GENETIC ANALYSIS OF RESISTANCE TO RICE BACTERIAL BLIGHT OF ASOMINORI CULTIVAR

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Bacterial blight of rice (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice. Since there is the no bactericide effective to the disease, introduction of resistant varieties is the most effective measure to control this disease. A rice cultivar Asominori conveyed a unique resistance to Japanese BB pathogen races. It showed not only qualitative resistance to some races but also quantitative resistance to the other races. The recombinant inbred lines (RILs) derived from the cross between Asominori and IR24 were used for the genetic analysis. The frequency distributions of lesion length caused by races I and V showed bimodal segregation with 2 resistant and susceptible groups. The segregation fits a 1:1 ratio, indicating that the resistance of Asominori to the both races I and V was under monogenic control. These results agreed with the previous finding of resistance genes Xa1 and Xa12 from a resistance donor Kogyoku. The alleles at Xa1 and Xa12 loci from Asominori were tentatively designated as Xa1-Aso and Xa12-Aso, respectively. On the other hand, the frequency distribution of lesion length to race III showed continuous variation. QTL analysis indicated that one QTL was detected on chromosome 4 near the region of Xa1-Aso and Xa12-Aso with LOD score of 10.4 and explained 48.5 percent of total variation. An F_2 population from the cross of IAS22/IR24 was inoculated by race I, and several F_2 plants showed homozygous and heterozygous for Xa1-Aso region were selected in F_3 generation. The locus of Xa1-Aso was mapped between RM6089 and RM1153, with genetic distances of 7.9cM and 5.8cM, respectively. The F_4 progeny from the homozygous F_2 plants for Xa1-Aso region was selected to evaluate quantitative nature of resistance to race III. The selected F_4 plants (XF_4 1 and 2) showed also the shorter lesion development when inoculated by race III. Quantitative nature of resistance conveyed by Asominori to the other Japanese race IV and Vietnamese races was also investigated using the F_4 progeny. Significant difference was not observed when compared with the susceptible check IR24.

Key words: CSSL, DNA markers, mapping, RILs, QTl,

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Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most destructive diseases of rice. Yield losses in severely infected fields range from 20 to 30 % and in some areas can be as high as 50% (Adhikari et al. 1995). The disease causes not only yield losses but also low grain quality of rice. Chemical control has not been effective, and the development of resistant varieties is considered to be the most effective way to control this disease (Fig 1). Race-specific interaction between rice and *Xoo* is thought to follow the classic gene-for-gene concept (Flor, 1971), in which the plant resistance gene can recognize or interact with an elicitor molecule, presumably encoded by an avirulence gene from the pathogen. The recognition and interaction lead to activation of a battery of defense responses and effectively inhibit pathogen invasion.

Fig 1. Rice field is affected by bacterial blight of rice

Currently, there are more than two dozen resistance genes to bacterial blight of rice have been identified and designated in a series from *Xa1* to *Xa27*, most of them providing vertical, race-specific resistance. Several of these genes have already been incorporated into improved rice varieties, which are now widely grown in many rice production countries to prevent and control bacterial blight disease effectively. A rice cultivar Asominori conveyed a unique resistance to Japanese Bacterial Blight pathogen races.

The study aims at to understand the genetic basis of resistance to various Japanese races of BB detected in Asominori.

The recombinant inbred lines (RILs) were developed by single seed descent method from the progeny of an F_2 population derived from a cross between a Japonica variety Asominori and an Indica variety IR24. 165 lines of F_7 generation were obtained from 227 original F_2 individual plants. From these lines, 71 individuals were randomly selected and used for genetic analysis (Fig. 3).

To develop of CSSLs and near-isogenic lines (NILs) for further analysis of resistance to bacterial blight of rice, the RILs were crossed and backcrossed with IR24. The whole genome survey was conducted to determine the genotypes of the promising plants for CSSLs and NILs. The candidate plants for the CSSL and NILs were selected by marker-assisted selection (MAS).

Bacterial strains and inoculation: representative bacterial blight strains of Japanese races: T7174, race I; T3133, race III; H75373, race IV; H8584, race V and Vietnamese races: G1, G2, G3, G4 were used for inoculation. (These strains were kindly provided by Dr. Furuya, Plant Pathology Laboratory, Kyushu University).

