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Genetic analysis of leaffolder resistance in rice

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Abstract

A double haploid (DH) population, which consists of 120 lines derived from anther culture of a typical *indica* and *japonica* hybrid 'CJ06'/'TN1', was used to investigate the genetic basis for rice leaffolder resistance. Using a constructed molecular linkage map, five QTLs for rolled leaves were detected on chromosomes 1, 2, 3, 4, and 8. The positive alleles from CJ06 on chromosomes 3, 4, and 8 increased the resistance to rice leaffolder, and the alleles from TN1 on chromosomes 1 and 2 also enhanced resistance to leaffolder. The interactions between QTLs were identified and tested, and four conditional interactions were acquired for resistance to rice leaffolder. These loci were located on chromosomes 2, 9, 10, and 11, respectively. QTL pyramiding indicated that the positive alleles affect resistance to leaffolder. The prospective application of this data in rice breeding was also discussed.

Keywords: rice (*Oryza sativa* L.); rice leaffolder; QTL analysis; conditional interaction

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Introduction

Rice (*Oryza sativa* L.) is one of the world's most important crops, providing a staple food for nearly half of the global population (FAO, 2004). Almost 90% of rice is grown and consumed in Asia (Khush et al., 2002). It feeds more than two billion people in the developing countries of Asia (FAO, 1995). In Asia, Africa, and Latin America, the demand for rice will increase due to the steady increase in population (Wang et al., 2005). As the population rises, China will need to produce approximately 20% more rice by 2030 to meet domestic requirements if rice consumption per capita remains at the current level (Peng et al., 2009). Meanwhile, the production of rice is constantly threatened by insect and disease (Heong et al., 1995). With a projected increase in world population to 10 billion over the next four decades, an immediate priority is to achieve maximum production of rice in an environmentally sustainable and cost-effective manner (Selvaraju et al., 2007).

Rice leaffolder (*Cnaphalocrocis medinalis*, Guenée) (RLF) is a destructive and widespread insect pest throughout the rice growing regions in South and South-East Asia (Gottfried et al., 1981). Formerly considered a minor pest, leaffolder appears to be more important with the spread of high-yielding rice varieties and the accompanying changes in cultural practices (Bautista et al., 1984). In

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particular, the heavy use of insecticides and fertilizer seems to favor the leaffolder population outbreaks. Leaffolders spin rice leaves longitudinally into a roll, by stitching together the opposite rims of the leaf. They then chew inside the fold by scraping the green matter; the scraped leaves become membranous, turn whitish, and finally withered. Some heavily infested fields appear scorched due to many folded leaves. This activity disturbs photosynthesis and plant growth. The infected plants are then predisposed to bacterial and fungal infection (Bashir et al., 2004). Leaffolder also induces rice sheath rot, difficulty in heading, and massive yield loss at the reproductive stage (Xiao, 1990; Rothschild, 1996). According to a report issued by the Chinese Ministry of Agriculture in 2003, rice leaffolder affected 23.1 million acres in China. In some fields, rice leaffolder caused more yield loss than rice blast and became a major pest (Xu et al., 2003; Wu et al., 2004).

Conventional chemical pesticides are used to protect rice against leaffolder; however, their use has caused concerns for food safety and environmental pollution. Moreover, due to their frequent immigration and long incubation period, it is difficult to forecast the occurrence of rice leaffolder and effectively prevent damage by rice leaffolder. Some groups have got the rice transformed with *Bt* genes to protect against rice leaffolder (Li et al., 2004; Yang et al., 2004), whereas, its potential ecological risk of transgenic plants could narrow the application in rice production (Song et al., 2002). Thus, breeding of resistant varieties of rice pest control is still the most cost-effective and safe methods (Jun et al, 2006).

In recent years, with the development of RFLP, AFLP, and microsatellite markers in rice, genetic linkage maps have been constructed. Construction of double haploid (DH) populations provides a new method for the analysis of quantitative trait loci (QTLs), the number of loci, and the genetic effects of each QTL. Molecular markers have been used widely in QTL analysis for important agronomic traits in rice (McCouch et al., 1997; He et al., 1999; Zeng et al., 2002; Hu et al., 2004), as well as in molecular marker-assisted breeding (Cheng et al., 2004).

A double haploid (DH) population derived from anther culture of TN1/CJ06, as well as its molecular linkage map, was used in this study to analyze the genetic basis for rice leaffolder resistance. We also explored the molecular mechanism and its application in breeding.

Materials and methods

Population development

A DH population comprising 120 DH lines, which was established in our laboratory, was used in this study. This population was developed by anther culture of an F_1 hybrid between the typical *indica* susceptible variety TN1 and the typical *japonica* resistant variety CJ06.

Evaluation for rice leaffolder resistance

The appraisal of naturally induced rice leaffolder resistance was undertaken in the field of the China National Rice Research Institute (Hangzhou, China) in 2006. The 120 DH lines, and the two parents, were planted in a randomized complete block design with three reduplications. Six-row plots were planted with six plants per row with spacing of 20 × 20 cm. No pesticide was used during the whole growing season. Damage by leaffolder was investigated when the damage was about 60% in the leaves of the susceptible control (TN1) at the active tillering stage. The area of damage in the leaves of all the test lines was measured in grades of 0–3. Based on these measurements, the damage grade and damage scale were calculated. Sixteen plants in the middle of the block were selected for further analysis.

QTL analysis

A linkage map containing a total of 178 SSR and STS markers, distributed evenly on 12 chromosomes of rice, was selected to construct a rice linkage map using Mapmaker/EXP version 3.0 (Lu et al., 1996; Xu et al., 1998). The map spanned approximately 1,674.8 cM, with an average interval of 9.4 cM between markers. Interval QTL mapping was conducted using the software Mapmaker/QTL version 1.1 to analyze the QTLs for rice leaffolder resistance (Lincoln et al., 1992). A likelihood of odds (LOD) threshold of 2.5 was used to declare the presence of a putative QTL in a given genomic region. The contribution to the phenotypic variance and additive effect of each QTL for relative traits were also calculated. The QTL nomenclature followed was that of McCouch et al. (1997).

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