THESIS

DETECTING DURABLE RESISTANCE TO RICE BACTERIAL BLIGHT

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ABSTRACT

DETECTING DURABLE RESISTANCE TO RICE BACTERIAL BLIGHT

The productivity of rice, a staple crop worldwide, is limited by pathogens such as *Xanthomonas oryzae* pv *oryzae* (*Xoo).* Controlling yield loss to the resulting disease, bacterial blight, is most effective through growing genetically pathogen resistant rice varieties. However, widespread deployment of varieties containing single gene resistance to bacterial blight places an immense selection pressure on *Xoo* to evolve virulence. The major virulence factors employed by *Xoo* to drive infection are transcription activator like (TAL) effectors. TAL effectors are secreted into the host cells where they target the transcription of particular host susceptibility genes to favor infection. Previous TAL effector research indicates that not all TALs are created equal and some are crucial to the virulence of *Xoo.* By breeding for resistance genes targeting necessary TAL effectors we may find more durable resistance as selection pressure on the pathogen will result in loss of the TAL effector function and therefore a decrease in virulence and pathogen fitness. In the present study, we characterized a novel and widespread TAL effector through quantitative trait loci (QTL) mapping. We used the *indica* rice Multi-Parent Advanced Generation Inter-Cross (MAGIC) population to screen for resistance to the cloned TAL effector, TAL7b, and the Philippine race 6 *Xoo* strain PXO99A. Our results confirm that TAL7b is a virulence enhancing factor and that the MAGIC population contains six loci targeting resistance to TAL7b.We also identified another seven resistance QTL to the highly virulent *Xoo* strain, PXO99A.

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Introduction

Over 40 resistance (R) genes have been identified to control bacterial blight disease of rice (*Oryza sativa*), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Verdier et al. 2011). These R genes elicit a strong, usually race-specific, resistance response that results in very short lesions, localized cell death, or lack of susceptibility. The problem, however, is that after deployment of these R genes, the pathogen populations rapidly evolve, sometimes within a few years, to overcome the resistance. Finding sources of durable resistance is a continuing challenge for effective control of bacterial blight.

Understanding the molecular mechanisms of plant/pathogen interactions that lead to resistance may provide insights into how to identify new sources of resistance, including those that might be more durable upon deployment. In R gene-mediated resistance, the plant R proteins either directly recognize specific effectors or indirectly recognize the activity of the effectors, and this recognition leads to elicitation of a resistance response. This type of resistance is pathogen race-specific, and depends on the pathogen's repertoire of effectors. In the interactions of *Xoo* and its host, rice, resistance results from host R gene recognition of Transcription Activator Like (TAL) virulence effectors (Kameswara Rao et al. 2002; Zhang and Wang 2013).

Xoo TAL effectors are interesting proteins because of their unusual structure and functions. They possess a nuclear localization signal, a transcriptional activation domain and a central repeat domain. The central repeat domain consists of many 33-35 amino acid repeats that differ only at the 12^{th} and 13^{th} position, referred to as the repeat variable diresidues (RVD) (Deng et al. 2014). The nuclear localization domain directs the TAL effector to nucleus where the

central repeat domain binds to particular sequences in the promoter of the host genome and activates transcription of the target gene. The promoter sequences recognized by the RVD are called effector binding elements (EBE). The central repeat domain wraps helically around the EBE with the 12th amino acid stabilizing the interaction, and the 13th repeat interacting with the nucleotide (Deng et al. 2012). The RVD to DNA specificity is known and target EBE sequences can be predicted from the RVD sequence (Boch et al. 2009; Doyle et al. 2012; Noël et al. 2013; Deng et al. 2014; Yang et al. 2014).

TAL effectors target the expression of host genes to influence their regulation in favor of pathogen development. Although, occasional differences exist, typically TALs are virulence effectors that activate host genes called susceptibility genes by binding to EBE in target gene promoters. Examples of susceptibility genes include copper and sugar transporters, transcription IIA subunits, and bZIP transcription factors (Iyer and McCouch 2004; Sugio et al. 2007; Yuan et al. 2010; Streubel et al. 2013; Hutin et al. 2015b). However, hosts have evolved diverse and clever resistance mechanisms to combat the action of TAL effectors. Several of the host defenses include genetic mutations in the TAL target genes or their promoters. Mutations in the EBEs of susceptibility genes, for example, result in resistance because the mutations block activation of the genes. Two well-characterized R genes to *Xoo, xa13* and *xa25,* are recessive resistance genes with such EBE mutations (Chu et al. 2006; Yuan et al. 2009; Zhou et al. 2015). Other TALs directly activate resistance genes that regulate cell death (sometimes called death genes). TAL effector AvrXa27, for example, binds to an EBE in the promoter of the resistance gene, *Xa27*, and triggers a hypersensitive response (Gu et al. 2005). Current knowledge of how TAL effectors interact with their target genes to control plant responses has been recently reviewed (Hutin et al. 2015a).

Genome sequencing studies have confirmed that *Xoo* strains contain large numbers (up to 28) of TAL effector genes (Mew et al. 1992; Lee et al. 2005; Salzberg et al. 2008; Scholze and Boch 2011; Sebra et al. 2015), but it is clear that some TAL effectors are more important for pathogen virulence (activation of susceptibility genes). Inactivation of TAL effectors AvrXa7, PthXo1, PthXo2, PthXo3,Tal5 and TalC in *Xoo* causes severe reduction in pathogen virulence, and these are considered major virulence contributors (Yang et al. 2000; Bai et al. 2000; Yang and White 2004; Yu et al. 2011; Streubel et al. 2013). Other TAL contribute less or not at all to virulence. The variation in TAL effector numbers, the activity of the TAL and the variety of mechanisms that plants have evolved to avoid or react to TAL effectors supports the importance of TAL effector function in plant disease resistance.

