</sub>F<sub>2</sub> lines for each blast isolate. Three segregation ratios, 1:1, 3:1, and 7:1, between heterozygously resistant and homozygously susceptible lines indicated one, two, and three genes controlling each resistance. Each BC<sub>1</sub>F<sub>2</sub> line was analyzed for its pattern of reaction to the blast isolates and the kind of gene conferring the resistance was identified.

### Allelism tests using a differential variety

Nine IRR1 rice cultivars, IR34 (VG 1b), IR24 (VG 2b), IR36 and IR60 (VG 3), PSBRc1 (VG 4), IR74 (VG 5), IR56 and IR70 (VG 6), and IR64 (VG 7b), were crossed with differential varieties, lines, and IRR1 cultivars, which already had a known blast resistance gene.

F<sub>2</sub> populations derived from each cross combination were used to identify the genes. Selected blast isolates avirulent to these genes were inoculated to the seedlings of the same populations. The differential varieties Toride 1 for *Piz-t*; BL1 for *Pib*; Yashiromochi, C105TTP2L9, and C101PKT for *Pita*; Tsuyuake for *Pik-m* and Kanto 51 for *Pik*; and IR24 for three genes, *Pib*, *Pik-s*, and *Pi20*, were used. The allelic relationship of the genes was determined based on the segregation of the F<sub>2</sub> progenies of a cross to a particular blast isolate. If the segregation did not include susceptible plants, it was assumed that the same allele was included in both parental varieties.

### Inoculation and evaluation

Pregerminated seeds of each population were sown in a 26 × 35-cm plastic tray. Ten grams of ammonium sulfate was applied to each tray as basal fertilizer and 1 g was added 1

week before inoculation. In all cases, susceptible cultivars CO39 and Lijiangxintuanheigu (LTH) were included in each tray to check the success of inoculation and the virulence of the blast isolates used.

Several blast isolates with known avirulence (Yanoria et al 2000) were used for inoculation. Seedlings at the fourth-leaf stage were sprayed with 40–50 mL of spore suspension adjusted to 10<sup>5</sup> spores mL<sup>-1</sup> in each tray. Trays were placed inside wet jute sacks for 18–24 hours, and transferred to an air-conditioned glasshouse room with 23 ± 3/30 ± 5 °C night and day temperature. Blast incidence on each seedling was examined 6–7 days after inoculation. The reaction was classified on a 0–5 scale with slight modifications as described previously by Mackill and Bonman (1992). Seedlings rated 0–2 were considered resistant (R), 3– moderately resistant (MR), 3+ moderately susceptible (MS), and 4–5+ susceptible (S).

## Results

### Segregation analysis of the BC<sub>1</sub>F<sub>2</sub> population

A total of six varieties were investigated for segregation in BC<sub>1</sub>F<sub>2</sub> populations, and the genotype of the resistance genes was identified (Table 1).

In the IR34 population, a total of 104 BC<sub>1</sub>F<sub>2</sub> lines were examined for segregation to five isolates, M39-1-3-8-1, M64-1-3-9-1, BN209, V850196, and B90002. Against M39-1-3-8-1 and M64-1-3-9-1, the lines segregated into 48 heterozygous-resistant and 56 homozygous-susceptible lines, and a co-segregation for resistance was recognized between these isolates. The segregation fitted the 1:1 ratio for single dominant gene control. Both isolates were avirulent to *Piz-t* and *Pita*, but IR34 in VG 1 was estimated to not have *Pita*. These results indicated that the segregation for resistance was governed by *Piz-t*. To isolate BN209, the segregation of heterozygous-resistant and homozygous-susceptible lines fitted to a 3:1 ratio, suggesting that two dominant genes controlled the resistance. Although one of the genes was estimated as *Pib*, because this gene was incompatible with BN209, the other one could not be identified based on the pattern of reaction to blast isolates. It was also found that the resistance to isolate V850196 was controlled by two dominant genes. One of these genes was estimated as *Pik-s*, but the other one could not be identified. Using isolate B90002 avirulent to *Pia*, which could not be estimated directly under the presence of *Pi20*, *Pita*, or *Pik<sup>t</sup>*, we examined the segregation, and all lines showed resistance. Tsunematsu et al (2000) indicated that CO39 had *Pia*. These results suggested that IR34 was also confirmed to have *Pia*. Thus, IR34 has *Pia*, *Pib*, *Pik-s*, *Piz-t*, and at least one unknown gene.

IR36 and IR60 were analyzed in VG 3 for three genes, *Pita*, *Pib*, and *Pik-s*, that were not previously estimated by reaction pattern analysis. The 61 BC<sub>1</sub>F<sub>2</sub> lines showed a single dominant gene segregation to IK81-3, which was avirulent to *Pita*. A segregation controlled by two dominant genes was shown to isolate BN209, which is avirulent to two resis-

**Table 1. Segregation in BC<sub>1</sub>F<sub>2</sub> population derived from the cross between an IRRI-bred variety and a susceptible variety, CO39, as the recurrent parent.**

Cultivar (group)	Isolate	No. of lines				$\chi^2$ value			P (df = 1)	Estimated gene
		RR <sup>a</sup>	R-	-	Total	1:1 <sup>b</sup>	3:1	7:1		
IR34 (VG 1b)	M39-1-3-8-1	-	48	56	104	0.62 <sup>a</sup>	-	-	0.43	<i>Piz-t</i>
	M64-1-3-9-1	-	48	56	104	0.62 <sup>a</sup>	-	-	0.43	<i>Piz-t</i>
	BN209	-	79	25	104	-	0.02	-	0.89	<i>Pib</i> , one unknown
	V850196	-	77	27	104	-	0.02	-	0.89	<i>Pik-s</i> , one unknown
	B90002	104	-	-	104	-	-	-	-	<i>Pia</i>
IR36 (VG 3)	IK81-3	-	35	26	61	1.32	-	-	0.25	<i>Pita</i>
	BN209	-	45	16	61	-	0.05	-	0.82	<i>Pib</i> , one unknown
	V850196	-	47	14	61	-	0.13	-	0.72	<i>Pita</i> , <i>Pik-s</i>
	IK81-3	-	36	17	53	-	1.41	-	0.24	<i>Pita</i> , one unknown
IR60 (VG 3)	M64-1-3-9-1	-	40	13	53	-	0.02	-	0.89	<i>Pita</i> , <i>Piz-t</i>
	BN209	-	48	5	53	-	-	0.46	0.50	<i>Pib</i> , two unknown
	V850196	-	37	16	53	-	0.76	-	0.38	<i>Pita</i> , <i>Pik-s</i>
IR74 (VG 5)	PO6-6	-	34	42	76	0.84 <sup>b</sup>	-	-	0.36	<i>Pik</i> <sup>†</sup> , <i>Pi20</i>
	Ca89	-	34	42	76	0.84 <sup>b</sup>	-	-	0.36	<i>Pik</i> <sup>†</sup> , <i>Pi20</i>
	M36-1-3-10-1	-	36	40	76	0.22	-	-	0.64	<i>Pi20</i>
	BN111	-	51	25	76	-	2.52	-	0.11	<i>Pik</i> <sup>†</sup> , <i>Pi20</i>
	BN209	-	54	22	76	-	0.63	-	0.43	<i>Pib</i> , <i>Pik</i> <sup>†</sup>
B90002	76	-	-	76	-	-	-	-	<i>Pia</i>	
IR46 (VG 7a)	IK81-25	-	59	41	100	3.24 <sup>c</sup>	-	-	0.07	<i>Pita</i>
	M64-1-3-9-1	-	59	41	100	3.24 <sup>c</sup>	-	-	0.07	<i>Pita</i>
	BN111	-	58	42	100	2.56 <sup>d</sup>	-	-	0.11	<i>Pi20</i>
	M36-1-3-10-1	-	58	42	100	2.56 <sup>d</sup>	-	-	0.11	<i>Pi20</i>
	V850196	-	79	21	100	-	0.85	-	0.36	<i>Pita</i> , <i>Pik-s</i>
	BN209	-	81	19	100	-	1.92	-	0.17	<i>Pib</i> , one unknown
IR64 (VG 7a)	IK81-25	-	41	31	72	1.39	-	-	0.24	<i>Pita</i>
	BN111	-	37	35	72	0.06	-	-	0.81	<i>Pi20</i>
	M64-1-3-9-1	-	51	21	72	-	0.67	-	0.41	<i>Pita</i> , <i>Piz-t</i>
	BN209	-	52	17	69	-	0.08	-	0.78	<i>Pib</i> , one unknown

<sup>a</sup>RR = homozygously resistant, R = heterozygously resistant, - = homozygously susceptible.

<sup>b</sup>a, b, c, and d indicate co-segregation among isolates.

tance genes, *Pib* and *Pik*<sup>†</sup>. It was clarified that *Pik*<sup>†</sup> was not included in VG 3 in previous research. From these results, *Pib* was estimated to be one of the two genes, but the other gene could not be identified. The two genes, *Pita* and *Pik-s*, were identified in the analysis of V850196 based on segregation controlled by two dominant genes and by the reaction pattern. Finally, it was concluded that the four genes, *Pib*, *Pita*, *Pik-s*, and one unknown gene, were included in IR36.

In the analysis of IR60 using 53 BC<sub>1</sub>F<sub>2</sub> lines, segregation controlled by two and three genes was observed for three isolates, IK81-3, M64-1-3-9-1, and V850196, and one, BN209, respectively. IK81-3 was avirulent to *Pita* and *Pik*<sup>†</sup>, but the latter could not be estimated in VG 3. M64-1-3-9-1 has avirulence to *Pita* and *Piz-t*, V850196 to *Pita* and *Pik-s*, and BN209 to *Pib*; therefore, the three genes (*Pita*, *Pik-s*, and *Pib*) were related in the segregation. However, one gene with resistance to IK81-3 and two genes with resistance to BN209 could not be identified by reaction patterns. These results indicate that *Pib*, *Pita*, *Piz-t*, and *Pik-s*, and two kinds of unknown genes, were present in IR60.

In IR74 of VG 5, 76 BC<sub>1</sub>F<sub>2</sub> lines were analyzed for segregation to six blast isolates, PO6-6 and Ca89 for *Pik*<sup>†</sup>, M36-1-3-10-1 for *Pi20*, BN111 for *Pi20* and *Pik*<sup>†</sup>, BN209 for *Pib* and *Pik*<sup>†</sup>, and B90002 for *Pia*, *Pik*<sup>†</sup>, *Pi20*, *Pib*, and *Piz-t*. Resistance controlled by a single dominant gene was shown in segregation to the three isolates, PO6-6, Ca89, and M36-1-3-10-1, and a co-segregation was also observed between isolates PO6-6 and Ca89. *Pik*<sup>†</sup> and *Pi20* controlled the segregation to two isolates, PO6-6 and Ca89, and to one isolate, M36-1-3-10-1, respectively. A segregation controlled by two dominant genes was observed in isolates BN111 and BN209. One gene, *Pik*<sup>†</sup>, was common in the segregation to both isolates. The other genes avirulent to BN111 and BN209 corresponded to *Pi20* and *Pib*, respectively. In the analysis of B90002, all the lines were resistant, suggesting that IR74 carried the same gene as CO39. Since only *Pia* was included in the genetic background of CO39, this gene also exists in IR74. From these results, four resistance genes, *Pia*, *Pib*, *Pik*<sup>†</sup>, and *Pi20*, were identified in IR74.

Two varieties, IR46 and IR64, in VG 7a that included *Pi20* and *Pita* were analyzed. Six isolates were used for the

segregation analysis in IR46, and four isolates, IK81-25, M64-1-3-9-1, BN111, and M36-1-3-10-1, showed segregation controlled by a single dominant gene. Co-segregation for resistance between IK81-25 and M64-1-3-9-1, and between BN111 and M36-1-3-10-1, was observed. IK81-25 and M64-1-3-9-1 are avirulent to *Pita*, and BN111 and M36-1-3-10-1 to *Pi20*. Based on these results, the existence of *Pita* and *Pi20* in IR46 was confirmed. The segregation for resistance to isolates V850196 and BN209 indicated that two dominant genes control the resistance. V850196 is avirulent to *Pita*, *Pik<sup>†</sup>*, and *Pik-s*, and BN209 to *Pib* and *Pik<sup>†</sup>*. *Pik<sup>†</sup>* was not included in VG 7a based on the reaction patterns in previous research. These results indicated that *Pita* and *Pik-s*, and *Pib* and an unknown gene, were related to the segregation for resistance to V850196 and BN209, respectively. Thus, *Pib*, *Pik-s*, *Pita*, *Pi20*, and one unknown gene were identified in IR46.

In IR64, 72 BC<sub>1</sub>F<sub>2</sub> lines were analyzed using four isolates, IK81-25, BN111, M64-1-3-91, and BN209. Segregation controlled by a single dominant gene was observed for isolates IK81-25 and BN111, and two dominant genes for isolates M64-1-3-9-1 and BN209. Three isolates, IK81-25, BN209, and BN111, were avirulent to three genes, *Pita*, *Pib*, and *Pi20*, respectively. M64-1-3-9-1 was avirulent to *Pita* and *Piz-t*. *Pik<sup>‡</sup>* is not included in VG 7 cultivars. From these results, four genes (*Pita*, *Pib*, *Pi20*, and *Piz-t*) were estimated to control the segregation for resistance to the isolates used. However, one of the two resistance genes could not be identified in the segregation to BN209.

#### Allelism tests

A total of 24 F<sub>2</sub> populations derived from crosses among nine IRR1 varieties and differential varieties and lines (DV) were used for the allelism tests (Table 2).

In IR34 of VG 1b, two F<sub>2</sub> populations derived from the crosses with Toride 1 as a DV of *Piz-t*, and BL1 as a DV of *Pib*, did not show any plants susceptible to isolates M64-1-3-9-1, M39-1-3-8-1, and BN209. The first two isolates and the last one are avirulent to *Piz-t* and *Pib*, respectively. From these results, it was confirmed that IR34 carries *Piz-t* and *Pib*.

In IR24 of VG 2a, all F<sub>2</sub> plants derived from the cross with BL1 as a DV for *Pib* were resistant to BN209, which was avirulent to *Pik<sup>†</sup>* and *Pib*. These results indicated the presence of *Pib* in IR24. The existence of *Pib* in IR24 previously reported by Imbe et al (1997) was further supported by the present allelism test.

In IR36 of VG 3, the four kinds of F<sub>2</sub> populations derived from the crosses with the DVs, C105TTP2L9 and C101PKT of *Pita*, Toride 1 of *Piz-t*, and BL1 of *Pib*, segregated only plants resistant to isolate IK81-25, which was avirulent to *Pita*; M39-1-3-8-1 and M64-1-3-9-1, avirulent to *Pita* and *Piz-t*; and BN209, avirulent to *Pib*. These results confirmed that IR36 had *Pita*, *Piz-t*, and *Pib*. The presence of *Piz-t* was also confirmed from the segregation of the F<sub>2</sub> population derived from a cross between IR34 and IR36 using isolates M39-1-3-8-1 and M64-1-3-9-1. In the F<sub>2</sub> popu-

lation derived from a cross between IR24 and IR36, the allelism of *Pib* and *Pik-s* was confirmed using isolates BN209 and V850196, respectively.

The F<sub>2</sub> populations derived from the crosses of IR60 with IR34 and IR24 did not segregate any plants susceptible to isolate BN209 avirulent to *Pib* and *Pik<sup>†</sup>*, V850196 avirulent to *Pita*, *Pik-s*, and *Pik<sup>†</sup>*, and M39-1-3-8-1 and M64-1-3-9-1 avirulent to *Pita* and *Piz-t*. IR34 was confirmed with *Pia*, *Pib*, *Piz-t*, and *Pik-s*, and IR24 had *Pia*, *Pib*, *Pik-s*, and *Pi20*. IR60 was not previously estimated to have *Pik<sup>†</sup>*, whereas the resistance to M39-1-3-8-1 and M64-1-3-9-1 was related to *Piz-t*, which was estimated to also be in IR34. On this basis, the three genes (*Pib*, *Piz-t*, and *Pik-s*) were also confirmed in IR60 of VG 3.

In PSBRc1 of VG 4, the F<sub>2</sub> populations derived from a cross with IR56 previously estimated to have *Pik<sup>†</sup>* and *Pita* showed that all plants were resistant to isolate PO6-6 avirulent to *Pik<sup>†</sup>* and to BN111 avirulent to *Pik<sup>†</sup>* and *Pi20*. Since *Pi20* was not included in VG 4 by the reaction pattern in previous research, the resistance of the F<sub>2</sub> plants to these isolates suggested allelism of *Pik<sup>†</sup>* in PSBRc1 and IR56. However, upon inoculation with isolate IK81-25 avirulent to *Pita*, the F<sub>2</sub> plants segregated into 406 resistant and 126 susceptible, which fit the 3:1 ratio ( $\chi^2 = 0.51$ ,  $P = 0.43$ ). This indicated that a single dominant gene (*Pita*) estimated to be in IR56 governed the segregation.

In IR74 of VG 5, all F<sub>2</sub> plants derived from a cross with IR56 showed resistance to PO6-6 avirulent to *Pik<sup>†</sup>* and to BN111 avirulent to *Pik<sup>†</sup>* and *Pi20*, which confirmed that IR74 has the same *Pik<sup>†</sup>* allele of IR56. On the other hand, segregation to IK81-25 avirulent to *Pita* fit the 3:1 ratio ( $\chi^2 = 0.82$ ,  $P = 0.37$ ) expected for single dominant gene control. The segregation was due to *Pita* in IR56.

In VG 6, IR56 was crossed with five DVs, C101PKT and Yashiromochi for *Pita*, Kanto 51 for *Pik*, Tsuyuake for *Pik-m*, and IR70 for *Pita* and *Pik<sup>†</sup>*. Eleven kinds of segregation analysis were carried out using three isolates, IK81-25 avirulent to *Pita*, PO6-6 avirulent to *Pik<sup>†</sup>*, and BN111 avirulent to *Pik<sup>†</sup>* and *Pi20*. Of these, two F<sub>2</sub> populations derived from crosses with C101PKT and Yashiromochi showed single-gene segregation to PO6-6. The other nine populations did not segregate any susceptible plants, which confirmed that the same gene controlled the resistance. It was estimated that the segregation to PO6-6 was due to *Pik<sup>†</sup>* in IR56. Since the blast isolates from the Philippines could not classify the *Pik* alleles except *Pik-s*, no susceptible plants were obtained from the populations derived from the combinations among Kanto 51 for *Pik*, Tsuyuake for *Pik-m*, and IR56 for *Pik<sup>‡</sup>*. These results confirmed that *Pita* and *Pik<sup>†</sup>* were included in IR56.

In VG 7a, the F<sub>2</sub> populations derived from the crosses between IR64 and DVs C105TTP2L9 and C101PKT for *Pita*, Toride 1 for *Piz-t*, and IR24 for *Pi20* did not show any segregants susceptible to IK81-25, M39-1-3-8-1, M64-1-3-9-1, and BN111, respectively. These results indicated that IR64 had the same alleles of *Pita*, *Piz-t*, and *Pi20*. A similar reaction was shown to IK81-25 in the F<sub>2</sub> plants from a cross

**Table 2. Allelism test using an F<sub>2</sub> population derived from the cross between an IRRI-bred and differential variety.**

Cultivar (group)	Cross combination (expected gene)		Isolate	No. of plants (lines) <sup>a</sup>			$\chi^2$ (3:1)	P	Identified gene
				R	S	Total			
IR34 (CG 1b)	Toride 1 ( <i>Piz-t</i> ) BL1 ( <i>Pib</i> )	/ IR34 ( <i>Pia, Pib, Piz-t, Pik-s</i> )	M39-1-3-8-1	94	0	94	-	-	<i>Piz-t</i>
			M64-1-3-9-1	84	0	84	-	-	<i>Piz-t</i>
			BN209	437	0	437	-	-	<i>Pib</i>
IR24 (V 2b)	BL1 ( <i>Pib</i> )	/ IR24 ( <i>Pia, Pib, Pik-s, Pi20</i> )	BN209	161	0	161	-	-	<i>Pib</i>
IR36 (CG 3)	IR36 ( <i>Pib, Pita, Pik-s</i> ) Toride 1 BL1 IR34 IR24	/ C105TTP2L9 ( <i>Pita</i> ) C101PKT ( <i>Pita</i> ) IR36 IR36 / IR36 / IR36 / IR36	IK81-25	468	0	468	-	-	<i>Pita</i>
			IK81-25	167	0	167	-	-	<i>Pita</i>
			M39-1-3-8-1	184	0	184	-	-	<i>Piz-t</i>
			M64-1-3-9-1	168	0	168	-	-	<i>Piz-t</i>
			BN209	420	0	420	-	-	<i>Pib</i>
			M39-1-3-8-1	467	0	467	-	-	<i>Piz-t</i>
			M64-1-3-9-1	516	0	516	-	-	<i>Piz-t</i>
			BN209	287	0	287	-	-	<i>Pib</i>
			V850196	295	0	295	-	-	<i>Pik-s</i>
			IR60 (CG 3)	IR34 ( <i>Pib, Pita, Piz-t, Pik-s</i> ) IR24	/ IR60 ( <i>Pib, Pita, Piz-t, Pik-s</i> ) IR60	M39-1-3-8-1	302	0	302
M64-1-3-9-1	303	0	303			-	-	<i>Piz-t</i>	
BN209	360	0	360			-	-	<i>Pib</i>	
PSBRc1 (CG 4)	IR56 ( <i>Pita, Pik<sup>†</sup></i> )	/ PSBRc1 ( <i>Pik<sup>†</sup></i> )	P06-6	531	0	531	-	-	<i>Pik<sup>†</sup></i>
			BN111	526	0	526	-	-	<i>Pik<sup>†</sup></i>
			IK81-25	406	126	532	0.51	0.43	<i>Pita</i> in IR56
IR74 (CG 5)	IR46 ( <i>Pita, Pik<sup>†</sup></i> )	/ IR74 ( <i>Pi20, Pik<sup>†</sup></i> )	P06-6	560	0	560	-	-	<i>Pik<sup>†</sup></i>
			BN111	585	0	585	-	-	<i>Pik<sup>†</sup></i>
			IK81-25	442	135	577	0.82	0.37	<i>Pita</i> in IR56
IR56 (CG 6)	C101PKT ( <i>Pita</i> ) Yashimochi ( <i>Pita</i> ) IR56 IR56 IR56	/ IR56 ( <i>Pita, Pik<sup>†</sup></i> ) IR56 / Kanto 51 ( <i>Pik</i> ) / Tsuyuake ( <i>Pik-m</i> )	IK81-25	469	0	469	-	-	<i>Pita</i>
			P06-6	358	85	443	7.98	0.005	<i>Pik<sup>†</sup></i> in IR56
			IK81-25	463	0	163	-	-	<i>Pita</i>
			P06-6	370	127	497	0.08	0.78	<i>Pik<sup>†</sup></i> in IR56
			P06-6	440	0	440	-	-	<i>Pik<sup>†</sup></i>
			BN111	407	0	407	-	-	<i>Pik<sup>†</sup></i>
			P06-6	498	0	498	-	-	<i>Pik<sup>†</sup></i>
IR70 (CG 6)	IR56	/ IR70 ( <i>Pita, Pik<sup>†</sup></i> )	BN111	496	0	496	-	-	<i>Pik<sup>†</sup></i>
			P06-6	577	0	577	-	-	<i>Pik<sup>†</sup></i>
			BN111	558	0	558	-	-	<i>Pik<sup>†</sup></i>
			IK81-25	567	0	567	-	-	<i>Pita</i>
IR64 (CG 7a)	IR64 ( <i>Pib, Pi20, Pita</i> ) Toride 1 IR56 IR24	/ C105TTP2L9 ( <i>Pita</i> ) C101PKT IR64 / IR64 / IR64 / IR64	IK81-25	274	0	274	-	-	<i>Pita</i>
			IK81-25	132	0	132	-	-	<i>Pita</i>
			M39-1-3-8-1	124	0	124	-	-	<i>Piz-t</i>
			M64-1-3-9-1	133	0	133	-	-	<i>Piz-t</i>
			IK81-25	296	0	296	-	-	<i>Pita</i>
			P06-6	193	60	253	0.22	0.64	<i>Pik<sup>†</sup></i>
			BN209	222	0	222	-	-	<i>Pib</i>
			V850196	227	0	227	-	-	<i>Pik<sup>†</sup></i>
			BN111	302	0	302	-	-	<i>Pi20</i>

<sup>a</sup>R = resistant, S = susceptible.

between IR64 and IR56. However, there was single-gene segregation ( $\chi^2 = 0.22$ ,  $P = 0.64$ ) to P06-6 due to *Pik<sup>†</sup>* in IR56. Against V850196 and BN209, the F<sub>2</sub> population of a cross of IR64 with IR24 did not segregate any susceptible plants, indicating that the same genes (*Pik-s* and *Pib*) conferred resistance to both isolates. These results confirmed that *Pik-s* and *Pib* were also included in IR64.

The six resistance genes, *Pita*, *Pib*, *Pik-s*, *Piz-t*, *Pi20*, and *Pik*, were confirmed in the nine IRRI varieties by allelism tests.

## Discussion

In a previous study, Ebron et al (submitted) have estimated at least seven blast resistance genes, *Pib*, *Pik-s*, *Piz-t*, *Pi20*, *Pita*, *Pik*, and *Pii* or *Pi3(t)*, in 42 rice cultivars. Through two kinds of genetic analysis, we have confirmed seven blast resistance genes, *Pia*, *Pib*, *Pik-s*, *Piz-t*, *Pi20*, *Pita*, and *Pik*. Four of these genes, *Pia*, *Pib*, *Pik-s*, and *Piz-t*, were identified in some varieties but were not previously estimated by reaction patterns (Table 3).



**Table 3. Resistance genes identified in IRRI-bred rice varieties.**

Cultivar group <sup>a</sup>	Variety	Resistance genes		
		Reaction pattern <sup>b</sup>	BC <sub>1</sub> F <sub>2</sub> analysis	Allelism test
VG 1	a	IR20, IR28, IR30, IR45, IR66	<i>Pib, Pik-s</i>	-
	b	IR29 IR34	<i>Pib, Pik-s, Piz-t</i>	- <i>Pia, Pib, Pik-s, Piz-t,</i> one unknown
VG 2	a	IR8, IR22, IR26, PSBRc30 IR24	<i>Pi20, Pib, Pik-s</i>	- <i>Pi20<sup>b</sup>, Pia<sup>b</sup>, Pib<sup>a</sup>, Pik-s<sup>b</sup></i>
	b	IR43	<i>Pi20, Pib</i>	-
	c	PSBRc2 <i>Piz-t</i>	<i>Pi20, Pib, Pik-s,</i>	-
VG 3		IR5, IR32, IR38, IR40, IR42, IR44, IR50, IR52, IR54, IR58, IR62, IR65, IR68, IR72, IR52, PSBRc4	<i>Pita, Pib</i>	-
		IR36	-	-
		IR60	<i>Pita, Pib, Pik-s,</i> one unknown	<i>Pib, Pita, Pik-s,</i> <i>Piz-t</i>
VG 4	PSBRc1	<i>Pik<sup>†</sup></i>	-	<i>Pik<sup>†</sup></i>
VG 5	IR74	<i>Pi20, Pik<sup>†</sup></i>	<i>Pi20, Pik<sup>†</sup>, Pia, Pib</i>	<i>Pik<sup>†</sup></i>
VG 6	IR56	<i>Pita, Pik<sup>†</sup></i>	-	<i>Pita, Pik<sup>†</sup></i>
	IR70	-	-	-
VG 7	a	IR46, IR48, PSBRc28 IR46	- <i>Pi20, Pita, Pib</i>	- <i>Pi20, Pita, Pib, Pik-s,</i> one unknown
		IR60	-	<i>Pi20, Pita, Pib, Piz-t,</i> one unknown
		IR64	-	-
	b	PSBRc10, PSBRc18, PSBRc20	<i>Pi20, Pita, Pib,</i> <i>Pii (or Pi3)</i>	-

<sup>a</sup>Classification was carried out based on the presence of the three genes, *Pi20*, *Pita*, and *Pik<sup>†</sup>*, and resistance genes were estimated by the reaction patterns based on a differential system using 14 blast isolates from the Philippines (Ebron et al, submitted).

<sup>b</sup>The data of Imbe et al (1997) were modified.

Among the seven blast resistance genes, *Pib* and one *Pik* allele were identified in almost all the cultivars tested in previous research, and this was confirmed again in this study. *Pib* was previously estimated in variety groups (VG) 1, 2, 3, and 7. It was confirmed in the varieties IR34 (VG 1), IR24 (VG 2), IR36 and IR60 (VG 3), and IR46, IR60, and IR64 (VG 7) through genetic analysis. Other than these varieties, IR74 (VG 5) was similarly identified with *Pib*, whose presence was not previously expected. The genes *Pik-s* and *Pik<sup>†</sup>* were confirmed in VGs 1 and 2, and VGs 4, 5, and 6, respectively, based on genetic analysis of IR34, IR24, PSBRc1, IR74, and IR56. Additionally, *Pik-s* was identified in IR36 and IR60 of VG 3, and in IR46 and IR64 of VG 7a. The apparent wide distribution of these genes may be correlated with the breeding method for which these varieties had IR8, IR24, and IR36, or their hybrid progenies in the pedigrees as parental varieties. IR8 was bred from a cross between Indonesian variety Peta and Dee-geo-woo-gen from Taiwan. Peta and Dee-geo-woo-gen were estimated to be carrying

*Pib* and *Pik-s* for Philippine isolates, respectively (unpublished data). IR8 and its progenies were included in the pedigrees of IR24 and IR36. Three varieties, Dawn, Tadukan, and Tetep, could also be found in the pedigrees of IRRI cultivars. They were estimated to have a *Pik* allele (other than *Pik-s*) (unpublished data). Kiyosawa (1981) reported that *Pik-h* was identified in these varieties. On the basis of these findings, Dee-geo-woo-gen, and the three varieties, Dawn, Tadukan, and Tetep, could be the sources of *Pik-s* and *Pik-h*, respectively. These results suggest that most IRRI varieties have *Pik-s* or *Pik-h*. It will be necessary to confirm the genotype of *Pik<sup>†</sup>* using effective blast isolates, which can classify the four kinds of *Pik* alleles, *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*.

Estimating *Pib*, *Pik-s*, *Piz-t*, and *Pia* was not easy whenever *Pi20*, *Pita*, and *Pik<sup>†</sup>* were carried in the varieties. The three genes, *Pik-s*, *Piz-t*, and *Pia*, showed a relatively wide spectrum of resistance, and they could mask the reactions of the other narrow-spectrum genes. Segregation analysis us-

ing backcross populations (BC<sub>1</sub>F<sub>2</sub> family lines) and an allelism test are necessary and effective in identifying these genes. For instance, *Pib* and *Piz-t* were confirmed from the segregation of F<sub>2</sub> plants derived from crosses with DVs BL1 for *Pib* and Toride 1 for *Piz-t*. The use of BC<sub>1</sub>F<sub>2</sub> analysis has been demonstrated in identifying *Pik-s* and *Piz-t* in IR36 and IR60 of VG 3 and IR46 and IR64 of VG 7, using isolates with specific avirulence to these genes.

Another gene, *Pia*, was also identified in IR34 in VG 1b and IR74 in VG 5. Imbe et al (1997) have already reported the presence of *Pia* in IR24. Many IRRI varieties included IR24 or its hybrid progenies as a parental variety in their pedigrees. The source of *Pia* was estimated to come from Blue Bonnet, which was one of the parental varieties for IR24 and was previously identified with *Pia* (Kiyosawa 1972). *Pia* may also be included in many IRRI varieties.

Previous genetic studies on blast resistance in some IRRI cultivars and lines were limited to the inheritance of resistance to particular isolates (Dekuku 1982, Flores-Gaxiola et al 1983, Yu et al 1987). The number of genes was estimated against specific blast isolates by segregation ratios, but these genotypes were not known. Apparently, the presence of several resistance genes in indica-type cultivars and the lack of a suitable differential system could have contributed to limited genetic studies in the tropics. We demonstrated the utility of the differential system for the gene analysis of indica-type rice cultivars bred at IRRI. The use of the differential system based on the selected isolates with known virulence genotypes could clarify and confirm several blast resistance genes in IRRI varieties. This information on the resistance genes in IRRI varieties should be important in breeding for a durable type of resistance.

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## Notes

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# Identifying blast resistance genes in elite indica-type rice varieties bred at the International Rice Research Institute

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The blast resistance genes in elite indica-type rice (*Oryza sativa* L.) varieties bred at the International Rice Research Institute (IRRI) were estimated following a differential system using Philippine isolates of the blast fungus *Pyricularia grisea* (Cooke) Sacc. based on the gene-for-gene theory. On the basis of the presence of three genes, *Pi20*, *Pita*, and one of the *Pik* alleles (other than *Pik-s*), the 42 varieties were classified into seven varietal groups. A group without these three genes included seven varieties that were bred from the progenies of the hybrids with IR24 as a parental variety. The biggest group characterized by *Pita* consisted of 17 varieties, including IR36 and its sister lines or progenies. The group characterized by *Pi20* had seven varieties that included IR8, IR24, and their hybrid progenies. Thus, most of the IRRI varieties were classified into these three groups that often included IR8, IR24, IR36, or their hybrid progenies in their pedigrees. A total of seven resistance genes—*Pib*, *Pita*, *Piz-t*, *Pik-s*, one of the *Pik* alleles (other than *Pik-s*), and one of the two genes, *Pii* or *Pi3*—were estimated to be present in these varieties. On the basis of the varieties used as parents in the ancestry of IRRI varieties identified to have one of the *Pik* alleles (other than *Pik-s*), the gene was assumed to be *Pik-h*. In some cases, genes such as *Pib*, *Pik-s*, and *Piz-t* could not be estimated because of the masking effect of *Pita*, the *Pik* allele, or *Pi20*. The number and kind of blast resistance genes in IRRI varieties were limited in comparison with previously reported blast resistance genes. Since *Pik-s* and another *Pik* allele were estimated in 17 varieties of five groups, and *Pib* in 38 varieties of four groups, these genes were considered to be widely distributed in IRRI varieties.

Blast, caused by *Pyricularia grisea* (Cooke) Sacc. (*P. oryzae* Cavara), teleomorph *Magnaporthe grisea* (Hebert) Barr, is one of the most destructive diseases of rice (*Oryza sativa* L.) worldwide. The threat is especially serious in temperate and subtropical rice production areas (Bonman 1992). In the tropics, blast is severe in upland and rainfed lowland environments. Susceptible varieties can be severely damaged by blast even under irrigated conditions (Bonman and Mackill 1988). Control of blast disease can be achieved by growing resistant varieties (Yu et al 1991, Bonman et al 1992). Hence, breeding for blast resistance is a major objective in a rice improvement program.

Resistance to rice blast has been explained by the gene-to-gene theory between the resistance gene in the host and the avirulence gene in *P. grisea* (Kiyosawa 1972, Silue et al 1992). To date, around 40 blast resistance genes and their chromosomal locations have already been reported (RGC 1998). In spite of the wide distribution of many known genes in rice varieties grown in different countries, genetic studies of blast resistance are limited in the tropics. This is partly

attributed to the lack of a suitable differential system for the efficient identification of those genes. Additionally, the presence of several resistance genes in indica-type varieties makes genetic studies of blast resistance genes difficult (Mackill et al 1985, Yu et al 1987).

The information regarding blast resistance genes in indica-type varieties has been limited as well in IRRI-bred varieties and lines. Several resistance genes, such as *Pita* (Kiyosawa 1966), *Pita-2* (Kiyosawa 1967), *Pik-h* (Kiyosawa and Murty 1969), *Pik-p* (Kiyosawa 1969), *Piz-t* (Yokoo and Kiyosawa 1970), *Pib* and *Pit* (Yokoo et al 1978), *Pi6(t)* (Yu 1991), *Pi11(t)[Piz-h]* (Zhu et al 1993), *Pi8* (Pan et al 1996), *Pi16(t)* (Pan et al 1999), and *Pi17(t)* (RGC 1996), have been identified in indica-type varieties. Regarding IRRI-bred varieties, Flores-Gaxiola et al (1983) reported two complementary genes and one dominant gene in the genetic analysis using hybrid progenies derived from a cross between IR8 and Tetep. Yu et al (1987) showed that the resistance in five varieties, IR36, IR46, IR54, IR56, and IR60, bred at the International Rice Research Institute (IRRI), and four tradi-

tional varieties, Carreon, Paikantao, Pankhari 203, and Tetep, was controlled by one or two dominant genes. These authors also recognized a recessive gene that controlled resistance in IR54 against one blast isolate, but the genotypes in each variety were not clarified. Yamaguchi et al (1996) reported that IR50 had two resistance genes, *Pia* and *Pib*, from analysis using Japanese blast isolates. Imbe et al (1997) reported that four resistance genes, *Pia*, *Pib*, *Pik-s*, and *Pi20*, were included in IR24 based on genetic analysis using blast isolates from the Philippines. Genotypic information in IRRI-bred and indica-type varieties was quite limited.

The lack of a differential system for blast resistance genes has been examined recently using blast isolates from the Philippines. Yanoria et al (2000) examined the pathogenicities of around 150 blast isolates from IRRI's stock collection using differential varieties and lines. Several isolates showed distinct reaction patterns to known resistance genes, and these were selected for use in a differential system. Moreover, Tsunematsu et al (2000) studied in detail the pathogenicities of 12 isolates, which were selected by Yanoria et al (2000), using 29 monogenic lines that were targeting 23 known blast resistance genes and containing a single gene in each genetic background. The set of monogenic lines and selected blast isolates from the Philippines can be used as a differential system to clarify the pathogenicity of the isolates and genotype of the rice varieties.

