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A Trial for the Identification of Brown Planthopper (*Nilaparvata lugens* Stål) Resistance Alleles in Two Rice Lines, Norin-PL3 and Norin-PL4, Using Next-generation Sequencing and Simple Graphical Genotyping Methods

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Abstract

Norin-PL3 and Norin-PL4 are rice (*Oryza sativa* L.) breeding lines that carry a brown planthopper (BPH) resistance gene. Norin-PL3 was bred by introgressing *BPH1* derived from the Indica Group variety Mudgo into Japonica Group rice, whereas Norin-PL4 was bred by introgressing *BPH2* derived from the Indica Group variety IR 1154-243 into Japonica Group rice. To detect the alleles derived from resistance donor varieties in these lines, we developed a simple method to create graphical genotypes using a part of the InDel information obtained by a short-read next-generation sequencing system. In Norin-PL3, chromosomal regions derived from Mudgo were found on chromosome 12 and two other locations on the short arm of chromosome 1. In Norin-PL4, the chromosomal regions derived from IR 1145-243 were found on chromosomes 5 and 12, the short arm of chromosome 4, and chromosome 8. Among these regions derived from resistant donors, chromosomal regions contributing to resistance were identified. This method could also assist in creating graphical genotypes of the other rice lines with both Japonica Group and Indica Group varieties as parents. Moreover, the results of the graphical genotype and DNA marker information of Norin-PL3 and Norin-PL4 will be useful for rice breeding research on BPH resistance.

Discipline: Crop Science
Additional key words: *BPH1*, *BPH2*, insertion–deletion (InDel), resistance gene

Introduction

A graphical genotype portrays the parental origin and allelic composition of a chromosome (Young & Tanksley 1989). This information is important for the use of breeding lines for functional genetic analyses and further breeding. Moreover, when performing gene expression analysis using RNA-seq data, graphical genotype information would be useful for mapping genes accurately on the chromosome. Furthermore, when creating a knockout transformant using CRISPR/Cas9 to analyze gene function, it would be useful to predict the allele type of a target gene by referring to its graphical genotype. In general, DNA markers have been used to create graphical genotypes. Restriction-fragment length polymorphism (RFLP) markers have been used to create early graphical genotypes for gene mapping (Tamura et al. 1999, Murata et al. 1998). DNA markers in which polymorphisms can be easily analyzed through polymerase chain reaction (PCR) and electrophoresis, such as simple sequence repeat (SSR) markers, have been subsequently developed (McCouch et al. 2002, International Rice Genome Sequencing Project 2005); they have been used to create graphical genotypes (Takai

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