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Genome-Wide Association Study of Leaf Blast Resistance in MAGIC *indica* Population of Rice (*Oryza sativa* L.) †

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Abstract: Rice blast is one of the most widespread diseases threatening rice production and crop damage account for a major loss to rice farmers worldwide. To identify genetic regions associated with leaf blast resistance, a genome-wide association study was performed with 391 MAGIC *indica* rice accessions developed at IRRI, Philippines. Evaluation of leaf blast severity was performed in the uniform blast nursery (UBN) during 2016 and 2017 at ICAR-IIRR, Hyderabad, India. Genome Wide Association Study was performed using six different statistical models with GBS data on 27041 single nucleotide polymorphisms (SNPs) distributed on all 12 rice chromosomes. Seven common associated SNPs on chromosome 3, 8 and 12 were identified with all the models. Farm CPU and Blink gave similar results and detected 31 associated SNPs on chromosome 3, 8 and 12. Highest number of annotated genes were identified on chromosome 12 followed by 8 and 3. Chromosome 12 harboring a major cluster of blast resistant genes and at least 20 blast resistant genes were mapped on this chromosome. Candidate genes identified in associated SNP regions on chromosome 12 in our study are encoding disease resistance genes; stripe rust resistance protein, MLA12, NBS-LRR disease resistance protein, NB-ARC domain containing protein, CC-NBS-LRR resistance protein MLA13, indicating the possibility of developing casual SNPs/QTNs for leaf blast resistance in rice from these regions.

Keywords: Blast resistant genes; GWAS; Leaf blast; MAGIC; Oryza sativa; Single nucleotide polymorphisms

1. Introduction

Rice blast disease is one of the most potent threats of rice production and crop damage account for a major loss to rice farmers worldwide. *Magnaporthe grisea*, the hemibiotrophic fungus is the causative organism for rice blast disease. The fungus is able to develop resistance to both chemical treatment and genetic resistance is a great threat to the effectiveness of blast-resistant rice varieties. However, host plant resistance/varietal resistance is the economical, environmentally safer method of control the disease. We conducted GWAS to identify marker trait association for blast resistance using 391 MAGIC *indica* rice accessions developed at IRRI, Philippines (Bandilo 2013).

2. Materials and methods

Three hundred and ninety-one MAGIC *indica* lines were screened for blast resistance in UBN (Uniform Blast Nursery) during 2016 and 2017 using HR12 and Tetep as susceptible and resistant checks. HR12, a local susceptible variety was sown in border rows on all sides of the bed, and after every five (5) test entries to help in disease spread. Scoring was done after 15 days of post infection as per the standard evaluation system (SES) 0–9 scale IRRI, 2013.

All these 391 line were subjected to GBS and 27041 high quality efficient polymorphic SNPs with <30% missing value and 0.05 MAF were found from the filtered raw reads. GAPIT was used to run GWAS using six different models- GLM, MLM, SUPER, MLMM, BLINK, FarmCPU (Figure 1).

