Identification of a RFLP Marker Tightly Linked to the Panicle Blast Resistance Gene, *Pb1*, in Rice

Kiyoshi Fujii¹⁾, Yuriko Hayano-Saito²⁾, Koji Saito²⁾, Naoki Sugiura¹⁾, Nagao Hayashi³⁾, Takako Tsuji¹⁾, Toshihiko Izawa¹⁾ and Mabito Iwasaki²⁾

¹⁾ Aichi Prefectural Agriculture Research Center (AARC), 1-1 Sagamine, Yazako, Nagakute, Aichi 480-1193, Japan

²⁾ Hokkaido National Agricultural Experiment Station, 1 Hitsujigaoka, Toyohira-ku, Sapporo, Hokkaido 062-8555, Japan

³⁾ Mountainous Regional Agriculture Research Institute, AARC, 11 Susogaeto, Inahashi, Inabu, Aichi 441-2513, Japan

We precisely mapped the *Pb1* locus for the panicle blast resistance of a quantitative nature on rice chromosome 11 using RFLP markers. Based on the cosegregation of the resistant genotypes of *Pb1* and *Stvb-i*, a rice stripe virus (RSV) resistance gene derived from an Indica cultivar Modan, we examined the linkage relationships between *Pb1* and 13 *Stvb-i*-linked RFLP markers located on the long arm of chromosome 11. The *Pb1* locus was mapped in the Modan-derived chromosomal region in the middle part of the long arm of chromosome 11. *Pb1* was located on the telomere side in relation to the *Stvb-i* locus. The *Pb1* gene was closely located at 1.2 cM from three RFLP markers: S723, CDO226, C189. Then, we examined the graphical genotypes of 34 Modan-derived RSV-resistant cultivars with or without panicle blast resistance, and 12 susceptible Japonica cultivars, using 21 RFLP markers. Among them, cultivars with panicle blast resistance were classified into four types: A, B, C and D, and those without it into six types: E, F, G, H, I and J. In all of the panicle blast-resistant cultivars, the Modan-type bands were observed in S723, CDO226 and C189. On the other hand, in all the panicle blast-susceptible cultivars, the Japonica-type band was observed in S723, whereas the Modan-type bands were noticed in CDO226 and C189 in Type E cultivars. Consequently, only the genotypes of the S723 locus completely coincided with the genotypes of the *Pb1* locus. Although, it was determined, based on linkage analysis, that S723, CDO226 and C189 were located at the same locus, graphical genotyping analysis, using many progeny cultivars derived from Modan, revealed that S723 was the closest marker to *Pb1* among the three.

Key Words: *Oryza sativa*, *Pyricularia grisea*, resistance gene, genetic map, linkage analysis, graphical genotyping analysis, RFLP.

Introduction

Rice blast, caused by *Pyricularia grisea* Sacc. is one of the most widespread and destructive diseases of rice. The disease is classified into two forms: leaf blast and panicle blast. Panicle blast occurs after heading, and directly reduces the number of mature panicles, the weight of individual grains and the quality of brown rice. It consequently causes severe damage under disease-conducive conditions. Most of the leading rice cultivars with superior eating quality in Japan are susceptible to panicle blast, and many fungicides have been applied to control the disease. Therefore, growing resistant cultivars is one of the most effective measures for controlling this disease and for reducing the use of fungicides. Genetic studies on panicle blast resistance had been hampered by the inaccuracy and difficulty in evaluating the level of resistance because the disease incidence is not uniform in experimental fields and fluctuates every year. We developed a reliable method to assess the degree of panicle blast resistance (Tooyama *et al.* 1993). Consequently, blast resistance of a quantitative nature with a weak expression for leaf blast and strong one for panicle blast, was found in Japonica cultivars harbouring the rice stripe virus (RSV) resistance gene, *Stvb-i*, derived from an Indica cultivar Modan (Koumura *et al.* 1985, 1987, Fujii *et al.* 1988, 1999a). Panicle blast resistance is controlled by a newly found major gene, *Pb1*, which is linked to the *Stvb-i* gene with a recombination value of $5.2 \pm 1.5\%$ (Fujii *et al.* 1999b).