For inoculation preparation, the bacteria were transferred to the slant of potato semi-synthetic agar medium and incubated at 30^oC for 3 days. Inoculums were prepared by suspending the bacterial mass with sterilized water at a concentration of about 10^9cells/mL. Rice leaves were inoculated by the leaf-clipping method (Kauffman et al. 1973) at booting stage of rice growing. Disease reaction was assessed at 18 days after inoculation by lesion length measurement and classified into two categories, resistance and susceptible.

Linkage map and QTL analysis: The genetic map for RILs population was constructed using the total of 375 RFLP (restriction fragment length polymorphisms) markers distributed across the 12 rice chromosomes (Tsunematsu et al., 1996). The resulting map spanned 1275.4 cM with an average distance between adjacent markers to locus was 4.4 cM. All of the 12 linkage groups were assigned to their respective chromosomes and no major discrepancy was found compared with the previous map (Saito et al. 1991, Kurata et al. 1994). For QTL analysis, simple interval mapping and composite interval mapping method were used by Window QTL cartographer version 2.0 (Wang et al. 2004). A critical threshold value of the likelihood of odd (LOD) score was calculated by conducting 1,000 of permutation test at significant level of 0.05.

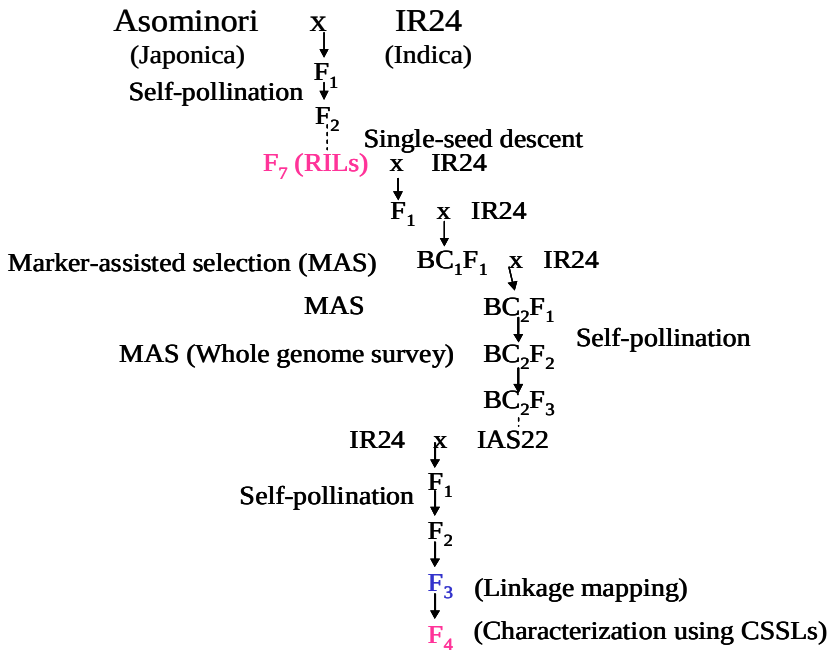


Fig. 2. Breeding scheme for developing RILs, CSSLs and segregating population.

Frequency distribution of lesion length caused by races I and V for resistance to bacterial blight of rice

The frequency distributions of lesion length to bacterial blight of rice to race I and race V in the RILs population, are shown in Fig. 3. The resistance in Asominori to race I and race V showed bimodal segregation with 2 resistant and susceptible groups. The segregation fits a 1:1 ratio, indicating that the resistance of Asominori to the both race I and race V was under monogenic control. The linkage between the resistance gene and DNA markers showed that the gene was located on chromosome 4. These results agreed with the previous finding of resistance genes *Xa1* and *Xa12* from a resistance donor Kogyoku. The alleles at *Xa1* and *Xa12* loci from Asominori were tentatively designated as *Xa1-Aso* and *Xa12-Aso*, respectively.

Frequency distribution of lesion length caused by race III to bacterial blight of rice