More than 15 years ago, Vera Cruz et al. (2000) demonstrated that durability of disease resistance genes in the field is related to the function of pathogen TAL effectors. They predicted that durability of resistance is related to the importance of effectors to pathogen fitness and virulence, i.e., the more necessary the effector is to the processes infection, the longer the resistance targeted to that effector lasts. Using both laboratory and field experiments, they demonstrated that loss of effectiveness of one rice bacterial blight R gene, *Xa7,* was correlated with mutations in the corresponding *Xoo* TAL effector gene *avrXa7*, and that the mutations in *avrXa7* resulted in reduced pathogenic fitness, as measured by reduced aggressiveness on susceptible rice cultivars (Bai et al. 2000; Vera Cruz et al. 2000; Ponciano et al. 2004). By contrast, mutation of the effector gene *avrXa10* did not affect pathogenic fitness, and the corresponding R gene, *Xa10,* was rapidly overcome in the field (Bai et al. 2000; Vera Cruz et al. 2000). Thus, selection on the pathogen population imposed by the *Xa7* gene, but not the *Xa10* gene, resulted in loss of *avrXa7* function and reduced pathogenic fitness (Bai et al. 2000;

Ponciano et al. 2004), and confirmed that pathogen effectors can be predictors of R gene effectiveness and durability (Vera Cruz et al. 2000; Leach et al. 2001).

Given that R gene durability is linked to the relative importance of effectors to pathogen virulence, one strategy to select for longer lasting resistance is to screen for sources of resistance that target important virulence factors. In this study, we test that approach by searching for resistance to a novel TAL virulence effector TAL7b. The *tal7b* gene was originally cloned from PXO86 (Hopkins et al. 1992). This effector was originally called *ab4.5* and was shown to be important for *Xoo* virulence through insertional mutagenesis (Bai et al. 2000). *tal7b* is of particular interest to this study because it is also present in the sequenced *Xoo* strains C8 and PXO99A (Salzberg et al. 2008; Pérez-Quintero et al. 2013), and is similar to a TAL found in *Xoo* strains MAFF311018 and KACC10331 (Ochiai et al. 2005; Lee et al. 2005), see Table 1 for RVD sequence similarity. Due to the highly conserved nature of this TAL effector and its demonstrated importance to virulence, we proposed that resistance genes targeting TAL7b would be durable in the field.

The first step to testing this hypothesis was to identify sources of resistance to TAL7b. Typically, resistance genes are identified by screening for a resistant rice variety or wild relative donor, and creating backcross or nearly isogenic lines (NIL) segregating only in phenotypic resistance to certain races of *Xoo* (Ogawa et al. 1991). While this method is advantageous for fine mapping (resolution of a few genes), it only considers one resistance gene in one genetic background, and ignores potential genetic interactions. It is also time consuming, labor intensive and serves only the purpose of identifying and cloning a single gene. Newer methods of discovering resistance genes targeting TAL effectors as well as the targets of TAL effectors include expression analysis during the course of *Xoo* infection in combination with EBE

prediction models and transgenic overexpression or knockout of finely mapped genes (Yang et al. 2006; Bogdanove et al. 2010; Hutin et al. 2015b). We chose to instead perform a mapping analysis to identify genomic regions significantly involved in the virulence of our novel TAL effector. We chose this more applied method of characterization to increase the impact our results; not only did we have the opportunity to use the extensive genetic resources available in rice to examine genes involved in TAL7b infection, but to also identify quantitative trait loci (QTL) conferring resistance to bacterial blight.

In our study, we used an *indica* rice Multi-Parent Advanced Generation Inter Cross (MAGIC) population (Bandillo et al. 2013), because these novel genetic populations possess unique advantages to other mapping populations. Commonly used biparental populations have limited genetic diversity because they are developed from only two parent lines. The *indica* MAGIC was developed with eight parents, providing the potential of up to eight different alleles at each locus. Biparental populations differ phenotypically, but interesting QTL may not be mapped because there were not contrasting alleles at all loci. Since the *indica* MAGIC population was intercrossed three times, compared to once for a biparental or Nested Association Mapping populations, more recombination events may have occurred, and therefore, smaller linkage disequilibrium and higher mapping resolution can be achieved. The parental or founder lines used to create the *indica* MAGIC populations are widely used varieties or elite germplasm, resulting in a population that is amenable to cultivation and lines that could be easily refined into varieties for release. Finally, QTL instability in different genetic backgrounds is a major concern of plant breeders. Screening a relatively diverse population such as MAGIC helps to mitigate this risk.

The overall goal of this study was to identify a source of resistance that targets the *Xoo* virulence effector TAL7b. In the process, we confirmed the virulence contribution of TAL7b to *Xoo*. By screening a subset of the rice *indica* MAGIC population with a strain of *Xoo* harboring the cloned *tal7b* gene (PXO99A-pHM1-*tal7b*) or the empty cosmid vector (PXO99A-pHMI), we identified six loci with putative novel sources of resistance targeted at Tal7b as well as loci conferring resistance to the highly virulent *Xoo* strain PXO99A.