The current method for evaluating panicle blast resistance in paddy fields is laborious, and can be applied only once a year. Hence, we are using RSV resistance as a marker for indirect selection of the panicle blast resistance conferred by *Pb1* in the rice breeding program at Aichi Prefectural Agriculture Research Center (AARC) (Fujii *et al.* 1993, 1999b). This is because we have developed a reliable mass screening method of RSV resistance, enabling the screening of several thousands entries throughout the year. However, in some cases, RSV-resistant cultivars without panicle blast resistance have been developed (Ohya *et al.* 1996, Fujii *et al.* 1999a), because the distance of the two loci is not close enough. Therefore, marker-assisted selection (MAS) (McCouch and Tanksley 1991) using molecular marker(s) tightly linked to the *Pb1* gene should be an

Communicated by C. Kaneda

Received October 6, 1999. Accepted March 23, 2000.

*Corresponding author (e-mail: kfujii@agri-rc.pref.aichi.jp)

accurate method for indirect selection of panicle blast resistance.

Recently, the *Stvb-i* gene has been mapped on the long arm of chromosome 11 (Hayano-Saito *et al.* 1998). The linkage relationship between *Pb1* and *Stvb-i* suggests that the *Pb1* gene is located near the *Stvb-i* gene on chromosome 11. However, the precise location of the *Pb1* gene on chromosome 11 has not been elucidated, because it remains to be determined whether *Pb1* is located on the centromere side or the telomere side in relation to the *Stvb-i* locus. Fine mapping of the *Pb1* locus and identification of molecular marker(s) closely linked to the gene are essential for MAS and map-based cloning of the *Pb1* gene. For precise mapping of the *Pb1* locus, we carried out a linkage analysis between the *Pb1* gene and *Stvb-i*-linked restriction fragment length polymorphism (RFLP) markers in the Modan-derived chromosomal region. Then, in order to determine the flanking markers associated with the *Pb1* locus, we performed a graphical genotyping analysis (Young and Tanksley 1989) using many progeny cultivars of Modan with or without panicle blast resistance.

Materials and Methods

Plant materials

In order to identify the closest RFLP marker to the *Pb1* locus, we used 46 cultivars for graphical genotyping analysis (Table 1). Among them, 18 cultivars were resistant to both RSV and panicle blast, 16 were RSV-resistant and panicle blast-susceptible, and 12 were susceptible to both RSV and panicle blast. All the RSV-resistant cultivars were derived from the Indica donor cultivar Modan.

Field test of panicle blast resistance

We carried out field tests of panicle blast resistance using the improved method (Tooyama *et al.* 1993). Fourteen seedlings of each cultivar were transplanted with two replications in paddy fields under conditions conducive to blast fungus infection both in Inabu and Nagakute in Aichi Prefecture located in the central part of Japan. Rice blast fungal races 007 and 037 virulent to all the cultivars tested were dominant in the assay fields. The average percentage of grains of each cultivar with blast disease was calculated at 3–4 weeks and 5–6 weeks after heading in Inabu and Nagakute, respectively. Panicle blast resistance of each cultivar was evaluated by the resistance score based on the average percentage of diseased grains and heading date (Table 1, cf. Fujii *et al.* 1999b).

Linkage analysis between *Pb1* locus and RFLP markers

For linkage analysis between the *Pb1* gene and *Stvb-i*-linked RFLP markers on chromosome 11, the segregation data of 119 F₂ individuals developed from a cross between Koshihikari (susceptible to both panicle blast and RSV) and Asanohikari (resistant to both diseases) reported by Fujii *et al.* (1999b) and Hayano-Saito *et al.* (1998) were used in this

study. Genotype of *Pb1* of each F₂ individual was classified as homozygous resistant, heterozygous or homozygous susceptible, based on the disease reaction of the corresponding F₃ line (Fujii *et al.* 1999b). Genetic distance (Kosambi 1944) and the best-fit gene order were determined using computer software MAPL ver. 3.0 (Ukai *et al.* 1995).

