Screening of Rice Blast Resistance Genes from Aromatic Rice Germplasms with SNP Markers

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Rice blast is one of the serious devastating diseases. This study was carried out to determine the genetic diversities of blast resistance (R) genes form 86 accessions of aromatic rice with eight SNP markers, zt4792, zt4792, zt60510, zt6057, k6415, k6411, k39575 and t256, which showed the close-set linkage to 6 major genes, Piz, Piz-t, Pik, Pik-m, Pik-p, and Pit. Four accessions of indica type, Mayataung, Yekywin Yinkya Hmwe, Basmati 6129 possessed a gene on chromosome 1 was detected with t256 marker and Basmati 5854, showed the positive amplicons of six genes, rice blast resistance, and has been one of the most serious diseases of rice because of their pathogenic complexity related pathogen, host, and micro weather (Kwon and Lee, 2002; Lee, 1994; Li et al., 2007; Ou, 1985; Teng et al., 1991).

Especially, the blast fungus is highly variable and numerous; races of blast fungus are present in most field conditions (Ou, 1979; Xia et al., 1993; Valent and Chumley, 1994). The identification and isolation of additional host R genes and pathogen avirulence gene are now required to deepen understanding of molecular mechanisms involved in the host-pathogen interaction (Valent, 1990).

So far, about 50 major rice blast R genes and 35 avirulence (Avr) genes have been reported. A great number of genes with complete or partial resistance to blast in rice have been mapped and developed so far, which included Pi1(t), Pi2(t), Pi4(t), Pi5(t), Pi6(t), Pi9(t), Pi10(t) and Pi11(t) (Causse et al., 1994), Pi5(t) and Pi7(t) (Wang et al., 1994), Pia, Pib, Pik, Pit, Pita, Pi12(t), Pi17(t), Pi18(t), Pi19(t), Pi20(t), Pi23(t), Pi62(t), and Pi157(t) (Kiyosawa, 1981; Mackill and Bonman, 1992; Xia et al., 1993; Valent, 1990). The identification and isolation of additional host R genes and pathogen avirulence gene are now required to deepen understanding of molecular mechanisms involved in the host-pathogen interaction (Valent, 1990).

Recently, many rice varieties with complete resistance to M. grisea have been developed, but in many cases this resistance has been breakdown within a few years of the initial cultivation owing to the emergence of stronger virulent isolates of rice blast fungus (Bonman et al., 1986; Han et al., 2001; Kiyosawa, 1981; Mackill and Bonman, 1992; Yaegishi, 1994). Partial or field resistance of rice blast has received much attention as a means of effective control of a parasite under natural field condition and conferring durable blast resistance when exposed to new races of that parasite (Hittalmani et al., 2000; Liu et al., 2005; Wang et al., 1994).

This study was carried out to acquire information for genetic diversities of resistance genes against rice blast...
disease in aromatic rice germplasms for improving aromatic rice breeding efficiency using PCR-based markers including several candidate SNP (single nucleotide polymorphisms) markers.

Materials and Methods

Rice plant materials and DNA extraction. In our previous studies, we described the origin and characteristics of 260 accessions of aromatic rice germplasms introduced from 20 origins and preserved in Korean RDA genebank. Eighty six of 260 aromatic rice accessions were evaluated with agronomic traits, pshycochemical characteristics, and analysis of aromatic compounds in 2007 (Kim et al., 2008a and Kim et al., 2008b). The eighty six aromatic rice accessions were offered from National Agrobiodiversity Center of RDA, Korea, in Table 2. Five rice seeds of each accession were disinfected with 2% NaOCl solution at 4 hrs and were washed with tap water at overnight and placed on moist filter paper laid on the petri-dish at 30°C for one-week in growth chamber.

Genomic DNA was extracted from frozen young leaves of one-week-old seedlings by an improved CTAB (hexadecyl trimethyl ammonium bromide) method based on the procedure described by Murray and Thompson (1980). The extracted genomic DNA was estimated on 1% agarose gels staining of ethidium bromide for quality test. The quantity of extracted DNA was measured by Nano Drop system (Thermo, U.S.A.) and they were diluted to 10 ng/μl with the sterilized distill water and stored at 4°C.

SNP markers specific for rice blast R gene. Table 1, eight PCR based allele-specific SNP marker set (z4792, zt4792, z60510, z4615, k6441, k39575 and t256) of 6 major rice blast resistance (R) genes, Piz, Piz-t, Pik, Pik-m, Pik-p and Pit, were previously reported another studies (Cho et al., 2007; Hayashi et al., 2004; Hayashi et al., 2006). Two of blast resistance genes, Piz and Piz-t genes, were reported that closed to same position of on chromosome 6, and related to four SNP marker, z4792, zt4792, z60510, and zt6057 (Hayashi et al., 2004).

We used the eight SNP markers for amplification of DNA fragments linked to rice blast resistant genes based on each previous research papers. All of the rice blast resistance markers were synthesized at the oligo synthesis facility of Bioneer Co., in Korea.

Polymerase chain reaction (PCR) analysis. The PCR analysis for the SNP allele-specific markers were conducted based on procedures described by Bioneer PCR Pre-mix kit manual (Bioneer Co. Ltd, Korea). The PCR reaction mixture contained 50 ng of genomic DNA, 5 pmol of each primer set, 2.5 mM of each dNTPs, 1.5 mM MgCl$_2$, 1X PCR buffer (10 mM Tris-HCl, pH 9.0, and 30 mM KCl), and 1 unit of Taq DNA polymerase in 20 μl PCR reaction volume. PCR amplifications were carried out in a My-Genie96 Thermal cycler (Bioneer Co. Ltd, Korea), and programmed that template DNA was initially denatured at 95°C for 4 min, followed by 30 cycles of PCR amplification steps with the following parameters; a 30 sec denaturation at 94°C, 30 sec primer annealing at from 42 to 62°C, and a 60 sec primer extension at 72°C allowed for completion of primer extension, with a final extension at 72°C for 10 min. Initially 4 μl of the amplified products were electrophoretically resolved on a 1.5% agarose gel in 0.5X TAE (Tris-acetate-EDTA) buffer (pH 8.0) and visualized under UV light after staining with 0.1 ug/ml of ethidium bromide (Et-Br). The amplified fragment using SNP markers were scored as presence (1) or absence (0) of amplicon linked each gene DNA fragment.

Results

MAS of rice blast resistance accessions of aromatic rice germplasm. Application of molecular markers will enhance the efficiency of rice evaluation and improvement