Materials and methods

Bacterial strains and plasmids

Xoo strain PXO99A (Hopkins et al. 1992) is a Philippine race 6 strain that is virulent on most rice varieties, except those harboring the bacterial blight resistance genes *xa13, Xa27* and *Xa21* (Vera Cruz et al. 1992). *X. oryzae* (*Xo*) strain X11-5A is deficient of TAL effectors, and is weakly virulent on most rice varieties (Jones et al. 1989). *Xo* and *Xoo* strains were grown on PSA (10 g/L peptone, 10 g/L sucrose, 16 g/L agar, 0.5g/L L-glutamic acid monosodium salt, 50 µg/L) (Tsuchiya et al. 1982) at 28°C. *Escherichia coli*, strain DH5α, was grown on Luria Agar with appropriate antibiotics at 37°C. All bacteria were stored in a 30% glycerol solution at -80°C.

tal7b, corresponding to the *Bam*HI fragment referred to *aB4.5* (Bai et al. 2000), was cloned from the cosmid pXO6-33 (Hopkins et al. 1992) derived from *Xoo* strain PXO86. The central repeat region of *tal7b* was cloned as a *Sph*I fragment into the single *Sph*I site of the entry vector pCS466, a derivative of the Gateway entry vector pCR8-GW (Invitrogen) that contains a truncated form of the *X. oryzae* pv. *oryicola* BLS256 *tal1c* gene, from which the *Sph*I fragment that comprises the repeat region had been removed (Verdier et al. 2012). *tal7b* was then transferred to the broad host-range destination vector pKEB31(Cermak et al. 2011) Addgene plasmid 31224, (www.addgene.org), using Gateway LR Clonase (Invitrogen), to create pKEB31-*tal7b* for constitutive expression in *Xanthomonas.* Finally, *tal7b* was cloned into the low-copy cosmid vector pHM1 by digesting pKEB31-*tal7b* with *Hind*III HF (New England Biolabs, Ipswich, MA) to extract the *tal7b* central repeat region flanked by the *tal1c* N and C terminal domains (Verdier et al. 2012). The resulting plasmid, pHM1-*tal7b* was transformed into *Xoo*

PXO99A and *Xo* X11-5A by electroporation (Choi and Leach 1994) and transformants were selected on nutrient agar containing 50 µg/L streptomycin, 50 µg/L spectinomycin and X-gal/IPTG. Colony PCR was performed with primers F4: CGCAATGCACTGACGGGTGC and R2458: CATGCAAAGACGCCTGATCCGG to further confirm the presence of the *tal7b* gene. The PCR program included an initial denaturation step at 96°C for 4 min, followed by 25 repeats of a 15 second 96°C denaturation, a 30 second 58°C annealing, and a 45 second 70°C elongation, with a final 70°C elongation step for 4 min. Finally, integrity of the *tal7b* gene was confirmed by Sanger sequencing of the fragment.

Experimental design and growth conditions

The *indica* MAGIC parental lines, see Bandillo et al. 2013 for parental line information, were screened prior to the population screen to determine compatibility of strains with the population. The parental lines were grown in single replication and inoculated with PXO99A, PXO99A-pHM1, X11-5A, PXO99A-pKEB31-*tal7b* and PXO99A-pHM1-*tal7b* (see Table 2 for strain descriptions) by leaf clip at 6 weeks of age. PXO99A-pHM1 was chosen as a control strain for PXO99A-pHM1-*tal7b* and as a proxy for PXO99A.

A subset of 330 of the advanced inbred lines (AILs) from the *indica* MAGIC population (Bandillo et al. 2013) along with the founder lines and two control lines Nipponbare (susceptible) and WAB-56-125 (resistant). The lines were grown in triplicate using an incomplete random block (IRB) design. The IRB design was implemented to ensure that a given line, but separate plant, was inoculated with PXO99A-pHM1 and PXO99A-pHM1- *tal7b* on the same day. Planting dates in 2014 were: Replication 1 May 27-29, Replication 2 June 3–5, Replication 3 June 24–26. Three seeds were sown into a 1” square plastic pot filled with a mixture containing 1 part volume Pro Mix ® BX Mycorrhizae (Premier Tech Horticulture,

Québec, Canada), 1 part volume peat moss, 0.25 part volume playground sand. Seedlings were thinned to one plant per pot at 3 weeks after sowing. Iron chelate was applied to the surface at two weeks after sowing. Plants were watered at least once daily from the bottom by filling the trays with water; fertilizer was added during watering twice per week starting at one month after sowing. Plants were treated for root aphids with Mantra ® 1G (Nufarm Americas Inc., Illinois, USA). Plants were grown in Colorado State University Greenhouses and replications were in separate areas of the greenhouse.

Bacterial inoculations and phenotyping

Rice plants were inoculated at 6 weeks after sowing by a leaf clip inoculation method (Kauffman et al. 1973). The two youngest, completely unfurled leaves of each plant were inoculated. Technicians were randomly assigned to day, block and strain to inoculate. Inoculations were conducted over three days for a given rep to correspond with blocks. Plate *Xoo* strains were flooded with sterile water, and bacterial suspensions were adjusted to $OD_{600} = 2.0 \pm 0.3$ in a spectrophotometer for use as inoculum. Lesion lengths were measured in centimeters at 14 days after inoculation.

Genotyping

Single nucleotide polymorphism markers were generated through genotyping by sequencing conducted at Cornell University. Raw reads were trimmed and aligned to the Nipponbare reference genome at the International Rice Research Institute. Founder lines were sequenced at 10x depth and AILs at 1x depth. Genotyping resulted in 396,361 SNP sites. Data filtration was conducted with TASSEL version 5.0.2. All heterozygous SNP sites were changed to N to indicate no SNP call. SNP calls with < 0.05% minor allele frequency were also converted to N because these were likely to be erroneous reads and not true genetic variation. The SNP

sites of the MAGIC population were filtered to allow up to 20% missing data, resulting in 24,742 SNPs. The MAGIC population SNPs were further filtered to exclude any sites not found in the parental haploid maps, resulting in 14,561 SNPs. The taxa (AILs) were then filtered so that all AILs must have calls for at least 70% of the sites, resulting in the loss of 14 lines and 316 remaining lines. Upon intersect join of the hapmap file and the phenotype data, one more line was excluded for PXO99A-pHM1 analysis and two lines for the PXO99A-pHM1- *tal7b* analysis due to missing phenotype data.