Graphical genotyping analysis of Modan-derived cultivars

Genomic DNA for RFLP analysis was extracted from young leaves of each cultivar by the modified CTAB method (Hayano-Saito *et al.* 1998). The purified DNA was digested with five restriction enzymes: *Bam*HI, *Bgl*III, *Eco*RI, *Eco*RV or *Hind*III, separated by gel electrophoresis in 0.8% agarose gel and transferred onto positively charged nylon membranes (Hybond N+, Amersham-Pharmacia) by the capillary transfer method.

In this graphical genotyping analysis, we used 13 *Stvb-i*-linked RFLP markers (Hayano-Saito *et al.* 1998) and eight additional RFLP markers on the long arm of rice chromosome 11: seven RFLP markers (prefix XNpb and gmZ) cited by Saito *et al.* (1991), five RFLP markers (prefix CDO and RG) provided by Cornell University (McCouch and Tanksley 1991), eight RFLP markers (prefix C, G, R and S) provided by the Rice Genome Research Program (RGP) of Japan (Kurata *et al.* 1994, Harushima *et al.* 1998) and ST10 (Hayano-Saito *et al.* 1998). Among the additional eight markers, six RFLP markers: XNpb181, XNpb376, RG1109, C50, C133B and C1003A, were located at the same locus of S723/CDO226/C189 markers or on the telomere side in relation to the site of these markers (Kurata *et al.* 1994, Chen *et al.* 1997, Harushima *et al.* 1998).

Results

RFLP mapping of *Pb1* gene

Linkage relationships between the 13 *Stvb-i*-linked polymorphic RFLP markers and the *Pb1* gene were detected. *Pb1* was mapped into the Modan-derived chromosomal segment in the middle part of the long arm of chromosome 11 (Fig. 1). Also, the *Pb1* locus was located on the telomere side from the *Stvb-i* locus at 5.8 cM. Consequently, the two genes were mapped near both ends of the introgressed segment. *Pb1* was most closely located at 1.2 cM from the three RFLP markers: S723, CDO226 and C189 (Fig. 1B). These three markers were located at the same locus in this linkage analysis.

Graphical genotypes in Modan-derived cultivars with or without the panicle blast resistance

Among the additional eight RFLP markers used for the graphical genotyping analysis, RG1109 gave the Modan-type bands in four cultivars: Modan, St. No. 1, Chugoku 31 and Mineyutaka, and Japonica-type bands in the remaining cultivars. C1003A showed the same polymorphic pattern as that of CDO226 and C189. C133B gave the same polymorphic pattern as that of gmZ410, CDO365, CDO534,

Fig. 1. RFLP map around *Pb1*

A. Graphical genotype of rice chromosome 11 in Asanohikari (from Hayano-Saito *et al.* 1998). The shaded portion indicates the segment derived from Modan, the Indica donor cultivar. CEN: centromere. S and L: short and long arm, respectively.

B. Integrated RFLP map of the genomic region around the panicle blast resistance gene, *Pb1*, on rice chromosome 11. The RFLP marker ST10 indicates the *Stvb-i* locus (Hayano-Saito *et al.* 1998).

C1172 and R3341. On the other hand, the three RFLP markers: XNpb181, XNpb376 and C50, gave no polymorphism between the panicle blast-resistant cultivar St. No. 1 derived from Modan and the susceptible cultivar Norin 8.