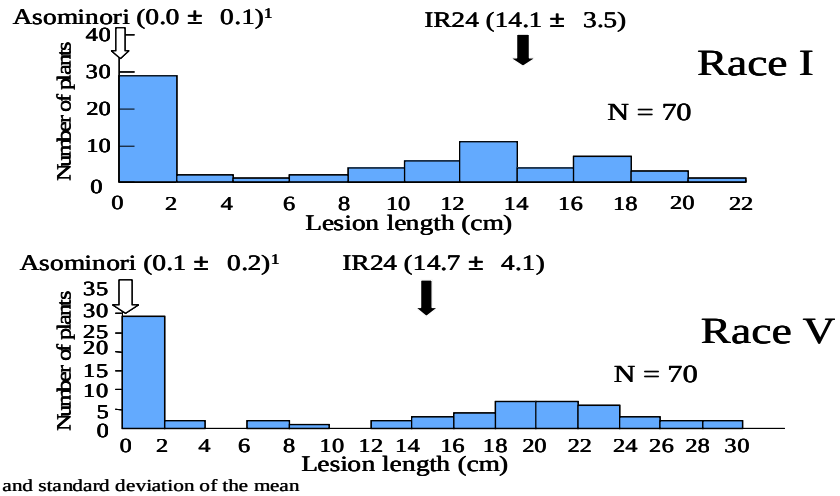
The frequency distribution of lesion length to bacterial blight of rice to Japanese strain, race III showed a pattern of continuous variation and approximately normal distributions. Result also showed that frequency distribution of lesion length in RILs had phenotypic values greater than the highest parent involving to transgressive segregation, indicating that the resistance of Asominori to race III was controlled by quantitative inheritance (Fig. 4).

Identification of QTLs of resistance to bacterial blight of race III

QTL analysis using the RILs population was performed to identify QTLs affected to bacterial blight resistance of rice to race III. By SIM and CIM method for QTL analysis, one QTL was detected on chromosome 4 near the region of *Xa1-Aso* and *Xa12-Aso* with LOD score of 10.4 and explained 48.5 percent of total variation (Table 1 and Fig. 5) with marker interval of C600 and Xa-1.

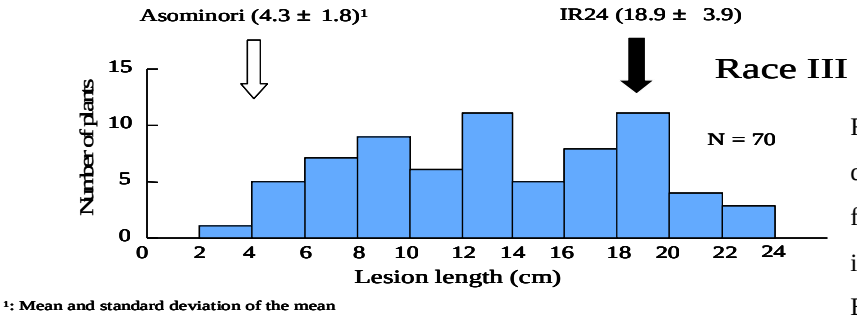
Linkage analysis of *Xa1-Aso* locus

The genetic distance between RM6909, RM6089 and RM1153 to *Xa1-Aso* locus were 0.0cM, 7.9cM and 5.8cM, respectively. Therefore, *Xa1-Aso* locus was completely linkage to the SSR marker, RM6909 on the chromosome 4 (Fig. 9). This linkage information is useful for marker-assisted selection of *Xa1-Aso* region in rice breeding and for fine dissection of *Xa1-Aso* region.



¹: Mean and standard deviation of the mean

Fig. 3. The frequency distributions of 70 RILs for lesion length, inoculated by Japanese BB strains, races I and V.



¹: Mean and standard deviation of the mean

Fig. 4. The frequency distribution of 70 RILs for lesion length, inoculated by Japanese BB strain (Race III).

Table 1. QTL for resistance to bacterial blight in RILs of Asominori and IR24 cross based on SIM and CIM

Chromosome	Marker interval	SIM			CIM		
		Peak LOD score	Variation (%)[a)]	Additive effect[b)]	Peak LOD score	Variation (%)[a)]	Additive effect[b)]
4	C600 - Xa-1	10.4	48.5	-3.6	10.4	48.5	-3.6

[a)] Percentage of total variation explained by the QTL
[b)] Negative value indicates effect from Asominori