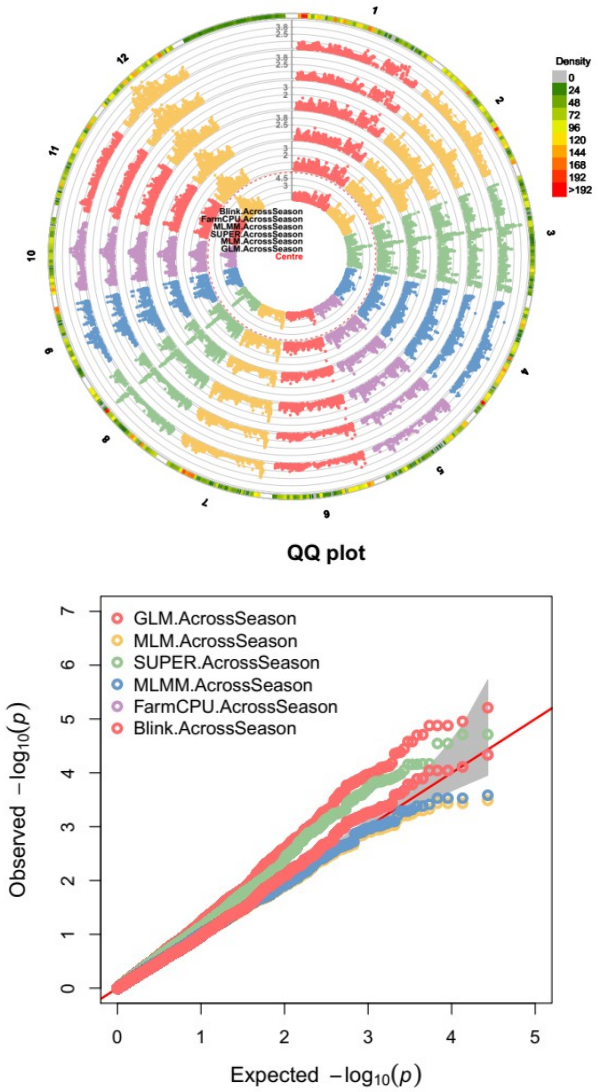

Figure 1. (**a**) GWAS for Blast over seasons (**b**) QQ plots for combined seasons for all the GWAS models.

3. Results and Discussions

Across seasons, around 50 significant SNPs were identified to be associated with blast resistance with P values ranging from 4.62E-05 to 9.85E-04, having upstream, downstream and synonymous variants. SNP S12_10207076 identified in LOC_Os12g17820 gene as 3_prime_UTR_variant. Six SNPs were downstream_gene_variants encoding for five genes, out of which one gene annotated as cystathionine gamma-synthase (CGS) on chromosome 3. CGS is reported to be involved in methionine biosynthesis. Null mutant deficient for methionine production was reported as methionine auxotrophic mutant, showing reduced pathogenicity on rice (Saint-Macary et al. 2015). Eight synonymous variant SNPs encoding seven genes of which two (LOC_Os03g25840 and LOC_Os03g25869) were annotated as amino acid permease family proteins reported to be expressed differentially for blast resistance in control *vs* stress genotypes in rice and barley. High affinity amino acid permease AGP2 (MGG_13334.6) and an amino acid permease GAP1 (MGG_07606.6) expressed differentially and they are co-regulated during fungal invasive growth (Mathioni et al., 2011). One synonymous variant SNP from Chr 3 (S3_14739507) encoding LOC_Os03g25760 annotated as Calmodulin binding protein (CBP) reported to be involved in plant defense mechanism. *Oryza sativa Calmodulin binding transcription factor* (*OsCBT* gene) which encodes CBP, when over expressed showed significant resistance to fungal pathogens (Koo et al. 2009).

Eight missense_variant genomic regions were significantly associated with blast resistance, among which, S3_14767464 and S12_13663603 encoding LOC_Os03g25790 and LOC_Os12g24040, annotated as glycosyl hydrolases family 17 and glycosyl hydrolase family 9 respectively. Glycosyl hydrolases are reported to be differentially expressed in rice, barley in carbon and nitrogen deficient nutrient medium infected with *Magnaporthe grisea* fungus (Mathioni et al., 2011). Two missence _variants SNPs (S8_20084173 and S8_20084133) encoding no apical meristem (NAM) (LOC_Os12g22940) is an integral part of NAC transcription factor reported to be involved in positive regulation of defense related genes under rice blast fungus stress conditions. Over expression of OsNAC111, elevates the expression of several defense-related genes including pathogenesis-related (PR) genes when compared with control plants. The brown pigmentation at the infected sites was lowered in plants with overexpressed OsNAC111 plants during early infection stages (Yokotani et al. 2014). Cellulose synthase gene (LOC_Os03g26044) was prominently associated with SNP (S3_14933779) and reported to be involved in blast resistance mechanism as they are down regulated in cell wall tissue expression experiments. Cellulose synthase genes OsCESA5 and OsCESA6 were severely affected during rice blast fungus infection and were down regulated (Jiang et al. 2017).

In conclusion the present study is a prominent GWAS of rice resistance to *Magnaporthe grisea* showing varied phenotype responses to disease infestation. The study across seasons has identified crucial genes involved in rice blast disease resistance that were also reported in different research studies. The significantly associated genes and their SNP effect along with annotations can be further studied for their validation in control and stressed environments using qRT-PCR or RNA-seq experiments.

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