IRRI-bred varieties have been distributed worldwide and used by farmers and breeders as important parental varieties in breeding programs. One IRRI variety, IR8, released in 1966, triggered the Green Revolution in tropical countries of Asia (Hossain 1995). IR36 once dominated rice production in several Asian countries in the 1970s (IRRI 1982). Released in 1985, IR64 has been widely accepted as a high-quality rice variety in many countries because of its desirable combination of intermediate amylose content and intermediate gelatinization temperature (Khush 1987). A more recent variety, IR72, has high yield potential, shorter growth duration, and improved resistance to several diseases and insect pests (Kropff et al 1990). Other than these outstanding traits, IRRI-bred varieties may be carrying important blast resistance genes useful for deployment in blast-prone environments or for breeding blast-resistant rice varieties. However, this has not been well understood. For the effective use of these genes, it is necessary to elucidate the genetic constitution for blast resistance of these varieties. This study tried to identify the kinds of blast resistance genes in IRRI-bred varieties using a differential system developed by Yanoria et al (2000) and Tsunematsu et al (2000).

## Materials and methods

### IRRI-bred varieties

A total of 42 indica-type varieties bred at IRRI were used for estimating blast resistance genes. These included, among others, IR8, IR24, IR36, IR64, and IR72, which have been widely distributed and used in many countries because of their outstanding traits. Eight varieties—PSBRc1, PSBRc2,

PSBRc4, PSBRc10, PSBRc18, PSBRc20, PSBRc28, and PSBRc30—developed by IRRI and designated by the Philippine Seed Board were also included.

### Inoculation and evaluation

Pregerminated seeds were sown in a 20 × 35-cm plastic tray, with seven seedlings for each variety replicated two times. Ten grams of ammonium sulfate was applied to each tray as basal fertilizer and 1 g was added 1 week before inoculation. In all cases, two susceptible varieties, CO39 and Lijiangxintuanheigu (LTH), were included in each tray to check the success of inoculation and virulence of the Philippine blast isolates used.

Fourteen blast isolates previously selected by Yanoria et al (2000) were used for inoculation. Virulence genotypes of these isolates have been confirmed and diverse pathogenicities have been shown. At the fourth-leaf stage, each tray of seedlings was sprayed with 40–50 mL of spore suspension adjusted to  $10^{15}$  spores mL<sup>-1</sup>. Trays were placed inside wet jute sacks for 18–24 h and transferred to an air-conditioned glasshouse room with  $23 \pm 3$  °C/30 ± 5 °C night and day temperature. Blast incidence on each seedling was examined 6–7 days after inoculation. The reaction was evaluated using a 0–5 scale with slight modification as described by Mackill and Bonman (1992). The values 0 to 2, 3–, 3+, and 5 were evaluated as resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S), respectively.

### Estimation of the genotype of the resistance gene

The genotype of the blast resistance gene in the IRRI variety was estimated based on the differential system developed by Yanoria et al (2000) and Tsunematsu et al (2000). The estimation was carried out based on the reaction patterns of nine kinds of monogenic lines to 14 blast isolates from the Philippines (Table 1). Each monogenic line carried a different single resistance gene—*Pi20*, *Pita*, *Pik-s*, *Pia*, *Pib*, *Piz-t*, *Pii*, *Pi3*, and one of the *Pik* alleles (other than *Pik-s*). In the pathogenicity test of blast isolates, *Pik-s* could be distinguished from the other four *Pik* alleles, *Pik*, *Pik-h*, *Pik-m*, and *Pik-p*. However, no isolates were available to classify the four alleles (Tsunematsu et al 2000, Yanoria et al 2000). The four alleles were designated tentatively as *Pik*<sup>†</sup> in this study.

The IRRI varieties were classified into seven variety groups with distinct reaction patterns. The differences in reaction patterns of the groups were based mainly on the presence of three genes—*Pi20*, *Pita*, and *Pik*<sup>†</sup>. These three genes showed a relatively wider spectrum of resistance than the other estimated genes. These demonstrated incompatibilities with several blast isolates but were easily determined by reactions to three isolates, M36-1-3-10-1, IK81-25, and PO6-6. Each of these isolates was known to possess avirulence to only one of these three genes, that is, M36-1-3-10-1 (avirulent to *Pi20*), IK81-25 (avirulent to *Pita*), and PO6-6 (avirulent to *Pik*<sup>†</sup>). Hence, the presence or absence of the genes *Pi20*, *Pita*, and *Pik*<sup>†</sup> was estimated by an R (resis-

**Table 1. Classification of IRR1 rice cultivars and the estimated blast resistance genes for each cultivar group following the reaction patterns of the monogenic lines to Philippine blast isolates of *Pyricularia grisea* Sacc.**

Isolate	Monogenic line										Variety group (VG)												
	IRBL20-IR24 (Pi20)	IRBLta-CP1 (Pita)	IRBLk-Ka (Pik <sup>k</sup> ) <sup>a</sup>	IRBLks-F5 (Piks)	IRBLa-A (Pia)	IRBLb-B (Pib)	IRBLzt-T (Piz-t)	IRBLi-F5 (Pii)	IRBL3-CP4 (P13)	1		2		3		4		5		6		7	
										a	b	a	b	a	b	a	b	a	b	a	b	a	b
PO6-6 <sup>a</sup>	S	S	R	S	S	S	S	R <sup>c</sup>	R	S	S	S	S	S	S	R	R	R	R	R	R	S	R
IK81-25 <sup>a</sup>	S	R	S	S	S	S <sup>c</sup>	R-M	R	R	S	R-M	S	S	S	R	S	S	S	S	R	R	R	R
M36-1-3-10-1 <sup>a</sup>	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	M	S	S	S	R	R	R	R
IK81-3 <sup>b</sup>	S	R	R	S	S	S	R-M	R	M	R-M	S	S	S	S	R	M	R	R	R	S	R	R	R
BN111 <sup>a</sup>	R	S	R	S	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
Ca89 <sup>a</sup>	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	S	S
V850256 <sup>a</sup>	S	R	R	S	S	S	S	S	R-M	S	S	S	S	S	M	R	R	R	R	R	R	M	M
M39-1-3-8-1 <sup>b</sup>	S	R	S	S	S	R	M	S	S	R	S	R	R	R	R	S	S	S	S	R	R	R	R
M64-1-3-9-1 <sup>a</sup>	S	R-M	S	S	S	R	S	S	S	S	M	R	R	R	S	S	S	S	S	R	R	R	R
M39-1-2-21-2 <sup>b</sup>	R	S	S	S	S	S	R-M	R	R	S	R	R	R	R	S	S	S	S	S	R	R	R	R-M
BN209 <sup>a</sup>	S	S	R	S	S	S	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
V850196 <sup>a</sup>	S	R	R	R	S	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B90002 <sup>a</sup>	R	R	R	S	R	R	S	S	S	R	M	R	R	R	R	R	R	R	R	R	R	R	R
C923-49 <sup>a</sup>	R	S	R	S	R	R	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Variety										IR20	IR29	IR34	IR8	IR43	PSBRc2	IR5, IR32	PSBRc4	IR74	IR56	IR46	PSBRc10		
										IR28	IR22	IR24	IR22		IR36, IR38				IR70	IR48	PSBRc18		
										IR30	IR26	IR26	IR24		IR40, IR42					IR64	PSBRc20		
										IR45	PSBRc30	PSBRc30			IR44, IR50								
										IR55					IR52, IR54								
															IR58, IR60								
															IR62, IR65								
															IR68, IR72								
															PSBRc4								
Additional estimated gene										Pib	Piks	Piz-t	Pib	Pib	Piks	Pib	Unknown	-	-	Pib	Pib	Pib	Pil or P13
										Pib	Piks	Piz-t	Pib	Pib	Piks	Pib	Unknown	-	-	Pib	Pib	Pib	Pil or P13
										Pib	Piks	Piz-t	Pib	Unknown	Piz-t	Pib	Unknown	-	-	Pib	Pib	Pib	Pil or P13
										Pib	Piks	Piz-t	Pib	Unknown	Piz-t	Pib	Unknown	-	-	Pib	Pib	Pib	Pil or P13

<sup>a</sup>Tsunematsu et al (2000). <sup>b</sup>Additional blast isolates used by our group. <sup>c</sup>New data obtained using advanced generation of the materials. R = resistant, S = susceptible, M = moderately resistant, R-M = resistant to moderately resistant. <sup>d</sup>Also includes monogenic lines carrying other *Pik* alleles, *Pik-p*, *Pik-h*, and *Pik-m*, showing similar reaction pattern with IRBLk-Ka. <sup>e</sup>Genotype of three kinds of blast resistance genes, *Pi20*, *Pita*, and *Pik\**.

tant) or S (susceptible) reaction to M36-1-3-10-1, IK81-25, and PO6-6 in a particular variety. The other isolates used in the study possessed different combinations of avirulence to known blast resistance genes other than *Pi20*, *Pita*, and *Pik<sup>t</sup>*.

Reclassification of the groups into subgroups was based on the presence of *Pib*, *Pik-s*, *Piz-t*, and *Pii* or *Pi3*. Isolates M36-1-3-10-1, IK81-25, and PO6-6 were virulent to *Pib*, *Pik-s*, and *Piz-t*; hence, reactions to other isolates were analyzed to estimate these genes; this included resistance to BN209, V850196, B90002, C923-49, M39-1-3-8-1, and M64-1-3-9-1. One of the two genes, *Pii* or *Pi3*, was estimated based on the reaction to PO6-6 and Ca89. Both isolates had similar pathogenicities, except virulence to *Pii* and *Pi3*.

## Results

Seven variety groups, VG 1 to VG 7, were formed in classifying the 42 IRRI rice varieties. In some cases, subgroups within a group were also formed whenever the variety reacted differently to a particular isolate. Three variety groups, VG 1, VG 2, and VG 7, were subdivided into two, three, and two subgroups, respectively. The number of varieties in each group varied from 1 to 17 (Table 1).

### VG 1

This group was characterized by the absence of the three genes, *Pi20*, *Pita*, and *Pik<sup>t</sup>*. Seven varieties, which showed an S reaction to M36-1-3-10-1 and PO6-6, were included in this group. Except for IR29 and IR34, the other five varieties also showed susceptibility to IK81-25. The R reaction of IR29 and IR34 to IK81-25 was estimated to be conferred by an unknown gene. Other than with IK81-25, *Pita* is also incompatible with IK81-3 and V850256. IR29 and IR34 showed an R-M reaction to IK81-3 but an S reaction to V850256, indicating the absence of *Pita*. In addition, three genes, *Pib*, *Pik-s*, and *Piz-t*, were included in this group and the varieties were reclassified into two subgroups, VG 1a and VG 1b. Two genes, *Pib* and *Pik-s*, were estimated to be common in both subgroups based on their resistance to BN209, BN90002, C923-49, and V850196. Although *Pita* and *Pik<sup>t</sup>* were also incompatible among these four isolates, resistance to BN209, B90002, and C923-49 was otherwise estimated to be due to *Pib*, and *Pik-s* was incompatible to V850196. Five varieties, IR20, IR28, IR30, IR45, and IR66, in VG 1a showed an S reaction to two blast isolates, M39-1-3-8-1 and M64-1-3-9-1, whereas IR29 and IR34 in VG 1b were resistant. Based on the reaction pattern, *Piz-t* and *Pita* were incompatible with these isolates. Since *Pita* was absent in this group, resistance to M39-1-3-8-1 and M64-1-3-9-1 was estimated to be due to *Piz-t*.

### VG 2

Seven varieties, IR8, IR22, IR24, IR26, PSBRc30, IR43, and PSBRc2, were included in this group characterized by *Pi20*. These showed R and S reactions to isolate M36-1-3-10-1, which is avirulent to *Pi20*, and to two isolates, IK81-

25 and PO6-6, which were virulent to *Pi20*. Additionally, all varieties showed an R reaction to three isolates, BN209, B90002, and C923-49, that were avirulent to *Pib*. These results indicated that all varieties in VG 2 probably had *Pib*.

VG 2 was divided into three subgroups, VG 2a, VG 2b, and VG 2c, differentiated by reactions to three isolates, M39-1-3-8-1, M64-1-3-9-1, and V850196. Two subgroups, VG 2a and VG 2c, showed resistance to V850196 and VG 2b was moderately resistant to it. *Pita* and *Pik<sup>t</sup>* were not included among VG 2's varieties, and the R reaction to V850196 was estimated to be due to *Pik-s*. The moderate resistance to the same isolate in VG 2b could be due to an unknown gene. Variety PSBRc2 in VG 2c was resistant to both isolates, M39-1-3-8-1 and M64-1-3-9-1, and was estimated to have *Piz-t*. VG 2b showed resistance to only M39-1-3-8-1 and moderate resistance to M64-1-3-9-1. These kinds of resistance could not be associated with the reaction patterns in the differential system using the 14 isolates.

### VG 3

This group had the largest number of varieties characterized by the presence of *Pita*. Seventeen varieties, IR5, IR32, IR36, IR38, IR40, IR42, IR44, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68, IR72, and PSBRc4, were included. In addition to resistance to IK81-25, the presence of *Pita* in VG 3 varieties was supported by resistance to isolates M39-1-3-8-1, M64-1-3-9-1, and IK81-3. All varieties in this group also showed an R reaction to two isolates, BN209 and C923-49. Since these isolates were avirulent to *Pib*, this gene was also estimated to be VG 3.

### VG 4

This group was characterized by *Pik<sup>t</sup>* and only one variety, PSBRc1, was classified. PSBRc1 was moderately resistant to M36-1-3-10-1 but was susceptible to M39-1-2-21-2 and IK81-25, indicating that PSBRc1 did not carry the other genes, *Pi20* and *Pita*. It also showed moderate resistance to isolate IK81-3. It was estimated that additional unknown gene(s) conferred the moderate resistance in this variety.

### VG 5

Only IR74 was included in this group, which was estimated to have two genes, *Pik<sup>t</sup>* and *Pi20*. Its S reaction to IK81-25 indicated the absence of *Pita* in the variety.

### VG 6

This group was characterized by *Pita* and *Pik<sup>t</sup>* and included two varieties, IR56 and IR70. Although the resistance of IR56 and IR70 corresponded approximately to the reaction patterns of *Pita* and *Pik<sup>t</sup>*, the reaction among the varieties to isolate V850256 ranged from M to R.

### VG 7

This group, characterized by *Pi20* and *Pita*, was divided into two groups, VG 7a and VG 7b. All seven varieties also showed an R reaction to isolate BN209, suggesting that VG 7 varieties had *Pib*. The two subgroups were differentiated



by the reaction to isolate PO6-6. VG 7a was susceptible and VG 7b was resistant to the isolate. The R reaction was estimated to be due to one of the two genes, *Pii* or *Pi*.

## Discussion

The 42 IRRI rice varieties were classified into seven groups based on the estimated presence of three resistance genes, *Pi20*, *Pita*, and *Pik<sup>t</sup>*. Moreover, the presence of other additional genes, *Pib*, *Pik-s*, *Pik-t*, and *Pii* or *Pi3*, was estimated according to the differential system by using the reaction patterns of the monogenic lines to blast isolates from the Philippines.

Among them, 15 varieties with *Pi20* were classified into three groups, VG 2, VG 5, and VG 7. IR8 and its derived progeny IR24 were included in VG 2. All the other varieties in VG 2 were bred from the crosses with IR8, IR24, or Peta. In the other groups, IR8, IR24, and Peta and their progenies were similarly found in the pedigrees of the varieties. Other than Peta, variety Sigadis was also included in the pedigree of IR24. Peta and Sigadis were estimated to carry *Pi20*, based on an analysis using the differential system with the Philippine blast isolates (unpublished data). Moreover, Imbe et al (1997) found *Pi20* in IR24. These results suggested that *Pi20* was introduced from Peta or Sigadis in the early stage of IRRI breeding activities.

*Pita* was included in the largest group of varieties, VG 3. IR36, once the most popular semidwarf variety (IRRI 1982), and its sister lines were found in the pedigrees of these varieties. IR36 was selected from the progeny of IRRI breeding line IR2071, derived from crosses of the progenies of IR24 and IR8. The five varieties (IR32, IR38, IR40, IR42, and IR44) were also selected from IR2071 and IR2070 that had the same parental line, CR94-13. The other varieties, IR50, IR52, IR54, IR58, IR60, IR62, IR65, IR68, and IR72, have IR36, IR2071, or IR36 hybrid progenies in their pedigrees. Several more recent varieties from other groups containing *Pita*, such as IR56 and IR70 in VG 6, and IR64, PSBRc10, PSBRc18, PSBRc20, and PSBRc28 in VG7, also have IR36 or its progenies in their pedigrees. IR36 was probably responsible for transmitting *Pita* into these varieties. CR94-13 was believed to carry *Pita*, which is effective against Philippine blast isolates (unpublished data). Kiyosawa (1966) reported that a rice variety from the Philippines, Tadukan, had *Pita*. Tadukan was also included in the breeding of IR36. From these findings, CR94-13 or Tadukan could be the source of *Pita* in IRRI varieties.

Kiyosawa (1972) and Kiyosawa and Ando (1997) reported that five alleles, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, and *Pik-s*, were found in the *Pik* locus. Although *Pik*, *Pik-h*, *Pik-m*, and *Pik-p* could not be distinguished from each other using blast isolates from the Philippines, one of them was found in PSBRc1 of VG 4, IR74 of CG 5, and IR56 and IR70 of CG6. Dawn, Tadukan, and Tetep were included in the pedigrees of these IRRI varieties. Kiyosawa (1981) reported that *Pik-h* was identified in Dawn, Tadukan, and Tetep. On the basis of this, the gene of the *Pik* allele in VGs 4, 5, and 6 was

estimated to be *Pik-h*.

In the development of IRRI rice varieties, Peta was used in a cross with semidwarf variety Dee-geo-woo-gen (DGWG), from which IR8 was selected. Peta and its derivatives such as IR8 were used in the subsequent development of many IRRI varieties. With the use of blast isolates from the Philippines, Peta and DGWG were estimated to carry *Pib* and *Pik-s*, respectively (unpublished data). These findings suggested that Peta and DGWG were the sources of *Pib* and *Pik-s* of IRRI varieties.

Yokoo and Kiyosawa (1970) reported that *Piz-t* showed a broad resistance spectrum against the races of blast fungus and was transmitted by an Indian variety, TKM1. With the use of blast isolates from the Philippines, *Piz-t* was estimated in TKM6, which was one of the lines in the TKM breeding series (unpublished data). The presence of *Piz-t* was estimated to be in IR29 and IR34 of VG 1b and PSBRc2 of VG 2c. These three varieties have TKM6 and its progeny in their pedigrees. Although IR28 of VG 1a is a sister variety of IR29 and IR34, it failed to inherit *Piz-t*. Thus, TKM6 was estimated to be a source of *Piz-t* of these varieties.

Among the seven resistance genes, two genes, *Pib* and one of the *Pik* alleles, *Pik-s* or *Pik-h*, were included in almost all varieties. The genes of the *Pik* allele were estimated in 17 varieties of five groups and those of *Pib* in 38 varieties of four groups. These results indicate that the *Pib* and *Pik* alleles' genes are commonly included in IRRI-bred varieties.

The gene *Pia* may also be included, as shown by the resistance of the varieties to avirulent blast isolates B90002 and C923-49. However, *Pia* was masked by the presence of *Pik<sup>t</sup>*, *Pi20*, or *Pita* in the varieties. Blue Bonnet, a variety from the United States, was introduced in the development of IR24 and was identified to carry *Pia* (Kiyosawa 1972). IR24 could have inherited *Pia* from Blue Bonnet and likely transmitted the gene to its progenies. Although *Pib* was postulated in the varieties in VGs 1, 2, 3, and 7, it may also be included in VGs 4, 5, and 6. *Pik-h* and *Pi20* in the varieties might have masked the effect of *Pib*; this gene was thus not estimated. *Pik-s* was included in VGs 1 and 2, whereas the other *Pik* alleles were postulated in VGs 4, 5, and 6. *Pik-s* was also possibly included in VGs 3 and 7 for the same reason that *Pib* was included. In our study based on the differential system using blast isolates from the Philippines, the existence of these genes in IRRI varieties could not be confirmed.

Around 40 blast resistance genes have already been reported (RGC 1998). Comparatively, we could postulate a limited number of blast resistance genes in the IRRI varieties probably because of the genetic relatedness among these varieties. In the development of IRRI varieties, IR8, IR24, and IR36 or their progenies were often included. Because of this breeding methodology, many IRRI varieties showed similar reaction patterns to the blast isolates. This study is the first in elucidating the genetic constitution for blast resistance of important indica-type rice varieties in the tropics. Previous studies on a few IRRI varieties and lines fo-



cused on the inheritance of resistance to particular blast isolates from the Philippines. The number of genes conferring the resistance to the isolates was determined by segregation ratios. However, the genotypes of the blast resistance genes were unknown. In our study, at least seven kinds of blast resistance genes were estimated in the varieties by analyzing the reaction patterns to well-characterized blast isolates. We could also demonstrate the ability of the differential system to estimate the genotypes of multiple genes for blast resistance carried in a variety. Using reaction pattern data, on the other hand, we faced limitations because resistance may result from the masking of some genes. Masked genes in some varieties are difficult to infer by reaction patterns and will require genetic analysis to clarify them. However, their genotypes in a variety may be presumed by examining the avirulence genotypes of the isolates used. In this study, we used isolates that have been clarified for their avirulence genotypes, thus revealing the kinds of blast resistance genes to which they exhibited incompatible reactions. We also examined the pedigrees of the varieties to relate the estimated genes (masked genes included) to the parental varieties involved in developing these varieties. Although pedigree data may not be conclusive evidence, nevertheless, they are also important in inferring the presence and the kind of genes in the varieties. Genetic analysis on the basis of segregation and allelism analyses will be necessary to confirm the presence of estimated and masked gene(s). Wang et al (1999) and Jia et al (2002) published the sequence information of two genes, *Pib* and *Pita*, and molecular markers that can directly detect these genes have been developed. These molecular markers will also be useful for confirming and identifying the resistance genes. The reaction patterns to well-characterized blast isolates from the Philippines were particularly useful in estimating blast resistance genes in the varieties. More importantly, the information produced from this study should also be quite useful in rice blast resistance breeding using IRRI-bred rice varieties.

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## Notes

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# The reaction pattern of quantitative trait loci for agronomic traits under different regions for temperate and tropical rice

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Heading date is a most important trait for adaptation to different cultivation regions and cropping seasons. Quantitative trait loci (QTL) analysis for heading date in rice (*Oryza sativa* L.) has been done using recombinant inbred (RI) lines in Japan and the Philippines. We tried to identify chromosomal regions associated with heading date and to investigate genotype by environment interactions at individual QTLs under different conditions from temperate to tropical regions. A total of 190 RI lines that were developed from a cross between indica-type Milyang 23 (M23) and japonica-type Akihikari (AK) by the single-seed descent (SSD) method were used. The RI populations were cultivated at nine sites in Japan from 1995 to 2000 and at Los Baños in five dry and wet seasons from 2000 to 2002, and a total of 33 replications were carried out. The RI population at each site showed a wide variation in days to heading, including transgressive segregation in late heading. The averages and distributions of RI segregation varied depending on the differences in cultivated site and season (year, dry and wet). A total of 216 QTLs were detected on all chromosomes of rice except for chromosomes 5 and 8. Some 143 and 70 QTLs showed late heading with M23 and AK homozygous genotypes, respectively. Two QTLs, in R7 and R11-2, were particularly characterized by the strong influence of and a conflicting relationship with heading date depending on the cropping conditions as one of the typical cases of QTL reactions. In the investigations at nine sites of Japan in 1999 and at Los Baños in the dry and wet season of 2000, the higher-latitude site showed a later heading date in the average value and distribution of RI segregation. Two QTLs, in R7 and R11-2, showed a unique reaction depending on the latitude. In the temperate region, the effect of the QTL in R7 indicated that the lower-latitude site showed a higher LOD and phenotypic variance explained ( $R^2$ , %). The QTL in R11-2 indicated the opposite effect, that is, the higher-latitude site showed a higher LOD value. In Los Baños, these QTLs in R7 and R11-2 were detected only in the wet season of 2002 and dry season of 2002, respectively. Eight other QTLs were detected in different dry seasons. These results suggest that heading date is controlled mainly by two QTLs in R7 and R11-2, with a contrasting relationship in the temperate region, and the reaction patterns of QTLs in the tropical zone were completely different from those found in the temperate zone. The QTL reaction of the dry season was unique in comparison with the wet season at Los Baños and at the temperate sites.

Heading date is an important agronomic trait and it is strongly associated with the regional adaptability of rice (*Oryza sativa* L.) cultivation (Kikuchi 1979). Genetic analysis for heading date of rice has been carried out and many genes have been identified. At present, 23 major genes controlling heading date have been reported in rice, and 13 of them were determined to be located on the chromosome (Ichitani et al 1998a, Kinoshita 1998).

Recently, many chromosome regions affecting heading date have been reported using the genetic analysis of quantitative trait loci (QTL) with molecular markers (Li et al 1995, 2003, Lin et al 1995, 1998, Xiao et al 1995, 1996,

1998, Prince et al 1997, Yano et al 1997, Lu et al 1997, Doi et al 1998).

Yano et al (1997) found five putative QTLs, *Hd1*, *Hd2*, *Hd3*, *Hd4*, and *Hd5*, for heading date on the centromeric regions of chromosome 6, the long arm of chromosome 7, the short arm of chromosome 6, the centromeric regions of chromosome 7, and the short arm of chromosome 8, respectively, using an  $F_2$  population derived from the cross between japonica variety Nipponbare and indica variety Kasalath. Yamamoto et al (1998) confirmed that *Hd1* inherited as a Mendelian factor based on segregation analysis using chromosome segment substitution lines developed

by backcross breeding and marker-aided selection (MAS) with DNA markers. Ichitani et al (1998b) suggested that *Hd1* and *Hd4* corresponded with *Se1* and *E1*, respectively, from the results of allelism analysis using the hybrid populations derived from the crosses between Kasalath and 11 tester lines with different genotypes of four photoperiod-sensitivity loci, *E1*, *E2*, *E3*, and *Se1*. Yokoo et al (1980) and Ichitani et al (1998b) suggested that *Se1* and *E1* loci were strongly associated with the regional differentiation of rice cultivars because *Se1* had been known to have multiple alleles (Yokoo and Kikuchi 1977) and these two loci had strong effects when compared with the other photoperiod-sensitivity loci.

Lu et al (1997) detected four QTLs, *hd-1*, *hd-8*, *hd-10a*, and *hd-10b*, on the long arm of chromosome 1, short arm of chromosome 8, and centromeric region and long arm of chromosome 10, respectively, using a doubled-haploid (DH) population established from a cross between indica variety Zhai-Ye-Qing 8 and japonica variety Jing-Xi 17. They tried to evaluate the interactions between genotype and environment ( $G \times E$ ), that is, the phenotypic expression level of QTLs in different environments based on investigations at three sites with different degrees of northern latitude in China, and suggested that environmental factors such as daylength and temperature might affect the phenotypic expression of heading date. On the basis of QTL analysis, Li et al (2003) also tried to reveal the  $G \times E$  interactions of heading date in nine Asian environments using a DH population with indica variety IR64 crossed with Azucena. They could detect 20 QTLs for heading date on all 12 rice chromosomes and indicated that the phenotypic expression of almost all QTLs was influenced by environmental differences. These two studies were carried out under a restricted number of investigation environments or replications at the same site. However, although the existence of  $G \times E$  interactions could be proved, these did not explain the relationships between the genes (QTL) and environmental factors, and the association of genes with the regional differentiation of rice cultivars in detail.

Fukuta et al (1999) developed recombinant inbred (RI) lines derived from a cross between indica variety Milyang 23 (M23) in Korea and japonica variety Akihikari (AK) in Japan. In this study, we try to identify the key gene(s) of heading date for association with the regional differentiation of Asian rice cultivars, based on the reaction analysis for the detected QTLs through various investigations under different environments, geographical sites, cultivation years or seasons, and fertility conditions in the field, using M23 and AK cross RI lines.

## Materials and methods

### Investigation of RI populations

A total of 190 RI lines that were developed from a cross between indica variety Milyang 23 (M23) and japonica variety Akihikari (AK) by the single-seed descent (SSD)

method were used to investigate days to heading (DTH) (Fukuta et al 1999).

The RI populations were cultivated in irrigated field conditions (Table 1). A total of 33 replicated investigations were carried out at nine different sites, Ohmagari (39°N, 140°E) in 1999; Sendai (38°N, 140°E) in 1996, 1997, 1998, and 1999; Niigata (37°N, 138°E) in 1996 and 1999; Joetsu (37°N, 138°E) in 1995, 1996, 1997, 1998, 1999, and 2000; Tsukuba (36°N, 140°E) in 1997, 1998, and 1999; Tottori (35°N, 134°E) in 1999; Fukuyama (34°N, 133°E) in 1997 and 1999; Fukuoka (33°N, 130°E) in 1997, 1998, and 1999; and Kagoshima (31°N, 130°E) in 1999 during the normal Japanese cropping season, May to October, and at IRRRI, Los Baños (14°N, 121°E), Laguna, Philippines, in the dry season from January to April of 2000, 2001, and 2002, and in the wet season from June to October of 2000 and 2001. This was done continually at five sites, Joetsu, Sendai, Tsukuba, Fukuoka, and Los Baños, and at all nine Japanese sites in 1999. Additionally, the RI population was cultivated under different field conditions (no fertilizer and normal fertilizer according to each site's original method) at Joetsu and Sendai. At Joetsu and Sendai, fertilizer was applied as 70 kg N ha<sup>-1</sup> in 1996, 1997, and 1998, and as 100 kg N ha<sup>-1</sup> in 1997 and 1999.

Days to heading (DTH) after transplanting in irrigated paddy fields was investigated at each site and in each season. DTH was defined as the time when 50% of the plants in a plot exerted at least one spikelet.

### QTL analysis

Based on the genotypic data and a linkage map consisting of 182 restriction fragment length polymorphism (RFLP) markers (Fukuta et al 1999), QTLs for heading date were estimated by interval mapping with a 1.0 cM interval in the computer program *Qgene* (Nelson 1997). An LOD score of 2.0 was used as the threshold for detecting QTL locations in the program, and the peak of the LOD score indicated the position of the QTL.

## Results

### Segregation of days to heading

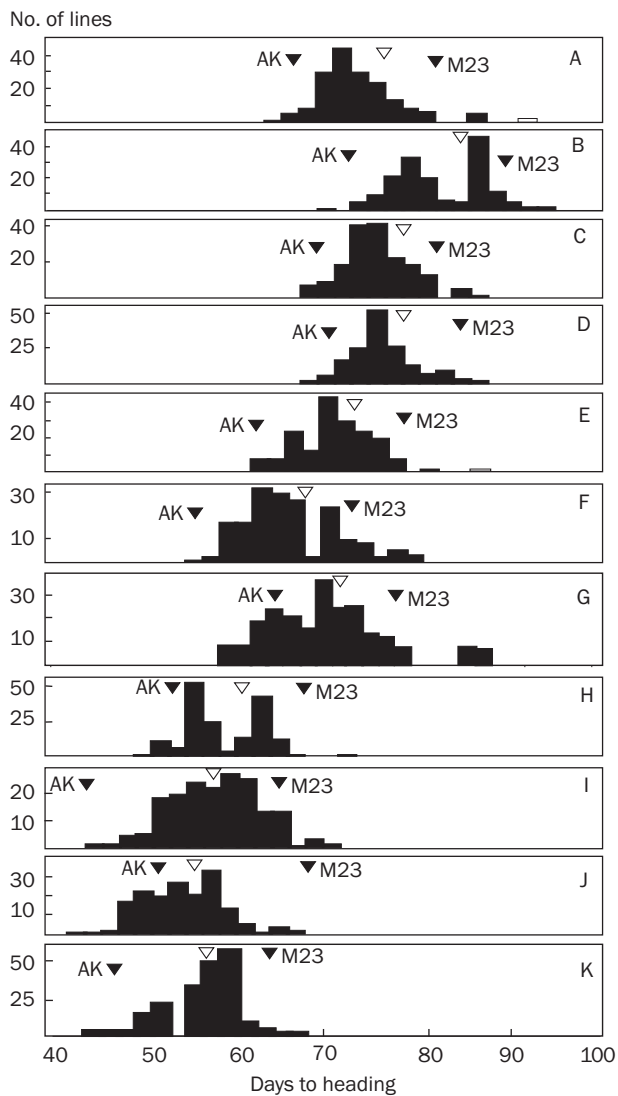
The RI population at each site showed a wide variation, including the transgressive segregation in late heading. The averages and distributions of RI segregation varied depending on the differences of the cultivated site, season (year, dry and wet), and fertilization conditions (Table 1).

The higher-latitude site showed a later heading date in the average value of RI segregation at nine sites in 1999 and at Los Baños in the dry and wet season of 2000. The segregation at Sendai, Tsukuba, Tottori, Fukuyama, and Fukuoka, and in the wet season of Los Baños showed distributions with two peaks (Fig. 1). These results suggested that DTH of the RI population changed dramatically according to the cropping sites, and few genes played an important role in this segregation. In addition, the effects of

**Table 1. Segregation of days to heading in Milyang 23/Akikhikari recombinant inbred lines.**

Site (latitude, longitude)	Research conditions		No. of lines used	Days to heading					
	Year or season	N fertilizer (kg ha <sup>-1</sup> )		Milyang 23	Akikhikari	Minimum	Maximum	Av	Standard deviation
Ohmagari (39°N, 140°E)	1999		190	81.0	65.0	65.0	95.0	74.7	5.2
Sendai (38°N, 140°E)	1996	100	164	101.0	78.0	77.0	108.0	91.8	6.6
	1997	100	191	98.0	75.0	77.0	127.0	93.0	7.1
	1997	0	190	97.0	75.0	76.0	113.0	89.5	6.4
	1998	100	191	97.0	75.0	76.0	113.0	89.5	6.4
	1999	100	190	93.0	70.0	70.0	102.0	83.7	5.4
	1999	0	190	90.0	70.0	73.0	109.0	85.5	6.2
Average		Normal		97.3	74.5	75.0	112.5	89.5	
		None		93.5	72.5	74.5	111.0	87.5	
Niigata (37°N, 138°E)	1996		153	93.0	67.0	69.0	99.0	85.2	6.7
	1999		187	97.0	84.0	84.0	109.0	94.6	4.3
Average				95.0	75.5	76.5	104.0	89.9	
Joetsu (37°N, 138°E)	1995	70	187	93.0	72.0	71.0	126.0	85.4	7.6
	1996	70	187	88.0	73.0	70.0	119.0	82.3	7.9
	1996	0	190	90.0	73.0	70.0	117.0	82.3	7.3
	1997	70	190	84.0	66.0	69.0	102.0	77.1	4.9
	1997	0	190	84.0	66.0	65.0	105.0	77.4	5.1
	1998	70	190	82.0	65.0	62.0	89.0	74.9	5.3
	1998	0	190	80.0	65.0	67.0	98.0	75.5	5.2
	1999	70	188	85.0	68.0	68.0	98.0	77.3	4.4
	2000	70	188	80.0	63.0	63.0	95.0	75.9	5.0
	Average		Normal		85.3	67.8	67.2	104.8	78.8
		None		84.7	68.0	67.3	106.7	78.4	
Tsukuba (36°N, 140°E)	1997		186	74.0	61.0	58.0	82.0	68.5	3.8
	1998		190	92.0	83.0	71.0	95.0	79.8	3.8
	1999		185	75.0	62.0	62.0	89.0	71.6	4.2
Average				80.3	68.7	63.7	88.7	73.3	
Tottori (35°N, 134°E)	1999		190	72.0	54.0	55.0	80.0	66.4	5.0
Fukuyama (34°N, 133°E)	1997		185	78.0	61.0	63.0	80.0	72.0	3.8
	1999		187	76.0	58.0	59.0	90.0	70.4	5.0
Average				77.0	59.5	61.0	85.0	71.2	
Fukuoka (33°N, 130°E)	1997		186	69.0	55.0	54.0	85.0	64.5	4.1
	1998		190	61.0	47.0	47.0	71.0	55.6	4.2
	1999		190	67.0	52.0	51.0	75.0	59.2	4.4
Average				65.7	51.3	50.7	77.0	59.8	
Kagoshima (31°N, 130°E)	1999		190	64.0	43.0	45.0	71.7	57.9	5.0
Los Baños (14°N, 121°E)	2000 Dry	50	188	67.0	50.0	38.0	68.0	54.4	4.9
	2000 Wet	50	190	62.0	44.0	33.0	67.0	56.3	4.1
	2001 Dry	50	190	59.0	29.0	26.0	63.0	46.5	7.4
	2001 Wet	50	182	51.0	24.0	30.0	59.0	45.4	4.9
	2002 Dry	50	182	60.0	28.0	28.0	69.0	50.8	7.7
Average		Dry		62.0	35.7	30.7	66.7	50.6	
		Wet		56.5	34.0	31.5	63.0	50.9	





**Fig. 1. Segregation of days to heading in recombinant inbred lines derived from a cross between Milyang 23 and Akihikari. Data are collected at nine sites of Japan in 1999 and at Los Baños, in the dry and wet season of 2000. A = Ohmagari, n = 190; B = Sendai, n = 190; C = Niigata, n = 187; D = Joetsu, n = 188; E = Tsukuba, n = 185; F = Tottori, n = 190; G = Fukuyama, n = 188; H = Fukuoka, n = 190; I = Kagoshima, n = 190; J = Los Baños, dry season, n = 188; K = Los Baños, wet season, n = 190.**

genes for heading date were influenced by the cropping conditions.