The graphical genotypes for 46 cultivars were classified into ten types (Fig. 2). The list of the cultivars belonging to each graphical genotype is shown in Table 1. The panicle blast-resistant cultivars were classified as Types A, B, C and D. Type A, which showed the Modan-type bands in all the RFLP markers, included the Indica donor cultivar Modan and first bridge cultivars: St. No. 1, Chugoku 31, Mineyutaka. Type B, which was different from Type A only in the Japonica-type reaction in RG1109, included a second bridge cultivar Shimahashirazu. Type C, which was different from Type A in the Japonica-type reaction in XNpb202, C459B and RG1109, included many commercial cultivars derived from St. No. 1. Type D, which was different from Type A in the Japonica-type reaction in XNpb202, C459B, XNpb254, XNpb257 and RG1109, included Aichi 21 (ST) which was a progeny of St. No. 1.

RSV-resistant cultivars without panicle blast resistance were classified as Types E, F, G, H and I. Type E was different from Type C only in S723 in the Japonica-type reaction. Types F and G showed the Japonica-type bands in the telomere side markers and included three progenies of Chugoku 31: Chugoku 56, Tamahonami and Chugoku 51.

Fig. 2. Graphical genotypes in panicle blast-resistant/susceptible cultivars.

Schematic representation of ten graphical genotypes of the long arm of rice chromosome 11 among 46 cultivars with or without panicle blast resistance. The designation of the graphical genotypes is listed on the left side. The disease reaction to panicle blast is shown on the right side: r, resistant in a quantitative sense; s, susceptible in a quantitative sense (see Table 1). Marker designations are placed on top, and the genetic distance is given in cM at the bottom: *, from Fig. 1B and Hayano-Saito *et al.* (1998); **, from Harushima *et al.* (1998); ***, from Chen *et al.* (1997). The approximate position of *Pb1* is shown schematically. CEN: centromere. The black areas represent the introgressed segments from the Indica donor cultivar Modan. The white areas represent the Japonica regions. The list of the cultivars belonging to each graphical genotype is shown in Table 1.

Besides, all of the cultivars susceptible to both RSV and panicle blast were classified as Type J.

All the panicle blast-resistant cultivars showed the Modan-type bands in the RFLP markers S723, CDO226, C189 and C1003A which were linked to *Pb1* at 1.2 cM (Fig. 2). RG1109 which was the other flanking marker of *Pb1*, was not considered to be linked tightly to *Pb1*, because panicle blast-resistant cultivars of Types B, C and D showed the Japonica-type bands in RG1109 (Fig. 2). It was noteworthy that the cultivars in Type E without panicle blast resistance showed the Japonica-type bands in S723, and the Modan-type bands in CDO226, C189 and C1003A (Fig. 2). Consequently, only the genotypes of S723 completely coincided with the genotypes of *Pb1*. Therefore, it was considered that S723 was the closest to the *Pb1* locus of all the markers.

Table 1. Rice cultivars classified into ten graphical genotypes by the 18 RFLP markers on the long arm of chromosome 11 and their reaction to RSV and panicle blast

Type ¹⁾	Cultivar	Reaction to RSV	Reaction to panicle blast ²⁾
A	Modan, St. No. 1, Chugoku 31, Mineyutaka	Resistant	Resistant
B	Shimahashirazu		
C	Aoisora, Tsukinohikari, Asanohikari, Aichi 67, Akanezora, Aoinokaze, Maturibare, Asahinoyume, Aichi 100-1406, Aichi 100-1414, Chubu 89, Koigokoro		
D	Aichi 21 (ST)		
E	Akei 429, Iku 726, Haresugata (1), Haresugata (2)	Resistant	Susceptible
F	Chugoku 56, Tamahonami		
G	Chugoku 51		
H	Akei 584, Aikei-bio 6, Aikei-bio 8-186, Aikei-bio 8-188, Aikei-bio 8-189, Aikei-bio 8-194		
I	Aichi 21 (A), Aichi 21 (B), Aichi 21 (C)		
J	Norin 8, Wasekirakogane, Koganebare, Hokuriku 103, Koshihikari, Mineasahi, Aichinokaori, Aichi 65, Hitomebore, Sachikaze, Nipponbare, Kanto 51	Susceptible	Susceptible

¹⁾ Symbols of types are the same as in Fig. 2.

