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QTL Mapping and Gene Cloning of Durable Resistance to Blast in Rice

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Short Commentary

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INTRODUCTION

Rice blast, caused by *Pyricularia grisea* Sacc., is a destructive disease of rice in most rice growing areas around the world. Breeding efforts have been made to introduce genes for blast resistance into desirable genetic background. Resistance to rice blast is categorized two types, complete (true) and incomplete (field resistance). Complete resistance is a hypersensitive reaction, often a complete form of resistance, and is characterized by a resistant infection type. More than 20 loci for complete blast resistance have been identified. Although cultivars with complete resistance were developed, the resistance has rapidly been broken down by compatible races of the pathogen. Field resistance, incomplete and usually polygenic, is a susceptible infection type that allows effective control of a parasite under natural field conditions and may be durable when exposed to new races of blast ^[1].

Mapping of QTL for Field Resistance to Rice Blast

QTL for field resistance to rice blast was analyzed using the progeny derived from the cross between Japanese upland and paddy rice cultivars. The frequency distribution of field resistance was continuous and the score ranged from 1 to 10. Owarihatamochi (resistant, upland) and Nipponbare (moderately susceptible, paddy), used as parents differed significantly in the blast resistance scores which were 2.8 and 8.0. QTL analysis was carried out in F_4 progeny lines from the cross between Nipponbare and Owarihatamochi using a total of 118 RFLP and SSR markers. The analysis with MAPMAKER/QTL revealed four QTLs located on three chromosomes with LOD scores higher than 2.0. Two QTLs were detected on chromosome 4 and one QTL were detected on chromosomes 9 and 12. The phenotypic variation explained by each QTL ranged from 7.9 to 45.7% and four QTLs explained 66.3% of the total phenotypic variation. Backcrossed progeny lines were bred to transfer the QTL with largest effect on the resistance using the susceptible cultivar Aichiasahi as a recurrent parent. Among 82 F_3 lines derived from the backcross, resistance segregated in the expected ratio of resistant 1: heterozygous 2: susceptible 1. The average score for blast resistance measured in the field was 4.2 ± 0.67 , 7.5 ± 0.1 and 8.2 ± 0.66 , for resistant, heterozygous and susceptible groups. The resistance gene, designated *pi21*, was mapped on chromosome 4 as a single recessive gene between RFLP marker loci G271 and G371 at a distance of 5.0 cm and 8.8 cm, respectively ^[2].

Positional Cloning and Function of *Pi21*

To identify the molecular basis of *Pi21*-mediated resistance and to settle the long-term debate about the cause of associated undesirable characteristics, we undertook map-based cloning of *pi21*, a recessive allele conferring field resistance to rice blast. By means of high-resolution mapping, we delimited the *Pi21* locus to a 1705-base pair (bp) region containing a single gene. The deduced amino acid sequence forms a protein containing a heavy metal transport/detoxification protein domain in the N-terminal region, implying that metal transport by *Pi21* might be associated with defense. Comparing the 1705 bp region between susceptible and resistant cultivars, seven nucleotide polymorphisms were identified, Two of them were located in the open reading frame associated with the phenotype. The resistant *pi21* allele had deletions of 21 and 48 bp from one susceptible *Pi21* allele and of 12 and 48 bp from the other allele in the proline-rich region. A 4.7kb *Xba*I fragment containing susceptible *Pi21* allele or resistant *pi21* allele was inserted into the multiple cloning site of binary vector pPZP2H-lac (S5). The construct for *Pi21* allele was prepared using Nipponbare (a reference cultivar of Rice Genome Project in Japan) PAC clone. pZ-N*Pi21* was introduced

into the resistant line carrying *pi21* allele and pPZ-Opi21 was introduced into the susceptible cultivar carrying *Pi21* allele by *Agrobacterium*-mediated transformation. T1 plants containing pPZ-Opi21 did not confer resistance, whereas T1 plants containing pPZ-NPi21 increased susceptibility to blast. These results suggest that the susceptible allele negatively regulates resistance and that the resistant allele carries a loss-of-function mutation. Increased resistance by suppression of susceptible *Pi21* expression by means of RNAi was confirmed, providing evidence that a gene encoding a protein with a heavy metal transport/detoxification protein domain has a role in plant defense. The possible defense mechanism of *pi21* is the protection from invasion of hyphae from penetrated cells into adjacent cells after inoculation of rice blast (**Figure 1**).

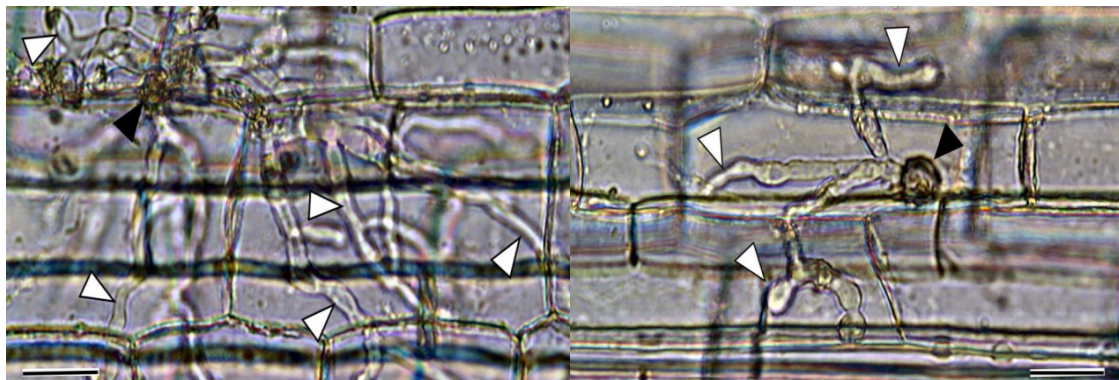


Figure 1. Possible defense mechanism of *pi21* allele.

DISCUSSION

Incompleteness, in addition to non-race specificity, may be a component of durable resistance. The response in resistant *pi21* plants after pathogen attack is not as fast or as strong as the *R* gene response. This slower induction of defense may be another type of incompleteness that contributes to the durability of resistance in rice. This report indicated that QTLs conferring incomplete field resistance to rice blast were firstly identified and one of QTLs, *pi21*, caused by a loss-of-function mutation, has potential for improvement of field resistance to rice blast. The durability of a resistance gene needs to be proved when cultivars carrying that gene alone maintain prolonged resistance under natural field conditions. Monitoring of newly released cultivars provides further evidence to confirm the durability of *pi21*-mediated resistance in rice. Moreover, *pi21* has been used for upland rice breeding in Africa Rice Center and its durability will be monitored in rice cropping areas in Africa.

REFERENCES

1. Fukuoka S, Okuno K (2001) Theor. Appl. Genet.103:185-190.
2. Fukuoka S, et al. (2009) Science 325:998-1001.