The distributions of the RI population at Joetsu from 1995 to 2000, at Sendai from 1996 to 1999, and at Tsukuba and Fukuoka from 1997 to 1999 showed significant differences among the years studied at each site. The average value and range of segregation in the RI population at Sendai, Joetsu, Tsukuba, and Fukuoka were 89.5 and 83.7–93.0, 78.8 and 75.9–85.4, 73.3 and 68.5–79.8, and 59.8 and 55.6–64.5, respectively, in normal soil conditions. Two or three peaks of segregation were observed in many investigations, but the distribution patterns were not similar among years at each site.

The reactions of RI populations to different fertility conditions were also investigated. In the two fertility conditions (0 kg N ha<sup>-1</sup> and 70 or 100 kg N ha<sup>-1</sup>), the RI population was cultivated at Joetsu in 1997 and 1998, and at Sendai in 1997 and 1999. The differences in average and ranges of segregation in the RI population were smaller than those among sites and years.

Among the nine sites in Japan, the averages and ranges of DTH in RI segregation were 59.8–87.5 and 55.6–91.8, respectively. At Los Baños, the values for the dry and wet season were 50.6 and 46.5–54.4, and 50.9 and 45.4–56.3, respectively, and significant differences between them were not recognized. The values at Los Baños were remarkably lower than those at the Japanese sites. These results indicated that the conditions at Los Baños in the tropical zone were completely different from those in Japan in the temperate zone, and the reaction of the RI population might have changed.

These investigations of site, year, and fertility conditions showed that DTH was changed conspicuously by cropping season and site, but not by field soil conditions. These results suggested that the environmental conditions, temperature, and length of day during the time of RI cultivation might influence most the segregation.

#### QTL analysis

From the results of 33 replications, a total of 213 QTLs were detected on all chromosomes of rice except for chromosomes 5 and 8. These QTLs were detected repeatedly in 13 chromosome regions on the long arm of chromosome 1 (R1), near the centromere (R2-1) and long arm (R2-2) of chromosome 2, on the short and long arm of chromosome 3 (R3), near the centromere (R4-1) and on the long arm (R4-2) of chromosome 4, on the long arm of chromosome 6 (R6), near the centromere of chromosome 7 (R7), on the short arm of chromosome 9 (R9), near the centromere of chromosome 10 (R10), and on the short arm (R11-1) and near the centromere (R11-2) of chromosome 11. Among these 213 QTLs, 143 and 70 showed late heading with the M23 and AK homozygous genotype, respectively. These QTLs in 10 chromosome regions—R2-1, R2-2, R3-1, R3-2, R4-1, R4-2, R6, R7, R9-1, and R9-2—and four regions—R1, R10, R11-1, and R11-2—showed late heading with the M23 and AK alleles, respectively (Fig. 2).

Among the chromosome regions that detected QTLs, the two QTLs in R7 and R11-2 were characterized by a strong influence on and mutual relationship with DTH depending on the investigated conditions as one of the typical cases of QTL reactions. The QTL in R7 was detected between RFLP markers *XNpb33* and *XNpb91* near the centromere, and the M23 allele showed late heading. The QTL in R11-2 was also located between two RFLP markers (*XNpb202* and *XNpb257*) near the centromere, with the AK allele showing late heading. In the temperate region, the function of the QTL in R7 showed that the lower-latitude site had a higher LOD value. The QTL in R11-2 indicated the opposite, that is, the higher-latitude site had a higher

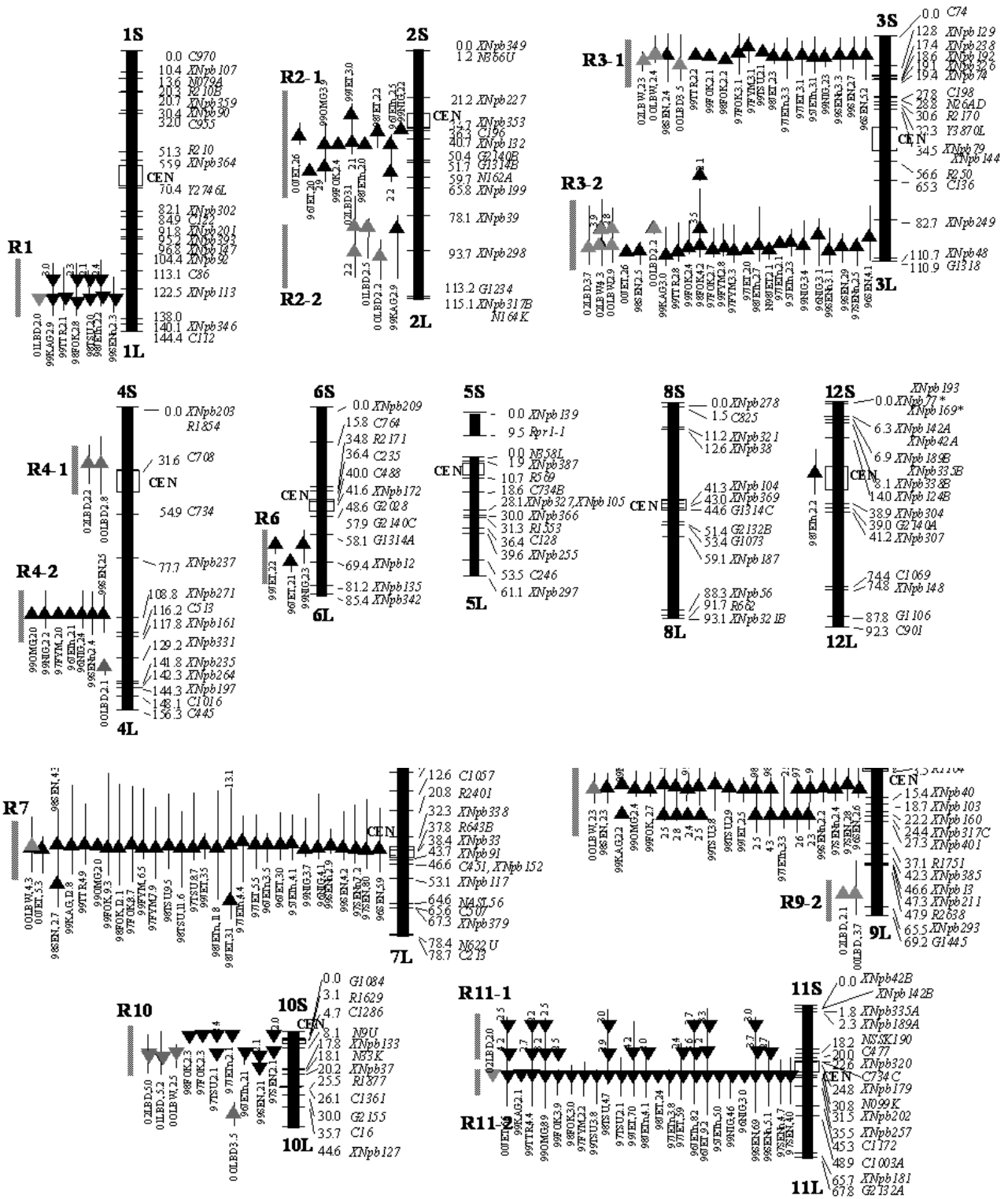


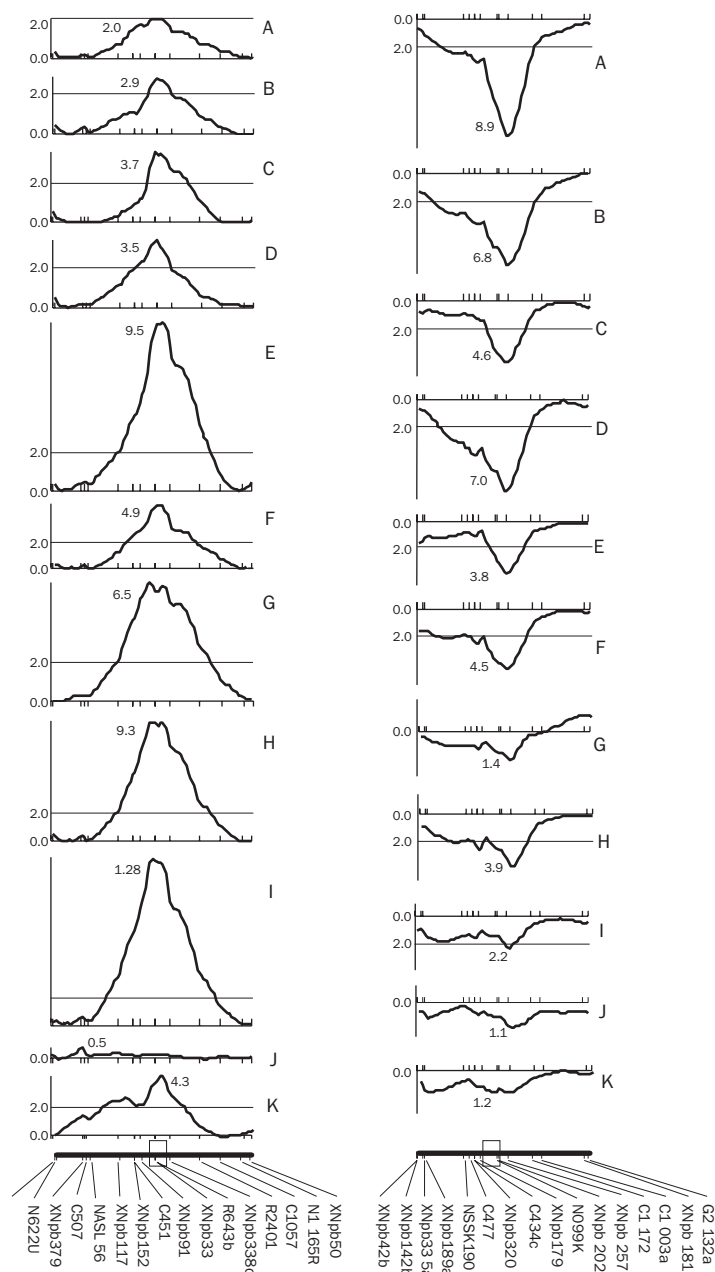
Fig. 2. Putative QTLs for days to heading in Milyang 23/Akihikari cross recombinant inbred lines. Investigations were carried out at nine sites of Japan from 1995 to 1999 and at Los Baños, Philippines, in the dry and wet season of 2000. Almost all QTLs were detected in several chromosome regions repeatedly, which were designated by R followed by a number. Triangles and bars indicate the position of peaks and the range of LOD scores over 2.0. Two kinds of triangles ( $\blacktriangle$  and  $\blacktriangledown$ ) indicate the effects on late heading of Milyang 23 and Akihikari genotypes, respectively. 990MG = Ohmagari in 1999, 96SEN = Sendai in 1996, 97SEN = Sendai (100 kg N ha<sup>-1</sup>) in 1997, 97SEN = Sendai (0 kg N ha<sup>-1</sup>) in 1997, 99SEN = Sendai (0 kg N ha<sup>-1</sup>) in 1999, 99SEN = Sendai (100 kg N ha<sup>-1</sup>) in 1999, 96NIG = Niigata in 1996, 99NIG = Niigata in 1999, 95JET = Joetsu in 1995, 96JET = Joetsu in 1996, 97JET = Joetsu (70 kg N ha<sup>-1</sup>) in 1997, 97JET = Joetsu (0 kg N ha<sup>-1</sup>) in 1997, 98JET = Joetsu (70 kg N ha<sup>-1</sup>) in 1998, 98JET = Joetsu (0 kg N ha<sup>-1</sup>) in 1998, 99JET = Joetsu in 1999, 00JET = Joetsu in 2000, 97TSU = Tsukuba in 1997, 98TSU = Tsukuba in 1998, 99TSU = Tsukuba in 1999, 97FYM = Fukuyama in 1997, 99FYM = Fukuyama in 1999, 97FOK = Fukuoka in 1997, 98FOK = Fukuoka in 1998, 99FOK = Fukuoka in 1999, 99TTR = Tottori in 1999, 99KAG = Kagoshima in 1999, 00LBD = Los Baños in 2000 dry, 00LBW = Los Baños in 2000 wet, 01LBD = Los Baños in 2001 dry, 01LBW = Los Baños in 2001 wet, 02LBD = Los Baños in 2002 dry. The RFLP linkage map (Fukuta et al 1999) was modified and used.

LOD value. In Los Baños, the QTL in R7 was detected only in the wet season of 2000, and the QTL in R11-2 could not be detected in both the dry and wet seasons except for the dry season of 2002. The two QTLs in R7 and R11-2 showed a mutual relationship, but any tendency or relationship among QTLs in the other chromosome regions was not recognized according to the latitude (Fig. 3). The effects of R7 and R11-2 changed according to the geographical differences. These relationships between the QTLs of R7 and R11-2 were detected only in Japan (temperate zone) and not in Los Baños (tropical zone).

These effects of QTLs in R7 and R11-2 also showed wide variations among years or seasons at each site (Table 2). In the normal fertility conditions of four sites, Sendai from 1996 to 1999, Joetsu from 1995 to 2000, Tsukuba from 1997 to 1999, and Fukuoka from 1997 to 1999, the LOD scores of the QTL in R7 were 2.9–4.2, 3.0–13.1, 8.7–11.6, and 8.7–12.1, respectively. The phenotypic variance explained ( $R^2$ , %) of the QTLs at Sendai, Joetsu, Tsukuba, and Fukuoka also ranged widely (6.8–17.5, 7.1–27.2, 19.3–24.4, and 19.4–25.3), respectively. The LOD scores and  $R^2$  (%) of the QTL in R11-2 at Sendai, Joetsu, Tsukuba, and Fukuoka were in the ranges of 1.5–6.9 and 4.2–15.3, 2.3–9.2 and 5.5–20.2, 3.8–4.7 and 9.0–10.7, and 2.0–3.9 and 4.8–9.0, respectively. These results suggested that the effects of the QTLs in R7 and R11-2 were changed by the cropping year or season. These effects of detected QTLs in the other chromosome regions also showed remarkable changes depending on years and seasons. In other words, segregation in the RI population and QTL effects changed dramatically depending on environmental conditions such as temperature or daylength during the growth stage.

In Los Baños, the QTLs in R7 and R11-2 were detected only in the wet season of 2000 and dry season of 2002, respectively. The QTL effects of R7 and R11-1 were also not so high (LOD = 4.3 and  $R^2$  = 9.9 %, and LOD = 2.0 and  $R^2$  = 5.0 %), respectively, compared with those of Japan. Eight specific QTLs were detected only in the dry season of Los Baños in three chromosome regions—R2-2 (two QTLs), R4-1 (two), and R9-2 (two)—and on the long arms of chromosomes 4 (flanking markers *XNpb331*–*XNpb235*) and 10 (*C16*–*XNpb127*). These results indicated that the reaction patterns of QTLs in Los Baños (tropical zone) were completely different from those found in Japan (temperate zone), and the dry season's reactions were unique in comparison with the wet season of Los Baños and the temperate zone.

These patterns and LOD scores and  $R^2$  (%) in interval mapping analysis were not so different between the normal



**Fig. 3. Interval mapping of days to heading on chromosomes 7 and 11 on the basis of data obtained at nine sites of Japan in 1999 and at Los Baños in the dry and wet seasons of 2000. Upper and lower LOD scores from horizontal bars indicate the positive effects for late heading in Milyang 23 and Akihikari alleles, respectively. The peak values of LOD scores are indicated on each interval map. A = Ohmagari, B = Sendai, C = Niigata, D = Joetsu, E = Tsukuba, F = Tottori, G = Fukuyama, H = Fukuoka, I = Kagoshima, J = dry season at Los Baños, K = wet season at Los Baños.**

and nonfertility conditions compared to those of years and sites. These results indicate that the influence of fertility conditions on QTL reaction is smaller than that of site and season.

These results suggest that the heading date in the RI population was strongly influenced by environmental factors (temperature and daylength), and two QTLs in R7 and

**Table 2. Two putative QTLs for days to heading on chromosomes 7 and 11.**

Cultivation conditions		Chr 7 (R7, XNpb33–XNpb91 <sup>a</sup> )				Chr 11 (R11-2, XNpb202–XNpb257 <sup>a</sup> )			
Site	Season and amount of N (kg ha <sup>-1</sup> )	Distance from short-arm terminal (cM)	R <sup>2b</sup> (%)	LOD	A <sup>c</sup>	Distance from short-arm terminal (cM)	R <sup>2b</sup> (%)	LOD	A <sup>c</sup>
Sendai	1996	36	15.1	5.8	2.7	34	4.2	1.5	-1.5
	1997	34	17.5	7.9	3.2	34	9.5	4.1	-2.3
	1997 (0)	36	16.1	7.2	2.7	34	11.0	4.8	-2.2
	1998	36	16.1	7.2	2.7	34	11.0	4.8	-2.2
	1999	38	6.8	2.9	1.5	34	15.3	6.9	-2.2
	1999 (0)	38	9.5	4.1	1.9	34	11.6	5.1	-2.2
Joetsu	1995	34	9.6	4.1	2.6	34	11.7	5.1	-2.7
	1996	39	7.1	3.0	2.2	34	20.2	9.2	-3.7
	1996 (0)	36	8.2	3.5	2.2	34	17.9	8.2	-3.3
	1997	36	8.2	3.5	2.2	34	13.2	5.9	-1.9
	1997 (0)	41	10.1	4.4	1.7	34	8.8	3.8	-1.6
	1998	40	27.2	13.1	3.0	34	5.5	2.3	-1.3
	1998 (0)	35	24.8	11.8	2.8	34	9.4	4.1	-1.7
	1999	38	8.2	3.5	1.3	34	15.8	7.0	-1.9
	2000	36	12.1	5.3	1.9	34	15.0	6.6	-2.1
Tsukuba	1997	35	19.3	8.7	1.8	34	9.0	3.8	-1.2
	1998	35	24.4	11.6	2.0	34	10.7	4.7	-1.3
	1999	36	21.1	9.5	2.1	34	9.1	3.8	-1.3
Fukuoka	1997	38	19.4	8.7	1.8	34	4.8	2.0	-0.9
	1998	36	25.3	12.1	2.2	35	7.1	3.0	-1.2
	1999	36	19.9	9.2	2.1	35	9.0	3.9	-1.4
Los Baños	2000 Dry	36	0.6	0.3	0.4	34	2.5	1.1	-0.8
	2000 Wet	37	9.9	4.3	1.3	34	2.8	1.2	-0.7
	2001 Dry	36	1.1	0.4	0.8	34	4.1	1.7	-1.6
	2001 Wet	36	1.4	0.6	0.6	34	0.1	0.0	-0.6
	2002 Dry	36	1.7	0.7	1.1	34	5.0	2.0	-1.8

<sup>a</sup>Markers flanking the detected QTL. <sup>b</sup>Phenotypic variance explained by each QTL. <sup>c</sup>Additive effect of the allele from Milyang 23 compared with that of Akihikari. R7 and R11-2 are chromosome regions where QTLs were detected.

R11-2 played the most important roles in segregation in the RI population, with a contrasting relationship in the temperate zone. Completely different QTL sets were functional between Los Baños (tropical zone) and Japan (temperate zone), and the dry and wet season in the tropical zone.

## Discussion

A total of 213 QTLs for heading date were detected, but the QTL corresponding to a photoperiod-sensitive gene, *Se1*, on chromosome 6 (Yano 1997, Yamamoto 1998) was not detected in this study. It was estimated that these two parents have the same allele on the locus. This means that the QTL analyses were carried out without the influence of a strong photoperiod-sensitive gene, *Se1*, and we could efficiently detect other QTLs for heading date.

The results of QTL analyses indicate that two QTLs in R7 and R11-2 controlled segregation of the RI population

in the temperate region. Instead of these two QTLs, the other QTL sets controlled segregation in the tropical zone. Another eight QTLs were detected in R2-2, R9-2, and the long arm of chromosomes 4 and 10 in the dry season of Los Baños. This means that heading date in a tropical zone, especially in the dry season, is controlled by a completely different genetic mechanism.

The QTLs in R7 and R11-2 had important roles in controlling days to heading in the temperate zone especially. The QTL in R7 may correspond to a photoperiod-sensitive gene, *E1*, because the detected QTL was located in the same chromosome region where Ichitani et al (1998a,b) found it. Yokoo et al (1980) and Ichitani et al (1998b) suggested that the two photoperiod-sensitivity loci, *Se1* and *E1*, were highly associated with the regional differentiation of rice cultivars because *Se1* had been known to have multiple alleles (Yokoo and Kikuchi 1977) and these two, *Se1* and *E1*, had strong effects compared with other genes. Both the QTL in



R11-2 and the QTL in R7 had a strong effect and mutual relationship. We conclude that the QTL in R7 might also be associated with the regional differentiation and adaptation of Asian rice cultivars. In the same chromosome region of R11-2, no QTL had been detected in almost all QTL analyses (Li et al 1995, Lin HX et al 1995, Xiao et al 1995, 1996, 1998, Prince et al 1997, Yano et al 1997, Lu et al 1997, Doi et al 1998, Lin SY et al 1998) because these investigations were carried out in a limited area, with no replications, or cross combination of hybrid populations. Li et al (2003) could detect only one QTL from the analyses using the IR64/Azucena DH population in the Indian environment, but the mutual relationship with *El* on chromosome 7 was not shown. In our investigations through a wide latitude from south to north, the effects of the QTL in R11-2 in a higher latitude were stronger than those of the lower latitude. In replications at Sendai from 1996 to 1999, at Joetsu from 1995 to 2000, at Tsukuba from 1997 to 1999, and at Fukuoka from 1996 to 1991, the effect of the QTL in R11-2 at each site, where the same daylength occurred, had a mutual relationship with the QTL in R7 among years. From these results, it can be hypothesized that the QTL in R11-2 is not a photoperiod-sensitive gene and the other genetic factor is related to vegetative growth.

Lin et al (1995) identified seven QTLs on chromosomes 1, 3, 6, (two), 8 (two), and 12 using two kinds of F<sub>2</sub> populations developed by indica and japonica line crosses. Li et al (1995) detected three QTLs, *QHd3a*, *QHd8a*, and *QH9a*, for heading date on chromosomes 3, 8, and 9, respectively, using hybrid progenies derived from tropical japonica variety Lemont, and indica variety Teqing. Prince et al (1997) found three QTLs on the short arm of chromosome 3, the distal region on the short arm of chromosome 8, and the centromeric region of chromosome 10 using the F<sub>2</sub> population derived from a cross between upland japonica variety Azucena and indica variety Bala. Lu et al (1997) detected four QTLs, *hd-1*, *hd-8*, *hd-10a*, and *hd-10b*, on the long arm of chromosome 1, the short arm of chromosome 8, and the centromeric region and long arm of chromosome 10, respectively, using a DH population established from a cross between indica variety Zhai-Ye-Qing 8 and japonica variety Jing-Xi 17. In previous reports, several QTLs were detected commonly on the same chromosome: the long arm of chromosome 1, the short arm of chromosomes 3 and 8, and the centromeric region of chromosome 10. In our analyses, QTLs for heading date were also detected in these chromosome regions, except for chromosome 8. These findings suggest that some genes with important roles for controlling heading date in Asian rice cultivars are located on these chromosome regions.

Yano et al (1997) and Lin et al (1998) detected five putative QTLs, *Hd1*, *Hd2*, *Hd3*, *Hd4*, and *Hd5*, and another three QTLs, *Hd6*, *Hd7*, and *Hd8*, by using the hybrid populations derived from crosses between Kasalath and Nipponbare. Yamamoto et al (1998, 2000, respectively)

confirmed the single-gene segregation and these detailed effects of *Hd1* and two QTLs, *Hd6* and *Hd7*, as Mendelian factors using chromosome segment substitution lines (CSSL) developed by backcross breeding and marker-aided selection (MAS) with DNA markers. These CSSL or near-isogenic lines for targeting the detected QTLs are useful for clarifying the detailed characters of the QTLs. We need to characterize the QTLs detected in detail and clarify the relationships between QTLs for heading date and other traits by using these CSSL or isogenic lines developed by backcross breeding using MAS.

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## Notes

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# **Crop management**

# Nutritional aspects of rice grain yield

G. Wada, P.C. Sta. Cruz, and H. Ando

Variation in nitrogen uptake pattern under different cultural practices and the varietal response of nitrogen were investigated for low to moderate nitrogen levels. IR varieties with different growth duration (GD) were evaluated in terms of N uptake and N-use efficiency.

The amount of N in the plant increased exponentially ( $y = ab^x$ ) at the early growth stage and linearly ( $y = a + bx$ ) at the middle and late growth stage. The crossing point of two equations coincided with the maximum-tillering stage (MT). No varietal difference was observed in nitrogen absorption pattern. Narrow spacing shortened the exponential phase and increased N uptake at the early growth stage (NAE). A higher basal N application increased N uptake at the early growth stage, but did not increase N uptake after MT. Parameter  $b$  in the linear phase was not affected by spacing and rate of fertilizer application, but it varied with the kind of basal fertilizer and soil fertility. Substantial differences occurred in total N in the plant at 5 weeks after transplanting, but not after MT. Generally, test varieties had consistent N absorption characteristics at different growth stages regardless of N level and cropping season.

Total N in the plant at the critical growth stage increased with GD, which can be attributed to N absorption pattern. Potential sink size (PS) was correlated with plant N at the late stage of spikelet initiation and with GD. Diminished sink size and unripened grains were correlated with GD. Therefore, sink size and yield were explained by a quadratic function of GD.

Total N in the plant at 30 days after transplanting (DAT) was positively correlated with PS, sink size, grain yield, contribution of plant N to sink formation, and N harvest index (NHI), but not with spikelet reduction or ripening percentage of short- and medium-duration varieties. The contribution of plant N to sink formation and yield is increased by higher basal N and narrow spacing for the short-duration varieties used. This suggests that NAE is an important determinant of sink size and grain yield of short- and medium-duration varieties.

Yield and yield-determining processes are markedly affected by the amount of nitrogen (N) absorption at each growth stage. In contrast with phosphorus and potassium, even a small excess of N immediately decreases ripening percentage and grain yield (Matsushima 1976). The rice plant requires 1.5 to 1.7 kg of N to produce 100 kg of paddy (Wada et al 1986).

Recently, the remarkable increase in rice yield has been attributed to high-yielding varieties, the use of N in commercial forms, and better cultural practices. However, the natural soil fertility of paddy fields is sometimes ignored by farmers, who prefer to use chemical fertilizers in large quantities, forgetting that a large amount of N absorbed by the plant is derived from the soil. Therefore, to obtain higher yield, how to increase the amount of N absorption by the plant without disturbing growth and how to increase plant

N-use efficiency are of utmost importance. This paper will deal with the nutritional aspects of yield and yield-determining processes as worked out in research from 1985 to 1989.

## Nitrogen absorption pattern

The nitrogen absorption pattern of rice varieties grown under different cultural practices and different cropping seasons was investigated. In both the dry and wet season (DS and WS), the amount of N in the plants was closely related to the number of days after transplanting (DAT). Exponential equations ( $y = ab^x$ ) were obtained during the early growth stage and linear equations ( $y = a + bx$ ) during the middle and late growth stages (Figs. 1, 2, and 3). There was no difference in N absorption pattern between short- and medium-dura-

Amount of N ( $\text{g m}^{-2}$ )

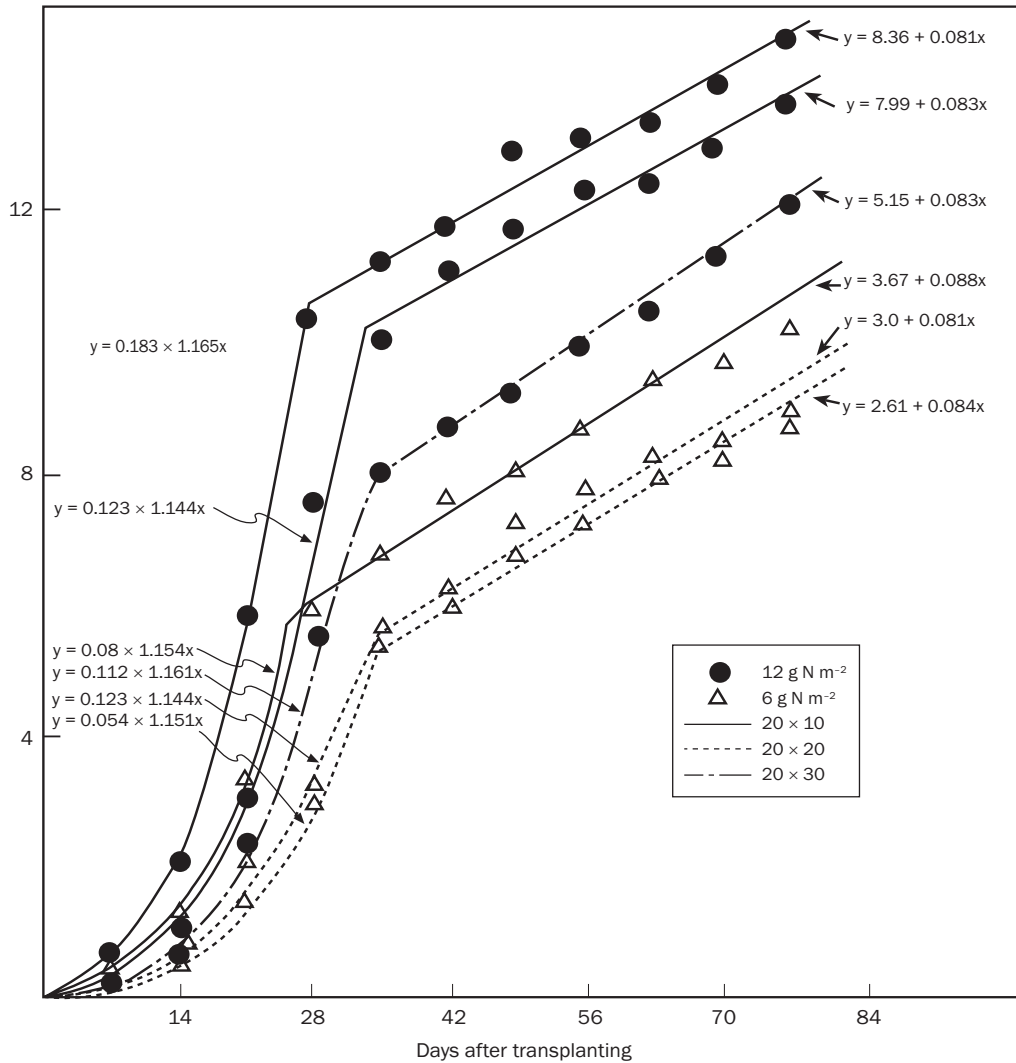


Fig. 1. Nitrogen absorption pattern of rice plants grown under different cultural practices (experimental fields, IR64, 1986 wet season).

tion varieties. Exponential equations were obtained during the period when  $\text{NH}_4\text{-N}$  was found in the plow layer. Linear equations were observed when the  $\text{NH}_4\text{-N}$  concentration in the plow layer was constantly low (Fig. 4). Earlier findings showed a similar trend of N absorption on accumulated effective thermal index (AETI) (Hanyu and Uchijima 1962, Takahasi et al 1973). In the tropics, there is no great difference in temperature during the period from transplanting to maturity. Hence, DAT is equivalent to AETI in the temperate area. The crossing point of the exponential equation and linear one generally coincided with the maximum-tillering stage (MT). The absorption of basal N usually terminated at MT.

The exponential phase was shortened by narrow spacing but not affected by the rate of N. Parameter b in the exponential equation was affected by spacing and rate of basal N. The application of slow-release fertilizer (urea coated with plastic resin) compared with ammonium sulfate resulted

in a longer duration of the exponential phase. Varietal differences in N absorption were observed only during the exponential phase. Parameter b in the linear equation did not differ with a varying rate of basal N and plant spacing. However, it was affected by soil chemical characteristics and kind of fertilizer. Slow-release fertilizer increased the rate of N uptake during the linear phase. The amount of N absorbed during the linear phase was correlated with the amount of mineralized N in fresh soil calculated by Michaelis-Menten's equation. Ando and Shoji (1986) obtained exponential equations for the mineralization of soil organic N compounds, which consist of rapidly and slowly mineralizable soil organic compounds. Rapidly mineralizable soil N compounds were mineralized at the early growth stage of the plant. The soil condition before transplanting affected the mineralization of soil organic N compounds. The longer the duration of drying, the greater the amount of soil organic N compounds that was mineralized irrespective of the soil's par-

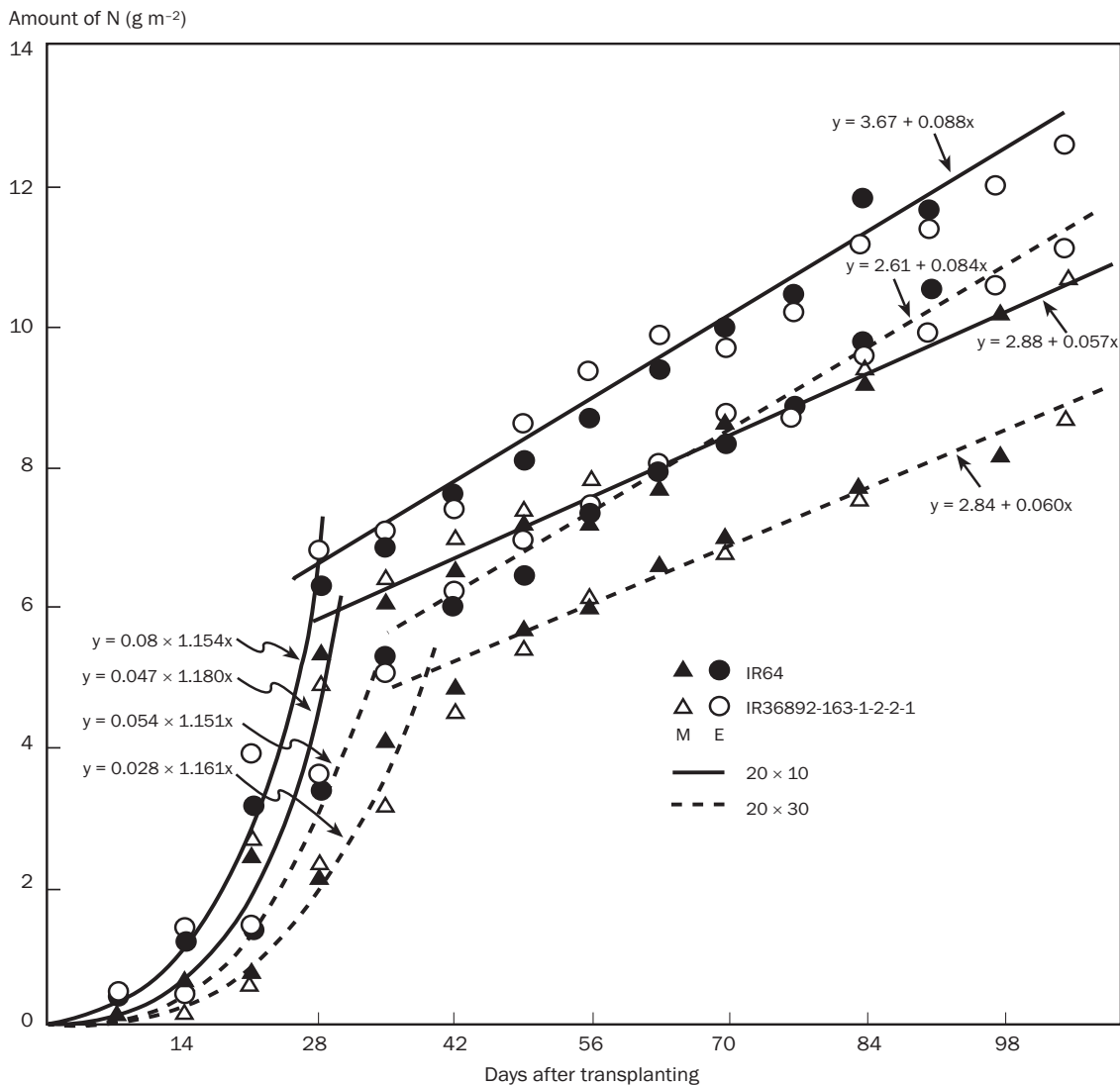


Fig. 2. Nitrogen absorption pattern of rice plants grown under different soils (1986 wet season). Note: M and E are experimental fields.

ent materials (dry effect). The dry effect of soil on the mineralization of soil organic N compounds was limited during the early growth stage of the plant (Ando et al 1989).

