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## Map-based cloning and molecular breeding of *pi21*, a non-race specific resistance gene to blast

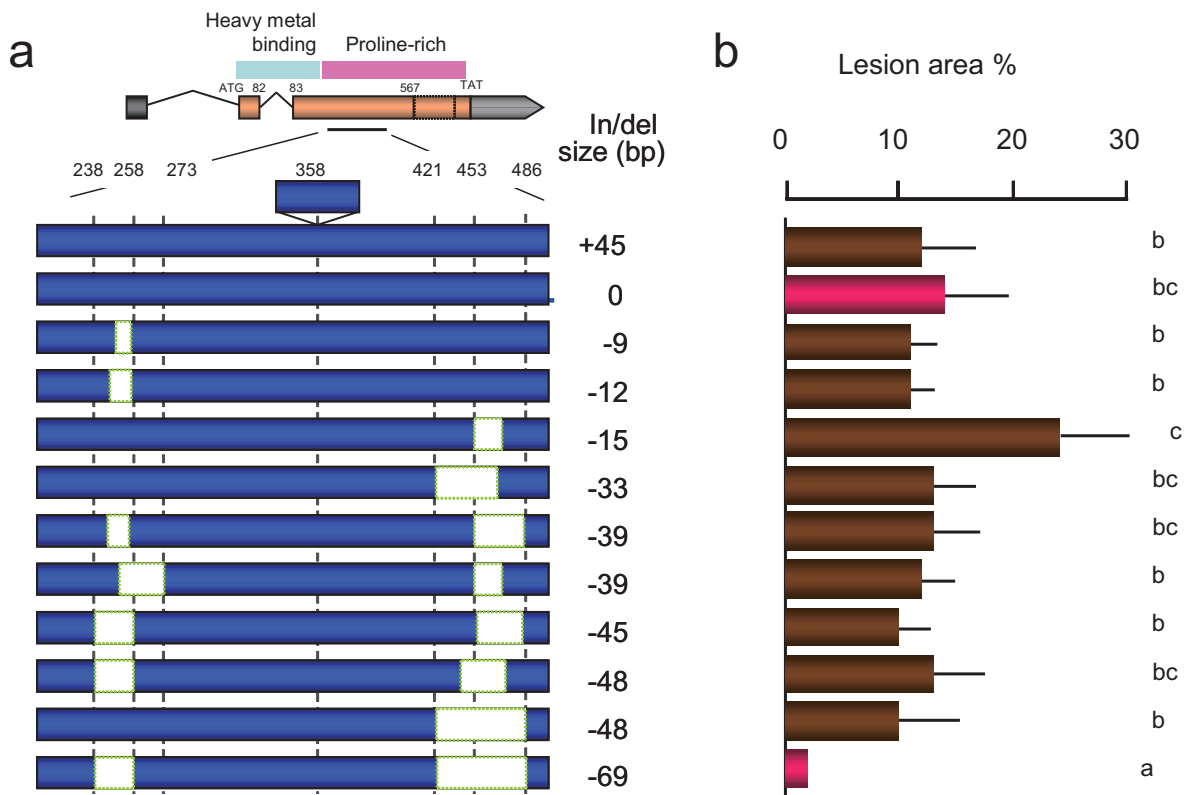
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Japanese upland rice (*Oryza sativa* L.) is a potential gene donor for durable resistance to blast disease caused by *Magnaporthe oryzae*. *Pi21* is a major quantitative trait locus (QTL), for which an allele, *pi21*, from a Japanese upland rice, induces resistance by restricting development of lesions without involvement of a hypersensitive response. High-resolution mapping and complementation testing of *pi21* identified a loss of function mutation of a heavy metal-transport/detoxification protein domain gene, Os04g0401000, encoding a protein structurally different from previously identified resistance proteins.

Sequence analysis of the *Pi21* gene from a number of worldwide cultivars identified 12 alleles. These alleles were discriminated based on insertion-deletion polymorphisms in the region containing proline-rich motifs that might be involved with the protein's function. Evaluation of a series of chromosome segment substitution lines (CSSLs), each possessing one of the *Pi21* alleles in the genetic background of a susceptible cultivar, clearly demonstrated that all except one were susceptible, and suggested that two deletions in the resistant allele are required to confer resistance (Fig. 1a). The analysis also revealed that the resistance allele was present in some strains of *japonica* rice, suggesting that it could improve blast resistance of rice worldwide (Fig. 1b).

Japanese upland rice has been used as a source of blast resistance in conventional breeding program since the 1920's, but the *pi21* allele has not been used in irrigated rice cultivars, possibly due to co-introduction of resistance and undesirable grain characteristics. The cause of this troublesome association could be either tight linkage of genes controlling independent traits, known as "linkage drag," or pleiotropic effects of the target gene on other traits; these two cases cannot be discriminated unless the linkage can be broken. Fine genetic analysis around *Pi21* locus identified gene(s) associated with inferior eating quality within 40-kb of *pi21*. Desirable recombinants between *pi21* and the genes conferring inferior eating quality were successfully selected from a breeding population over 6000 plants by using DNA markers for the region around *Pi21*. Field evaluation confirmed that the resistance allele does not penalize agronomic traits and that the cause of the association is tight linkage with genes causing undesirable effects. Map-based cloning of *pi21* identified a novel gene that negatively regulates plant defense and provides a breakthrough to durably resistant cultivars without any penalties on agronomic traits, which has not been achieved by conventional rice breeding.



**Fig. 1** Natural variations in *Pi21*  
 (a) The insertion/deletion-size variation of *Pi21* among Asian cultivated rice. In/del sizes of respective alleles from the Nipponbare-allele are indicated on right. (b) The average lesion areas caused by blast infection in backcrossed lines carrying the respective *Pi21* alleles. The lesion area followed by different letters differ significantly according to Tukey' s HSD test at 5%.

**Reference**

Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, Hayashi N, Takahashi A, Hirochika H, Okuno K, Yano M (2009) Loss of function

of a proline-containing protein confers durable disease resistance in rice. *Science*, **325**: 998-1001.