²⁾ Resistant: cultivars with resistance score from 2 to 4. Susceptible: cultivars with resistance score from 6 to 8 (Fujii *et al.* 1999b).

Discussion

We identified close linkages between *Pb1* and each of the three RFLP markers: S723, CDO226 and C189, located in the middle part of the long arm of chromosome 11, in the F₂ population consisting of 119 individuals. No recombination was detected among the three markers in the linkage analysis. While, on the other hand, since the graphical genotyping analysis revealed that only the genotype of the S723 locus completely coincided with the differences in disease reaction to panicle blast in all the cultivars, S723 was found to be the closest to *Pb1* of all the tested markers. The difference between the two results may be due to the limitation in the F₂ population size used for the linkage analysis. Thus, graphical genotyping analysis using many progeny cultivars obtained from a single resistance donor was considered to be a useful method to identify the closest marker of the target gene.

We confirmed that the S723 probe was useful for MAS of the panicle blast resistance conferred by *Pb1* in a rice breeding program, and developed a couple of STS markers and a CAPS marker for practical use based on the genomic sequence of the cDNA clone S723 (Tooyama *et al.* 1998). These molecular markers are currently used to develop rice cultivars with panicle blast resistance and a near-isogenic line for this gene in AARC. MAS can be very efficient for identifying the *Pb1* gene, since the expression of the *Pb1* gene is influenced by the environment and is phenotypically difficult to evaluate. Furthermore, in a backcross breeding program, we can select desired B_nF₁ individuals for use for further backcrossing before flowering by MAS. We could not choose them before flowering by the conventional field assay method, because the panicle blast resistance was evaluated at the ripening stage of each plant. We can also identify resistant heterozygous B_nF₁ plants accurately using MAS even in the off-season of field assays for panicle blast

resistance. These advantages enable to perform backcross cycles efficiently. In addition, since conventional evaluation of panicle blast resistance is still laborious, MAS may enable to solve this problem as well.

The linkage and graphical genotyping analyses in this study revealed that *Pb1* had been incorporated into Japanese cultivars with *Stvb-i* from the Indica cultivar Modan. St. No. 1 and Chugoku 31, which were developed from the same cross (Modan/6*Norin 8) and classified as Type A (Table 1, Fig. 2), are the first Japanese cultivars to be resistant to both panicle blast and RSV. Most of the RSV-resistant progeny cultivars from St. No. 1 are also resistant to panicle blast (Fujii *et al.* 1999a), while, all of the three RSV-resistant progenies from Chugoku 31 are susceptible to panicle blast. This genealogical difference can be attributed to the difference in the genotype around the S723 locus associated with *Pb1*.

This gene does not confer any true resistance with hypersensitive reactions, and the protecting ability of the gene against panicle blast is sufficient for commercial rice production in Japan except in environments highly conducive to the disease (Fujii *et al.* 1999a, 1999b). True resistance to blast controlled by a major gene often breaks down within a few years after the dissemination of cultivars with the true resistance gene (Ujihara *et al.* 1955, Kiyosawa 1972, Ou 1979), due to the occurrence of blast fungal race(s) adapted to the gene (Kiyosawa 1982, Bonman *et al.* 1986). Although panicle blast resistance is also conferred by a major gene, the breakdown of this resistance has not been reported for more than 15 years since the beginning of commercial cultivation of rice cultivars with *Pb1* in Japan (Fujii *et al.* 1999a). Therefore, since *Pb1* is considered to be a gene for durable resistance, it is useful in rice breeding and is also an important gene in plant protection. The isolation of the gene would be essential for studies on the gene function and gene-pathogen relationship. In the present study,

we detected a closely linked RFLP marker located on the centromere side in relation to the *Pb1* locus. However, a tightly linked marker located on the telomere side in relation to *Pb1* has not yet been detected. Further studies to detect flanking markers and more precise mapping of the *Pb1* locus should be conducted to facilitate map-based cloning of the gene.