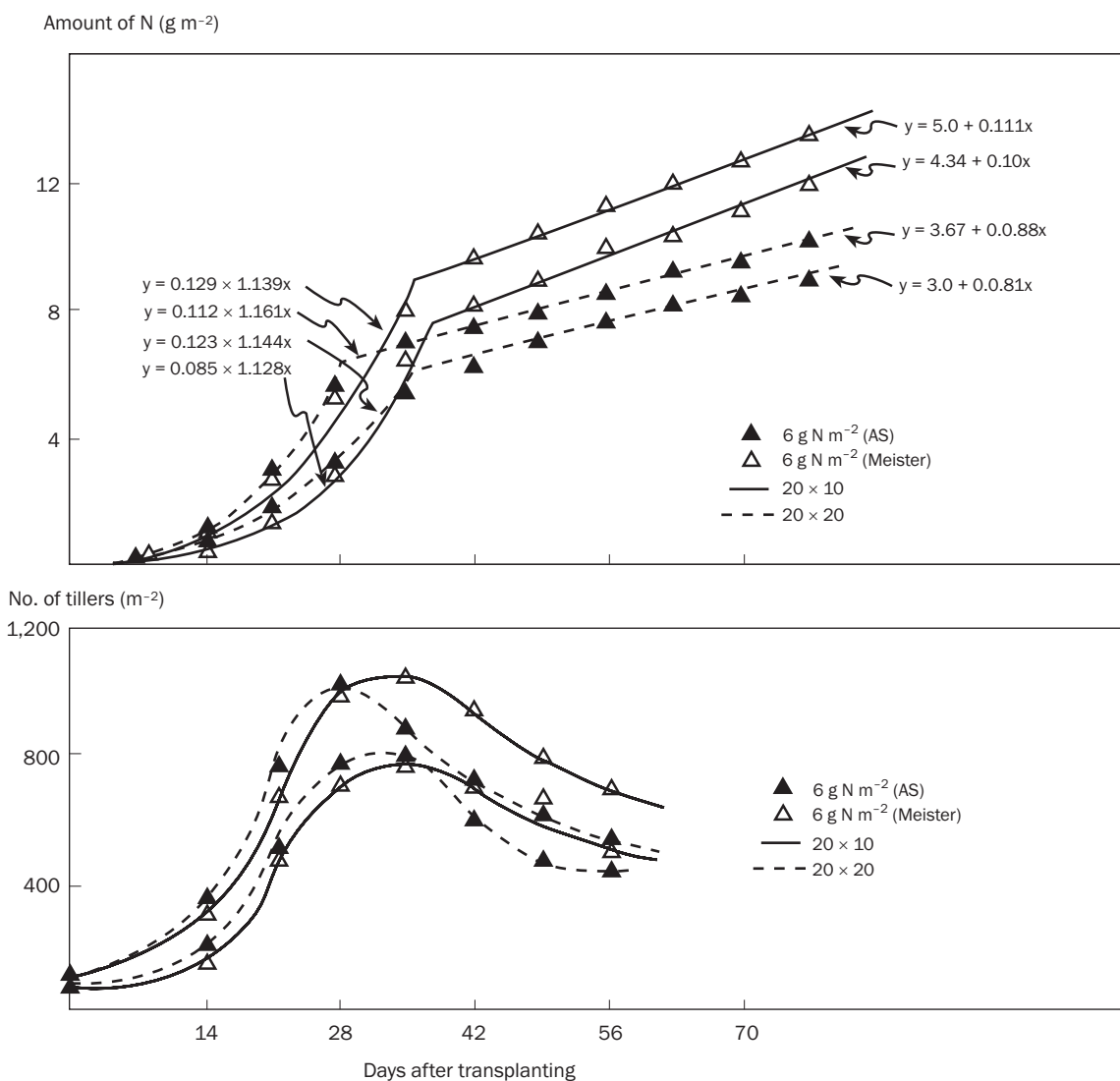
At the early growth stage, plants absorbed mainly basal N and rapidly mineralizable organic N and partly absorbed slowly mineralizable organic N (Table 1). At the middle and late growth stages, plants absorbed slowly mineralizable organic N and topdressed N. After topdressing, the rate of N absorption increased rapidly. When the  $\text{NH}_4\text{-N}$  derived from topdressed fertilizer was no longer available in the plow layer, the N absorption rate returned to the normal rate before topdressing (Wada et al 1986). No varietal difference in N absorption of soil N and topdressed N during the linear phase was observed.

During the exponential phase, the absorption of basal N and rapidly mineralizable soil N can be increased by a higher dose of basal N and narrow spacing. In contrast, there

is a very limited chance to increase the absorption of slowly mineralizable soil N by cultural practices.

Panicle primordia initiation (PI) is observed almost on the same day in identical varieties within a cropping season. Therefore, the duration of the vegetative lag phase (VLP) of the plant was affected by plant spacing and growth duration of the plant. Varietal difference in the N absorption pattern based on the DAT was not apparent. Based on the growth stage, however, varietal difference in the N absorption pattern was noted between short- and long-duration varieties. Within a variety, the N absorption pattern was affected by plant spacing. Subsequent N-use efficiency was affected by VLP. The contribution of plant N to sink size (S/N) and potential sink size (PS/N) increased with an increase in VLP for short-duration varieties; however, there was no relationship among VLP and S/N and PS/N in the case of long-duration varieties. Narrow spacing and higher basal N





**Fig. 3. Nitrogen absorption pattern and pattern of tillering of rice plants grown under different kinds of nitrogen fertilizer (experimental fields, IR64, 1986 wet season). AS = ammonium sulfate.**

application increase the contribution of plant N to PS/N and S/N, particularly for short-duration varieties (Table 2).

### Varietal differences in N response

Recent studies pointed out the importance of varietal differences in N absorption and the contribution of plant N to yield-determining processes (Broadbent et al 1987, Cregan et al 1984, Miyagawa 1981). To determine the effect of growth duration (GD) and N uptake on yield-determining processes, 60 IR varieties and lines were planted under two N levels. The amount of N in the plant at critical growth stages was positively correlated with GD (Fig. 5). Varietal differences in N absorption were apparent during the tillering stage, whereas insignificant differences were noted after MT (Table 3). Since the MT stage occurs simultaneously in all the varieties grown under the same cultural conditions, the

amount of N in the plant at the critical growth stages increases with an increase in GD. Potential sink size was correlated with GD (Fig. 6) and amount of N in the plant at flowering ( $r = 0.76^{**}$ ,  $y = -511 + 180x$ ). The contribution of N in the plant at flowering to PS (PS/N) was explained by the quadratic function of GD (Fig. 7). PS/N increased with GD up to 125 days (equivalent to an increase in VLP up to 15 days). The low contribution of plant N of short-duration varieties can be explained by the contribution of N absorbed at different growth stages and N absorption patterns. The PI of short-duration varieties occurred before or simultaneously with the MT stage. Therefore, a large amount of N is still absorbed from PI to the late stage of spikelet initiation. The N absorbed after PI until the late stage of spikelet initiation has less effect on differentiation of spikelets than before PI (Wada 1969). The coefficient of variance (CV) of PS/N decreased with an increase in GD.

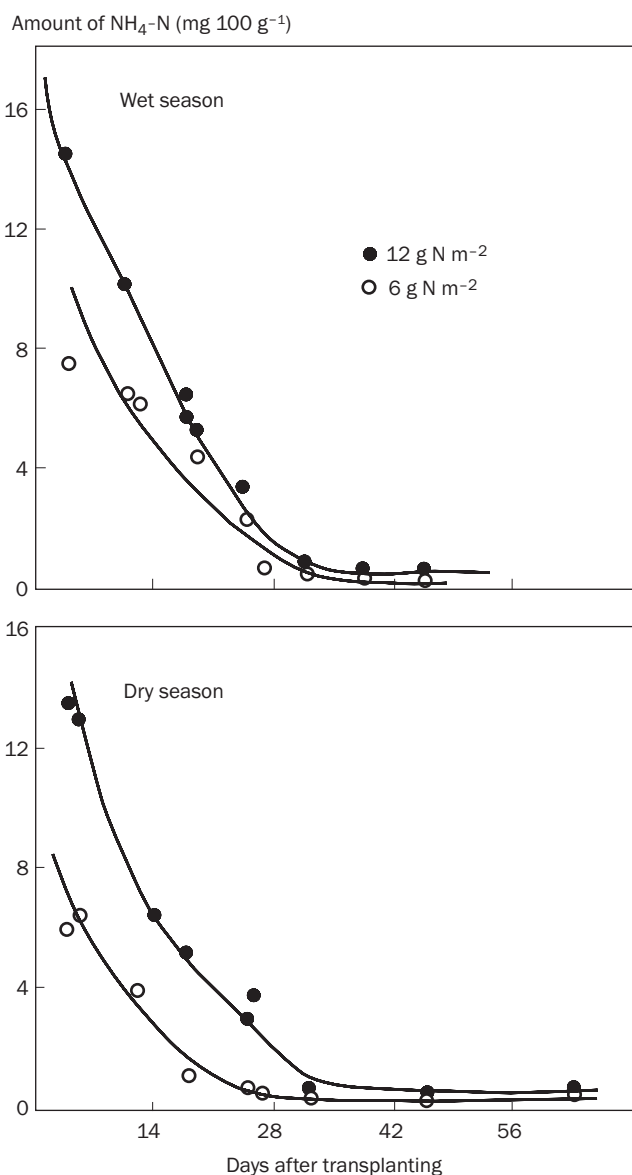


Fig. 4. Behavior of  $\text{NH}_4\text{-N}$  in plow layer (1986 wet season, 1987 dry season).

Diminished sink size was positively correlated with GD (Fig. 8). Variations in diminished sink size were smaller in short-duration varieties than in long-duration varieties. The rate of diminished sink size increased with a decrease in N content in laminae during the reduction division stage of PMC (Wada 1969). Sink reduction can be overcome by shortening the GD in the field, excluding the nursery (Wada 1985b).

Sink size (S) was determined by the relationship between PS and diminished sink size and was represented as a quadratic function of GD (Fig. 9). Sink size was also positively correlated with the amount of N in the plant at flowering ( $r = 0.822^{**}$ ,  $y = -97 + 99x$ ). However, the contribution of N to sink formation (S/N) differed with GD. Maximum sink size was associated with specific GD. The average optimum GD for sink size was 127 days (CV = 8.7% for 3 seasons and 2 N levels,  $20 \times 20$  cm spacing). Variation in optimum GD between the DS and WS was 2–3 days, with 7 days between N levels. Optimum GD was shorter in the DS and had a high N level compared with the WS and its low N level. When GD was shorter than optimum, the low sink size was mainly due to low PS caused by a low amount of N at the late stage of spikelet initiation. Another possible cause is the low PS/N and S/N. In this case, it is important to increase N absorption at the early growth stage and to induce longer VLP by manipulating some cultural practices to increase the contribution of plant N to spikelet differentiation. When GD was longer than optimum, low sink size was attributed to more diminished spikelets. To increase sink size, it is necessary to prevent spikelet reduction by enhancing N absorption from the late stage of spikelet initiation to flowering (Wada 1969).

Grain yield was positively correlated with sink size, and was a quadratic function of GD (Figs. 10 and 11). The optimum GD for grain yield was 122 days (CV = 9% for 3 seasons and 2 N levels,  $20 \times 20$  cm spacing). Variation in optimum GD between the DS and WS was 6 days, with 5 days between N levels. The optimum GD for grain yield was

Table 1. Amount of N ( $\text{g m}^{-2}$ ) in plant from different origins.

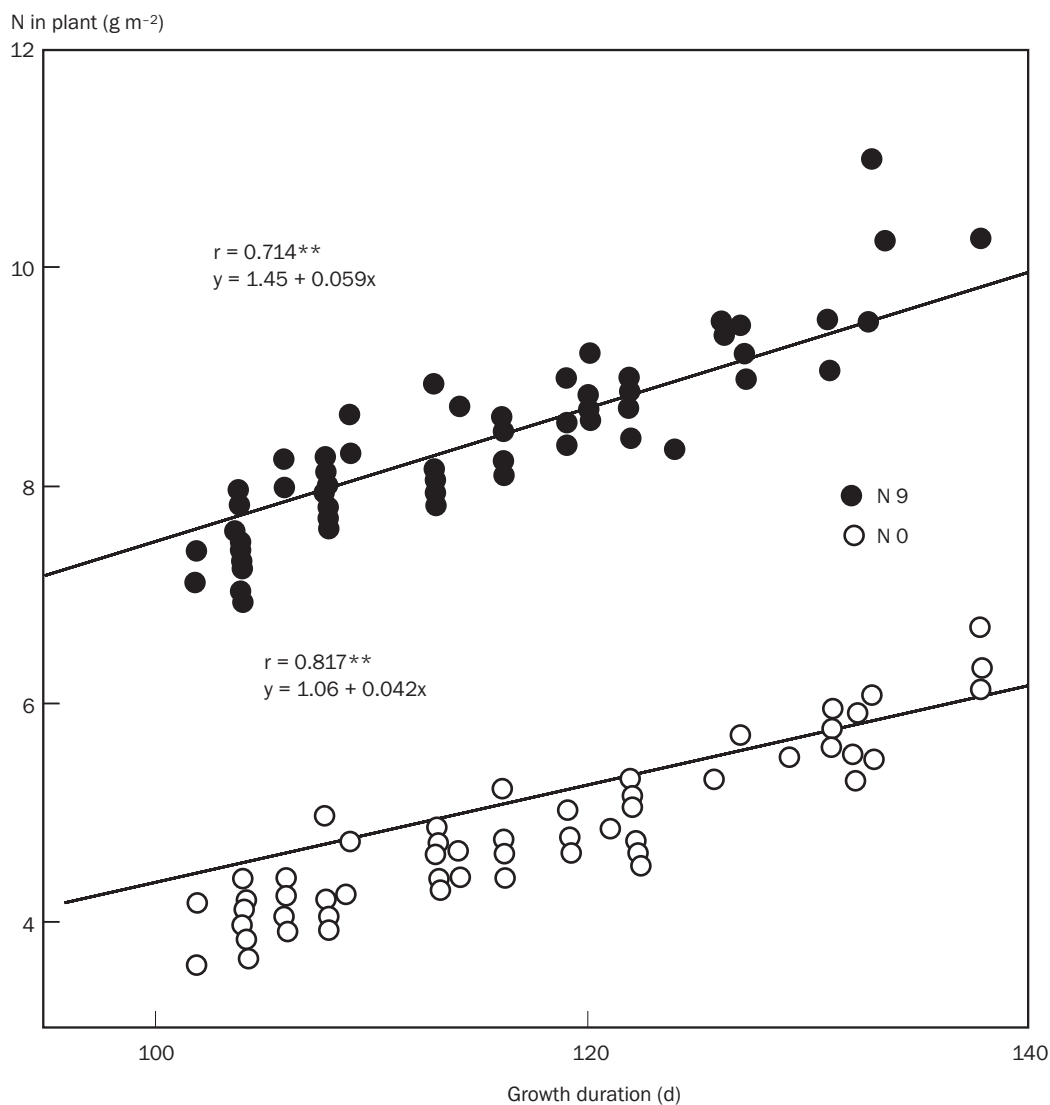
Season	Field	Spacing (cm)	5 WAT <sup>a</sup>			Flowering		
			Basal N	Soil N <sup>b</sup>		Basal N	Soil N <sup>b</sup>	
				R	S		R	S
1986 WS	E	$20 \times 10$	1.9	4.4	0.5	1.9	4.4	3.2
		$20 \times 20$	1.7	3.8	0.4	1.8	3.8	3.2
		$20 \times 20^c$	-	3.2	0.3	-	3.2	3.1
	M	$20 \times 10$	1.8	3.9	0.4	1.8	3.9	2.5
		$20 \times 20$	1.7	3.0	0.3	1.8	3.0	2.5
1987 DS	E	$20 \times 10$	1.7	4.0	0.4	1.7	4.0	3.0
		$20 \times 20$	1.5	2.5	0.3	1.7	2.5	3.2
		$20 \times 20^c$	-	2.0	0.2	-	2.0	3.1
	M	$20 \times 10$	1.8	3.9	0.4	1.8	3.9	2.5
		$20 \times 20$	1.7	3.0	0.3	1.8	3.0	2.5

<sup>a</sup>5 weeks after transplanting. Rate of basal N =  $6 \text{ g }^{15}\text{N m}^{-2}$ . <sup>b</sup>R = rapidly mineralizable soil N, S = slowly mineralizable soil N. The amounts of R and S are roughly estimated by the equation of mineralization of organic N (Ando and Shoji 1986). <sup>c</sup>No nitrogen.

**Table 2. Effect of spacing and N level on the contribution to sink (1986 wet season).**

Variety	N level (g m <sup>-2</sup> )	Spacing (cm)	Vegetative lag phase (d)	PS <sup>a</sup> /N <sup>b</sup>	S <sup>c</sup> /N
IR64	0	20 × 10	10	96	79
		20 × 20	5	85	70
		20 × 30	-1	77	66
	6	20 × 10	10	100	76
		20 × 20	5	97	73
		20 × 30	-2	90	70
IR36892-163-1-2-2-1	0	20 × 10	31	115	76
		20 × 20	26	117	77
		20 × 30	20	123	77
	6	20 × 10	31	117	76
		20 × 20	26	115	75
		20 × 30	20	115	78

<sup>a</sup>PS = potential sink size. <sup>b</sup>N = amount of N in plant at flowering stage. <sup>c</sup>S = sink size.

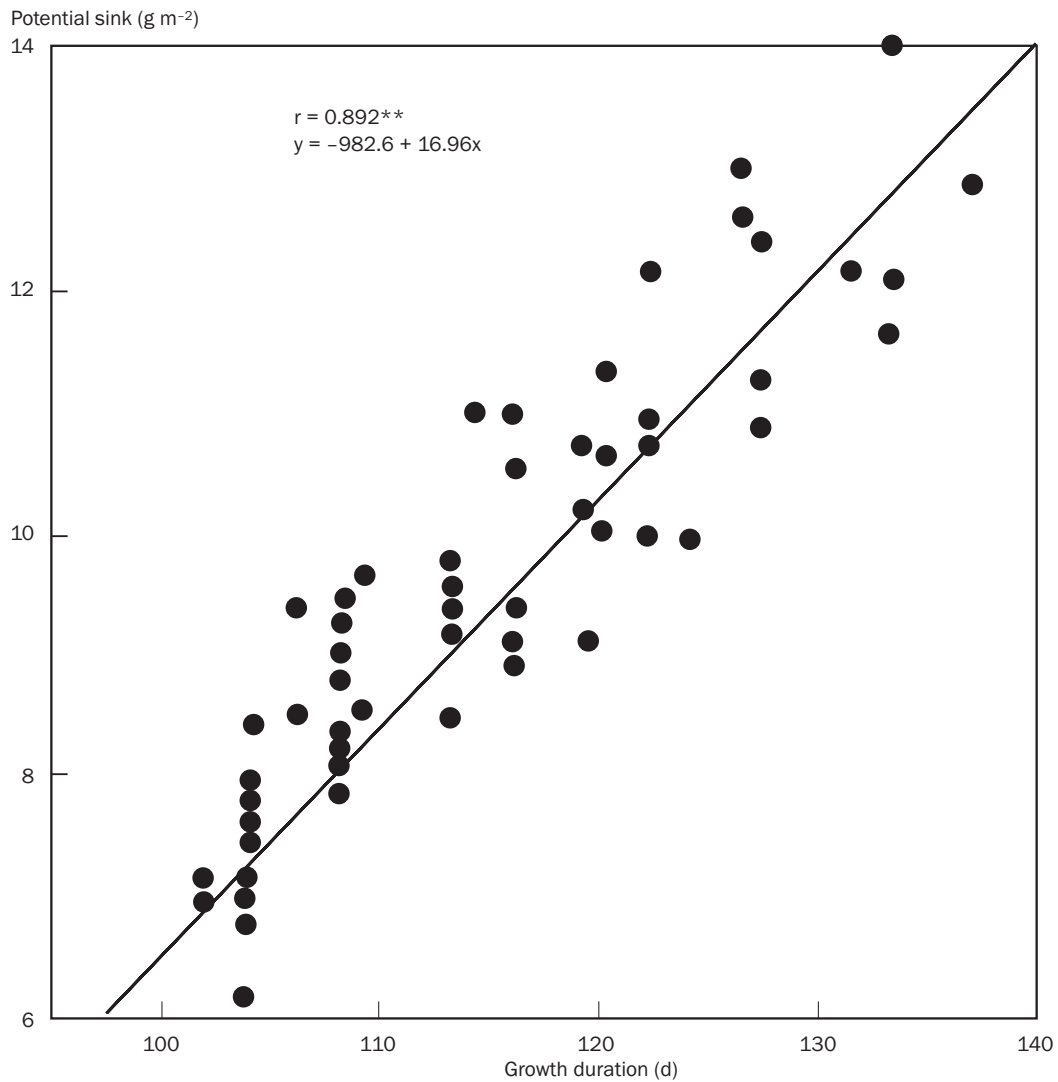


**Fig. 5. Relationship between the amount of N in the plant at flowering and growth duration (1987 dry season).**

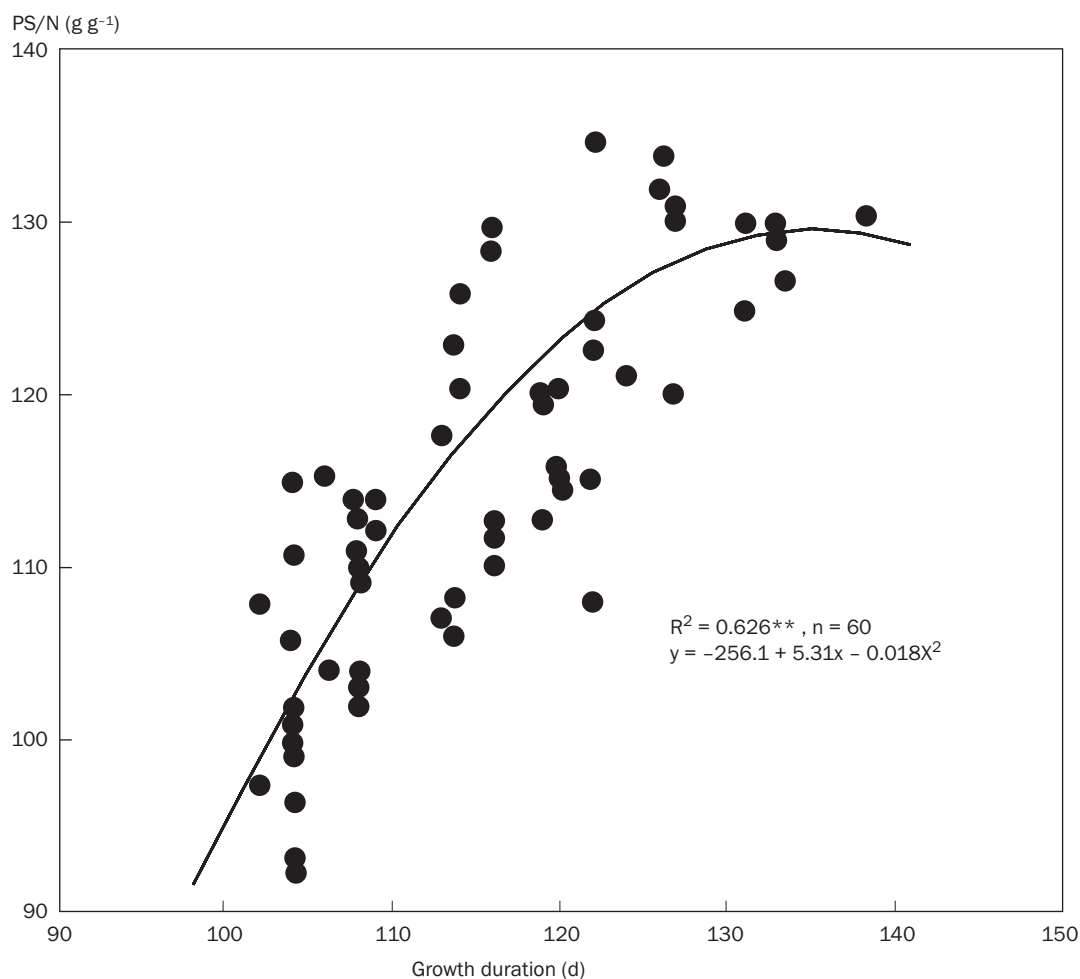
**Table 3. Varietal differences in nitrogen absorption of short-duration varieties/lines at different growth stages (1986 dry season).<sup>a</sup>**

Variety/line	Amount of nitrogen absorbed (g m <sup>-2</sup> )						
	Active tillering (N1)	dN1 (N2 - N1)	Maximum tillers (N2)	dN2 (N3 - N2)	Flowering (N3)	dN3 (N4 - N3)	Maturity (N4)
IR36	1.4 bc	1.2 c	2.6 c	7.3 ab	9.9 bc	2.7 a	12.6 bc
IR25588-7-3-1	1.6 b	1.6 b	3.2 b	7.2 ab	10.4 b	2.8 a	13.2 b
IR25261-135-1-1	2.0 a	2.4 a	4.4 a	7.5 a	11.9 a	2.5 a	14.4 a
IR29658-69-2-1-2	1.5 b	1.3 bc	2.8 bc	7.2 ab	10.0 bc	2.4 ab	12.4 c
IR29692-94-2-1-3	1.4 bc	1.1 cd	2.5 cd	7.2 ab	9.7 c	2.6 a	11.3 d
IR29725-109-1-2-1	1.3 c	1.3 bc	2.6 c	7.3 ab	9.9 c	2.7 a	12.6 bc
IR31868-64-2-3-3-3	1.3 c	1.2 c	2.5 cd	7.3 ab	9.8 c	2.7 a	12.5 c
IR32419-81-2-3-3	1.2 c	0.9 d	2.1 e	7.1 b	9.2 d	2.4 ab	11.6 cd
IR32429-47-3-2	1.3 c	0.9 d	2.2 de	7.6 a	9.8 c	2.2 b	12.0 c
IR32429-122-3-1-2	1.5 b	1.1 cd	2.6 c	7.4 ab	10.0 bc	2.4 ab	12.4 c

<sup>a</sup>Growth duration = 111 days. Numbers followed by common letters are not significantly different by Duncan's multiple range test at P = 0.05.



**Fig. 6. Relationship between potential sink and growth duration, 1987 dry season.**



**Fig. 7. Effect of growth duration on the contribution of N in the plant to potential sink size (PS/N).**

generally shorter than for sink size. This is related to ripening percentage, which generally decreased with an increase in GD.

Yield was correlated with sink size and the amount of N in the plant at flowering and maturity (Wada 1968). However, the contribution of N in the plant to grain yield and nitrogen harvest index (NHI) varied with GD. Lower yields were observed in short- and long-duration varieties; hence, the occurrence of optimum GD. NHI represents an exponential function of GD. Harvest index is negatively correlated with GD (Wada 1968). Therefore, there is a difference in the contribution to grain yield between dry matter production and N absorption.

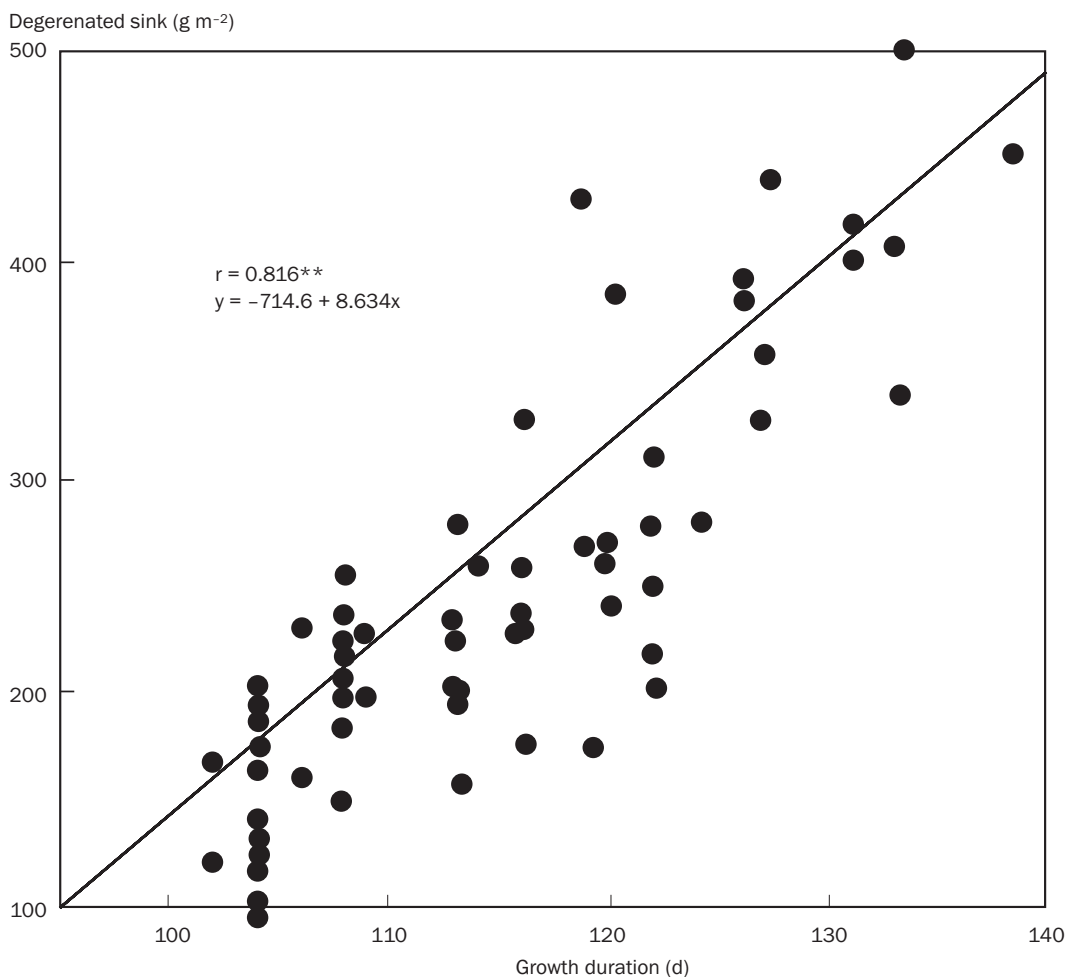
Previous research identified the presence of the optimum GD (Kawano and Tanaka 1968, Vergara et al 1964, 1966, Yamakawa and Nishiyama 1958). Since yield is primarily governed by sink size (Wada 1969, 1985a, Yoshida 1981), the optimum GD should be discussed from the viewpoint of sink formation. Vergara et al (1964, 1966) and Yamakawa et al (1958) pointed out that, when GD is shorter than the optimum, low yield is caused by a shortage of vegetative growth, lower number of panicles, and lesser num-

ber of spikelets. When GD is longer than the optimum, this is caused by a lesser number of spikelets and long VLP (Vergara et al 1966, Wada 1981). This evidence suggests that the low yield of varieties having a shorter or longer GD than the optimum is explained primarily by small sink size. Moreover, the optimum GD for yield as a function of cultural practices can be attributed to sink size.

#### Importance of N absorption at the early growth stage

Because there were significant differences in N uptake at the early growth stage, a follow-up study was made using 60 IR varieties and lines with varying GDs to determine the effect of N uptake at the early growth stage (NAE) on yield-determining processes. Positive correlation coefficients were obtained between high and low nitrogen levels, and between the DS and WS in terms of plant N content at the early growth stage (30 DAT), flowering, and maturity (Table 4). Generally, the test varieties have consistent N absorption characteristics at different growth stages regardless of N level and cropping season. Furthermore, NAE was highly corre-





**Fig. 8. Relationship between degenerated sink and growth duration, 1987 dry season.**

lated with the amount of N in the plant at flowering and maturity in varieties classified under the same GD group (Table 5). These results suggested that N absorption ability of varieties is a genetically stable characteristic (Miyagawa 1981).

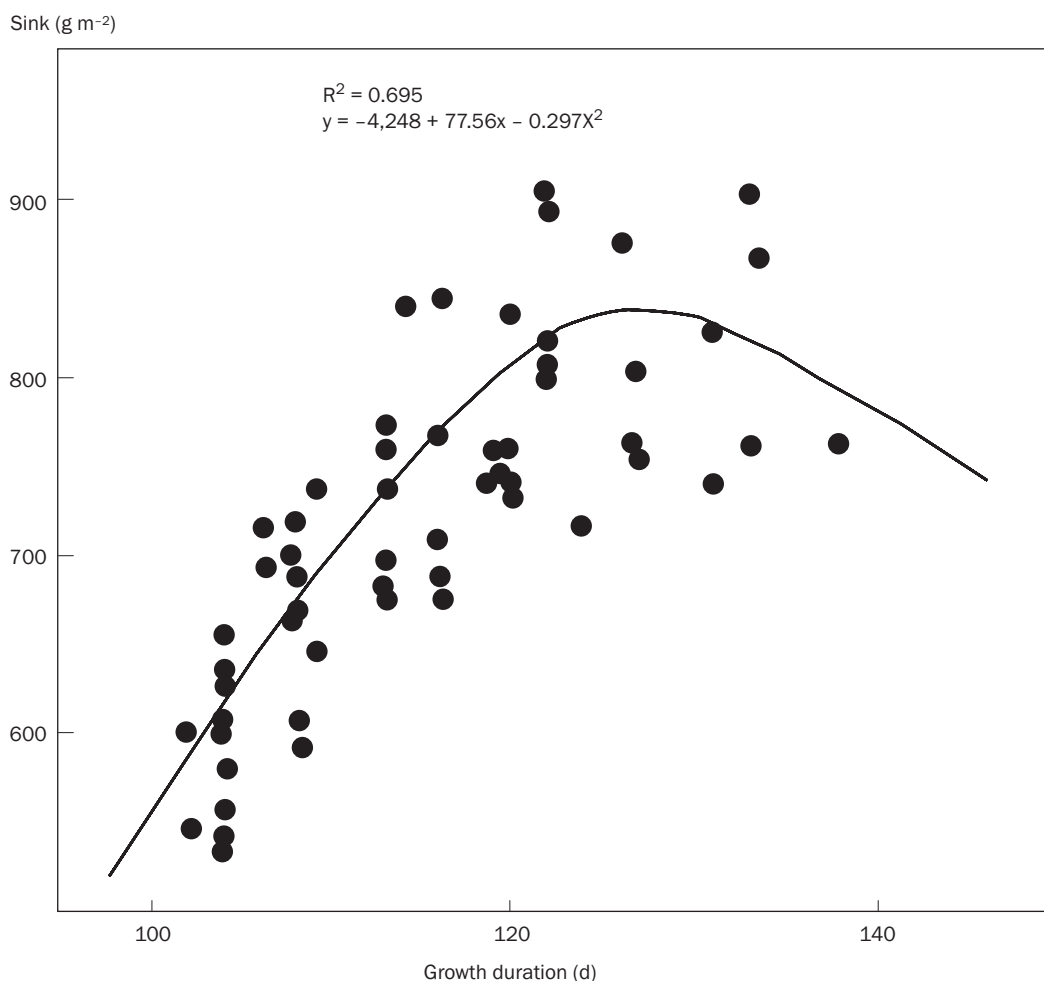
Similarly, yield and sink size of tested varieties were consistent under different N levels and cropping seasons. This was demonstrated by the positive correlations between high and low levels, and between the DS and WS cropping in terms of yield and sink size (Table 4). These results imply that varietal differences in yield and sink size are stable at different N levels and in different cropping seasons.

The contribution of N in the plant at flowering to sink size and yield is affected by GD. Likewise, the contribution of N in the plant to yield and yield components varied with different growth stages. Therefore, the effect of NAE on yield and yield components may vary with GD, cultural practices, and cropping season.

Potential sink size was correlated with NAE in all GD groups under DS and WS cropping. Sink reduction, however, was correlated with GD in medium- and long-duration groups only. Sink size was correlated with NAE in short-

duration varieties. No significant relationship was observed between ripening percentage and NAE in any GD group. Since grain yield is highly associated with sink size, significant correlation coefficients between grain yield and NAE in short-duration varieties are logical (Table 5). From these observations, we can infer that NAE is an important factor in yield, sink size, and PS formation, particularly in short-duration varieties. Sink reduction and the occurrence of unripened grains disturbed the NAE-yield relationship of long-duration varieties.

From this study and previous reports, the effect of GD on yield-determining processes is schematically presented in Figure 12. Potential sink size is a linear function of GD, whereas sink size and yield are a quadratic function of GD. The optimum GD for sink size is longer than for yield. When GD is less than optimum, NAE is highly associated with grain yield. On the other hand, when GD is more than optimum, the association of NAE with grain yield is relatively low because other factors, such as sink reduction and ripening, which are highly affected by N nutrition during the middle and late growth stage (Wada 1969), have a greater effect on yield-determining processes.



**Fig. 9. Relationship between sink size and growth duration (1987 dry season).**

Nitrogen harvest index (NHI) and yield were correlated with NAE in short-duration varieties. Therefore, NAE is an important characteristic in increasing the yield and N-use efficiency of short-duration varieties.

The effects of spacing and N level on N absorption and the contribution of plant N to sink size and yield are shown in Table 6. The amount of N absorbed by the plant at the early growth stage is affected by the dose of basal fertilizer and plant spacing. Higher basal dressing and higher plant density resulted in higher N absorption at the early growth stage. The S/N, PS/N, NHI, and N absorption rate increased with higher basal N and plant density by affecting VLP and NAE in short-duration varieties. In long-duration varieties, the N-use efficiency parameters were also affected by basal N level and spacing, but at a relatively lower magnitude. Therefore, aside from high N absorption ability of a variety, NAE could be compensated by narrow spacing and a high N level.