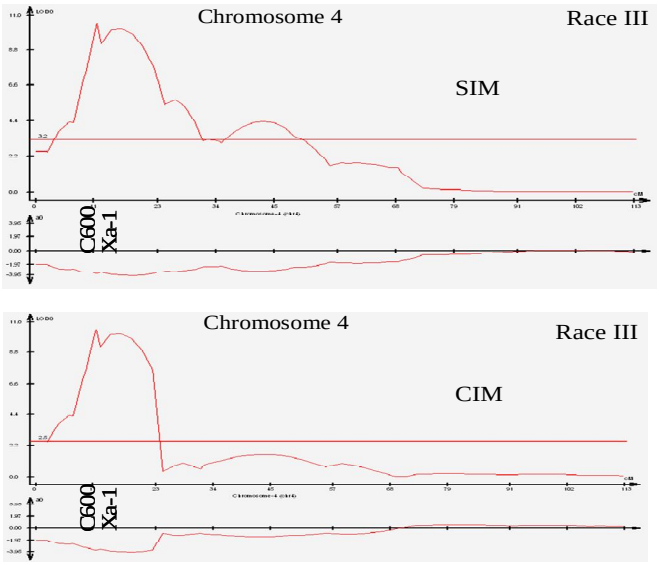


Fig. 5. The LOD score curve based on SIM and CIM of RILs

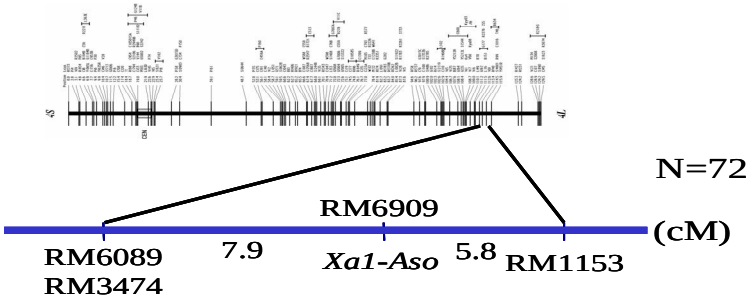


Fig. 6. Linkage mapping between *Xa1-Aso* locus and SSR markers

The QTL analysis of physiological traits, which reveal inheritance basis of such complex characters and provide important information is to apply marker-assisted

selection for plant breeding program. Information on QTL epistasis, QTL x environmental interaction effects, gene action of QTL in the new genetic background, and DNA markers closely linkage to target QTLs is particularly lacking (Li, 2001). The results of this study showed that resistance in Asominori conveyed a unique resistance to Japanese BB pathogen races. It showed qualitative resistance to both races I and V, and exhibited quantitative resistance to race III. This resistance pattern was long recognized as genetic resource to resistant improvement for rice breeding. Multiple resistance specificity from a single locus is highly desirable in breeding program for durable resistance. Currently, two hypotheses could explain the molecular basis of this resistance. One hypothesis suggests that these loci are composed of a cluster of tightly linked functional genes, each of which recognizes unique pathogen elicitors produced by pathogen avirulence genes. The majority of R genes reside at complex loci, and the structure of these may influence the rate of R-gene diversification as well as resistance specificity (Hulbert et al. 2001). Another hypothesis speculates that these loci carry single resistance genes capable of recognizing diverse pathogen elicitors. The latter possibility can be explained by the recently proposed "guard" hypothesis that R proteins might guard a limited set of key cellular targets of pathogen virulence factors (Dangl and Jones 2001). From this study, Asominori showed qualitative and quantitative resistance to various Japanese BB pathogen races at the same locus. We are still unclear whether the locus contains multiple functional members with each possessing different resistance specificity or whether it harbors a single gene that confers broad resistance. Transgressive segregation is the term used to describe the phenomenon in which individuals in segregating populations out-perform the parents. It has been observed in the progeny of inter-and sub-specific crosses of rice, but the underlying genetic basis of this phenomenon has not been experimentally determined. The accumulation of the positive QTL alleles from both parents would be the genetic basis of the transgressive segregation (de Vicente and Tanksley, 1993).

Many rice BB resistance genes (*Xa1*, *Xa2*, *Xa12* and *Xa14*) have previously been mapped to the region of long arm on chromosome 4 (Ogawa et al. 1978; Taura et al. 1987; Yoshimura et al. 1994). It has been proposed that a disease resistance locus is made up of many alleles that can recombine to produce a locus with a novel specificity (Pryor 1987; Ronald et al. 1992). It is speculated that duplications and rearrangements in the plant genome may have given rise to the complexity and race specificity observed in many disease resistance loci. It would be very interesting to sequence and compare these regions from different cultivars carrying different resistance genes.

The availability and utilization of the genomic sequence of *cv.* Nipponbare greatly accelerated the fine-scale genetic mapping of the *Xa1-Aso* locus. The high-resolution genetic map and closely linked markers will facilitate the isolation of the broad spectrum *R* gene by positional cloning.