Data analysis

SAS® software version 9.4 (SAS Institute Inc., 2013) was used for statistical analysis of phenotype means. The two leaves per plant were treated as technical replications and simple means calculated. Biological replications were averaged using Least Square means (LSmeans) function in PROC MIXED. Bacterial strain, line, and bacterial strain * line interaction were treated as fixed effects (type III). Block and replication were treated as random effects. p-values for difference in LSmeans between strains and AILs were adjusted using Tukey's method for multiple comparisons.

GWAS was conducted with TASSEL version 5.0.2. A kinship matrix was generated for the MAGIC population, excluding parents, to account for population structure. Statistics and effects were generated in TASSEL with both the general linear model (GLM) and mixed linear model function (MLM). False discovery rate from GWAS was controlled with q-value package (Storey 2003) in R. The default mixed linear model settings were used, where compression level was calculated using the optimum level and the variance component estimated using the P3D method. Manhattan plots of GWAS results were generated using qqman R package (Turner 2014).

Interval mapping was conducted in R Fire Safety version 3.2.2 with mpMap package (Huang and George 2011). The linkage map (mpcross) was generated from the pedigree information (with six generations of self-pollination after crossing) for each AIL, the founder genotypes and the AIL genotypes. Genotypes were filtered using TASSEL and the same as those used in the GWAS. Interval mapping was conducted independently for PXO99A-pHM1 and PXO99A-pHM1-*tal7b* LSmeans estimates of lesion lengths with the arguments of true marker positions, no covariates and step size of 1 cM. One cM was set as 250,000 bp as per previous linkage disequilibrium calculations on the *indica* MAGIC population (C. Raghavan, *personal communication*). The QTL interval was calculated as the 95% confidence interval, corresponding to the markers that are 2 LOD less significant than the most significant marker in the QTL peak.

Local linkage disequilibrium around significant markers was calculated with the Pearson coefficient of correlation squared (r^2) in TASSEL v5.0.2 for pairs of SNPs. MAGIC populations present a challenge for linkage disequilibrium calculation because the r^2 is influenced by uneven allele frequency between marker pairs. To control this only the r^2 for pairs with similar allele frequencies were kept. The rate of LD decay was calculated as described Mackay et al., 2014; the pairwise marker distances were plotted by their respective r^2 values and fitted with a locally weighted linear regression with the Lowess function in R. LD was estimated to end when the Lowess line fell below r^2 = 0.2.

Results and discussion

MAGIC parents segregate for PXO99A +/- TAL7b resistance

The virulence of *Xoo* strains PXO99A, PXO99A-pHM1, X11-5A and PXO99A-pKEB31-*tal7b* and PXO99A-pHM1-*tal7b* to the eight *indica* MAGIC parental varieties was assessed to identify if the parents might harbor resistance to the strains. X11-5A was not virulent to any of the MAGIC parents and was therefore not used in the MAGIC population screen. PXO99A-pKEB31-*tal7b* was also excluded from the large screen, because the high copy nature of pKEB31 lead to increased and artificial resistance responses from some hosts. PXO99A-pHM1 and PXO99A-pHM1-*tal7b* were chosen for the MAGIC population screen based on the criteria that some of the parents had significantly different responses to PXO99A-pHM1, that some parents had significantly different responses between PXO99A-pHM1 and PXO99A-pHM1-*tal7b*, and that PXO99A-pHM1-*tal7b* increased virulence of some of those parents. In the preliminary screen of PXO99A-pHM1 and PXO99A-pHM1-*tal7b* on the MAGIC parents, IR45427-2B-2-2B-1-1 was the most susceptible parent to both strains with LL > 17.7 cm while PSBRc82 and IR4630-22-2-5-1-3 were the most resistant with LL < 7.6 cm. Sanhuangzhan-2 was more resistant to PXO99A-pHM1-*tal7b* than PXO99A-pHM1 (p-value = 0.069) and IR77298-14-1-2-10 was more susceptible (p-value = 0.083), see Table 3 for all parental responses to PXO99A-pHM1 +/- *tal7b* in the preliminary screen.

Overall, during the MAGIC population screen both PXO99A-pHM1 and PXO99A-pHM1-*tal7b* were more virulent to the parents than during the preliminary parental screen, see Table 3 for comparison of parental responses in preliminary and population screen. During the population screen, PSBRc158 was the most resistant parent to PXO99A-pHM1-*tal7b* (LL = 11.5

cm) and Samba Mahsuri-*sub1* was the most resistant parent to PXO99A-pHM1 (LL = 9.8 cm). IR45427-2B-2-2B-1-1 was still the most susceptible to both strains, but became the only parent significantly more susceptible to PXO99A-pHM1 than PXO99A-pHM1-*tal7b* (p-value 0.098). Of the five parents with significantly different LL between the two strains, four parents were more susceptible to PXO99A-pHM1-*tal7b* than PXO99A-pHM1. The difference in the parental responses suggests that resistance to PXO99A-pHM1-*tal7b* and PXO99A-pHM1 is influenced by environment as the preliminary screen was conducted in greenhouses during spring and the MAGIC population screen conducted during the summer. Furthermore, in the MAGIC population screen, both replication and nested blocks were significantly different for the AILs as well, see Table 4 for ANOVA results with replication and blocks as random effects.

MAGIC population segregates for resistance to PXO99A +/- TAL7b

Although no strong resistance (LL < 5 cm) was evident in the founder lines during the preliminary screen, the evidence of mild resistance (LL < 10 cm) to the two strains along with the significantly increased resistance of Sanhuangzhan-2 to PXO99A-pHM1-*tal7b* encouraged us to screen a subsample of the *indica* MAGIC population for resistance and susceptibility to PXO99A-pHM1 and PXO99A-pHM1-*tal7b*. Screening of 330 advanced inbred lines (AILs) from the MAGIC population with PXO99A-pHM1 and PXO99A-pHM1-*tal7b* revealed transgressive segregation for resistance to both strains (Figure 1B and C). Taken with the normal distribution of phenotypic disease to each strain and the lack of strong resistance, our data indicate that the MAGIC population has various small and moderate effect resistance QTL. Of particular interest to breeding efforts, 24 of the MAGIC population AILs were more resistant to PXO99A-pHM1-*tal7b* than the most resistant parent (Sanhuangzhan-2) and 32 AILs were more resistant to PXO99A-pHM1 than the most resistant parent (Samba Mahsuri-*sub1*). These lines

are likely to have a favorable concentration or combination of disease resistance QTL and therefore could be incorporated into breeding programs.