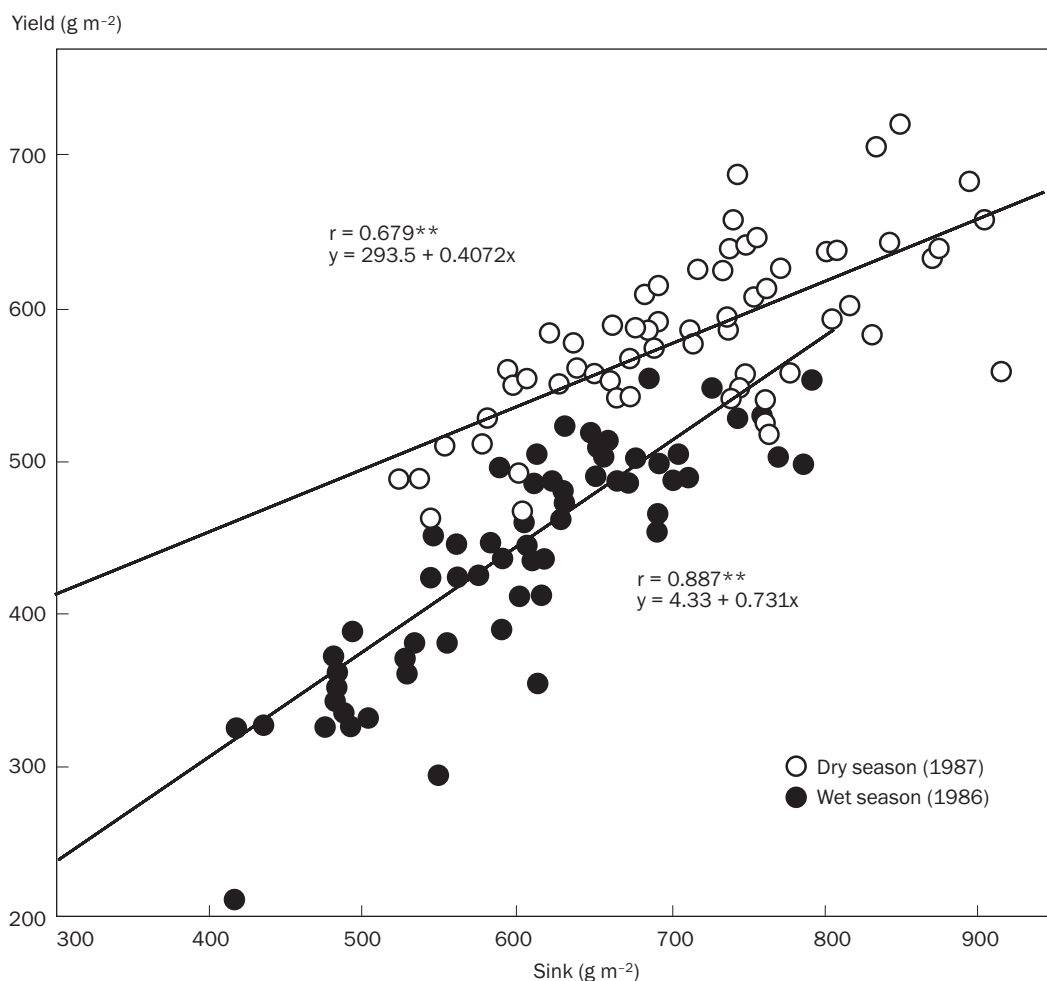
#### Physiological aspects of short-duration varieties

From the abovementioned evidence, the low yield of short-duration varieties has been attributed to the low amount of

N in the plant at the late stage of spikelet initiation and lower contribution of N in the plant to sink formation. To evaluate a response of short-duration varieties to cultural practices and N level, field trials were carried out using six short-duration varieties and one each of medium- and long-duration varieties as a control. Under narrow spacing, higher plant N was obtained at panicle primordial initiation (PI), flowering, and maturity; however, under wider spacing, a larger amount of N was absorbed during the period from PI to the late stage of spikelet initiation or flowering. After the late stage of spikelet initiation, there was no difference in N absorption under different spacing (Table 7).

Potential sink size was correlated with the amount of N in the plant at PI. Therefore, PS was higher in narrow-spacing plants than in wide-spacing plants, and higher in a long-duration variety than in a short-duration one. The percentage of sink reduction was very low and was not affected by spacing, in spite of a big difference in PS. Since the diminished sink was very small compared with PS, sink size was correlated with the amount of N at PI and flowering (Fig. 13).

Ripening percentage was always higher in low-N application than in high-N application because of a big differ-



**Fig. 10. Relationship between yield and sink.**

ence in sink size between them. However, very little difference in ripening percentage in different plant spacing was noted, in spite of a big difference in sink size. A higher ripening percentage was observed in panicle number type than in panicle weight type (Table 8). One reason why the lower ripening percentage was in panicle weight type and why the ripening percentage of narrow spacing did not decrease can be explained by the difference in panicle characteristics (Wada 1968).

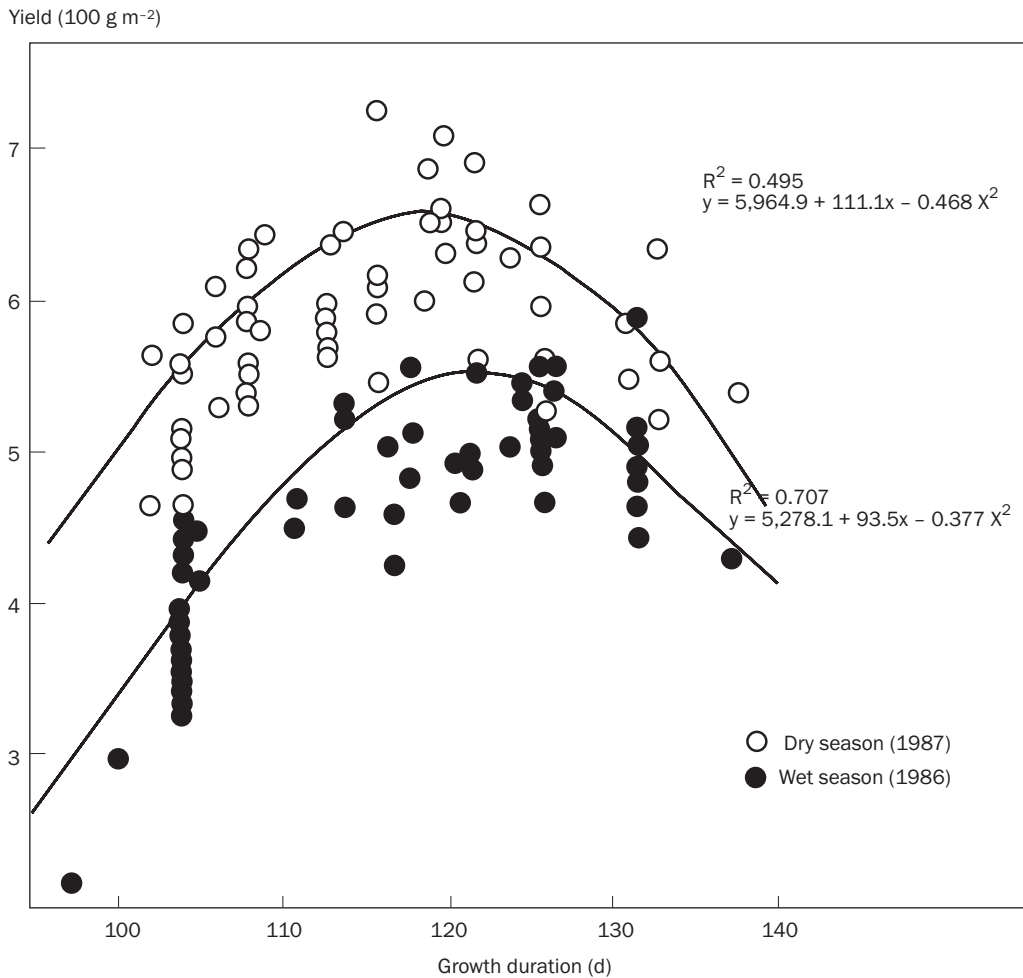
Yield was correlated with PS and sink size; however, the contribution of sink size or PS to yield varied with vegetative growth duration and cropping season. The contribution of PS to yield was much higher in short-duration varieties than in long-duration varieties and was also higher in the DS than in the WS.

Yield was correlated with the amount of plant N at maturity (Fig. 14). However, three linear equations explained the relationship of yield with the amount of plant N at maturity. The first one was on topdressed plots at the late stage of spikelet initiation and the second one was on nontopdressed plots in the 1986 DS. The third one was on both the 1986 WS and 1987 DS. The two differing equations in the 1986 DS have a similar trend for the relationship between sink

size and the amount of N in the plant at the late stage of spikelet initiation. In the 1986 DS, a higher amount of N was absorbed during the ripening period. The effect of higher N absorption of short-duration varieties during the period from the late stage of spikelet initiation to flowering and during the ripening period on yield was very small. The contribution of plant N at maturity was the lowest in the first group and highest in the third group. Therefore, since short-duration varieties are characterized by lower potential sink reduction and relatively high ripening percentage, increasing N absorption at the early growth stage and increasing the contribution of plant N to PS are the most important considerations for increasing grain yield. The coefficient of variation (CV) of yield and sink size was higher in short-duration varieties than in long-duration varieties (Table 8). Within the short-duration varieties, the CV was higher in the panicle weight type than in the panicle number type.

### Response to plant spacing

Since yield-determining processes are affected by plant spacing as reported, field experiments were carried out with a



**Fig. 11. Relationship between yield and growth duration.**

wider range of plant spacing and by using different ages of seedlings. Under extremely narrow spacing, the exponential phase of N absorption was shortened markedly (Aragones and Wada 1989). Narrow spacing and younger seedling age at transplanting (or seeding) decreased FLW (number of days from seeding to 50% flowering), promoted tillering, and decreased the number of days from seeding to the MT stage (Table 9, Fig. 15). As a result, narrow spacing and younger seedlings brought about longer VLP. Longer VLP decreased the percentage of bearing tillers. VLP was lengthened by narrow spacing, and by the use of younger-aged seedlings.

Narrow spacing increased in both varieties; the effect was higher in the short-duration varieties than in the long-duration varieties. Seedling age at planting also affected yield, particularly under extremely narrow spacing, where young seedlings decreased yield. There was a small difference in ripening among treatments within the same variety; hence, yield was governed by sink size. Under extremely narrow spacing, young seedlings reduced sink size by increasing degenerated spikelets and nonbearing tillers, especially in long-duration varieties. The large number of degenerated spikelets and nonbearing tillers is caused by a

long VLP and low N content (dry basis) in the plant (Wada 1968, Wada et al 1986).

PS is generally correlated with GD of plants grown under the same cultural conditions and amount of plant N at the flowering stage; however, under direct seeding (high plant density, 400 hills m<sup>-2</sup>), no relationship between PS and GD was observed. The GD-PS relationship under direct seeding can be assumed to be disturbed by the uncounted reduced sink size because of a large number of nonbearing tillers. Under direct-seeding conditions, grain yield was higher for short-duration varieties than for long-duration varieties. Optimum GD for grain yield was observed at 95 days (Aragones and Wada 1989).

The yield ratio of narrow spacing (20 × 10 cm) to wider spacing (20 × 20 cm) decreased with an increase in GD. Similar trends were observed in sink size ratio and PS ratio. This decreasing trend of ratio with GD was attributed to the difference in N absorption pattern and plant N-use efficiency caused by a difference in VLP. The optimum GD for grain yield was shorter for narrow spacing than for wider spacing (Sta. Cruz 1990).

**Table 4. Correlation coefficients for yield, sink size, and amount of N in the plant in N0 and N9 treatments, and dry season (DS) and wet season (WS) croppings (n = 60, average of two replications).<sup>a</sup>**

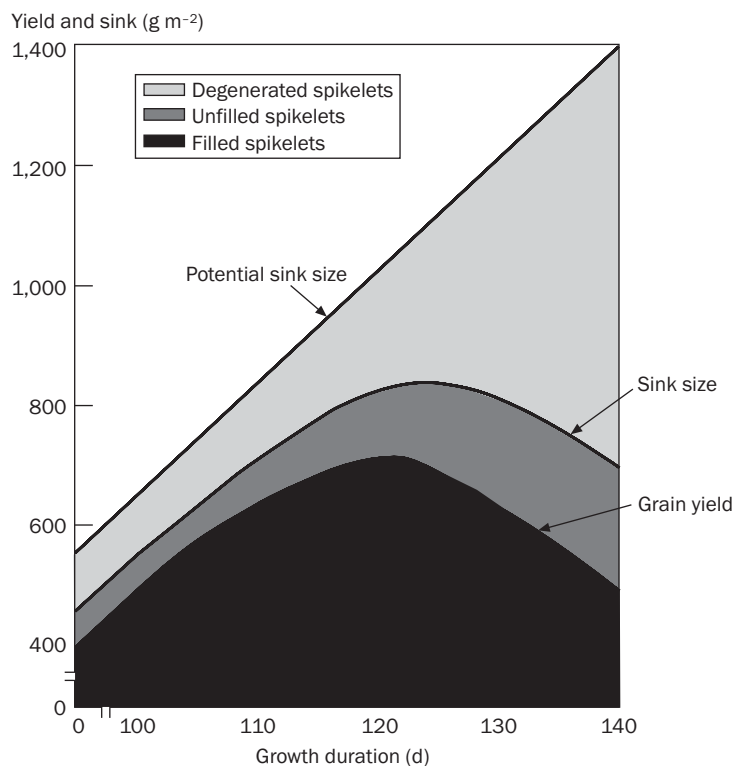
	Yield		Sink		NAE		N1		N2	
	DS	WS	DS	WS	DS	WS	DS	WS	DS	WS
N0:N9	0.611	0.827	0.783	0.872	0.835	0.850	0.905	0.889	0.887	0.781
DS:WS	N0	N9	N0	N9	N0	N9	N0	N9	N0	N9
	0.553	0.556	0.640	0.614	0.830	0.815	0.880	0.771	0.883	0.841

<sup>a</sup>NAE = amount of N in plant at 30 DAT. N1 = amount of N in plant at flowering. N2 = amount of N in plant at maturity. N0 = no nitrogen. N9 = 90 kg N ha<sup>-1</sup>. All values are significant at 1% level.

**Table 5. Correlation coefficients for NAE<sup>a</sup> and yield-determining factors.**

Growth duration (d)	Y <sup>b</sup>	S	PS	Red. S	S/N1	PS/N1	R	N1	N2	NHI
Dry season										
102-104	0.551**	0.858**	0.905**	0.696**	0.728**	0.850**	-0.264 ns	0.834**	0.850**	0.841**
108-109	0.580**	0.723**	0.730**	0.299 ns	0.716**	0.704*	-0.449 ns	0.865**	0.785**	0.831**
113-116	0.600**	0.897**	0.922**	0.593 ns	0.862**	0.722*	-0.394 ns	0.957**	0.940**	0.938**
119-122	0.708 ns	0.920**	0.839**	0.669 ns	0.593 ns	0.728*	-0.529 ns	0.859**	0.905**	0.972**
124-127	0.660 ns	0.839**	0.927**	0.936**	0.422 ns	0.736*	-0.170 ns	0.970**	0.965**	0.040 ns
>130	0.528 ns	0.534 ns	0.971**	0.885**	0.387 ns	0.472 ns	-0.538 ns	0.946**	0.950**	0.041 ns
Wet season										
104	0.726**	0.817**	0.751**	0.339 ns	0.760**	0.778**	-0.319 ns	0.833**	0.845**	0.796**
111-118	0.685*	0.894**	0.811**	0.428 ns	0.765*	0.765*	-0.665 ns	0.858**	0.825**	0.894**
117-122	0.948**	0.849**	0.920**	0.288 ns	0.773*	0.969**	-0.483 ns	0.940**	0.850**	0.839**
125-127	0.499 ns	0.623 ns	0.904**	0.725*	0.341 ns	0.864**	-0.361 ns	0.942**	0.930**	0.532 ns
>130	0.269 ns	0.291 ns	0.843*	0.849**	0.358 ns	0.314 ns	-0.073 ns	0.849**	0.858**	-0.283 ns

<sup>a</sup>NAE = amount of N absorbed by plant from transplanting to 30 days after transplanting. <sup>b</sup>Y = yield, S = sink, PS = potential sink, Red. S = reduced sink, R = percentage of ripened grains, N1 = amount of N in plant at flowering, N2 = amount of N in plant at maturity, NHI = nitrogen harvest index. \*, \*\* = significant at 5% and 1% level, respectively. ns = not statistically significant (P>0.05).



**Fig. 12. Differences in grain yield, sink size, and potential sink size of IR varieties and lines with varying growth duration, IRRI, 1987 dry season and wet season.**



**Table 6. Effect of spacing and N level on the amount of N in plant, vegetative lag phase (VLP), and contribution of N in the plant at flowering to potential sink size (PS/N), sink size (S/N), and nitrogen harvest index (NHI), 1987 dry season.**

Variety	N level (g m <sup>-2</sup> )	Spacing (cm)	Amount of N (g m <sup>-2</sup> )				PS/N	S/N	NHI
			3 WAT <sup>a</sup>	5 WAT	Flowering	VLP			
IR64	0	20 × 10	1.8	3.7	7.0	10	79.8	65.2	60.6
		20 × 30	0.6	2.7	6.7	-7	74.8	57.8	57.7
	6	20 × 10	2.4	5.2	7.9	10	84.8	64.5	63.7
		20 × 30	0.7	3.2	6.9	-7	78.8	62.6	59.0
	12	20 × 10	3.0	8.4	10.8	10	93.2	76.3	70.2
		20 × 30	0.9	5.0	8.5	-7	82.2	67.3	63.0
IR36892-163-1-2-2-1	0	20 × 10	1.8	3.9	8.4	31	124.4	83.4	57.4
		20 × 30	0.7	2.4	7.9	20	124.7	81.8	57.8
	6	20 × 10	2.2	6.1	10.6	31	128.3	79.4	64.0
		20 × 30	0.6	3.2	9.0	20	125.6	76.4	62.0
	12	20 × 10	3.3	8.6	13.7	31	129.1	79.9	64.0
		20 × 30	1.0	5.2	10.0	20	124.7	77.6	62.3

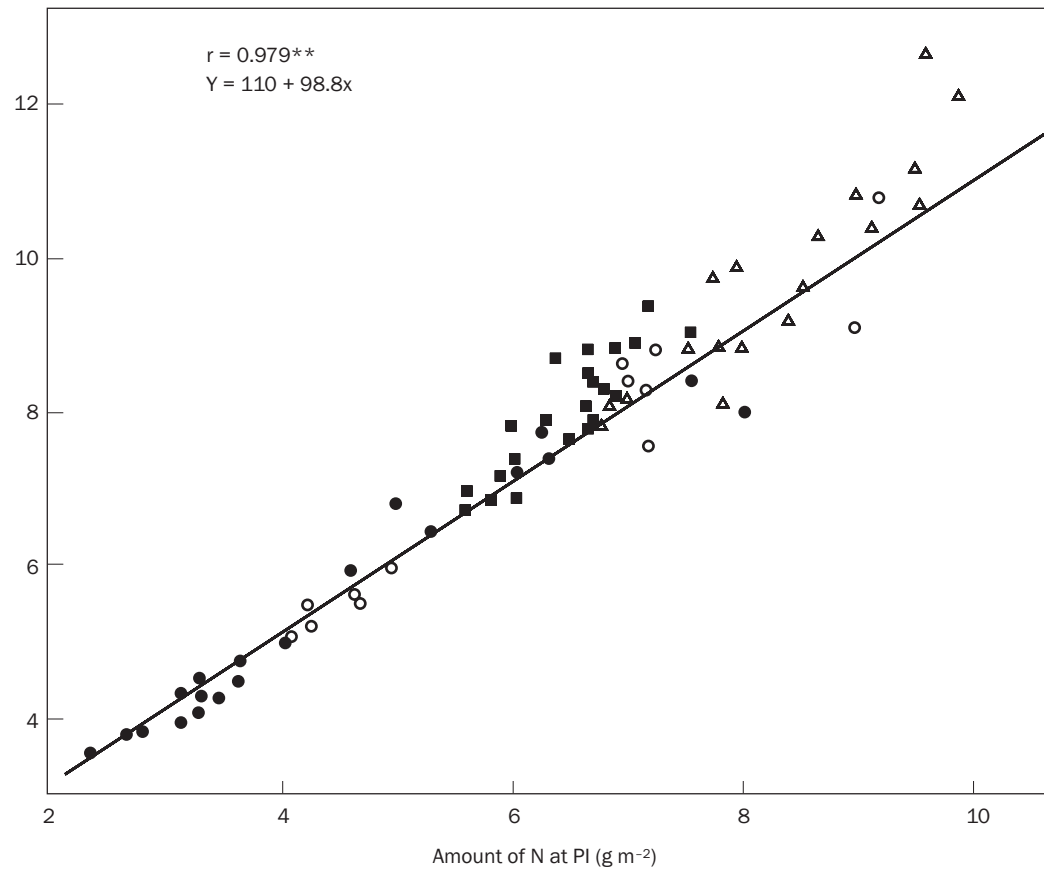
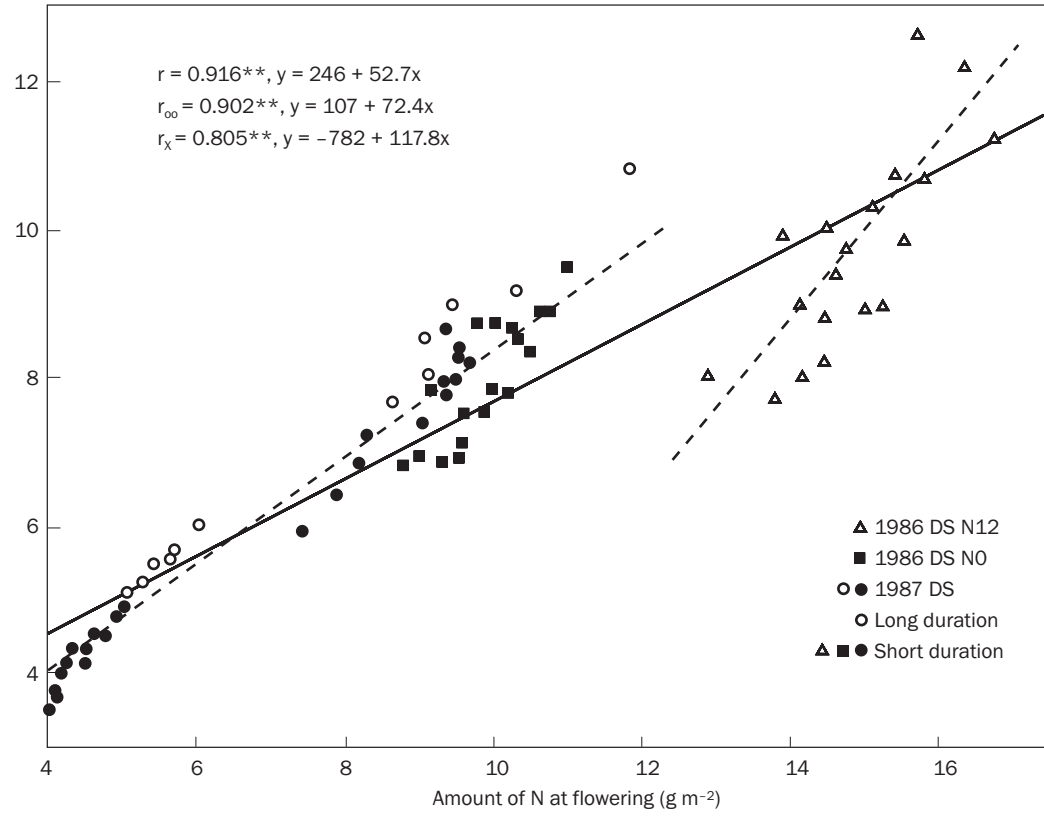
<sup>a</sup>WAT = weeks after transplanting.

**Table 7. Effect of spacing on N absorption and N-use efficiency.<sup>a</sup>**

Variety	N rate	Spacing	N1	N3	N4	PS/N3	S/N3	Y/N3	Y/N4		
1986 DS											
IR58	0	12 × 10	5.8	10.8	16.6	87.9	82.4	75.5	49.8		
		12 × 30	4.5	10.0	16.0	76.3	73.6	66.0	44.8		
	12	12 × 10	9.7	16.0	21.5	79.5	69.7	67.3	47.8		
		12 × 30	7.9	15.1	20.8	61.3	59.3	53.6	38.8		
IR25588-1-3-1	0	12 × 10	6.7	11.0	16.8	99.8	85.6	79.2	49.8		
		12 × 30	5.1	10.6	16.5	93.0	83.2	75.0	45.9		
	12	12 × 10	10.1	16.5	22.3	84.9	74.7	62.8	44.4		
		12 × 30	8.2	15.5	21.5	72.3	64.6	52.2	37.6		
1987 DS											
IR58	0	12 × 10	3.14	3.56	4.15	5.76	154.5	116.8	94.2	90.4	65.3
		12 × 30	2.66	3.30	4.05	5.55	157.0	102.7	86.9	83.7	61.0
	9	12 × 10	6.68	8.58	9.56	11.06	149.3	104.4	86.0	81.3	66.2
		12 × 30	4.60	6.65	7.41	8.98	155.7	96.6	78.9	74.0	61.0
IR25588-7-3-1	0	12 × 10	4.02	4.48	5.09	6.78	177.1	139.3	96.9	91.7	68.8
		12 × 30	3.26	4.02	4.81	6.54	179.7	121.8	93.7	87.9	64.5
	9	12 × 10	7.17	9.06	9.70	11.18	177.0	130.8	85.3	77.5	67.2
		12 × 30	6.12	8.48	9.17	10.62	182.4	119.2	82.4	74.7	63.5
IR36892-163-1-2-2-1	0	12 × 10	4.96	5.38	6.01	7.56	186.0	153.7	98.8	85.0	67.6
		12 × 30	4.27	4.76	5.35	7.08	186.0	148.4	102.8	87.4	66.1
	9	12 × 10	7.81	9.52	10.29	11.87	196.6	137.3	88.4	73.2	63.4
		12 × 30	6.21	7.85	8.72	10.26	192.7	137.3	86.3	73.6	63.2

<sup>a</sup>N1 = amount of N in plant at PI, N2 = amount of N in plant at late stage of spikelet initiation, N3 = amount of N in plant at flowering, N4 = amount of N in plant at maturity, PS = potential sink size, S = sink size, Y = yield.

Sink (100 g m<sup>-2</sup>)



**Fig. 13.** Relationship between sink size and the amount of N in the plant at panicle initiation and flowering stages under different cropping seasons and N levels.

**Table 8. Effect of spacing and N level on yield and yield components of rice varieties and advanced lines of different types,<sup>a</sup> IRRI, 1986 wet season (WS) and 1987 dry season (DS).**

N level	Spacing (cm)	Yield (g m <sup>-2</sup> )			Sink (g m <sup>-2</sup> )			Potential sink (g m <sup>-2</sup> )			Ripening (%)			Vegetative lag phase (d)		
		PN	I	Check	PN	I	Check	PN	I	Check	PN	I	Check	PN	I	Check
0	20 × 10	429	490	512	486	559	636	620	699	963	90	88	81	2 to 10	4	31 to 37
	20 × 20	381	433	455	434	503	570	551	621	864	89	87	80	-2 to 5	-1 to 0	27 to 30
	20 × 30	355	409	447	403	477	575	499	595	869	88	86	78	-4 to 3	-1	20 to 25
90	20 × 10	708	773	739	809	932	937	1,057	1,227	1,344	89	79	79	2 to 10	4	32 to 37
	20 × 20	620	649	630	721	783	808	921	1,035	1,241	86	78	78	-2 to 5	-1 to 0	27 to 30
	20 × 30	568	605	592	682	740	766	876	1,024	1,207	84	78	78	-9 to 2	-9 to -2	20 to 25
CV total		30.0	34.1	19.8	26.5	28.6	18.6	29.9	30.1	18.6	9.8	7.8	7.8			

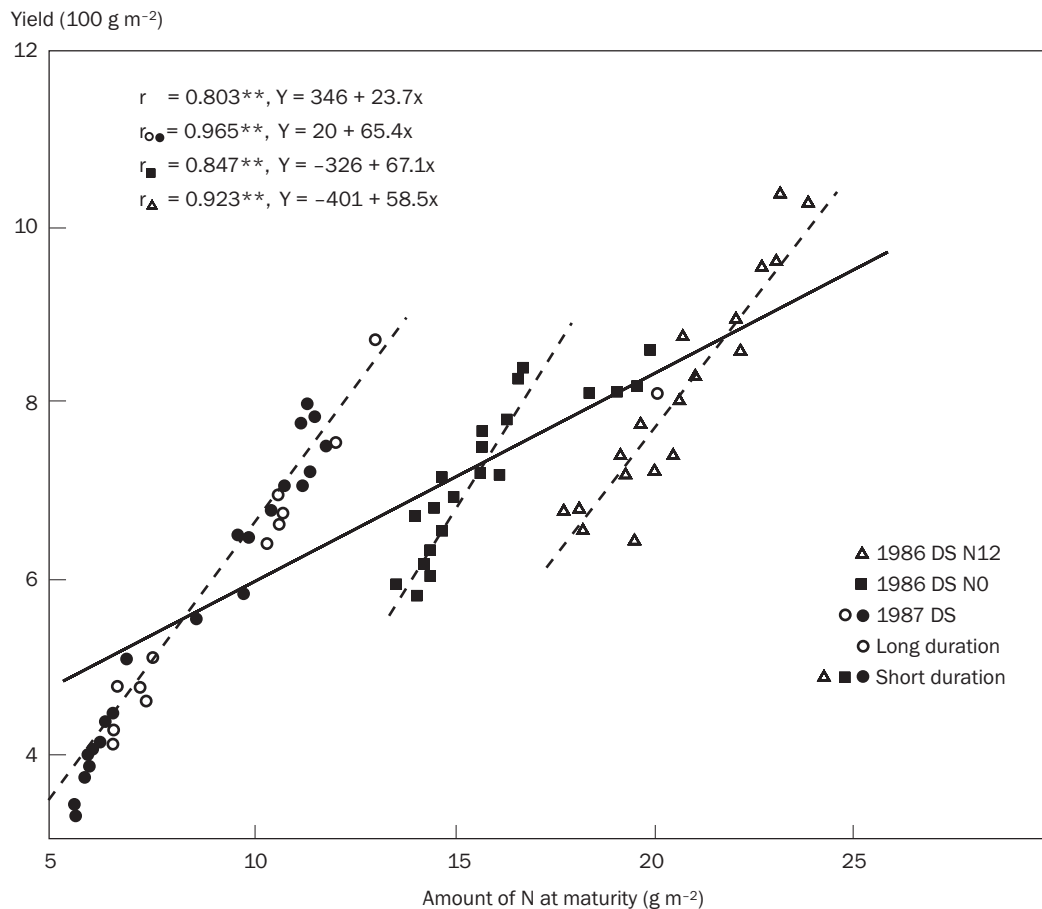
<sup>a</sup>Panicle number type (PN): IR58 (100–104 d in WS, 107–108 d in DS), IR64 (112 d in WS, 113–118 d in DS), intermediate type (I): IR25588-7-3-1, IR29262-65-2-3-3 (104–106 d in WS, 109–112 d in DS), Check: IR36892-163-1-2-2-1 (130 d in WS, 135–140 d in DS).

Under narrow spacing, the yield increase was higher for short-duration varieties than for long-duration varieties (Fig. 16). However, there was no identical trend in PS, which was increased by narrow spacing between short-duration and long-duration varieties. Therefore, there was a big difference in the effect of VLP, as increased by narrow spacing, on yield-determining processes between short-duration and long-duration varieties.

Through all the experiments, the optimum VLP for grain yield was observed to be 15 days. When VLP is shorter than 15 days, cultural practices that can increase VLP and PS should be employed. On the other hand, when VLP is longer than 15 days, cultural practices that will reduce sink size but increase ripening percentage are needed.

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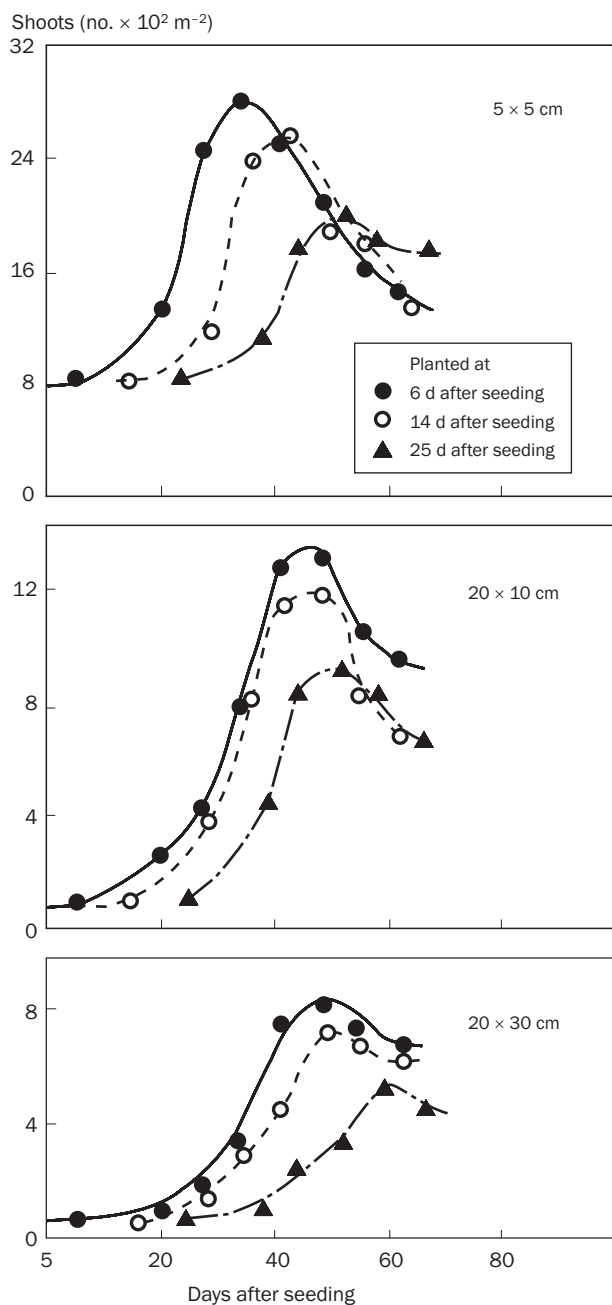


**Fig. 14. Relationship between yield and the amount of N in the plant at maturity in short- and long-duration varieties under different cropping seasons and N levels.**

**Table 9. Effect of seedling age and spacing on growth and yield of rice plants, IRRI, 1988 dry season.**

Spacing (cm)	Seedling age (d)	Days from seeding to flowering	Vegetative lag phase (d)	Panicles (no. m <sup>-2</sup> )	Sink (g m <sup>-2</sup> )	Potential sink (g m <sup>-2</sup> )	Yield <sup>a</sup> (g m <sup>-2</sup> )
<i>IR66</i>							
5 × 5	6	75	20	1,507	1,360	1,865	1,202 b
	14	79	14	1,160	1,528	2,031	1,293 b
	25	79	4	1,240	1,549	1,991	1,366 a
20 × 10	6	75	15	548	903	1,248	770 d
	14	79	11	547	901	1,260	743 d
	25	79	4	565	848	1,239	741 d
20 × 30	6	83	13	444	795	1,136	660 de
	14	83	9	382	785	1,110	645 de
	25	86	4	362	712	949	624 de
<i>IR36892-163-1-2-2-1</i>							
5 × 5	6	104	49	933	1,112	1,769	740 de
	14	104	39	893	1,216	1,845	944 c
	25	104	29	880	1,320	1,943	1,007 c
20 × 10	6	104	44	448	860	1,301	646 de
	14	104	36	381	850	1,246	660 de
	25	108	33	358	874	1,289	624 de
20 × 30	6	112	38	331	747	1,119	607 e
	14	108	34	294	773	1,130	624 de
	25	108	26	260	772	1,151	618 e

<sup>a</sup>Numbers followed by a common letter indicate no significant difference.