Acknowledgments

We are grateful to Dr. Y. Ukai for providing the computer software MAPL with technical advice, Dr. A. Saito for the gift of a set of RFLP markers, Dr. S.R. McCouch and Dr. S.D. Tanksley for the gift of some RFLP markers, the Rice Genome Research Program of the National Institute of Agrobiological Resources/Institute of Society Techno-innovation of Agriculture, Forestry and Fisheries in Japan for the gift of some RFLP markers and technical information, the rice breeding laboratories of the National Agriculture Research Center, Chugoku National Agricultural Experiment Station, Saitama Prefectural Agricultural Experiment Station, Tochigi Prefectural Agricultural Experiment Station and Miyagi Prefectural Furukawa Agricultural Experiment Station for the gift of some seeds of rice cultivars, Mr. T. Tooyama, Mr. T. Kato and Mr. N. Saka for their assistance in the field experiments, Dr. Y. Honda, Dr. H. Araki, Dr. A. Kato, Mr. A. Shumiya, Mr. M. Inoue, Dr. S. Koizumi, Dr. N. Kishimoto and Dr. M. Iwase for their assistance, and Dr. Y. Futsuhara and Dr. T. Koumura for the discussions.

Literature Cited

- Bonman, J.M., T.I. Vergel De Dios and M.M. Khin (1986) Physiologic specialization of *Pyricularia oryzae* in the Philippines. *Plant Dis.* 70: 767-769.
- Chen, X., S. Temnykh, Y. Xu, Y.G. Cho and S.R. McCouch (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 95: 553-567.
- Fujii, K., A. Shumiya, N. Saka, T. Ito and T. Kato (1988) Blast resistance of rice varieties with stripe disease resistance originated from St. No. 1. *Jpn. J. Breed.* 38 (suppl. 2): 406-407. (In Japanese)
- Fujii, K., A. Shumiya, S. Kudo, N. Saka, T. Tooyama and T. Ito (1993) Combination of eating quality derived from Koshihikari and panicle blast resistance linked with the stripe resistance originated from a parental line St. No. 1 in *japonica* rice. *Jpn. J. Breed.* 43 (suppl. 1): 162. (In Japanese)
- Fujii, K., T. Tooyama, N. Sugiura, N. Saka, T. Izawa, M. Inoue and A. Shumiya (1999a) Characteristics and genealogical analysis of panicle blast resistance in a RSV-resistant *japonica* rice cultivar Tsukinohikari and related cultivars. *Breed. Res.* 1: 69-76. (In Japanese with English summary)
- Fujii, K., Y. Hayano-Saito, N. Sugiura, N. Hayashi, N. Saka, T. Tooyama, T. Izawa and A. Shumiya (1999b) Gene analysis of panicle blast resistance in rice cultivars with rice stripe resistance. *Breed. Res.* 1: 203-210. (In Japanese with English summary)
- Harushima, Y., M. Yano, A. Shomura, M. Sato, T. Shimano, Y. Kuboki, T. Yamamoto, S.Y. Lin, B.A. Antonio, A. Parco, H. Kajiya, N. Huang, K. Yamamoto, Y. Nagamura, N. Kurata, G.S. Khush and T. Sasaki (1998) A high-density rice genetic linkage map with 2275 markers using a single F₂ population. *Genetics* 148: 479-494.
- Hayano-Saito, Y., T. Tsuji, K. Fujii, K. Saito, M. Iwasaki and A. Saito (1998) Localization of the rice stripe disease resistance gene, *Stv-b¹*, by graphical genotyping and linkage analyses with molecular markers. *Theor. Appl. Genet.* 96: 1044-1049.
- Kiyosawa, S. (1972) Genetics of blast resistance. In "Rice Breeding" Khush, G.S. (eds.), IRRI, Los Banos, 203-225.