The CSSLs produced in this study to evaluate quantitative nature of resistance in Asominori to bacterial blight of rice with a systematic way was developed for detailed QTL analysis and molecular mapping detection. Subsequently, the precise effect of the QTLs in the sets of CSSLs for resistance to bacterial blight of rice can be confirmed. Furthermore, it is possible to produce NILs and a large segregating population with a desirable genetic background for an individual QTL by one or two additional backcrossing using the CSSLs. The CSSLs may provide an integrated approach to quantitative genetics used together with the RILs and Molecular markers for mapping derived from a single cross combination of Indica-Japonica rice to produce the NILs carrying single bacterial blight resistance gene in a common genetic background allowed the efficient tagging and combining bacterial blight resistance genes.

Conclusion:

- Genetic analysis and QTL analysis revealed that the resistance in Asominori to both races I and V was under monogenic control and located at *Xa1-Aso* and *Xa12-Aso* region, respectively on chromosome 4.
- On the other hand, the frequency distribution of lesion length to race III showed continuous variation. QTL analysis has been conducted to indicate that one QTL was detected on chromosome 4 near the region of *Xa1-Aso* and *Xa12-Aso* with LOD score of 10.4 and explained 48.5 percent of total variation.
- The locus of *Xa1-Aso* was mapped between RM6089 and RM1153, with genetic distance of 7.9cM and 5.8cM, respectively.
- This phenomenon in both qualitative and quantitative natures of resistance is controlled by the same region. It should be considered for the further development of BB resistance genes in future breeding program.

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REFERENCES

Adhikari T. B, Vera Cruz C. M, Zhang Q, Nelson R. J, Mew T. W, Leach J. E (1995). Genetic diversity of *Xanthomonas* pv. *oryzae* in Asia. Appl. Environ. Microbiol. 61 (3): 966 – 971.

Dangl J, Jones J. D. G (2001). Plant pathogens and integrated defence responses to infection. Nature 411: 826-833.

De Vicente, M. C. and S. D. Tanksley (1993). QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134:585-596.

Flor H. H (1971). Current status of the gene-for-gene concept. Annu. Rev. Phytopathol 9: 275-296.

Hulbert S. H, Webb C. A, Smith S. M, Sun Q (2001). Resistance gene complexes: evolution and utilization. Annu. Rev. Phytopathol 39: 285-312.

Kauffam H. E, Reddy A. P. K, Hsien S. P. Y, Merca S. D (1973). An improved technique for resistance of rice varieties to *Xanthomonas oryzae*. Plant. Dis. Rep 57: 537 – 541.

Kubo T, Nakamura K, Yoshimura A (1999). Development of a series of Indica chromosome segment substitution lines in Japonica background of rice. Rice Genet. Newsl. 16: 104-106.

Li Z. K, Sanchez A, Angeles E, Singh S, Domingo J, Huang N, Khush GS (2001). Dominant and recessive plant disease resistance genes similar? a case study of rice R genes and *Xanthomonas oryzae* pv *oryzae* races. Genetics 159: 757-765.

Ogawa T, Morinaka T, Fujii K and Kimura T (1978). Inheritance of resistance of rice variety Kogyoku and Java 14 to bacterial group V of *Xanthomonas oryzae*. Ann. Newsl. 5: 106-107.

Pryor T (1997). The origin and structure of fungal disease resistance genes in plants. Trends Genet. 3: 157-161.

Ronal P. C, Albano B, Tabien R, Abenes L, Wu K, Mc Couch S, Tanksley S. D (1992). Genetic and phisical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol Gen Genet 236: 113 – 120.

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). Likage map of restriction fragment length polymorphism loci in rice. Breed. 41:665-670.

Taura, S, Ogawa T, Tabien R. E, Khush G. S, Yoshimura A and Omura T (1987). The specific reaction of Taichung Native 1 to Philippine race of bacterial blight and inheritance of resistance to race 5 (PXO112). Rice Genet. Newsl. 4: 101-102.

Tsunematsu, H., A. Yoshimura, Y. Harushima, Y. Nagamura, N. Kurata, M. Yano, T. Sasaki and N. Iwata (1996). RFLP framework map using recombinant inbred lines in rice. Breed. Sci. 46: 279-284.

Wakimoto S (1954). The determination of the presence of *Xanthomonas oryzae* by the phage technique. Sci. Bull. Fac. Agric. Kyushu Univ. 14: 485-493.

Wang S, Basten C. J, Zeng Z. B (2004). Windows QTL Cartographer 2.0. Raleigh, NC, Department of Statistics, North Carolina State University.

Yoshimura S, Kurata N, Sasaki T and Yoshimura A (1994). Genetic analysis of bacterial blight resistance genes in rice by using molecular markers. Resent Adv. Breed. 36: 33-36.