**Fig. 15. Difference in tillering pattern among treatments, IRRI, 1988 dry season.**

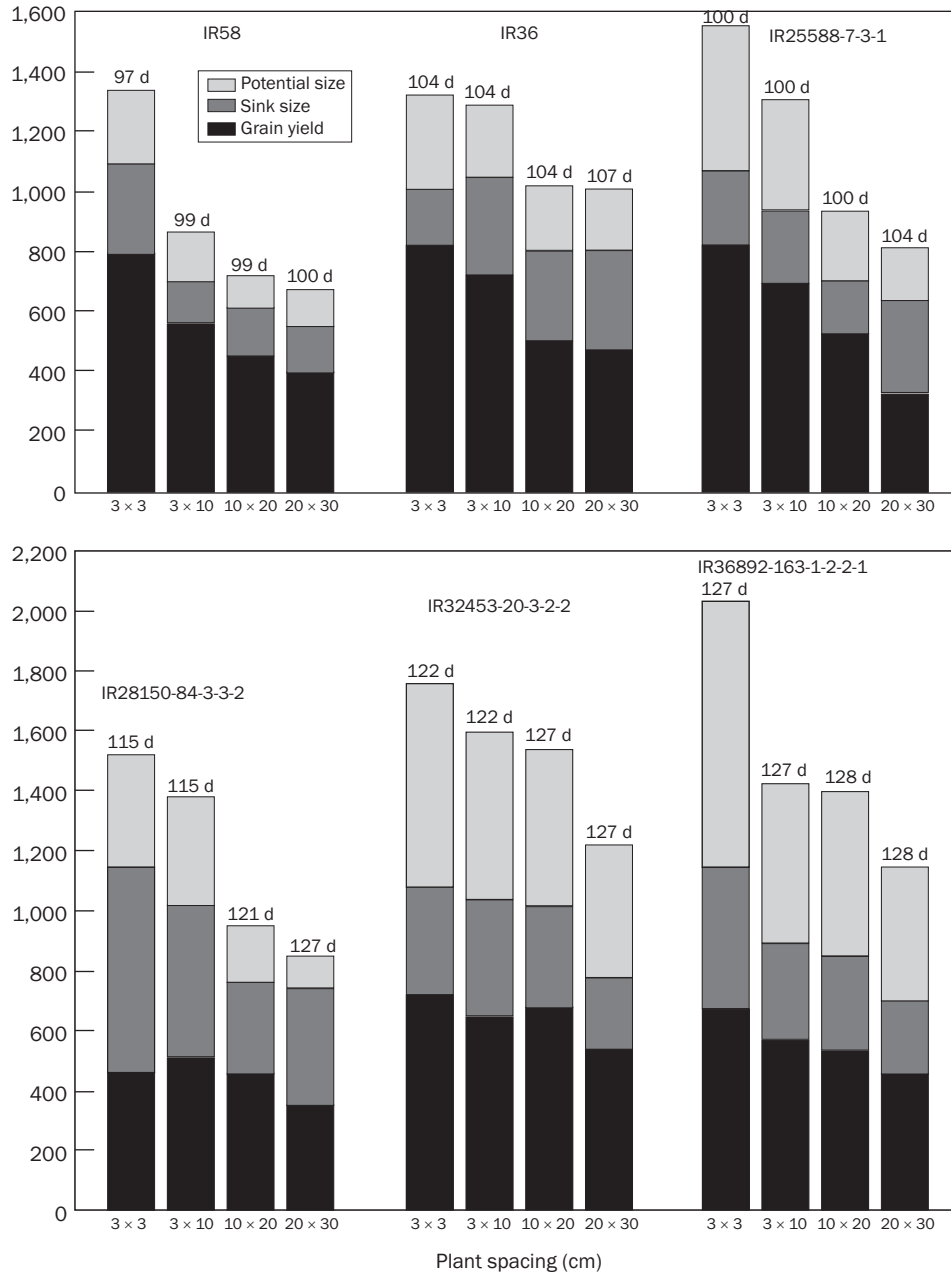
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## Notes

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Grain yield, sink size, and potential sink size ( $\text{g m}^{-2}$ )



**Fig. 16. Grain yield, sink size, potential sink size, and growth duration of six IR varieties and advanced lines under different plant spacings (1989 dry season).**

# Ecophysiology of rice cultivation under anaerobic direct sowing

M. Yamauchi

Direct sowing is becoming popular among rice farmers in the tropics, but inconsistent seedling establishment, susceptibility to lodging, and weed infestation constrain further adoption. Although rice is a crop that can germinate and establish seedlings in flooded anaerobic soil, this character has not been fully used in rice cultivation until now. We hypothesize that sowing rice seeds under the surface of puddled soil where the oxygen supply is limited may help to overcome various constraints associated with direct sowing. Screening germplasm that can establish seedlings in flooded soil was conducted and it was found that 3% of 2,300 accessions tested were superior over controls commonly used for transplanting. The reason superior cultivars grow better in flooded soil than the controls was that the coleoptile elongates faster and longer in hypoxia and is able to reach the soil surface where  $O_2$  is available. The importance of seed vigor was demonstrated in achieving successful seedling establishment in anaerobic soil. The selected cultivars performed better in anaerobic broadcasting (sowing pregerminated seeds just after puddling) and with drill sowing (use of seeder developed for drill sowing in puddled soil) in two locations each in the Philippines, Vietnam, and Myanmar. Anaerobic drill sowing was suitable for increasing lodging resistance. A study under various degrees of weed and water controls demonstrated that the technology would be useful for reducing herbicide use and increasing water productivity.

Farmers in the tropics are shifting rice crop establishment methods from transplanting to wet direct sowing. Farmers prefer direct sowing because it requires less labor and time than transplanting. The shift is prominent in Malaysia, Thailand, Vietnam, the Philippines, and Myanmar. In wet direct sowing, the land is puddled in the same way as in transplanting culture, and, after 1–2 d, germinated seeds are broadcast on the soil surface with (water sowing) or without standing water (wet sowing). It is generally believed that seeds covered by wet soil develop poorly or die. Therefore, farmers broadcast the seeds on the soil surface. Grain yield of direct-seeded rice is reported to be the same as, or higher than, that of transplanted rice.

Three major constraints, however, have to be overcome for increasing the adoption of direct sowing.

1. Seedling establishment is inconsistent (Hiraoka et al 1992). Causes include the destruction of seedlings by birds and rats and the failure of rice seedling development because of factors associated with excess or deficiency of water. Because farmers sow seeds on the soil surface, bird and rat damage is serious.
2. Weed infestation is more pronounced in direct-seeded rice fields than in transplanted fields (Moody 1993). Direct-seeded rice plants start to grow at the same time as the weeds, being exposed to stronger weed competition than transplanted ones.  
Herbicide use is most effective in weed control of direct-seeded rice fields. In addition, weed infestation can be reduced by intensive land preparation before sowing, improvement of seedling establishment and water control, an increase in seed rate, and the use of weed-competitive cultivars. Drill sowing instead of broadcast sowing provides the opportunity for mechanical and manual weeding.
3. Lodging is more common in direct-seeded rice than in transplanted rice. Because farmers are sowing seeds on the soil surface, plants are susceptible to culm-bottom-type lodging (Nishiyama 1985). In addition, a high seed rate leads to high plant density, which induces bending-type lodging. Deep sowing and a reduced seed rate are likely to increase lodging resistance, but they make seedling establishment unstable.

**Table 1. Rice germplasm tolerant of flooded anaerobic soil conditions at seedling establishment.**

Specification of rice germplasm subjected to the screening <sup>a</sup>	Number tested	Number selected	Percentage selection
<i>Oryza sativa</i> L. isozyme group 1, major group, indica	93	7	9
<i>O. sativa</i> L. isozyme group 2, minor group, aus	29	5	17
<i>O. sativa</i> L. isozyme group 3, satellite, deepwater	5	3	60
<i>O. sativa</i> L. isozyme group 4, satellite, deepwater	2	0	0
<i>O. sativa</i> L. isozyme group 5, minor group, basmati	31	0	0
<i>O. sativa</i> L. isozyme group 6, major group, japonica	90	5	6
<i>O. sativa</i> L. not classifiable in isozyme group	8	0	0
<i>O. sativa</i> L. not classified	509	5	1
<i>O. sativa</i> L. improved semidwarf cultivars (breeding lines)	404	8	2
<i>O. sativa</i> L. F <sub>1</sub> hybrids	61	1	2
<i>O. glaberrima</i>	111	9	8
<i>O. sativa</i> L. maturity less than 100 d	979	25	13

<sup>a</sup>Isozyme classification is according to Glaszmann (1987).

Source: Yamauchi (1995).

## Hypothesis

The failure of rice seedling establishment needs to be clarified by analyzing the seed microenvironment and plant tolerance of abiotic and biotic stresses. Seedling growth might be optimum when seeds are placed between the soil and atmosphere where O<sub>2</sub> and water are available. According to Chapman and Peterson (1962), rice seeds can develop even in water using dissolved O<sub>2</sub>. When seeds are placed on the top of drained soil and contact between seeds and soil is loose, the amount of water is insufficient for seed development. In addition, seedlings on the soil surface are not only flushed and prone to lodging by heavy rain but are also destroyed by birds and rats. When the soil is flooded, seedlings with poor anchorage float.

When seeds are sown deep in the soil, the seedlings grow poorly or die (Jones 1933). Rice, however, has an ability to germinate and develop a coleoptile in the absence of O<sub>2</sub>, though the root and leaf do not develop in the absence of O<sub>2</sub> and the elongation of these organs depends on O<sub>2</sub> concentration (Alpi and Beevers 1983). There are differences among cultivars in coleoptile, root, and leaf development at low O<sub>2</sub> concentration (Turner et al 1981). We need to clarify these differences in connection with seedling establishment.

Coating seeds with an O<sub>2</sub>-releasing chemical, calcium peroxide (trade name: Calper), makes sowing into anaerobic soil possible (Yamada 1952). Calcium peroxide, in contact with water, produces oxygen that would be available to the germinating rice seeds in anaerobic soil. Coating with Calper creates an aerobic environment around the seed.

If we were to develop a technology in which seeds were sown under the surface of flooded or water-saturated soil (anaerobic sowing), damage caused by birds and rodents, water stress, rain-splashing, and floating could be avoided. Such a technology would be relevant to the development of weed control and lodging prevention.

## Germplasm screening

An inexpensive mass-screening system was developed to identify cultivars tolerant of flooded anaerobic conditions at the establishment stage (Yamauchi et al 1993). Seeds that germinated for 2 d were placed on plastic seedling trays compartmentalized into 16 × 16 × 25 mm with 17 rows × 34 columns. One seed was placed in each compartment in a row and covered with 25 mm of sieved dry soil. The tray was then placed on the surface of flooded soil in a concrete block paddy with 20–50-mm water depth. The percentage of seedling establishment, height, leaf development, and mesocotyl length were determined 15 d after planting.

We identified anaerobic-tolerant cultivars more frequently in aus (an early summer rainfed) and deepwater rice from northeast India and Bangladesh (isozyme groups 2 and 3, Glaszmann 1987) than in indica (1) and japonica (6) rice (Table 1). The cultivar group with less than 100-d growth duration showed a high selection percentage. Anaerobic-tolerant cultivars were also identified in improved semi-dwarf cultivars, *Oryza glaberrima*, and F<sub>1</sub> hybrid rice.

Anaerobic-tolerant cultivars had a longer coleoptile than check cultivars in a gas flow of N<sub>2</sub> or air, or under various degrees of hypoxia induced in closed flasks (Yamauchi et al 1994). In a flooded soil, the coleoptile of tolerant cultivars emerged above the soil surface more than that of the check cultivar. Coleoptile emergence in the check cultivar was not followed by 1st-leaf emergence (Yamauchi and Biswas 1997). These findings could be explained by the fact that coleoptiles and mesocotyls of tolerant cultivars develop faster and longer than those of the check in soil under anaerobic conditions.

The differences in seedling establishment between the cultivars and between the Calper-coated and noncoated seeds were large when the seeds were sown deeper (Yamauchi and Chuong 1995). Calper coating was more effective in promoting seedling establishment than the use of anaerobic-tolerant cultivars.

## Seed vigor

Seed vigor, the potential for rapid uniform emergence, and the development of normal seedlings under a wide range of field conditions (Association of Official Seed Analysts 1983) vary according to seed age, cultivar, time of harvest, weather during maturation, nutrition, position of seeds on the panicle, specific gravity, mechanical integrity, and the presence of pathogens (Seshu et al 1988). In screenings, we often observed that the seed source changed the performance of a cultivar.

Subjecting seeds to an accelerated aging treatment significantly reduced anaerobic seedling establishment (Yamauchi and Tun Winn 1996). When seeds were kept for 0–9 d at 100% relative humidity at 43 °C, IR36 (check) and JC178 (tolerant) showed a lower percentage of germination and seedling establishment. A tolerant cultivar, ASD1, however, did not deteriorate during the aging treatment and maintained a high seedling establishment ability, suggesting that this cultivar is tolerant of seed aging as well. Because production and postharvest conditions vary among seed sources, seed vigor may differ even among the seed lots of an identical cultivar. The use of a cultivar tolerant of both seed aging and anaerobic conditions might be important to achieve consistent seedling establishment.

## Sowing method

The methods of placing seeds under the surface of puddled soil should be simple so that farmers can practice anaerobic sowing. The following methods were developed.

1. Anaerobic broadcast sowing. Most farmers broadcast germinated seeds when the soil surface becomes hard (1–2 d after land preparation). In anaerobic broadcast sowing, the seeds are broadcast before the soil surface becomes hard (mostly, on the day of land preparation) so that a thin soil layer covers broadcast-sown seeds.

During the dry season, seedling establishment was optimum when seeds were sown 1 d after land preparation (Yamauchi 1995) mainly because of the seed landing position: half of the seed body stays in the soil and the remaining half in the atmosphere. When seeds were sown on the day of land preparation, seeds sank under the soil surface and seedling establishment was reduced, resulting in a clear difference between cultivars. Sowing at 2–3 d after land preparation reduced seedling establishment because of poor contact between seed and soil.

During the wet season, sowing at 0–2 d after land preparation resulted in a similar level of seedling establishment, regardless of the cultivars, at the two locations in the Philippines (Yamauchi 1995). Because the seeds were splashed by rain, there was little difference in seedling establishment between the seeds sown on the day of land preparation (covered by soil at sowing but exposed to the soil surface by rain-splashing later) and those sown 2 d after land preparation (seeds stayed on the soil sur-

face at sowing but were covered by or mixed with soil by rain-splashing later).

In water sowing, farmers broadcast when the soil particles settle and water becomes clear. Because of buoyancy, many seedlings are detached from the ground and float. This problem is particularly serious when it is windy. Broadcasting seeds on the day of land preparation and covering them with a thin layer of soil could be advantageous in preventing the occurrence of floating seedlings. The sowing depth, however, was significantly affected by the timing of sowing after land preparation, soil properties, and intensity of land preparation, resulting in occasional failures of seedling establishment. In addition, the presence of snails in the standing water destroyed the seedlings. Thus, the technique of water sowing needs to be further developed.

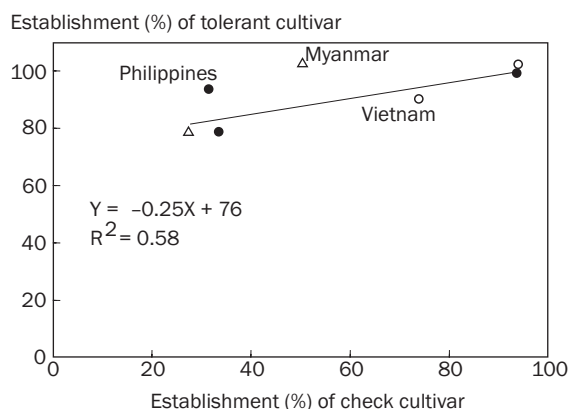
2. Anaerobic drill sowing. Commercial seeders developed for sowing Calper-coated seeds are useful for anaerobic drill sowing. At IRRI, we modified the IRRI drum seeder (designed for surface drill sowing) into an anaerobic seeder by attaching buoyant flotation wheels and furrow openers and closers (Borlagdan et al 1993, 1995). This seeder can be produced locally for about US\$150 per unit. The seeder was successfully operated in Los Baños and Muñoz, Philippines; Yezin and Kyaukse, Myanmar; and Hanoi, Vietnam.

## Crop establishment

Anaerobic drill sowing was tested in Los Baños and Muñoz, Philippines, and anaerobic broadcast sowing in Muñoz in the 1993 dry season (Yamauchi et al 2000). The percentage of seedling establishment, single-seedling weight, height, and biomass production of anaerobic-tolerant cultivars were significantly greater than those of the semidwarf high-yielding check cultivars. Cultivar performance varied between locations but not between sowing methods. Biomass production was controlled not only by the percentage of seedling establishment but also by single-seedling weight. To improve crop establishment, it is first necessary to increase the percentage of seedling establishment, and second to use cultivars with rapid initial seedling growth.

There was little difference in grain yield between anaerobic-tolerant and check cultivars at the two locations when cultivars of improved semidwarf plant type were compared. Although tolerance of anaerobic conditions was observed more often in traditional cultivars than in improved semidwarf cultivars (Table 1), a tolerance gene could be incorporated into semidwarf high-yielding plants.

Anaerobic broadcast and drill sowing were successfully tested also in the Mekong Delta (Chau and Yamauchi 1994) and Red River Delta (Chuong and Yamauchi 1994) in Vietnam and in Myanmar (Tun Winn et al 1997), leading to improved crop establishment and higher grain yield than with check cultivars. These experiments indicated that a cultivar that performed well at a given location did not al-



**Fig. 1. Seedling establishment of anaerobic-tolerant cultivars versus local check cultivars in the tropics. The data of 7 experiments conducted at 6 locations in 3 countries were analyzed. Each experiment involved 2 check cultivars and 5 to 10 anaerobic-tolerant cultivars. On the x axis, percentage establishment of the best check cultivars is shown. The y axis indicates percentage establishment of the best anaerobic-tolerant cultivar in the experiment.**

ways perform well at other locations. It seems that the performance is influenced by the interaction between the seed and local conditions, particularly soil. The relationship of seedling establishment between the local check and the best-performing anaerobic-tolerant cultivar was analyzed to understand the potential to stabilize seedling establishment with the introduction of anaerobic tolerance (Fig. 1) (Yamauchi 1999). The regression curve indicated that the introduction of an anaerobic-tolerant cultivar resulted in more than 80% seedling establishment while the performance of the local check cultivar ranged from 20% to 100%. We conclude that anaerobic tolerance stabilizes seedling establishment in the tropics.

## Weed control

The introduction of anaerobic drill sowing makes mechanical and manual weed control possible. This might be useful when a herbicide is not available or its use has to be reduced.

Crop establishment and weed control of anaerobic-tolerant cultivar IR41996-50-2-1-3 were compared with those of check cultivar PSBRc 4 under various combinations of sowing methods, water control, and weed control levels. IR41996-50-2-1-3 had higher yield across sowing methods and water controls and competed better with weeds than PSBRc 4 because of superior seedling establishment, initial growth, and panicle number (Pablico et al 1994).

The contamination of rice fields with noncultivated rice plants (weedy rice) is becoming serious, reducing grain yield and market price. Weedy rice was found to be anaerobic-tolerant (Yamauchi et al 1995). The introduction of anaerobic-tolerant cultivars may reduce the infestation of weedy rice.

Although herbicide application is an essential component of weed control in broadcast-sown rice fields, it must be reduced as much as possible through the integration of water control, tillage, and seedling establishment method. Anaerobic sowing with flooding 3 d after sowing allowed cultivars to sustain high yield and increased water productivity without having to use herbicide or with only half of the recommended herbicide rate (Tuong et al 2000).

## Lodging

We evaluated the lodging resistance of rice plants by visual observation and by using a push-gauge. A gauge that measures lodging resistance by evaluating a single hill was modified to determine the lodging resistance of a broadcast-sown canopy. The force required for a canopy of 30-cm width to exhibit 45° lodging at 15-cm height was closely correlated with the visual observation (coefficient of simple linear correlation  $-0.947$ , significant at the 1% level).

We evaluated the lodging resistance of transplanted, surface broadcast and drill-seeded, and anaerobic broadcast and drill-seeded rice at two locations in the Philippines (Yamauchi 1995). The lodging resistance of drill-seeded rice was equivalent to or slightly lower than that of transplanted rice, but significantly higher than that of broadcast-seeded rice. Anaerobic-tolerant cultivar IR52341-60-1-2-1 showed greater resistance to lodging at Los Baños than the others.

The analysis of interactions in lodging resistance among planting method, water management, and weed control suggested that weed infestation reduces lodging resistance the most (Yamauchi 1995). This was particularly true when we compared the lodging resistance of rice crops grown without herbicide with those grown with herbicide. Anaerobic sowing followed by advanced flooding resulted in a reduction in weed infestation and higher lodging resistance in the plots without herbicide application (Pablico et al 1996).

## Relevance to direct sowing in Japan

Rice has been produced in Japan by transplanting for more than 2,000 years. After achieving rice self-sufficiency in recent years, the government encouraged a shift from transplanting to direct sowing when it foresaw the need to reduce rice production costs. Although rice transplanting in Japan is well mechanized, the amount of labor and materials required for raising seedlings and transporting them from the nursery to fields is huge.

In the past, direct sowing was locally practiced in the form of water sowing in Japan. Pregerminated seeds were sown into the standing water after the settlement of soil particles. Floating of seedlings was the major problem that limited adoption.

Yamada (1952) found that seed coating with calcium peroxide improves seedling establishment in flooded anaerobic soil. Rice scientists in Japan used this finding and established the technology of seed coating with oxy-



gen-release chemical Calper. The technology is popular among rice scientists, extension workers, and policymakers in Japan. However, Japanese farmers have not yet adopted the technology as much as expected. Some farmers say that transplanting with a machine is much more labor- and time-saving, resulting in more reliable crop establishment than direct sowing with the seed-coating technology. Because the coating technology requires both labor for seed coating and the purchase of chemicals and a coating machine, and because the coated seeds have to be sown on time (thus, farmers lose flexibility of farm operations), it might be difficult to persuade farmers to adopt the technology.

This study emphasizes the importance of using the inherent physiological character “anaerobic tolerance” in lieu of coating seeds with an oxygen-release chemical. Nowadays, some studies in Japan demonstrate reducing the use of seed-coating material from the originally recommended two-times seed weight to one time to zero use. Some breeding programs propose germplasm screening for anaerobic tolerance. It seems that research on direct sowing in Japan is changing from depending on chemical use to depending on germplasm use.

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## Notes

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# Stabilizing rice production under water stress in rainfed and upland cropping

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In the face of global water scarcity, improvement of upland rice production under water-limited conditions is crucial for maintaining food security in the tropics. The major abiotic constraints that limit the productivity of upland rice are unstable water availability and low nutrient supply. To seek possible management options to overcome these two constraints, this study aimed to analyze water-nutrient interactions related to (1) soil N dynamics, (2) N-use efficiency for yield, and (3) water uptake by the plant, with particular emphasis on the morphology and functions of the root system. Field studies in diverse uplands in the Philippines, Thailand, and India showed that the causes of limited yield response to N inputs were a low N recovery because of high N leaching and/or low internal N-use efficiency because of water stress. A large amount of soil indigenous N was generally found in the early growth period, but the soil was subject to N leaching brought about by intensive rainfall.  $\text{NO}_3^-$ -absorbing capacity of the acidic Ultisols retarded N leaching. The use of controlled-release N was useful for improving N recovery. Internal N-use efficiency often decreased in uplands because of limited water uptake. Limited water capture by rice was first attributed to the plant's shallow root distribution. An adequate nutrient supply, particularly of P, in the surface layer had a significant effect on improving the water-capturing capacity from the layers below the surface by enhancing deep-root development. Strong environmental effects and genotype  $\times$  environment interactions in rooting depth indicated that it is important to optimize a combination of genotype and soil conditions to improve the deep-root system. A QTL analysis on root morphology showed the importance of phenotyping conditions and also suggested a possibility of marker-aided selection for deep-root traits. The water uptake rate decreased rapidly with decreasing soil water because of reduced root growth and limited water uptake rate per unit root length, which implied the possibility that a lower root conductance for water is responsible for the limited water capture by rice under the stress. Genotypic variations in the structures of sclerenchyma and xylems were found among the genotypes of tropical origin, which might be related to the water uptake ability under the stress.

Because of the global water scarcity, the improvement of rice productivity under water-limited conditions, such as in uplands, is becoming more important. To improve current low yield levels, we need to identify the constraints to yield and develop strategies and technologies for aerobic soil conditions. For abiotic stress, low nutrient supply, especially of N, and the high sensitivity of rice to drought are the critical constraints in most upland areas (Yoshida 1975, Widawsky and O'Toole 1996). To develop efficient N management options, it is important to identify the critical processes in the determination of a yield response to N inputs, which includes N supply from soil, N uptake by roots, and N-use efficiency for yield. Drought tolerance of the plant is a complex character consisting of physiological and morphological traits that confer three mechanisms, escape,

avoidance, and tolerance (Levitt 1980). Past research clarified that maintenance of high water potential in the shoot supported by water capture from deep soils is related to growth during the stress period in rice genotypes, which indicated significant genetic variation in avoidance ability (Yoshida and Hasegawa 1982, Liley and Fukai 1994). The genetic diversity and genetic control in root morphology, especially in the nodal root system, have been studied extensively since the 1970s for the genetic improvement of a large and deep root system (for example, Chang et al 1982, Yoshida and Hasegawa 1982, Yadav et al 1997). Compared to this progress in genetic aspects, the understanding of environmental effects and genotype  $\times$  environment interactions (G  $\times$  E interactions) on root development, which is essential for maximizing the expression of genetic poten-

tial, has been limited. In addition, the adaptive traits under lower soil water should be further clarified to identify the critical plant traits for improving water-capturing capacity and water-use efficiency. On the basis of this background, this project focused on

1. An evaluation of the effects of nutrient and water stress on yield and characterization of the dynamics of N in the soil in upland rice.
2. The root system as affected by the environment and genetic factors.
3. The characteristics of root growth and water uptake under water stress.
4. Genetic and environmental effects on the variation in carbon isotope discrimination and water-use efficiency.

Evaluating the effects of nutrient and water stress on yield and characterizing N dynamics in upland rice soil

### Effects of nutrient and water stress on yield and nitrogen-use efficiency

Final grain yield can be considered as a function of uptake of soil-N and applied-N and the internal N-use efficiency for grain production, both of which could be affected by water regimes. Therefore, to overcome the problems responsible for an unstable yield response to N, it is important to develop improved N and crop management (Kondo et al 1998, 2002a). To identify the critical factors for determining yield in diverse upland conditions, field experiments were conducted in major upland rice areas in India, Thailand, and the Philippines. To analyze the movement of water and N in soil profiles, a simulation model for the water balance and N leaching in the soil was developed using a simple one-dimensional model (Murty and Kondo 2001).

Yield without N application ranged from 0.85 to 1.92 t ha<sup>-1</sup> without irrigation, indicating a low yield potential without nutrient input. The yield gain from N application varied markedly among sites, years, and application methods for N and P. A large response to N was obtained in Hazaribag, India (1.48 t ha<sup>-1</sup> on average), whereas a limited yield gain (0.13 t ha<sup>-1</sup>) was obtained in Fang, Thailand. The variable response to N was attributed to the variation in N recovery and/or internal N-use efficiency among the sites. N recovery mainly determined the agronomic N-use efficiency (yield gain from N input) in Hazaribag, indicating the importance of improving N recovery (Fig. 1). On the other hand, in Matalom, Philippines, agronomic N-use efficiency increased as N recovery increased, but it remained at a relatively low level compared with the value obtained in flooded conditions without water stress. Agronomic N-use efficiency was quite variable against N recovery and low in Fang. These low and variable agronomic N-use efficiencies were mainly caused by water stress. The results across sites implied that the prioritized strategy to improve yield should differ among sites. The simulation on N movement in the soil in Hazaribag suggested that one factor for variable N recovery was considered to be N leaching as affected by rainfall. To improve

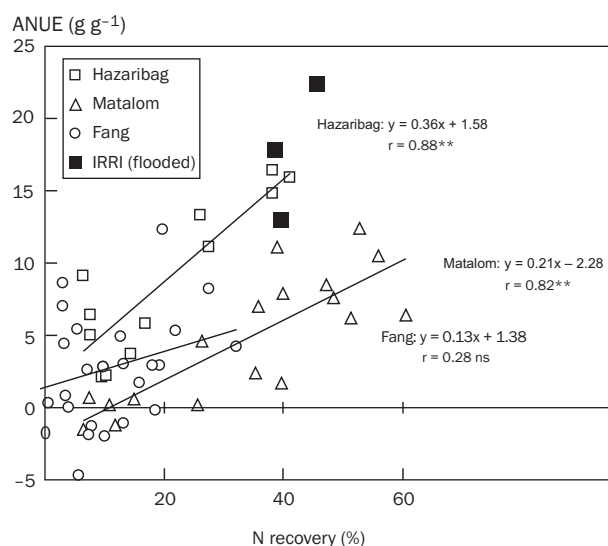


Fig 1. Relationship between N recovery from N application and agronomic N-use efficiency (ANUE) in Hazaribag, Matalom, and Fang under upland conditions and at IRRI under flooded conditions.

N recovery, the use of controlled-release urea was found to be effective compared with four split applications of soluble urea, especially under high rainfall.

On the other hand, in the areas where the internal N-use efficiency was low and/or variable because of water stress, such as Fang and Matalom, nutrient input combined with genetic/cultural improvement in water-capturing capacity by the roots is needed to increase the efficiency expected from nutrient input. In this connection, improvement of root development in genetic factors and soil environmental factors will be sought.

### N dynamics in upland soil as affected by rainfall and soil NO<sub>3</sub> absorption

To establish an N application method to meet the N demand of the crop, a proper understanding of the dynamics of soil N availability and an ideal pattern of plant N accumulation for maximum yield is essential. In upland rice soils in Cale (Haplustalfs, pH (H<sub>2</sub>O) 5.18) and Siniloan (Palehumults, pH (H<sub>2</sub>O) 4.50) in the Philippines, a large amount of available NO<sub>3</sub> (KCl-extractable), higher than 120 kg ha<sup>-1</sup> in 0–80-cm depth, was detected during the crop establishment period at both sites, which was probably due to mineralization at the onset of the wet season (Kondo et al 1998). Thereafter, NO<sub>3</sub> disappeared from the surface, then from the deeper layer. NO<sub>3</sub> decreased markedly, especially after the heavy rain in Cale, which indicated N loss by leaching. On the other hand, available NO<sub>3</sub> to 80-cm depth decreased only slowly with increases in the 40–80-cm layer during the crop season in Siniloan, which was at least partly due to retardation of N leaching by the NO<sub>3</sub> absorption capacity of the soil. Field experiments using <sup>15</sup>N showed that the different N-supplying patterns from the soils among the sites affected the optimum timing of N application. In the soil without NO<sub>3</sub> ab-

sorption, the use of N from the soil N pool at the early stage was important together with the application of a topdressing at later growth stages to supplement the decreasing soil available-N. In contrast, there was less difference in N recovery among the timing of N application in the acidic Ultisol because a large portion of applied  $^{15}\text{N}$  remained in the rooting zone until maturity. These results suggested that site-specific N management is necessary with consideration of the soil type as well as rainfall pattern to improve N recovery.

### The root system as affected by environmental and genetic factors in upland rice

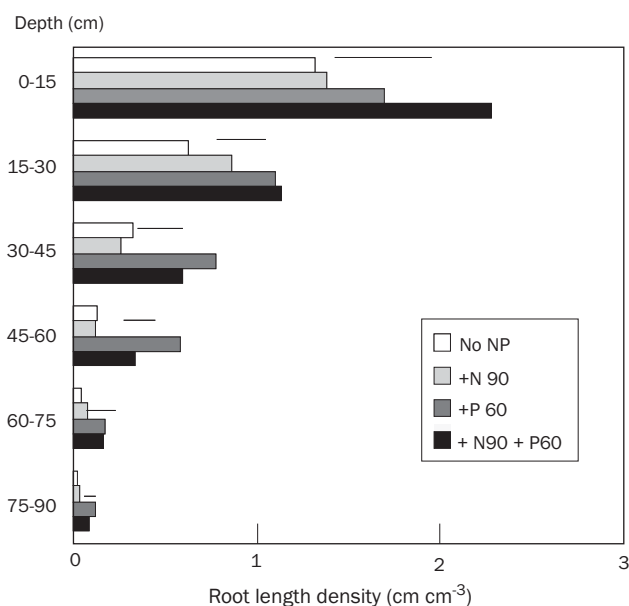
Although the importance of a deep rooting zone to ensure water capture under drought is established in upland rice in general, progress in developing a genotype possessing an efficient root system for water uptake has been relatively limited. The reasons for limited progress can be attributed to (1) a lack of knowledge on environmental factors that complicate varietal evaluation in root morphology, (2) the tedious screening procedure for root morphology, (3) an insufficient understanding of the genetic control of root traits, and (4) insufficient information on the value of root traits for different types of water stress. Considering this background, in this section we examine the emphasis on environmental (soil, water, nutrient management) and genetic factors and their interactions for root morphology because this is needed to develop genotypes adapted to the target environment and to identify suitable management for the expression of the potential of genotypes.

Developing linkage maps and identifying QTLs associated with root morphology help to develop marker-aided selection for better selection efficiency for root morphology and drought tolerance, and to understand the genetic control of root traits. To enhance this progress, further understanding is needed on the environmental effects on variations of QTLs associated with the deep root system trait and the underlying mechanisms.

### Characteristics of the root system and nutrient effects on root development in upland rice

The field data from the IRRI upland field showed that maximum rooting depth was attained at the early tillering stage. The rooting zone expanded both vertically and horizontally from crop establishment to the tillering stage and slightly more horizontally from tillering to panicle initiation. The change in root distribution was possibly related to the increase in shallower nodal roots emerging from late stems. The close linkage between the development of the stem and nodal roots is reflected in the spatial expansion of the rooting zone.

Root length density usually decreases exponentially along the depth. On average across experimental sites and management, 22% of the root was distributed below 30-cm depth in terms of length, indicating a general feature of shallow root distribution in rice, though considerable varia-



**Fig 2. Effect of N (90 kg ha<sup>-1</sup>) and P (60 kg ha<sup>-1</sup>) on root development at flowering stage, Siniloan, 1996.**

tion occurred (Kondo et al 1999a, 2001a). The results on the effect of nutrients on root growth and water uptake showed that an adequate nutrient supply, particularly of P, in the surface layer significantly improved the water-capturing capacity from the layers below the surface by enhancing deep-root development. The application of P led to deeper root distribution in P-deficient acidic soils (Fig. 2) because the nodal roots from lower internodes in early stems increased with a higher P level. Phosphorus status in the plant at the early stage affects root growth and distribution after the reproductive stage. It was found that a medium level of N (90 kg ha<sup>-1</sup>) generally increased root length density without a significant change in root distribution (Fig. 2). This is different from the case in submerged conditions, in which N application substantially inhibits the elongation of nodal roots to form a shallow root system (Kawata et al 1977). Regarding the timing of N, basal N enhanced root growth from early stems, which led to deeper distribution at the reproductive stage (Winn 1999).

### Effect of nitrogen-catch crops on root growth and nitrogen use in rice in rainfed lowland ecosystems

In rice-based rainfed lowland ecosystems in northeast Luzon, Philippines, intensive cropping systems with rice in the wet season and a cash crop in the dry season are commonly adopted. The introduction of an N-catch crop during the dry-to-wet transition period is expected to reduce N pollution of the groundwater that resulted from excessive N application for cash crop cultivation (Kondo et al 1999b). Planting of an N-catch crop may affect the root development of rice as well as scavenging soil N, which would affect water uptake.

In field experiments conducted in farmers' fields (Inceptisol) in Batac, Ilocos Norte, Philippines, N-catch

crops (indigo and maize) showed an almost uniform root length density along the depth below 20 cm and down to 70 cm, reflecting the high capacity to trap NO<sub>3</sub> leaching from the deep soil layer as N-catch crops (Shrestha 1997). The root system in rice after N-catch crops was larger and deeper with a higher root/shoot ratio compared with that with chemical N fertilizer after fallow. N supplied gradually from the residue of N-catch crops and physical amelioration of the soil may have enhanced root growth. The efficient rooting characteristics of indigo and maize for trapping NO<sub>3</sub> and enhancing root growth of rice in the succeeding crop suggested the advantage of N-catch crops over the fallow system. The results signified that the underground root interactions between rice and other component crops must be considered when selecting cropping systems and residue management in rainfed lowland rice.

### Genotypic variation and genotype × soil and N interactions in root development

Genotypic variation in root traits related to the size and distribution of the root system was examined with particular interest in the interaction with soil and N levels (0 and 90 kg N ha<sup>-1</sup>) using 11 rice genotypes grown in three different soils, Cale (Haplustalfs), Siniloan (Palehumults), and the experimental field of IRRI in Los Baños (Tropudalfs), in the Philippines, in the 1997 wet season. G × E interactions were analyzed by additive main effects and multiplicative interaction (AMMI).

Upland rice varieties tended to have a deeper root distribution than lowland rice varieties (Kondo et al 2001a). While deep distribution was commonly found among upland varieties, there were differences in root morphology among genetic groups. Japonica upland varieties had a large root dry weight associated with a high root/shoot ratio, high specific root weight, and a low number of nodal roots and stems. On the other hand, aus (Dular) and indica (Vandana) varieties showed deep root distribution with a medium root/shoot ratio and low specific root weight. It would be interesting to consider the possibility that those characters con-

tribute to the efficient exploitation of the rooting zone with the use of limited biomass because of a short growing period in the major cropping areas in eastern India and Bangladesh (Courtois and Lafitte 1999), where the duration of the cropping season is restricted by the limited rainy season. The variation in root thickness among the varieties tested raised the need to verify the significance of root thickness in water uptake. The thick root trait was suggested as a valuable root trait for drought tolerance to reduce axial resistance for water transport (Richards and Passioura 1981). However, a rigid evaluation of the significance of this trait for drought tolerance seems to be unavailable in rice (Yambao et al 1992).

Among root traits, the ones related to root size (root dry weight, root/shoot ratio, number of nodal roots) and thickness (specific root weight) had a larger effect with variety than with G × E interactions, indicating the dominance of genetic factors in those traits (Table 1). The number of nodal roots in particular had a high stability across environments, which was probably linked with the stable number of stems through the formation of phytomers. The larger effect of variety in root/shoot ratio than in root dry weight suggested that root/shoot ratio is regulated by genetic factors to a larger extent than root dry weight.

On the other hand, root depth was highly affected by site, but not by N level, indicating high sensitivity to edaphic conditions (Table 1). The difference in soil water conditions among the sites was possibly one factor that affected root depth because the growth of rice roots is very sensitive to soil water conditions (Fukai and Inthapan 1988). In addition, the results that root depth was closely correlated with stem size signified the involvement of a developmental factor in determining root distribution. The limited supply of carbon per root axis might have limited root elongation. High N increased root dry weight without a substantial change in the root/shoot ratio and root distribution, and the interaction of variety × N was relatively small in all examined root traits.