Variance of lesion lengths caused by PXO99A-pHM1 and PXO99A-pHM1-*tal7b* were significantly different (p-value < 0.001), indicating that potential susceptibility gene targets or resistance genes responsive to TAL7b were present in the *indica* MAGIC population, see Table 4 for ANOVA results. Although the variance of the line by strain interaction was not significant in the mixed model (Table 4), the t-tests comparing LSmeans of PXO99A-pHM1 to PXO99A-pHM1-*tal7b* individual AILs showed 63 lines with significantly different responses to the strains, nine with Tukey adjusted p-value < 0.001, and 54 with Tukey p-values of < 0.05 (data not shown). In the screen of the MAGIC population, data summarized in Figure 1A, the overall average lesion length calculated from the LSmeans estimate for PXO99A-pHM1 was 13.7 cm, compared to 17.1 cm for PXO99A-pHM1-*tal7b*. PXO99A-pHM1-*tal7b* caused greater disease on 96 AILs than it did on the founders, confirming the previous report that TAL7b is a virulence factor (Bai et al., 2000). PXO99A-pHM1 virulence did not increase on the population as compared to the founders, suggesting that IR45427-2B-2-2B-1-1, the most susceptible parent to PXO99A-pHM1, already contains all of the susceptibility QTL alleles to PXO99A-pHM1 present in the MAGIC population. Given that four of the five significantly different responses of the founder lines to the strains were increased susceptibility to PXO99A-pHM1-*tal7b* over PXO99A-pHM1 during the population screen, we conclude that Tal7b is indeed a virulence-enhancing factor.

MAGIC genotypes

After filtering, 14,561 SNP markers remained to cover the rice genome, with a marker density of one SNP marker per 25.6 kb (Figure 2). This estimate is based on release 7 of the

MSU Rice Genome, which does contain gaps and covers about 373 Mb of the estimated 430 Mb genome. Estimated LD decay of this population averages around 250 kb resulting in about 10 markers per LD block. Our markers were overall dense enough to detect most QTL associations. SNP markers were named descriptively of genomic location, i.e. the number following S is the chromosome number followed by the bp coordinate of the SNP in the MSU7 Nipponbare reference genome.

Marker analyses reveals genomic regions involved in PXO99A-pHM1 +/- TAL7b resistance

A major advantage of MAGIC populations is that both Genome Wide Association Studies (GWAS) and Interval Mapping (IM) can be performed because there should be little population structure from three intercrossing events and diverse founder lines. However, we tested for population structure that might confound our GWAS results by conducting Principle Component Analysis on the genotype data (data not shown). The Principal Component 1 for 2.6% of all variance in the population. Although PC1 was very small, the QQplot of the General Linear Model (GLM) revealed an exceptionally large divergence of expected p-values to observed, indicating that population structure was affecting association results (Figure 3). Consequently, a Mixed Linear Model (MLM), incorporating population structure through a kinship matrix calculated from the SNP genotypes, was run, and the QQplot of this analysis showed that most p-values adhered to the normal distribution except for those with very small p-values. A False Discovery Rate of 5% (q-value < 0.05) was applied to the p-values to further control false positive associations.

PXO99A-pHM1 reveals resistance QTL in the MAGIC population

Mixed linear model GWAS revealed just two QTL (on Chr 5 and 11) for PXO99A resistance, see Figure 3 and Table 5, while interval mapping detected six QTL (on Chr 1, 5, 7 10,

11 and 12), see Table 6. The Chr 5 regions from GWAS and interval mapping overlapped, but the Chr 11 regions were different. Interval mapping appeared to have more power to detect significant QTL than GWAS in this study. All IM QTL contained markers that were significant at the p-value 0.001 level in either the MLM or GLM GWAS, except the Chr 1 QTL marker that had a MLM p-value of 0.08 (see Table 6 for summary). Bandillo et al. (2013) also detected the Chr 1 (IM), Chr 5 (IM and GWAS), Chr 11 (GWAS) and Chr 12 (IM) QTL during the screening of the *indica* MAGIC S_4 population for PXO99A resistance. The Chr 5 and 10 QTL were also detected for PXO99A-pHM1-*tal7b* interval mapping. Detection of the same QTL in different subpopulations of the *indica* MAGIC population and in different environments substantiate the presence of important resistance genes to PXO99A in the MAGIC population and the efficacy performing mapping analyses on as little as 330 AILs from the MAGIC population for disease resistance.

The Chr 5 QTL was most significantly associated region to PXO99A-pHM1 resistance in both GWAS (p-value < 0.0001) and interval mapping (p-value < 0.0001). GWAS detected a larger region of significant markers, spanning 1.2 Mb from the beginning of Chr 5 to S5_1224178, than the QTL detected in IM which spanned 0.194 Mb between markers S5_347328 - S5_542193. LD decays in approximately 0.37 Mb around the Chr 5 markers, indicating that more than one QTL may exist in the 1.2 Mb region. The markers of the IM QTL directly flank the *xa5* locus, although *xa5* is not considered an effective resistance gene to PXO99A-pHM1. Finer mapping of this region will help resolve if *xa5* or yet unknown resistance gene is the source of PXO99A.