- Kiyosawa, S. (1982) Genetic and epidemiological modeling of breakdown of plant disease resistance. *Annu. Rev. Phytopathol.* 20: 93-117.
- Kosambi, D.D. (1944) The estimation of map distances from recombination values. *Ann. Eugen.* 12: 172-175.
- Koumura, T., A. Shumiya, I. Shaku, M. Takamatsu, T. Ito, S. Kudo, T. Kato and N. Saka (1985) A new stripe-resistant rice variety "Tsukinohikari". *Res. Bull. Aichi Agric. Res. Ctr.* 17: 1-16. (In Japanese with English summary)
- Koumura, T., A. Shumiya, T. Ito, I. Shaku, M. Takamatsu, T. Kato, K. Fujii, N. Saka and S. Kudo (1987) A new stripe-resistant rice variety "Asanohikari". *Res. Bull. Aichi Agric. Res. Ctr.* 19: 1-20. (In Japanese with English summary)
- Kurata, N., Y. Nagamura, K. Yamamoto, Y. Harushima, N. Sue, J. Wu, B.A. Antonio, A. Shomura, T. Shimizu, S.-Y. Lin, T. Inoue, A. Fukuda, T. Shimano, Y. Kuboki, T. Toyama, Y. Miyamoto, T. Kirihaara, K. Hayasaka, A. Miyano, L. Monna, H.S. Zhong, Y. Tamura, Z.-X. Wang, T. Momma, Y. Umehara, M. Yano, T. Sasaki and Y. Minobe (1994) A 300 kilobase interval genetic map of rice including 883 expressed sequences. *Nature Genetics* 8: 365-372.
- McCouch, S.R. and S.D. Tanksley (1991) Development and use of restriction fragment length polymorphism in rice breeding and genetics. In "Rice biotechnology" Khush, G.S. and Toennissen (eds.), CAB Int. and IRRI, Wallingford, Oxon, 109-133.
- Ohya, K., T. Kojima, K. Sato, T. Okubo, H. Ito, T. Saotome, T. Kodato, T. Fujii, K. Tochigi and S. Kobayashi (1996) A new rice cultivar 'Haresugata' with the resistance to stripe. *Bull. Tochigi Agr. Exp. Stn.* 44: 1-14. (In Japanese with English summary)
- Ou, S.H. (1979) Breeding rice for resistance to blast, A critical view. In "Proceedings of the Rice Blast Workshop" IRRI, Manila, 79-137.
- Saito, A., M. Yano, N. Kishimoto, M. Nakagahra, A. Yoshimura, K. Saito, S. Kuhara, Y. Ukai, M. Kawase, T. Nagamine, S. Yoshimura, O. Ideta, R. Ohsawa, Y. Hayano, N. Iwata and M. Sugiura (1991) Linkage map of restriction fragment length polymorphism loci in rice. *Jpn. J. Breed.* 41: 665-670.
- Tooyama, T., K. Fujii, A. Shumiya and S. Kudo (1993) A method for testing panicle blast resistance of rice varieties by sprinkling water in a warm plain. *Res. Bull. Aichi Agric. Res. Ctr.* 25: 95-102. (In Japanese with English summary)
- Tooyama, T., Y. Hayano-Saito, N. Sugiura, K. Fujii, M. Iwasaki, T. Izawa and H. Nakamae (1998) Development of PCR-based markers which linked to paddy rice panicle blast resistance gene *Pb1(t)*. *Res. Bull. Aichi Agric. Res. Ctr.* 30: 27-34. (In Japanese with English summary)
- Ujihara, M., T. Nishio and K. Tanabe (1955) Research on the breeding of rice cultivars with high blast resistance using foreign cultivars (preliminary report). *Bull. Aichi Agric. Exp. Stn.* 10: 135-144. (In Japanese)

- Ukai, Y., R.Ohsawa, A.Saito and T.Hayashi (1995) A package of computer programs for construction of DNA polymorphism linkage maps and analysis of QTL. *Breed. Sci.* 45: 139-142. (In Japanese with English summary)
- Young, N.D. and S.D.Tanksley (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. *Theor. Appl. Genet.* 77: 95-101.