**Table 1. AMMI analysis of variance for total root dry weight (TRM), root depth as root length depth index (RLDI), root/shoot ratio (R/S ratio), number of nodal roots, and specific root weight (SRW).**

Source	df	TRM (g m <sup>-2</sup> )		RLDI (cm)		R/S ratio (g g <sup>-1</sup> )		Root number (no. hill <sup>-1</sup> )		SRW (mg cm <sup>-1</sup> )	
		Sum of square	(%)	Sum of square	(%)	Sum of square	(%)	Sum of square	(%)	Sum of square	(%)
Variety (G)	10	28,182	34.2	17.9	1.8	0.076	39.1	134,417	75.7	0.110	63.8
Environment (E)	5	41,705	50.6	884.1	90.3	0.058	29.9	10,750	6.1	0.010	5.7
G × E	50	12,546	15.2	77.1	7.9	0.061	31.1	32,492	18.3	0.053	30.6
IPCA 1	14	9,566	76.2	34.4	44.6	0.040	66.2	11,493	35.4	0.029	53.9
IPCA 2	12	1,531	12.2	17.7	23.0	0.011	18.7	8,505	26.2	0.011	19.8
IPCA 3	10	907	7.2	16.4	21.3	0.006	10.1	7,975	24.5	0.007	12.9
IPCA 4	8	346	2.8	5.9	7.6	0.002	3.6	3,363	10.3	0.006	10.6
Residual	6	196	1.6	2.7	3.5	0.001	1.4	1,156	3.6	0.001	2.8
Total	65	82,434	100.0	979.1	100.00	0.195	100.0	177,658	100.00	0.173	100.0



Relatively large  $G \times E$  interactions in root dry weight, which accounted for 44% of variety factors, indicated the importance of understanding  $G \times E$  interactions in root growth. Analysis of adaptation to environments (site and N) by AMMI analysis showed that genetic groups had different patterns of adaptation to the soil. Japonica upland varieties displayed a positive interaction with the Siniloan site, an acidic soil under wet conditions, while Vandana and Dular showed a positive interaction with Cale, where the soil had a slightly acidic reaction with a deep profile. Edaphic factors such as available-P, exchangeable-Al, pH, bulk density, and soil water regime were suggested as being determining factors for observed variety  $\times$  site interactions.

The results of the variety  $\times$  site interactions in root growth implied that it is important to define the key soil characteristics for root development in the target area to optimize the deployment of genotypes for better root growth. The strong effect of soils on root distribution suggested the importance of edaphic factors and soil management to maximize rooting depth, together with genotype selection. Molecular markers would be a useful tool for helping to clarify the genetic factors and physiological mechanisms involved in responses of roots to soil environments.

#### **Genetic control of root morphology by QTL analysis under different N supply**

In most previous studies in QTLs associated with nodal root morphology, not enough attention has been paid to phenotype conditions and developmental interactions between root and shoot traits (Kondo et al 2001b). In this study, root system morphology in 101 doubled-haploid lines derived from parents having a contrasting root system—IR64, a lowland indica variety, and Azucena, an upland tropical japonica variety—was investigated and QTLs associated with root morphological traits, especially those related to a deep root system, were located under different N conditions ( $\text{NH}_4$ , 20 mg N L<sup>-1</sup>;  $\text{NO}_3$ , 20 mg N L<sup>-1</sup>;  $\text{NO}_3$ , 2 mg N L<sup>-1</sup>) in hydroponic culture. QTL analysis was performed on root and shoot traits in the plant grown for 38–40 days after germination in three different seasons.

For the average population, total dry weight and stem number were highest with  $\text{NH}_4$  at 20 mg L<sup>-1</sup> than with  $\text{NO}_3$ , but the average nodal root length and maximum root length were smaller by 20–27% in  $\text{NH}_4$  at 20 mg L<sup>-1</sup> than in  $\text{NO}_3$  at 20 mg L<sup>-1</sup> and a strong inhibition of root elongation occurred under  $\text{NH}_4$ . Treatment  $\times$  line interaction was significant for maximum root length, root dry weight, and root number. This indicated the importance of the interaction of genetic and N factors in root morphology.

QTLs were commonly detected across the treatments and only in specific N treatments. For plant height, most QTLs were commonly detected in more than two treatments. On the other hand, QTLs commonly detected across treatments were rather limited in root and shoot-root traits. The number of QTLs commonly detected across different seasons was also rather limited in root and shoot-root traits. For maximum root length, 3–5 QTLs were detected in individual

N treatments, of which one was commonly detected across treatments. Common QTLs explained 11.7–25.3% of the phenotypic variation. For average root nodal length, one common QTL was found across N treatments, which explained 14.6–29.1% of phenotypic variation.

Several QTLs were associated with plural traits. QTLs were associated with related traits such as number of nodal roots and average nodal root length with which the negative relationship was seen in phenotypic correlation. We need to confirm whether those QTLs affected root length directly or indirectly through the effect on number of roots.

The results here in hydroponic culture showed some similarities with the results on QTLs that were detected in the soil culture in the same population (Yadav et al 1997). QTLs associated with depth of the root system were commonly found in similar regions on chromosomes 2 and 9. QTLs on the short arm of chromosome 2 from Azucena increased root length, whereas QTLs on chromosome 9 had the opposite effect. QTLs associated with root size were detected on chromosomes 1 and 8 in both hydroponic and soil culture. The presence of those QTLs commonly found under various growing conditions signified the possibility for marker-aided selection for a deep and large root system. On the other hand, the diverse QTLs among N-supply conditions and growing seasons indicated the importance of selecting phenotyping environments for QTL analysis of root traits.

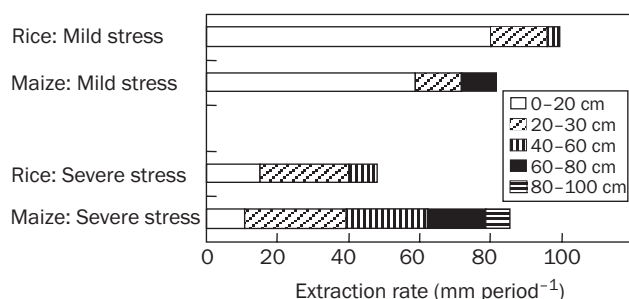
#### **Characteristics of water uptake under water stress in rice**

##### **Water uptake under water stress**

Root length density at depth is the primary factor that determines water uptake from the soil layer; hence, rooting depth is considered to be an important criterion for water capture under drought stress. However, past studies indicated that water uptake rate per unit root length is not simply determined by soil water (matric) potential in a given soil layer in rice and other crops when water stress is imposed (Hasegawa and Yoshida 1982). Understanding on the morphological and physiological adaptation of the root to drought stress may lead to more effective selection traits for breeding programs and strategies for better cultural management.

The field study showed that water extraction patterns were similar in rice and maize under mild stress conditions (Fig. 3). The most water was extracted from the 0–20-cm layer because of high root length density and possibly because of the lower axial resistance in the shallower layer. Under severe stress, rice showed a much larger suppression of dry matter production in the shoot compared with maize because of limited water uptake from the deep layers (Kondo et al 2000a). The difference in water uptake between maize and rice was attributed to (1) the low morphological response to the stress in increasing root density, especially in deep layers, and (2) the decreasing and limited specific water absorption rate per root length in the deep soil layer in





**Fig 3. Water extraction from soil layers under mild water stress and severe water stress in rice and maize.**

rice. The specific water absorption rate per root length increased temporarily below 20-cm depth as the water potential in the surface 0–20-cm layer decreased. However, the specific water absorption rate decreased markedly when the soil water potential became lower than  $-0.04$  to  $-0.05$  MPa in the layers below 20-cm depth in rice. A detailed study on the change in water potentials in the soil and plant suggested that the limited water uptake rate in the deeper layer under severe stress was probably caused by the decreased conductance for water in the root. It would be valuable to explore the genetic variation in morphological growth and conductivity and to verify the traits critical to improving water capture under drying soil.

#### Diversity in the anatomy of nodal roots in tropical rice varieties

As discussed in a previous section, the maintenance of root activity for water absorption and transport is an important trait for improving water uptake under drying soil conditions. The axial conductance is possibly related to xylem structure in the number and size of xylem vessels. The rice root system possesses several traits that are related to its semiaquatic nature such as developed sclerenchyma and aerenchyma. However, information is lacking on the role of those structures in the radial and axial transport of water. Genetic diversity for those traits and the advantages and drawbacks for water uptake and transport will be evaluated.

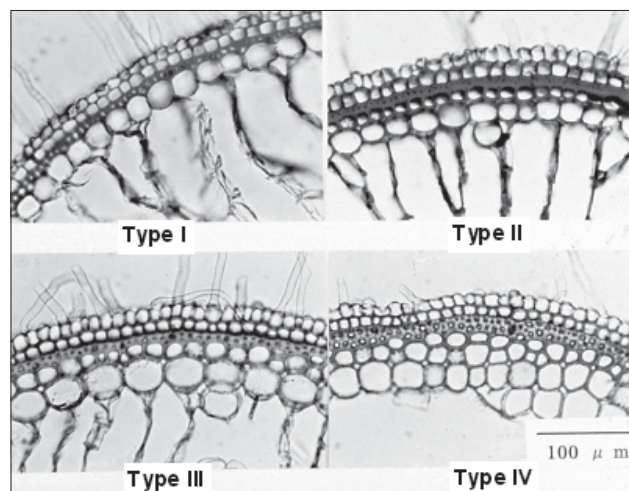
A comparison of 12 lowland and upland genotypes showed that traditional upland japonica varieties had the largest diameter of root, stele, and xylem vessel, followed by modern upland varieties (Okada et al 2002). The varietal difference in the ratio of stele to root diameter was associated with the genetic group with the highest value found in japonica varieties, rather than with the ecosystem. Axial conductance is proportional to equivalent xylem vessel diameter ( $De^4$ ), assuming that the water flow through the xylem vessel simply follows the Hagen-Poiseuille flow. The largest  $De^4$  was found in Moroberekan, which was 22.3 times larger than in IR20 with the smallest  $De^4$  in the roots on the main stem. The relatively consistent relationship between  $De$  and stele diameter across varieties, portion of root, and environments indicated that stele diameter would be more appropriate than root diameter for estimating conductance.

The larger stele relative to the root diameter resulted in a larger  $De$ /root ratio in japonica than in indica and aus.

Four distinctly different types of sclerenchyma anatomy were identified among the varieties (Fig. 4; Kondo et al 2000b). The japonica varieties had a higher frequency of the types that have a doubled cell layer in the sclerenchyma with a thick cell wall (types III and IV) than indica and aus. The difference among the genetic groups was nearly consistent across soil water regimes and aerobic and submerged soils, which implied that sclerenchyma development is mainly controlled by a genetic factor. Morphological differences in the sclerenchyma might be interesting when its function is considered. Prevention of apoplastic water movement, an increase in physical strength (Feldman 1984), and protection of the root from pest infection are based on the lignification or the suberization in the cell wall. The thick sclerenchyma might reduce water permeability of the roots and prevent collapse of the cortex when the soil is drying. Further study is desired to clarify the value of the traits found in traditional upland japonica varieties, which are the larger root and stele diameter, and  $De$  and thickened sclerenchymatous tissue, to improve their adaptability to upland conditions.

#### Genetic and environmental effects on variation in carbon isotope discrimination and water-use efficiency

Genotypic variations in water-use efficiency and carbon isotope discrimination ( $\Delta$ ) have been reported in rice (Dingkuhn et al 1991). Also, varietal differences in stomatal response to decreasing leaf water potentials have been indicated (Dingkuhn et al 1989). However, information on the linkage among water-use efficiency, stomatal response, and biomass productivity under various water regimes has been limited, but it is needed to clarify the value of  $\Delta$  for use as an



**Fig. 4. Different types of sclerenchyma in terms of thickening of cell wall in the outer cortical parenchyma and the number of sclerenchymatous cell layers.**

index for improvement of productivity in or adaptability to water-limited conditions. A field study was conducted (1) to assess the relative magnitude of environmental and genotypic variation in  $\Delta$  among rice genotypes and (2) to analyze the relationship among  $\Delta$ , gas exchange, stomatal behavior, single-leaf transpiration efficiency, and biomass production, using 11 rice genotypes grown in three different water regimes—flooded soil, aerobic soil without stress, and aerobic soil with stress—at the IRRI experimental farm during the 1997 dry season (potential evaporation 939 mm).

The genotypic mean of  $\Delta$  in the whole plant varied from 19.9‰ in IR65598-112-2 to 21.7‰ in IR20. Japonica genotypes tended to have a lower  $\Delta$ . Water regime significantly affected  $\Delta$ , with a higher  $\Delta$  under a higher water supply. For total biomass, genotypic factors were more dominant for the variation in  $\Delta$  across water regimes with a smaller genotype  $\times$  water interaction.

Genotypic and environmental variations in  $\Delta$  were explained by the changes in  $C_i/C_a$  and were positively related to single-leaf transpiration efficiency. Lower  $C_i/C_a$  or  $\Delta$  may result from either lower stomatal conductance ( $g_s$ ) or greater net photosynthetic rate ( $A$ ).  $\Delta$  had a positive correlation with  $g_s$  across genotypes in general, which indicated that genotypic variation in  $C_i/C_a$  was mainly related to  $g_s$ . The tendency of a lower  $g_s$  in japonica genotypes was consistent with other reports (Dighkuhn et al 1989). In aerobic soil conditions,  $A$  seemed to be also related to the genotypic variation in  $C_i/C_a$  and  $\Delta$  at late growth stages.

N application (90 kg ha<sup>-1</sup>) slightly but significantly lowered  $\Delta$  by 0.2‰ to 0.3‰ compared with no N with no significant N  $\times$  water or N  $\times$  genotype interaction. The lower  $C_i/C_a$  at higher N was mainly due to the higher  $A$  in flooded soil, which was probably caused by higher Rubisco activity. In aerobic soils, in contrast, the lower  $C_i/C_a$  was related to lower  $g_s$ , possibly resulting from magnified water stress caused by larger biomass production under aerobic soil conditions.

A consistent genotypic variation in  $\Delta$  across environments and its linkage with transpiration efficiency suggested that  $\Delta$  will be useful for screening genotypes that differ in water-use efficiency. On the other hand, the relationship between  $\Delta$  and biomass productivity is more complex. There was a positive relationship between biomass and  $\Delta$  in flooded conditions, whereas no positive relationship between biomass and  $\Delta$  was found in aerobic conditions. A rather negative relationship was found in aerobic conditions when one genotype was excluded. The association between lower  $\Delta$  and lower biomass indicated the drawback of higher water-use efficiency for productivity under favorable water supply, possibly because of limited stomatal conductance. On the other hand, the absence of a positive relationship between  $\Delta$  and biomass in aerobic soils signified the possibility for the simultaneous improvement of water-use efficiency and productivity. Genotypes that exhibited low  $g_s$  under flooded conditions tended to have low  $g_s$  and/or high  $A$  under aerobic soils, resulting in the consistent

genotypic ranking of  $\Delta$  across water regimes.  $A$  was not related to  $g_s$  at the flowering stage under aerobic soils, indicating that nonstomatal factors affected genotypic variation in  $A$  in later growth stages under water-limited conditions. The factors affecting genotypic variations in  $A$  and  $C_i/C_a$ , especially in later stages, should be further clarified under water-limited conditions.

## Future research

- Modeling on crop-growth-water-N will be necessary for the quantitative evaluation of the beneficial effects of improved N management for stabilizing and increasing rice productivity.
- Genetic variation in adaptation to severe water stress should be clarified, especially regarding water flow in the soil-root-shoot system, to identify valuable plant traits for enhancing the capture of water.
- Considering the limits of genetic diversity of rice in the putative traits for drought tolerance, valuable root and shoot traits, such as osmotic adjustment and stomatal response, should be combined and accumulated in genetic resources.

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## Notes

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# Nitrogen-use efficiency as affected by cultural and varietal differences under flooded soil conditions

K. Kakuda and T. Nozoe

The relationship between characteristics of rice roots and nitrogen-use efficiency of basal and topdressed N was examined under the flooded soil system. Treatments in this study consisted of the control (IR72 with 25 hills  $m^{-2}$ , CTR), high hill density (IR72 with 50 hills  $m^{-2}$ , HHD), and new plant type (IR68544-29-2-1-3-1-2 with 25 hills  $m^{-2}$ , NPT). Fertilizer-N recovery efficiency was investigated for basal N and topdressed N applied at midtillering (MT) and panicle initiation (PI) by using  $^{15}N$ -labeled urea. The cultivar used in the NPT had a longer growth duration and vegetative phase than IR72. Shoot dry weight and total N in plants at PI were the highest in the NPT among the treatments. However, in the NPT, the recovery rate of basal N was the lowest among the treatments though no difference in recovery rate of topdressed N was observed. In the HHD, the recovery rate of basal and topdressed N was comparable with the CTR. Root dry weight density in the plow layer was higher in the HHD and NPT than in the CTR. The average uptake rate (AUR) of basal N per unit root dry weight was comparable between the HHD and CTR, and the AUR of basal N in the NPT was one-third of that in the CTR. Results obtained in this experiment suggest that N recovery efficiency of basal N can be improved by increasing N absorption ability in roots, though the environmental factors associated with N-loss mechanisms in the flooded soil system are more important for the recovery efficiency of topdressed N than morphological and physiological characteristics of rice roots.

The rice plant must absorb a high amount of nitrogen (N) to obtain high yield. Assuming a target yield to be 10 t  $ha^{-1}$ , biomass production of rice must be 20 t  $ha^{-1}$  unless the harvest index declines. Therefore, a higher amount of absorbed N (ranging from 200 to 300 kg  $ha^{-1}$ ) might be required to obtain the target yield. Since mineralized N from the soil is not sufficient to meet the demand under normal cropping conditions, application of fertilizer nitrogen is required. In the flooded soil system, however, the recovery rate of fertilizer N is 20–40% for basal N (Savant and De Datta 1980) and 40–70% for topdressed N (Craswell and Velk 1979, Savant and De Datta 1982). Losses of fertilizer N from the flooded soil system are mainly caused by denitrification and  $NH_3$  volatilization (Keeney and Sahrawat 1986). One of the by-products in the denitrification reaction is nitrous oxide, which is considered to affect global warming (Mosier 1994). It is important to improve the recovery rate of fertilizer N to both increase economic efficiency and control global warming. In previous reports, it was observed that some methods of fertilizer application were effective for increasing the recovery rate of fertilizer N under flooded conditions, such as split applications (Norman

et al 1992, Peng and Cassman 1998), deep placement (Savant and De Datta 1980, Eriksen et al 1985), chemical application for suppressing nitrification (Minami 1994, Saad et al 1996), and the use of urea granules (Wilson et al 1994) or slow-release fertilizer (Kamekawa et al 1990, Wada et al 1991). However, these methods require additional labor cost and other expenses so that they may restrict the extension of these methods to farmers.

The new plant type (NPT) has been bred as a high-yielding variety at IRRI since the end of the 20th century. The NPT lines have short stature; a sturdy stem; dark green, thick, and erect leaves; reduced tillering; large panicles; and a vigorous root system (IRRI 1995). The bigger and deeper root system in upland crops has an advantage for water and N uptake (Garwood and Williams 1967, Fukai and Cooper 1995). Therefore, it is expected that cultivars with a vigorous root system have the ability to improve the recovery rate of fertilizer N under flooded conditions without any additional expenses. However, information is limited on applied N absorption associated with root characteristics under the flooded soil system. The objective of this study is to clarify the relationship between morphological and physi-



ological traits of rice roots and the nitrogen-use efficiency of basal and topdressed N under the flooded soil system using an NPT line.

## Materials and methods

A field experiment was conducted at the International Rice Research Institute (IRRI) farm, Los Baños, Laguna, in the dry season (DS, January-May) in 2002. The soil at IRRI was an Andaqueptic Haplaquoll with pH 6.1.

A semidwarf indica cultivar, IR72, and NPT breeding line that was established as a high-yielding variety (HYV) at IRRI, IR68544-29-2-1-3-1-2, were used in this experiment. Treatments in this study consisted of two rice cultivars and two hill spacings, the control (IR72 with 25 hills  $m^{-2}$ , CTR), the treatment of high hill density (IR72 with 50 hills  $m^{-2}$ , HHD), and the treatment of new plant type (IR68544-29-2-1-3-1-2 with 25 hills  $m^{-2}$ , NPT), as shown in Table 1. Fourteen-day-old seedlings were transplanted with two seedlings per hill on 29 January 2002. Hill spacing was 20 by 20 cm and 10 by 20 cm, equivalent to 25 and 50 hills  $m^{-2}$ , respectively. Nitrogen (40 kg  $ha^{-1}$  as urea), phosphorus (60 kg  $ha^{-1}$  as single superphosphate), and potassium (60 kg  $ha^{-1}$  as KCl) were incorporated to a depth of 15 cm 3 days before transplanting. The experimental field was flooded just after transplanting and the conditions were maintained throughout the experimental period. Pest and disease control were used as necessary. The experiment was carried out

in a randomized complete block design with four replications.

Fertilizer-N recovery efficiency was investigated for basal N and topdressed N by using  $^{15}N$ -labeled urea. Three microplots (0.8  $\times$  0.4 m) were established in each main plot (9  $\times$  8 m) prior to the growing season for the evaluation of fertilizer-N recovery efficiency for basal N. To reduce lateral flow and dilution of the applied fertilizer N, wooden barriers with a depth of 30 cm were set up for each microplot. Enriched urea fertilizer (3.06%  $^{15}N$ -atom excess) was uniformly incorporated into the microplots with a depth of 15 cm at 40 kg N  $ha^{-1}$  prior to transplanting. All plants inside the microplot (8 or 16 hills) were collected from each plot at midtillering (MT), panicle initiation (PI), and flowering (FL) for the evaluation of the fertilizer-N recovery efficiency for basal N. Topdressed N was applied at the MT and PI stages. Two quadrat boxes with no bottom (0.2  $\times$  0.2 m) were placed on the hill (or hills) having an average number of stems 1 week before MT and PI in each main plot. The enriched urea fertilizer mentioned above dissolved with water was topdressed in the quadrat box at 40 kg N  $ha^{-1}$  at MT and PI. Plants inside the quadrat box were collected two times after the topdressing to evaluate the fertilizer-N recovery efficiency of topdressed N. The dates of topdressing and plant collection were different among the treatments (Table 2) since the two cultivars used in this experiment differed in growth duration.

**Table 1. Rice cultivars and plant hill density in three treatments.**

Treatment	Cultivar (breeding line)	Plant hill density (hills $m^{-2}$ )
Control	IR72	25
HHD <sup>a</sup>	IR72	50
NPT <sup>b</sup>	IR68544-29-2-1-3-1-2	25

<sup>a</sup>HHD = high hill density. <sup>b</sup>NPT = new plant type.

**Table 2. Timetable of N application and plant collection for the evaluation of nitrogen-use efficiency.**

Cultivar and fertilizer	$^{15}N$ application	Plant collection		
		1st	2nd	3rd
(days after transplanting) <sup>a</sup>				
<i>IR72</i>				
Basal N	0	23 (23)	44 (44)	72 (72)
Topdressed at MT <sup>b</sup>	23	44 (21)	72 (49)	-
Topdressed at PI	44	51 (7)	72 (18)	-
<i>IR68544-29-2-1-3-1-2</i>				
Basal N	0	23 (23)	56 (56)	86 (86)
Topdressed at MT	23	56 (33)	86 (63)	-
Topdressed at PI	56	63 (7)	86 (20)	-

<sup>a</sup>Numbers in parentheses represent the duration from  $^{15}N$  application to plant collection. <sup>b</sup>MT = midtillering, PI = panicle initiation.

Four hills having an average number of tillers were collected from each main plot for the evaluation of total N in plants. Two soil blocks including rice roots with a depth of 15 cm were collected by using a quadrat core with no bottom ( $20 \times 20 \times 15$  cm) 23, 44, 58, 72, and 86 days after transplanting (DAT). The soil blocks were divided into two soil layers (depth of 0–7.5 and 7.5–15 cm) and roots inside the soil blocks were washed with tap water. Dry weight of shoots and roots was measured after oven-drying at 70 °C. Shoots were digested in sulfuric acid and total N was determined by the indo-phenol method (Scheiner 1976). Atom % of  $^{15}\text{N}$  in plants was determined by mass spectrometry.

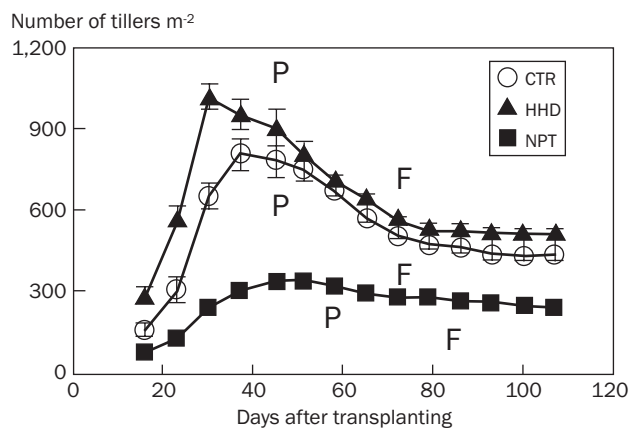
## Results and discussion

### Growth duration and timing of topdressed N

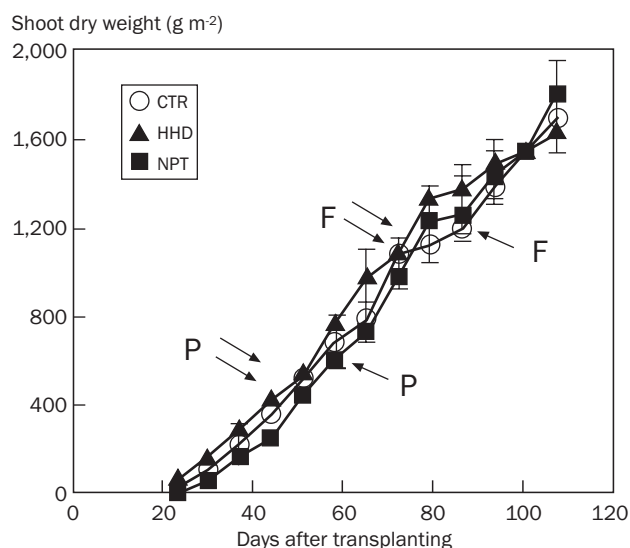
No difference in the date of FL was observed between the control (CTR) and the treatment of high hill density (HHD) since IR72 was used in both treatments. However, IR68544-29-2-1-3-1-2 used in the NPT treatment had a 2-week delay in FL vis-à-vis IR72 (Figs. 1, 2, 3, and 5). This means that the cultivar used in the NPT had a longer growth duration and vegetative phase than IR72. The timing of N topdressing for the reproductive phase is commonly decided by physiological plant growth stages. In this experiment, N was topdressed at MT and PI. For topdressing at MT, N was applied at the same time (23 DAT) in all the treatments. However, N at PI was topdressed at 44 DAT in the CTR and HHD, and at 58 DAT in the NPT owing to the different growth duration in the two cultivars used in this experiment.

### Physiological conditions of plants during the period of applied N absorption

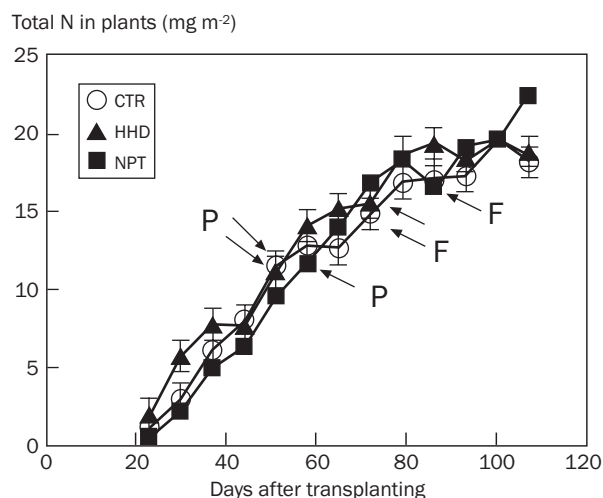
Different hill densities and cultivars affected the number of tillers per unit area (Fig. 1). The number of tillers was the highest in the HHD and lowest in the NPT throughout the



**Fig. 1.** Tiller number as affected by different varieties and hill densities. CTR = control, IR72 with 25 hills  $\text{m}^{-2}$ ; HHD = high hill density, IR72 with 50 hills  $\text{m}^{-2}$ ; NPT = new plant type, IR68544-29-2-1-3-1-2 with 25 hills  $\text{m}^{-2}$ . Letters P and F indicate panicle initiation and flowering. Bars represent standard error of the mean and are smaller than the data points in some cases.



**Fig. 2.** Shoot dry weight as affected by different varieties and hill densities. CTR = control, IR72 with 25 hills  $\text{m}^{-2}$ ; HHD = high hill density, IR72 with 50 hills  $\text{m}^{-2}$ ; NPT = new plant type, IR68544-29-2-1-3-1-2 with 25 hills  $\text{m}^{-2}$ . Letters P and F indicate panicle initiation and flowering. Bars represent standard error of the mean and are smaller than the data points in some cases.



**Fig. 3.** Amounts of nitrogen in plants as affected by different varieties and hill densities. CTR = control, IR72 with 25 hills  $\text{m}^{-2}$ ; HHD = high hill density, IR72 with 50 hills  $\text{m}^{-2}$ ; NPT = new plant type, IR68544-29-2-1-3-1-2 with 25 hills  $\text{m}^{-2}$ . Letters P and F indicate panicle initiation and flowering. Bars represent standard error of the mean and are smaller than the data points in some cases.

experimental period. At FL, the number of tillers was about 25% higher in the HHD and about 50% lower in the NPT than in the CTR. This result shows that the cultivar used in the NPT had low tillering ability, which is a typical characteristic in NPT lines.

There was no big difference in shoot dry weight among the treatments throughout the experimental period (Fig. 2). The shoot dry weight in the HHD was slightly higher than that in the CTR. No significant difference in shoot dry weight between the CTR and NPT was observed after 44 DAT. However, shoot dry weight at PI was the highest in the NPT



among the treatments (Fig. 2 and Table 3). The period from MT to PI was 21 days in the CTR and HHD, and 35 days in the NPT. Crop growth rate (CGR) during this period was 8.0, 10.2, and 9.5 g m<sup>-2</sup> d<sup>-1</sup> in the CTR, HHD, and NPT, respectively. The high level of dry matter accumulation at PI was attributed to the high CGR in the HHD. The high hill density and use of rice plants having longer growth duration can be effective for obtaining a larger biomass aboveground at PI. At MT, however, the NPT had the lowest shoot dry weight among the treatments (Table 3). The duration from transplanting to MT was 23 days in all the treatments. The low shoot dry weight of the NPT at MT could be attributed to the trait of low tillering ability of the cultivar used in the NPT.

No big difference in total N in plants was observed among the treatments throughout this experimental period (Fig. 3). There was no significant difference in the total N in plants between the HHD and CTR after 44 DAT. The NPT had a slightly larger amount of total N in plants than the CTR after 65 DAT. At the same growth stage, however, significant differences in total N in plants were observed among the treatments (Fig. 3 and Table 3). At MT, the total N in plants was the highest in the HHD and lowest in the NPT. The increasing order of total N in plants was reflected by the increase in shoot dry weight at MT. However, at PI, the amount of N in plants was the highest in the NPT and comparable between the CTR and HHD. The total N in plants was not reflected by the shoot dry weight at PI and MT. The rate of N absorption by plants during MT to PI was 0.34, 0.27, and 0.34 g m<sup>-2</sup> d<sup>-1</sup> in the CTR, HHD, and NPT, respectively. Since the rate of N absorption in the HHD was lower than that in the CTR during MT to PI, the HHD did not surpass the CTR in the total N at PI. Although the rate of N absorption in the NPT was comparable with that in the CTR, the longer vegetative phase of the cultivar used in the NPT reflected the larger amount of N in plants at PI.

### Fertilizer-N recovery efficiency of basal and topdressed N

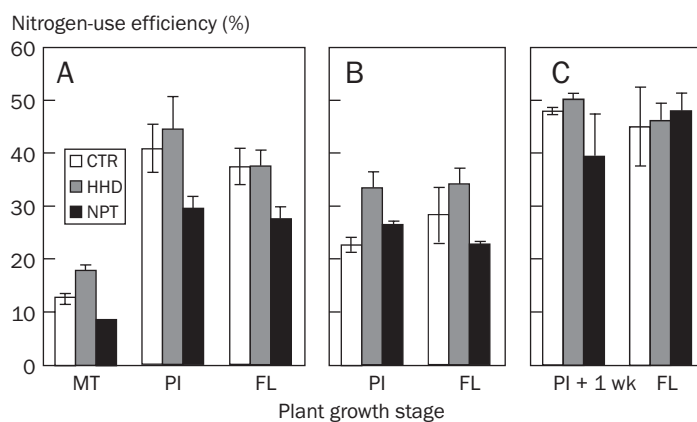
Fertilizer-N recovery efficiency of basal N was evaluated at MT, PI, and FL in this experiment (Fig. 4A). In all the treatments, the recovery rate of basal N did not increase from PI to FL. This result indicates that the plants finished absorbing basal N before PI. At MT, the recovery rate of basal N was the highest in the HHD and lowest in the NPT among the treatments. No difference in the recovery rate of basal N between the CTR and HHD was observed at PI and FL. In the NPT, the recovery rate of basal N at PI and FL was the lowest among the treatments though the NPT indicated the largest shoot dry weight and total N in plants at PI and FL. These results suggest that the increase in biomass accumulation above the current level and adoption of cultivars having a longer growth duration have no effect on fertilizer-N recovery efficiency of basal N.

Fertilizer-N recovery efficiency of N topdressed at MT was evaluated at PI and FL (Fig. 4B). In all the treatments, the recovery rate of N applied at MT did not increase after PI and ranged from 23% to 35% at FL. The HHD and NPT had no difference in the recovery rate of N applied at MT with the control at FL. Fertilizer-N recovery efficiency of topdressed N applied at PI was evaluated 1 week after the topdressing (PI + 1 wk) and at FL (Fig. 4C). In all the treatments, the recovery rate of N applied at PI did not increase after PI + 1 wk and ranged from 45% to 48% among the treatments at FL. The HHD and NPT had no difference in the recovery rate of N applied at PI with the control at both stages. These results indicate that the N uptake rate of plants became higher with plant growth, and rapid N absorption gave a higher recovery of applied N. Low N absorption ability of plants in the early growth stage causes low fertilizer-N recovery and increases the potential for fertilizer-N losses (Peng and Cassman 1998). The recovery rate of basal N was higher than that of N topdressed at MT. In this experiment,

**Table 3. Physiological conditions of rice plants at N topdressing.**

Item	Treatments		
	Control <sup>a</sup>	HHD <sup>b</sup>	NPT <sup>c</sup>
<i>Topdressing at MT<sup>d</sup></i>			
Number of tillers (m <sup>-2</sup> )	301 ± 21 <sup>f</sup>	571 ± 37	126 ± 8
Shoot dry weight (g m <sup>-2</sup> )	26.9 ± 0.7	59.9 ± 2.7	21.0 ± 0.3
Amount of N in plant (g m <sup>-2</sup> )	0.90 ± 0.03	2.03 ± 0.12	0.68 ± 0.03
<i>Topdressing at PI<sup>e</sup></i>			
Number of tillers (m <sup>-2</sup> )	784 ± 32	915 ± 59	316 ± 20
Shoot dry weight (g m <sup>-2</sup> )	367 ± 12.7	423 ± 5.3	613 ± 35
Amount of N in plant (g m <sup>-2</sup> )	8.01 ± 0.38	7.61 ± 0.09	11.76 ± 1.56

<sup>a</sup>IR72 with 25 hills m<sup>-2</sup>. <sup>b</sup>IR72 with 50 hills m<sup>-2</sup>. <sup>c</sup>IR68544-29-2-1-3-1-2 with 25 hills m<sup>-2</sup>. <sup>d</sup>MT = midtillering. <sup>e</sup>PI = panicle initiation. <sup>f</sup>Standard error of the mean.



**Fig. 4. Nitrogen-use efficiency of basal and topdressed N at different plant growth stages. A = basal N; B = topdressed N applied at midtillering; C = topdressed N applied at panicle initiation. CTR = control, IR72 with 25 hills m<sup>-2</sup>; HHD = high hill density, IR72 with 50 hills m<sup>-2</sup>; NPT = new plant type, IR68544-29-2-1-3-1-2 with 25 hills m<sup>-2</sup>. PI + 1 wk = 1 week after N topdressed at PI. Bars represent standard error of the mean.**

the pH of irrigated water was above 8 at MT and PI. A high pH in flooded water (above 8) favors N loss by volatilization (Mikkelsen et al 1978). Therefore, the low N absorption ability of plants and high pH in flooded water might have made the recovery of N applied at MT low in this experiment.

No difference in the recovery rate of topdressed N between the HHD and the CTR was observed at MT and PI, though the HHD had a larger biomass than the CTR at MT and PI. In addition, the NPT had no difference in the recovery rate of N topdressed at MT and PI with the control, though the NPT had a larger shoot dry weight than the CTR at PI. These results suggest that dry matter accumulation in shoots and the adoption of cultivars having a longer growth duration had no effect on increasing the fertilizer-N use efficiency of topdressed N.