Chromosome 11 contained two QTL, one detected in GWAS at S11_28483987 and another at the IM QTL between S11_17230702 - S11_17352369. The GWAS resistance allele

was present in all parents except Samba Mahsuri-*sub1* whereas Sanhuangzhan-2 contributed the greatest founder resistance effect in the IM analysis. The distance between the two QTL as well as the different parental donars strongly support that these QTL are independent of each other. The presence of two QTL on Chr 11 is not surprising given the high number of resistance genes on this chromosome (Ronald et al. 1992; Sun et al. 2004; Khush et al. 2006; Zheng et al. 2009; Hur et al. 2013; Wang et al. 2014a; Zhang et al. 2014; Wang et al. 2014b; Horgan and Henderson 2015; Kim et al. 2015). The Chr 11 GWAS marker is near a cluster of *Xoo* resistance genes, containing *Xa4*, *Xa3/Xa26*, *Xa22(t)* and *Xa40(t),* summarized in Table 7 (Niño-Liu et al. 2006; Xiang et al. 2006; Kim et al. 2015). The LD decay for Chr 11 in this population is approximately 1.4 Mb and several interesting candidate genes are near the Chr 11 IM QTL, see Table 8 for a summary. LOC_Os11g31540 is within 1 Mb of the Chr 11 IM QTL and shares 74% identity with the sorghum lead (Pb) transport gene, *SbLRR2* (Zhu et al. 2013). Overexpression of *SbLRR2* prevented lead accumulation in the plant and resulted in the activation of a lead detoxification pathway in arabidopsis. The lead detoxification pathway may be a target of TAL effectors given that a known TAL target, *Xa13,* is implicated in promoting pathogen growth by removing copper from xylem vessels (Yuan et al. 2010) and that LOC_Os11g31540 is differentially regulated upon PXO99A expression (Cernadas et al. 2014). Also within 850 kb of Chr 11 IM QTL is *OsSWEET14,* another nodulin MtN3 family protein and the target of TAL effectors AvrXa7 and PthXo3. While PXO99A does not contain either *avrXa7* or *pthXo3,* nor does it induce the expression of *OsSWEET14* (Antony et al. 2010; Streubel et al. 2013), the resistance allele of *OsSWEET14*, *xa41* (Hutin et al. 2015b), may confer resistance against PXO99A by an uncharacterized mechanism. About 2 Mb from the Chr 11 IM QTL is LOC_Os11g26790, a

dehydrin gene and the putative target of many TAL effectors, including some produced by PXO99A (Pérez-Quintero et al. 2013; Wilkins et al. 2015).

Both interesting and perplexing, are the candidate genes near the Chr 1 QTL revealed in the screen with PXO99A-pHM1. Within the marker interval of the Chr 1 QTL is *OsSBP,* a selenium binding protein that increases resistance to *Xoo* in overexpression rice lines (Sawada et al. 2004). The Chr 1 QTL is also 2 Mb from the target gene of PXO99A TAL effector PthXo7, *TF11Aγ-1*. Of particular interest for the QTL is LOC_Os01g68740, a keratin, type I cytoskeletal 9 gene that is the putative target of the PXO99A TAL (TAL7a) that shares an identical RVD sequence with TAL7b (Pérez-Quintero et al. 2013), see Table 1. This gene contains the top predicted EBE for TAL7b RVD and is upregulated in susceptible plants upon inoculation with PXO99A (Moscou and Bogdanove 2009). Peculiarly, this QTL does not appear in association with PXO99A-pHM1-*tal7b.*

The Chr 7 and 12 QTL were detected in previous pathogen resistance mapping studies in the *indica* MAGIC population (Bandillo et al. 2013) and the Chr 10 QTL was also detected in PXO99A-pHM1-*tal7b* resistance. No known resistance or susceptibility genes are near the Chr 12 QTL although the IM QTL contains 11 genes with disease resistance classifications, three NB-ARCs and three NBS-LRRs. The Chr 10 QTL is 700 kb from two genes that were not induced by PXO99A infection, but were implicated in host redox control by *Xoc* strain BLS256 (Cernadas et al. 2014). Although not induced by PXO99A, these genes may still play a role in PXO99A resistance and infection through other regulation mechanisms besides transcriptional activation. Within the Chr 7 QTL is LOC_Os07g47790, the putative target of *Xoc* TAL effectors; however, this gene did not appear to be involved in PXO99A resistance (Moscou and Bogdanove 2009; Pérez-Quintero et al. 2013).The stronger candidate gene is *SPIN6*, a player in rice innate

immunity (Liu et al. 2015) because the Chr 7 QTL was associated with rice blast resistance in the previous MAGIC study (Bandillo et al. 2013). Future work would shed light on which, if any, of the genes in these three genomic regions confer resistance to a broad spectrum of rice pathogens. It is possible that the already identified candidate genes may control resistance to PXO99A in addition to rice blast and BLS256, or there may be several distinct resistance genes clustered near these QTL.

PXO99A-pHM1-tal7b *reveals resistance QTL distinct from PXO99A-pHM1*

GWAS and interval mapping detected a total seven QTL for PXO99A-pHM1-*tal7b* resistance in the MAGIC population. The Chr 5 and 10 QTL overlapped with the PXO99A-pHM1 QTL, but all others were unique to PXO99A-pHM1-*tal7b* as indicated by distance and the parental resistance allele contributor (Tables 9). Interval mapping identified six QTL on chromosomes 3, 5, 8, 10, 11 and 12 (Table 10). Similar to PXO99A-pHM1, fewer QTL were detected in the GWAS analysis than interval mapping. The MLM method of GWAS for PXO99A-pHM1-*tabl7b* detected significant markers (q-value < 0.05) in the Chr 5 and 8 interval mapping QTL, but a unique Chr 12 region. Also similar to PXO99A-pHM1 mapping, markers within the IM QTL of the remaining QTL were almost significant in either the MLM or GLM GWAS methods, see Table 10 for corroboration of IM with GWAS.