#### Root dry weight density and ability of N absorption

A deep and wide root system in upland crops is beneficial for N uptake because the low water potential of surface soil restricts water and N uptake under dry conditions (Garwood and Williams 1967). In paddy soil, however, submerged conditions are maintained throughout the growing season except for the period of midseason drainage. In addition, ammonium N, which is the dominant form of inorganic N under anaerobic conditions, has a lower diffusion coefficient than nitrate N (Reddy et al 1980). Therefore, the amount of N lost by leaching is negligible under the flooded soil system (Buresh and Austin 1988). These facts suggest that flooded rice does not necessarily have a deep and wide root system to absorb applied N efficiently.

In this experiment, root dry weight density was investigated in the plow layer of 0–7.5- and 7.5–15-cm depth (Fig. 5). In the soil layer of 0–7.5 cm, root dry weight density ranged from 400 to 800 mg cm<sup>-3</sup> (Fig. 5A). The NPT had a

larger root dry weight density than the CTR in the soil layer. No difference in root dry weight density between the CTR and HHD was observed in the soil layer. In the layer of 7.5–15 cm, root dry weight density ranged from 130 to 370 mg cm<sup>-3</sup> (Fig. 5B). The HHD and NPT had a higher root dry weight density than the CTR in the soil layer.

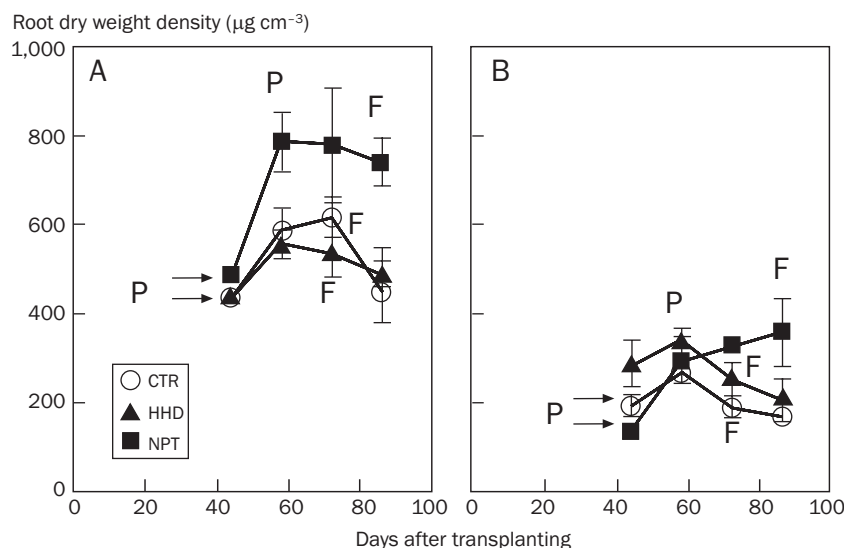
Since the basal N was incorporated into the plow layer before transplanting in this experiment, all roots in the plow layer of 0–15 cm would absorb basal N. On the premise that root dry weight increased linearly with time from transplanting to PI, the average uptake rate (AUR) of basal N per unit root dry weight during the period of basal N absorption can be estimated by the following equation:

$$\text{AUR (mg N g}^{-1} \text{ d}^{-1}) = \text{Nb}/[(\text{R1} + \text{R2}) \times \text{Dp}] \times \frac{1}{2} \quad (1)$$

where Nb is the amount of plant N derived from basal N at PI (mg m<sup>-2</sup>), R1 and R2 are the root dry weight in the soil depth of 0–7.5 cm and 7.5–15 cm at PI (g m<sup>-2</sup>), and Dp is days after transplanting at PI (d). Topdressed N is hard to diffuse from surface to subsurface soil under submerged conditions (Kakuda and Ando 1998). This fact suggests that the roots contributing to the absorption of topdressed N existed only in the surface soil. On the premise that root dry weight increased linearly with time from MT to PI, the AUR of N topdressed at MT during the period of the topdressed N absorption can be estimated by the following equation:

$$\text{AUR (mg N g}^{-1} \text{ d}^{-1}) = \text{Ntm}/[(\text{R1} + \text{R1}') \times (\text{Dp} - \text{Dm})] \times \frac{1}{2} \quad (2)$$

where Ntm is the amount of plant N derived from N topdressed at PI (mg m<sup>-2</sup>), R1' is the root dry weight in the soil depth of 0–7.5 cm at MT (g m<sup>-2</sup>), and Dm is days after transplanting at MT (d). Since the plants finished absorbing



**Fig. 5. Root dry weight density in different soil layers as affected by different varieties and hill densities. A = soil depth of 0–7.5 cm; B = soil depth of 7.5–15 cm. Letters P and F indicate panicle initiation and flowering. Bars represent standard error of the mean.**

N applied at PI for 1 week following the N application, the change in root dry weight would be negligible during the period. Therefore, the AUR of N topdressed at PI can be estimated by the following equation:

$$\text{AUR (mg N g}^{-1} \text{ d}^{-1}) = \text{Ntp}/(\text{R1} \times 7) \quad (3)$$

where Ntp is the amount of plant N derived from the topdressed N 1 week after the topdressing.

Table 4 shows the average uptake rate of fertilizer N calculated by equations 1, 2, and 3. In the basal-N absorption, the AUR in the HHD was comparable with that in the CTR, and the AUR in the NPT was one-third of that in the CTR. Since the recovery rate of basal N increased with an increase in AUR (Figs. 4 and 5A), N uptake ability per unit root dry weight would play an important role in the recovery of basal N. We suspect that the improvement of N uptake ability in roots is more effective for improving the recovery of basal N than increasing root dry weight in the plow layer and could become possible by adopting cultivars having a higher N uptake ability. In the absorption of N topdressed at MT and PI, the AUR was the highest in the HHD and lowest in the NPT. There was no relation between the AUR and recovery rate of topdressed N. These facts indicate that the increase in N uptake ability of roots had no effect on the recovery of topdressed N.

The results obtained in this experiment suggest that the environmental factors associated with N-loss mechanisms in the flooded soil system are more important for the recovery efficiency of topdressed N than the morphological and physiological characteristics of rice roots. However, in the case of basal N, N recovery efficiency can be improved by increasing the N absorption ability in roots. Genetic se-

lection for high yield potential often takes place with a high fertilizer-N input. However, high-N input conditions can mask efficiency differences among genotypes (Kamprath et al 1982). Information on physiological and morphological characteristics of rice roots involving N absorption under flooded soil conditions must be accumulated to make it possible to breed cultivars that can achieve high yield under low inputs.

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## Notes

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# Inhibition of rice growth in the field by water drainage in fallow

T. Nozoe

In a long-term rice cultivation experiment at the International Rice Research Institute (IRRI), water drainage in the field during the fallow season suppressed the growth of paddy rice, especially during the early stage of subsequent cultivation. This symptom became more prominent when no rice straw was applied, and when the amount of fertilizer applied was small. This growth suppression, which was observed in both the wet and dry seasons, was reduced by the application of a mixture of nitrogen, phosphorus, and potassium fertilizers. The amount of phosphorus in the growth-suppressed rice was smaller than that in rice without growth suppression. The findings suggest that one of the factors that suppresses rice growth is phosphorus deficiency. Growth suppression was associated with high soil pH and/or low Fe(II) content in the problem soil. The low Fe(II) content of the soil was attributed to the high soil pH because there was negative correlation between soil pH and Fe(II) content. Water drainage during fallow decreased the amount of Fe(II) in the soil because Fe(II) was oxidized to Fe(III) under oxidative conditions. During the subsequent cultivation season, this low Fe(II) content in the soil kept the soil pH high. The application of rice straw improved rice growth because of the decrease in soil pH. The production of CO<sub>2</sub>, which was generated as the terminal product of straw decomposition, presumably decreased the pH. The phosphorus deficiency of growth-inhibited rice was associated with a small amount of available phosphorus because of the high soil pH and/or low Fe(II) content in the soil.

The intensive rice ecosystem, producing two to three crops of rice per year, is an important agricultural production system for improving rice yield in the tropics. Especially in tropical Asia, irrigated lowland rice is the most important agricultural ecosystem because yields are higher on irrigated land than on rainfed and upland land. Soil submergence leads to unique biogeochemical processes, which govern the management of soil, water, and agricultural inputs and further influence ecosystem sustainability and environmental processes, such as carbon storage, nutrient cycling, and water quality. One of the characteristics of irrigated rice cultivation is the difference in oxidation-reduction status between fallow and cultivation periods. When the fallow period is rainy, there are only short stages in the field with the intensive rice-cropping system.

In the double-crop system in the tropics, there are two fallow seasons between cultivation in the wet season (WS) and dry season (DS). Each fallow season is around 2 to 3 months. It can be estimated that the water conditions during the fallow seasons of the WS (mainly October and November) and DS (mainly April and May) are different. After

the harvest, the field is generally plowed or left without introduction of water. Therefore, only weather conditions affect the oxidation-reduction status of the soil. Soil drying during the fallow season usually has beneficial effects on the following rice crop. Soil drying avoids excessive reduction of soil and that increases available soil nitrogen (N) by the air-drying effect on ammonification (Reichardt et al 2000). Applying rice straw in the soil increases the uptake of nutrient components by rice, which increases the amount of organic acids in the soil. The organic acids are toxins for rice growth (Tanaka et al 1990). Therefore, it is recommended that rice straw be decomposed in the previous fallow season under oxidative conditions that can be obtained with soil drying (Oik et al 2000). It is generally accepted that soil management to maintain oxidative status is preferable for rice cultivation. However, little research has been done on water management and rainfall in the fallow season with regard to the following rice crop. In this study, water drainage in the fallow season inhibited rice growth. In this report, first, the mechanisms of the inhibition were analyzed and, subsequently, the methods to improve this inhibition with



water, organic material, and fertilizer management were investigated.

## Materials and methods

IRRI has conducted a long-term experiment since 1972. In this field (field name: L2), the same water, organic material, and fertilizer management has been continued in each plot since the beginning of this experiment. For the current experiment, shoots and soil were sampled periodically in both the wet and dry season of 2001 and 2002. For all experiments of the 2001 wet season (WS), 2001 dry season (DS), 2002 WS, and 2002 DS, pregerminated seeds of IR72 were sown on seedling trays to produce uniform seedlings. Fourteen-day-old seedlings were transplanted on 11 Jan 2001, 26 Jun 2001, 8 Jan 2002, and 27 Jun 2002 as 2001 DS, 2001 WS, 2002 DS, and 2002 WS cultivation, respectively. Spacing was at 20 × 20 cm with four seedlings per hill. Cultivation periods in the 2001 DS, 2001 WS, 2002 DS, and 2002 WS were 96, 91, 93, and 90 days, respectively. The mid-tillering stage was at 20 days after transplanting (DAT) irrespective of season and amount of fertilizer. Panicle initiation (PI) stage in the F1 plot was at 44–46 DAT irrespective of season. This stage was 4 or 5 days earlier than that in the F0 plot. Flowering stage in the F1 plot was at 72–76 DAT irrespective of season. This stage was 4 or 5 days earlier than in the F0 plot. Groundwater was introduced to the field as irrigation water.

Eight plots (two for water management in fallow season × two for management of organic material × two for fertilizer management) were laid out in a randomized complete block design with four replications. The size of each plot was 5 × 6.5 m<sup>2</sup>. In the main plot of W0, irrigation water was not introduced to the field, whereas, in the W1 plot, the field was flooded throughout the fallow season. To analyze the effect of the application of organic materials, two plots were set up as subplots. In S0, all rice straw was removed from the field after harvesting of the previous crop. In S1, the straw was spread on the field and incorporated into the soil 1 month before transplanting. The average amount of

rice straw in the soil was approximately 150% of the grain yield. To analyze the effect of the amount of fertilizer, two plots were set up as sub-subplots. In F0, 50 kg ha<sup>-1</sup> of nitrogen (N) was applied irrespective of season. Neither phosphorus (P) nor potassium (K) was applied. In F1, 150, 30, and 40 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O were applied in the DS. Those components in the WS were 80, 20, and 30 kg ha<sup>-1</sup>, respectively. All fertilizer was applied with puddling.

Roots of collected plant samples were removed and shoot dry matter was weighed after drying. For the analysis, a part of the dried shoot was separated into leaf (leaf blade and sheath), stem, and panicle, which were ground. The amount of N, P, K, and zinc (Zn) in the plant samples obtained in the 2001 WS and 2002 DS was analyzed. The amount of N was determined with the Kjeldahl method and P, K, and Zn were analyzed with the Infra Analyzer 405 (Braun+Lube, Norderstedt, Germany) (Jimenez and Ladha 1995). In F0, soil was sampled by a core sampler (5 cm diam) and the soil 2 cm from the surface was removed. This soil sample was transferred to the laboratory immediately. Ferrous iron was extracted by acetate buffer (pH 2.8) and determined by a colorimetric method using *o*-phenanthroline (Kumada and Asami 1958). The soil pH was determined by a conventional method (Thomas 1996).

## Results

Figure 1 shows changes in dry weight in the 2001 WS and 2002 DS. In both seasons, the dry weights in F1 tended to be greater than those in F0. In each season, the differences in dry weight among the plots in F1 were smaller than those in F0. This tendency was observed in the 2001 DS and 2002 WS (data not shown). To analyze the effect of the application of rice straw and water management in the fallow season precisely, the changes in dry weight in F0 plots were replotted in Figure 2 on the basis of the data shown in Figure 1. In W0, in which the irrigation water was not introduced to the field during the fallow season, the dry weights were smaller than those in W1 plots where the fields were flooded during the fallow season. Especially in W0S0F0 plots, the

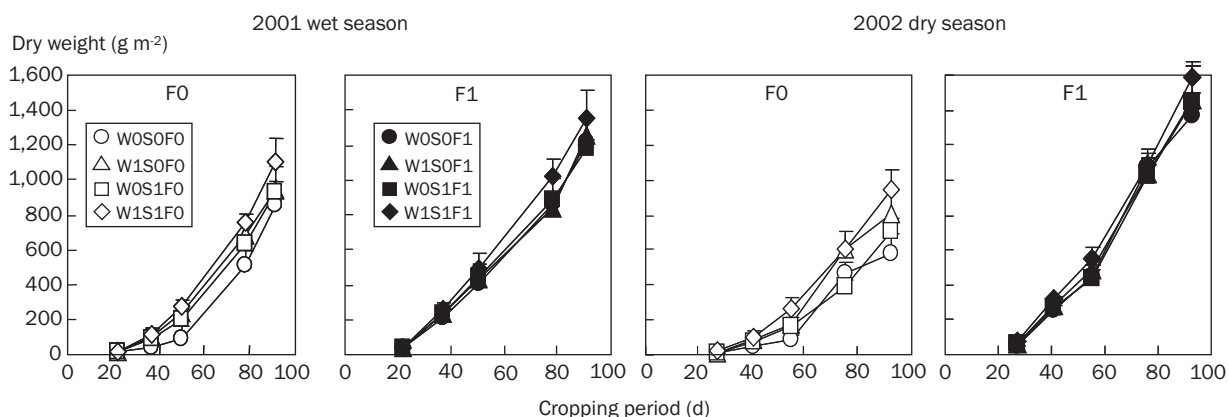
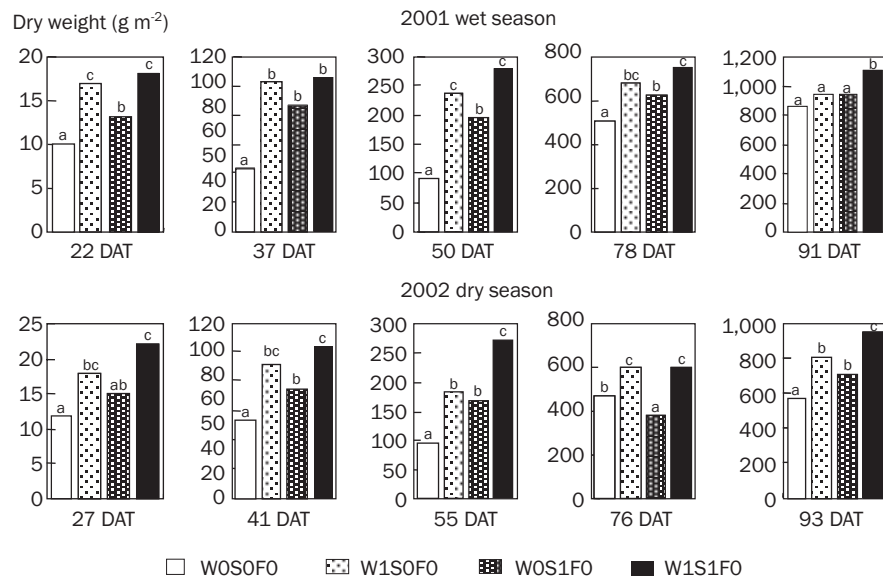
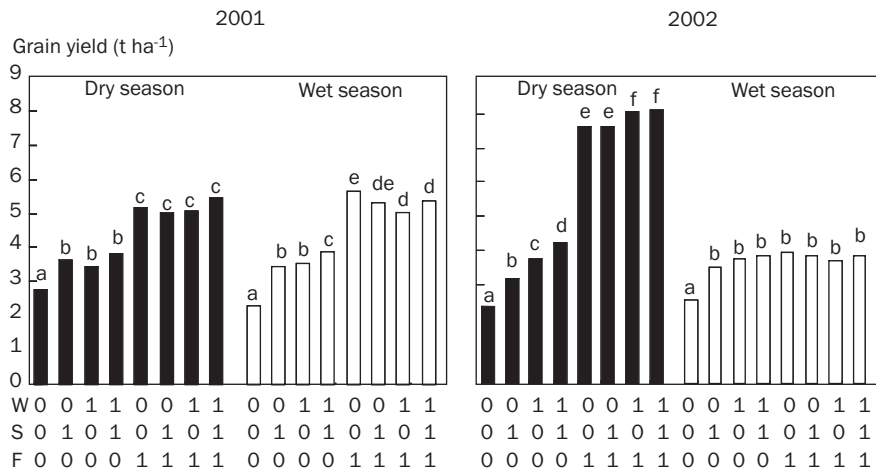


Fig. 1. Changes in dry weight. Vertical bars indicate standard deviation (as in Figs. 4 and 5).



**Fig. 2. Changes in dry weight in F0 plots. Symbols with different letters denote a significant difference at the 5% level (DAT = days after transplanting).**



**Fig. 3. Grain yield. Data were converted to grain yield at 14% water content. W, S, and F indicate plots.**

dry weights tended to be lower than those in other F0 plots. This inhibition in W0S0F0 plots continued from the beginning of cultivation to 50 to 60 DAT, and recovered after that. The final dry weights in W0S0F0 plots were lower than those in W1S1F0 plots. In many cases in F0 plots, dry weight was in the following order (Fig. 2): W1S1F0 > W1S0F0 > W0S1F0 > W0S0F0.

This phenomenon, which was also observed in the 2001 DS and 2002 WS (data not shown), suggests first that either the absence of straw or water drainage causes growth inhibition, and subsequently the presence of both of these factors makes the inhibition serious. In W0S0F0 plots, tiller number was low and leaf color was dark green, irrespective of season.

In the 2001 DS, 2001 WS, and 2002 DS, grain yields in F1 plots were greater than those in F0 plots because the amount of fertilizer in F1 plots was greater than that in F0 plots (Fig. 3). In all cropping seasons of current experiments, grain yields in W0S0F0 plots were significantly lower than in other F0 plots. In the 2001 WS and 2002 DS, the grain yields of W1S1F0 plots were significantly higher than those of other F0 plots. This finding indicates that the application of straw or flooded water in the fallow season caused growth inhibition to be overcome. In the 2001 DS and 2002 WS, this recovery was not observed.

Figure 4 shows the changes in nutrient concentration of N, P, K, and Zn in the 2001 WS and 2002 DS. Tendencies were the same in the nutrient concentration of stems (data

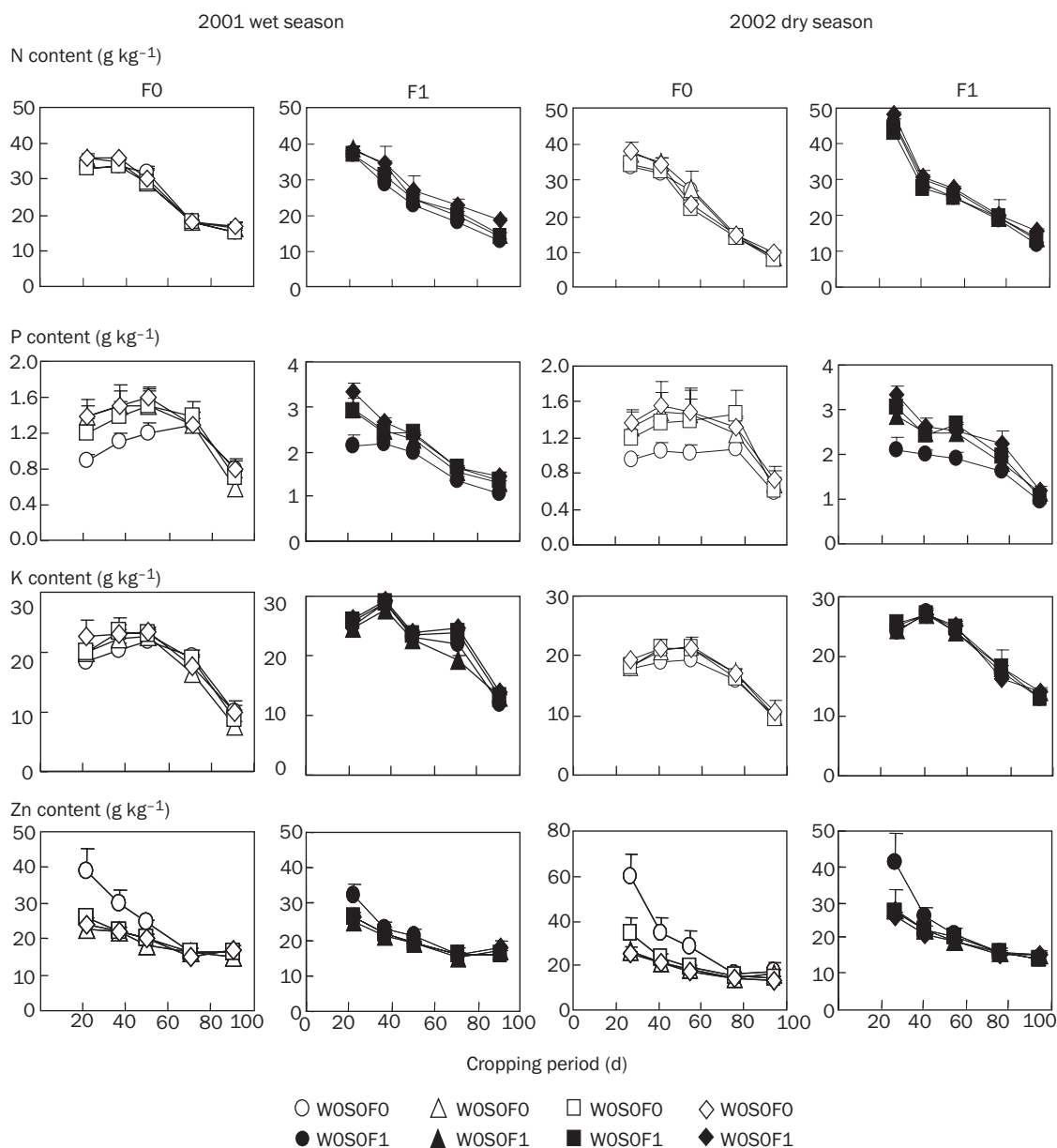


Fig. 4. Changes in N, P, K, and Zn contents in leaf.

not shown). The N concentration decreased gradually during the cultivation period, and differences among plots were barely observed. The difference in the amount of fertilizer and rice straw barely affected the N content. The P concentration in F1 plots was greater than in F0 plots because P fertilizer was applied in F1 plots. In both F0 and F1 plots, the P content in W0S0 plots was lower than in other plots. Although the K concentration was greater than in F0 plots, the effects of straw application and water management were not clear. The Zn concentration in W0S0F0 and W0S0F1 was higher than in other plots. The tendency of the changes in N, P, K, and Zn concentration in leaf was the same in the 2002 WS (data not shown).

Figure 5 shows the changes in soil pH and Fe(II) content in soil in the 2001 WS. This tendency was the same as in the 2002 WS (data not shown). The values of pH went above 7.0 except in the late stage of cultivation in the 2001 WS and a part of W1S0F0, W0S1F0, and W1S1F0. In all cropping seasons, the pH of W0S0F0 plots was greater than in other plots. Especially in the 2001 WS, the pH during the early stage of cultivation surpassed 8.0. Those findings suggest that the drainage of water during the fallow season increased the soil pH, while the application of rice straw decreased it. Especially, the soil pH during the early stage of cultivation in the WS was higher than in the DS. Except for the Fe(II) content in the 2001 DS, content in W0S0F0

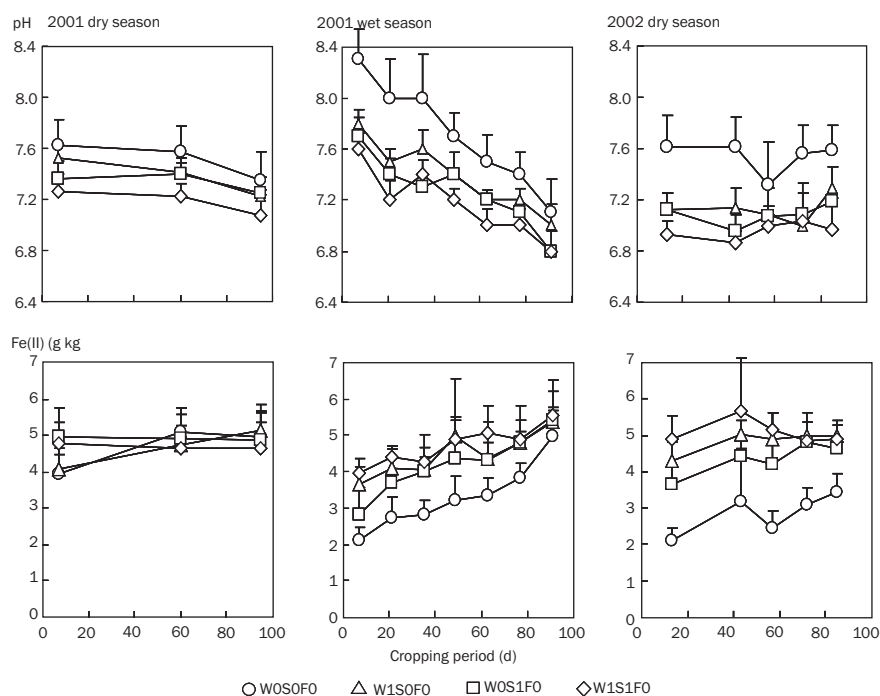


Fig. 5. Changes in amounts of soil Fe(II) and pH values in F0 plots.

was lower than in other plots. During cultivation, the soil pH tended to decrease, while Fe(II) content tended to increase.

## Discussion

### Growth inhibition of rice

Except for grain yield in the 2002 WS, yield of F1 was higher than that of F0 plots, suggesting that this difference was caused by the greater amount of fertilizer in F1 plots than in F0 plots (Fig. 3). In the 2002 WS, the application of fertilizer in F1 plots did not affect grain yield, presumably because of climatic factors. This remains to be analyzed. In all cropping seasons, grain yield in W0S0F0 plots was lower than in other F0 plots, whereas no significant differences occurred among grain yield in F1 plots. Growth inhibition in W0S0 plots was observed only in the F0 plots.

As shown in Figures 1 and 2, drainage of water during the fallow season inhibited rice growth during the cultivation period. This inhibition was overcome by the application of sufficient fertilizer (F1), the application of rice straw (S1), or the flooded soil during the fallow season (W1) (Fig. 2). In the W0S0F0 plots, although growth was recovered during the late stage of cultivation (Fig. 2), grain yield was lower than in other plots, especially in the 2001 WS and 2002 DS (Fig. 3). These findings suggest that growth inhibition in the early stage of cultivation decreased grain yield. The differences in growth inhibition between the WS and DS were not clear. As mentioned above, flooded soil (W1), the application of rice straw (S1), and/or application of fertilizer (F1) helped overcome growth inhibition. The appli-

cation of a large amount of fertilizer (F1) improved rice growth irrespective of the presence or absence of flooded water in the fallow season and the application of rice straw (Fig. 1). As shown in Figure 2, dry weight in F0 plots tended to be W1S1F0 > W1S0F0 > W0S1F0 > W0S0F0.

These findings indicate first that the effect of the flooded soil in the fallow season (W1) to overcome growth inhibition was greater than that of the application of rice straw (S1). Subsequently, the presence of both factors (W1 and S1) made the improvement more efficient because of the interaction of two factors.

### The relationship between nutrient components and growth inhibition

Rice growth was not inhibited in the F1 plots (Fig. 1), indicating that N, P, and/or K was associated with the inhibition. Dobermann and Fairhurst (2000a,b,c,d) show the critical level of deficiency using the concentration of nutrient in the leaf. According to them, the critical level of N is 25 g kg<sup>-1</sup> in midtillering and PI (Dobermann and Fairhurst 2000a). In all cropping seasons of our study, the midtillering stage was present at around 20 DAT irrespective of F0 and F1 plots. The PI stage of F1 plots at 44–46 DAT was 4–5 d earlier than that of F0 plots. The flowering stage of F1 plots at 72–76 DAT was 4–5 d earlier than that of F0 plots. Especially at the early stage of cultivation, in which growth was inhibited, N content was higher than the critical level, and no difference between the plots could be observed. These findings indicate that growth inhibition was not associated with N deficiency (Fig. 4). Phosphorus deficiency occurs in acidic or alkaline soil such as acidic latosol soil, acid sul-

fate-rich soil, and calcareous soil (Yoshida 1981). Dobermann and Fairhurst (2000b) reported that the optimal level of leaf P concentration from midtillering to PI is 2–4 g kg<sup>-1</sup>, and the critical level for P deficiency is 1 g kg<sup>-1</sup>. In our study, the leaf P concentration of WOSOF0 plots changed around 1 g kg<sup>-1</sup> in the early stage of cultivation. Therefore, growth inhibition in WOSOF0 plots was associated with P deficiency. In the early stage of cultivation of F0 plots, leaf P concentration was in the following order: W1S1F0 = W1S0F0 > WOS1F0 > WOS0F0.

This finding indicates that the P content in W0 plots was lower than in W1 plots. This tendency was similar to that in dry weight (Fig. 2). In WOS0F0 plots, the leaf was dark green and the number of stems tended to be small. Those symptoms corresponded to the characteristics of P deficiency. All of those tendencies support the presence of P deficiency (Fageria et al 1988). Sasaki and Hirata (1995) reported that rice plants require the uptake of P, especially in the early stage of growth. When the amount of P in the plant is not sufficient, the plant moves P from old to new tissue. This means that the plant barely moves into P deficiency during the late stage of growth if P uptake during early growth is sufficient. In our study, rice growth in WOS0F0 plots was inhibited in the early stage of cropping (Fig. 2). That was attributed to P deficiency during the early stage of cultivation.

Potassium deficiency occurs under a reduced condition of soil (Dobermann and Fairhurst 2000c). It is reported that the optimum concentration in the leaf during midtillering and PI is 18–26 g kg<sup>-1</sup>, and that the critical level for K deficiency is 15 g kg<sup>-1</sup>. In our study, although the K content in WOS0F0 plots was lower than in other plots, it changed around 20 g kg<sup>-1</sup>. Therefore, the possibility of the presence of K deficiency was small.

Zn deficiency occurred in alkaline soil, and that is one of the most serious problems in tropical soils (Dobermann and Fairhurst 2000d). The optimal concentration of leaf Zn is 25–50 mg kg<sup>-1</sup>, and the critical level of leaf Zn for deficiency is 20 mg kg<sup>-1</sup> (Dobermann and Fairhurst 2000d). In these conditions, the Zn concentration in the leaf was over 20 (mg kg<sup>-1</sup>), and the Zn concentration in WOS0F0 plots was higher than in other plots. Therefore, Zn was not involved in growth inhibition. Based on the results discussed above, growth inhibition, which was caused by the soil drying in the fallow season, was associated with P deficiency. No N, K, and Zn were involved in the inhibition.

### The relationship between soil pH and growth inhibition

It is reported that rice has a comparatively high tolerance of high pH (Ikehashi 1997), namely, a high soil pH barely inhibits rice growth directly, although high pH changes the nutrient availability of soil. In our study, the values of soil pH in WOS0F0 plots were higher than those in other plots in all cropping seasons (Fig. 5). Especially in the 2001 WS, the initial pH in WOS0F0 plots was over 8.3. The values of pH in WOS0F0 plots were high in the early stage of cultiva-

**Table 1. Relationship between pH and Fe(II) in soil (y = pH; x = amount of Fe(II) (g kg<sup>-1</sup> soil)).**

Season	n	r <sup>2</sup>
2001 dry season	12	0.18
2001 wet season	28	0.87 <sup>a</sup>
2002 dry season	20	0.67 <sup>b</sup>
2002 wet season	28	0.76 <sup>c</sup>

<sup>a</sup>Significant at 1% level. Regression equation: y = -0.38x + 8.98.

<sup>b</sup>Significant at 1% level. Regression equation: y = -0.20x + 8.05.

<sup>c</sup>Significant at 1% level. Regression equation: y = -0.18x + 8.31.

tion and decreased gradually (WS), or remained high during cultivation (DS). Rice growth in WOS0F0 plots was strongly inhibited during the early stage of cultivation (Fig. 2). Those findings suggest that the high values of soil pH in WOS0F0 plots were related to growth inhibition. In the 2001 DS, there were no differences in Fe(II) content in each plot (Fig. 5), whereas, in the 2002 DS, significant differences in each plot were observed. In the previous fallow season of the 2001 DS, the effect of soil drying was small because of rain. As a result of this insufficient soil drying, Fe(II) did not decrease even in the W0 plots. The characteristics of the changes in Fe(II) content were opposite to those in the values of pH. The amounts of Fe(II) in WOS0F0 plots were smaller than those in other plots except for those in the 2001 DS. In many cases, like in the 2001 WS, pH values increased, while the amount of Fe(II) decreased throughout the cultivation period.

To analyze the relationship between Fe(II) content and pH values, the data shown in Figure 5 and those of the 2002 WS were re-plotted in another figure (data not shown). The values of pH and Fe(II) content were plotted on the y and x axis, respectively. Table 1 shows the coefficients of determination (r<sup>2</sup>) and regression equations. On the basis of this analysis, there was a significant negative correlation between the values of pH and Fe(II) content in the 2001 WS, 2002 WS, and 2002 DS. Those findings indicate that, first, the amounts of Fe(II) affected the changes in pH, and subsequently the high pH in the WOS0F0 plot was attributed to low Fe(II) content. There was no significant correlation between the values of pH and Fe(II) content in the 2001 DS. In this season, the soil pH was affected by a factor other than Fe(II) content. We still need to analyze what conditions make the relation of those factors significant.

In the 2001 WS and 2002 DS, it can be estimated that the low Fe(II) content in WOS0F0 plots affected the initial oxidation-reduction status of soil in the cropping season. The water was not supplied to the W0 field except for rain in the fallow season, while the fields in W1 plots were flooded throughout the fallow season. With that management, the initial soil in the cropping season of W0 plots was reductive, whereas that in W1 plots was oxidative, and it became reductive during the cropping season. In a previous study in relation to single, double, and triple rice cropping in the tropics, Nozoe et al (2003) reported that the pH of those



soils decreased with the increase in the annual number of croppings. This indicates that the pH value tended to be high with the increase in the periods of fallow season, and with the increase in the intensity of drying. The results obtained in our study support those results. Ponnampereuma et al (1966) reported a positive correlation between the partial pressure of soil CO<sub>2</sub> and the pH value when the soil pH was high. Carbon dioxide is generated as a terminal product of the decomposition of organic materials and the soil organic materials serve as an electron donor for the soil reduction. As the soil reduction proceeds, the soil pH decreases because the partial pressure of CO<sub>2</sub> increases. Therefore, the partial pressure of CO<sub>2</sub> in oxidative soil is low, and the pH of this soil is high. In our study, the pH in F1 plots was low. This was presumably caused by the increase in CO<sub>2</sub> concentration in the soil.

In relation to the mechanism of the suppression of P uptake under high pH conditions, the high concentration of calcium (Ca) in the soil decreases the amount of available P (Dobermann and Fairhurst 2000b). Phosphate is adsorbed to Fe(III) oxides and the Fe(II) is oxidized to Fe(III) during the fallow season (Kirk et al 1990). During the following cropping season, the phosphate becomes available with the reduction of Fe(III) oxides to Fe(II). In our study, however, the mechanism of the P deficiency is not clear. The application of rice straw first increases the amount of P in rice from the rice straw, which subsequently improves the suppression of P uptake by decreasing soil pH.

In our study, soil drying in the fallow season inhibited the following rice growth. The application of P or rice straw improved the inhibition. Actually, the amount of P that is applied in the tropics is small (Herdt and Stangel 1984, Maene 1990). Much rice straw is removed or burned (Flinn and Marciano 1984). Our study indicated that soil management in the fallow season inhibited the following growth, especially under high pH conditions. It remains to be confirmed whether rice is inhibited outside of IRRI fields.

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## Notes

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