Two QTL for PXO99A-pHM1-*tal7b* resistance were detected on Chr 12. Interval mapping detected a QTL spanning the markers S12_19452481- S12_19836912 whereas the GWAS detected significant markers 3 Mb away at S12_23092043 and S12_23120151. Given that different parents contributed the resistance alleles along with the distance, we conclude that two QTL exist on Chr 12. The IM Chr 12 QTL is 2 Mb from *Os12N3/OsSWEET13*, a nodulin MtN3 family protein gene that is the susceptibility target of *Xoo* TAL effector PthXo2 (Liu et al.

2011; Cernadas et al. 2014; Zhou et al. 2015). *xa25* is the recessive resistance allele of *OsSWEET13* conferred by a SNP mutation in the promoter (Liu et al. 2011). Within 456 kb of the GWAS marker S12_23092043 is a calmodulin binding protein that was shown to be upregulated during PXO99A infection (Cernadas et al. 2014) and within 348 kb of S12_23120151 is *SPL11* (Tables 11 and 12 summarize candidate genes near the GWAS and IM QTL, respectively). Strikingly, SPL11 modulates rice resistance to *Xoo* and rice blast through ubiquitination of the immunity negative regulator SPIN6 (Liu et al. 2015) . *SPIN6* is a PXO99A-pHM1 Chr 7 candidate gene identified in this study. The markers near the Chr 7 QTL were detected for rice blast resistance in previous screening of the MAGIC population with rice blast (Bandillo et al. 2013), supporting the importance of this QTL in plant immunity signaling and suggesting that TAL7b may target this signaling pathway to suppress host immune response.

The Chr 3 and 10 QTL contained GWAS markers with p-value < 0.005 and 0.07, respectively. The Chr 3 QTL contained LOC_Os03g07540, a bHLH family protein that is the putative target TAL3c of *X. oryzae* pv. *oryzicola* (*Xoc*) strain BLS256 (Cernadas et al. 2014), but does contain a highly ranked EBE for TAL7b. bHLH proteins are a superfamily of transcription factors implicated in susceptibility and resistance (Kim et al. 2012; Muñoz Bodnar et al. 2013). For example, *upa20*, a bHLH gene targeted by TAL effector AvrxBs3 of *X. campestris* pv. *vesicatoria*, regulates host cell size genes (Kay et al. 2007). Though TAL7b likely does not target this locus, a non-genetic interaction may be important for TAL7b resistance.

The Chr 8 QTL contains the markers detected in GWAS and was also donated by the same parent as designated in GWAS, Sanhuangzhan-2. Within 1.4 Mb of the Chr 8 QTL is *OsSerk1* (LOC_Os08g07760) a brassinosteriod LRR-RLK. This gene is upregulated upon *Xoo* infection, is coexpressed with the broad spectrum HR gene *Xa39* (Zhang et al. 2015), and,

interestingly, contains EBEs for several TAL effectors including TAL7b. The role of *OsSerk1* in plant immunity is unclear, but this gene appears to be important in *Xoo* infection and future expression and silencing studies will shed light on *OsSerk1's* role in TAL mediated resistance.

The Chr 11 PXO99A-pHM1-*tal7b* QTL is 1.7 Mb upstream from the PXO99A QTL. The PXO99A-pHM1-*tal7b* Chr11 QTL contains LOC_Os11g26790, a dehydrin gene putatively targeted by multiple TAL effectors in *Xoo* and *Xoc* (Pérez-Quintero et al. 2013). This gene is targeted by TAL6a and Avrxa23 of PXO99A and two TAL effectors from MAFF311018 (Pérez-Quintero et al. 2013). The TAL effectors have unique EBE elements within this gene and the gene was upregulated between 3 and 5 fold upon inoculation of the given strains (Pérez-Quintero et al. 2013). This gene is also a candidate target of TALs in nine *Xoc* strains that are similar to TAL2g of *Xoc* strain BLS256 (Wilkins et al. 2015). Although this gene is undoubtedly crucial to *Xoo* and *Xoc* infection, it is unclear whether this QTL is truly unique to TAL7b given that two other PXO99A TALs are predicted to target this gene. TAL Effector-Nucleotide Targeter 2.0 does not rank LOC_Os11g26790 highly as a predicted target of TAL7b, i.e., it is ranked as # 95,795 (Doyle et al. 2012). While LOC_Os11g26790 may not be the direct target of TAL7b, this function of this gene or the ability of TALs to change its regulation may be dependent on TAL7b function. Another putative target of TAL7b is the keratin gene within the Chr 1 QTL detected for PXO99A-pHM1 resistance. Both keratins and dehydrins play roles in drought stress tolerance in plants (Yang et al. 2012; Hanin et al. 2011), but their roles in plant-pathogen interactions remain uncertain.

Screening the MAGIC population for TAL7b resistance genes yielded five unique QTL. Although the regions were large, interesting genes fell within or near the marker interval. Some of the candidate genes were susceptibility genes, indicating that recessive resistance may exist

against TAL7b. Fine mapping of these QTL along with expression studies will help clarify which genes near the QTL are involved in TAL7b resistance and resolve the role TAL7b plays in manipulating the host genome.

Interacting QTL

To test for interactions among the QTL, a linear model of the most signficant markers was run in SAS. The markers were set as interaction terms and each interaction term was within an independent model. None of the interaction terms were signficant at the 0.05 level. However, interactions are difficult to detect even in scenarios with relatively high power (Tian et al. 2011). These results did not identify interacting QTL, though true interactions may still exist.

Founder line PSBRc82, known to carry the resistance genes *Xa4* on Chr 11 and *xa5* on Chr 5, donated the Chr 5 resistant alleles and was one of seven parents that carried the resistant Chr 11 allele. All 32 AILs with PXO99A lesion lengths < 10 cm had the resistant Chr 11 marker. All 11 AILs with lesion lengths < 8.1 cm and eight of 21 AILs with lesion lengths between 8.2 and 10 cm had the Chr 5 resistant markers. The combination of the two QTL resulted in greater resistance, as the most resistant lines (LL < 8 cm) had both the Chr 5 and Chr 11 markers. This is consistent with previous work that demonstrated pyramiding of *Xa4* and *xa5* results in stronger resistance (Suh et al. 2013).

Conclusions

The *indica* MAGIC population was more susceptible to PXO99A when carrying the plasmid borne *tal7b* gene, confirming our hypothesis that TAL7b is a virulence enhancing factor. Additionally, unique and significantly different phenotypes and QTL were observed between PXO99A-pHM1 and PXO99A-pHM1-*tal7b,* indicating that novel TAL effectors can be characterized in strains containing TALs with identical RVD sequences. Seven and six QTL were detected in the MAGIC population for PXO99A-pHM1 and PXO99A-pHM1-*tal7b* resistance, respectively. Almost all of the QTL contained or were near a gene involved in *Xoo* or *Xoc* disease susceptibility or near markers previously associated with disease resistance, corroborating the legitimacy of our QTL. By using a MAGIC population for QTL study we observed the background dependent nature of many of the QTL, and future studies with these lines may reveal the molecular basis of how host genetic backgrounds can influence function of resistance alleles.

The identification of regions harboring *xa5* and *Xa4* as being most significant QTL to PXO99A-pHM1 resistance was surprising, as neither of these R genes is considered an effective resistance gene to PXO99A. However, although PXO99A virulence decreases slightly in the presence of *xa5,* PXO99A is one of the few *Xoo* strains to cause disease on *xa5* rice lines (Mew 1987). Likely, PXO99A's virulence on *xa5* is due to the activity its TAL effector PthXo7. PthXo7 increases the transcription of a TFIIAγ homolog on Chr 1 and restores virulence to *xa5* of other strains when transgenically introduced (Sugio et al. 2007). Interestingly, PXO99A TAL Avrxa27 recruits TFIIAγ-5 (Xa5) to activate *Xa27* which leads to a strong HR response and lines

homozygous for *xa5* fail to activate *Xa27* (Gu et al. 2009). Therefore, the fitness cost to PXO99A by *xa5* appears to be necessary to avoid *Xa27.*

Screening the MAGIC population with *Xoo* strains revealed new sources of resistance to PXO99A and helped to characterize the function of TAL7b. This method is useful for initial characterization of novel TAL effectors and locating existing resistance in widely used cultivars. Although no major effect QTL were identified for TAL7b or PXO99A, we now have genomic regions to explore as potential sources of quantitative resistance to this common virulence TAL effector. Future studies may scan more diverse germplasm such as a collection of landraces to find existing strong resistance alleles or alternatively, expression studies may identify the precise susceptibility gene(s) involved in TAL7b virulence that would enable EBE editing to create a resistant allele of this TAL's target. Rice provides a model to which downstream recognition of infection through changes in the susceptibility targets may offer another avenue of resistance engineering. Many of the QTL identified in this study are near known TAL targets, indicating that MAGIC interval mapping is a useful tool in identifying genomic regions containing TAL targets for susceptibility in addition to resistance genes. Furthermore, this method is contingent on naturally occurring contrasting alleles so any regions identified must contain a resistance allele. Susceptibility alleles turned to resistance alleles offer theoretically more durable resistance compared to ligand receptor mediated resistance.

Figures

Table 1: TAL7b is a common virulence effector in sequenced *Xoo* strains. The RVDs of PXO86 TAL7b, PXO99A TAL7a and TAL8a, and a C8 TAL are identical. KACC10331 and MAFF311018 also contain TALs with similar RVDs to TAL7b.

Strain	TAL (TAL accession number)	Repeat Variable Diresidue (RVD) Sequence																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
PXO86	TAL7b	NI	HG	NI	NI	NI	NN	HD	NS	NN	NS	NN	HD	NN	NI	HD	NN	NS	NG		
PXO99A	TAL7a/TAL8a (ACD59223.1)	NI	HG	NI	NI	NI	NN	HD	NS	NN	NS	NN	HD	NN	NI	HD	NN	NS	NG		
C8	(ACD11364.1)	NI	HG	NI	NI	NI	NN	HD	NS	NN	NS	NN	HD	NN	NI	HD	NN	NS	NG		
KACC10331	(AAW75382.1)	NI	HG	NI	NI	NI	NN	HD	NS	NN	NS	NN	HD	NN	NI	HD	NN	**NI**	NG	**HD**	**N***
KACC10331	(YP_200767.1)	NI	HG	NI	NI	NI	NN	HD	NS	NN	NS	NN	HD	NN	NI	HD	NN	**NI**	NG	**HD**	**NG**
MAFF311018	(YP_451027.1)	NI	HG	NI	NI	NI	NN	HD	NS	NN	NS	NN	HD	NN	NI	HD	NN	**NI**	NG	**HD**	**NG**

Table 2: Plasmids and strains in this study.

Strain	Species	Relevant Characteristics
PXO86	*Xanthomonas oryzae* pv *oryzae*	Philippine Race 2, donor of TAL7b
PXO99A	*Xanthomonas oryzae* pv *oryzae*	Philippine Race 6, carrier of TAL7a and TAL8a
PXO99A-pHM1	*Xanthomonas oryzae* pv *oryzae*	PXO99A control strain with empty vector pHM1
PXO99A-pHM1-*TAL7b*	*Xanthomonas oryzae* pv *oryzae*	PXO99A carrying pHM1 borne TAL7b
PXO99A-pKEB31-*TAL7b*	*Xanthomonas oryzae* pv *oryzae*	PXO99A carrying pKEB31 borne TAL7b
X11-5A	*Xanthomonas oryzae*	Weakly virulent United States strain, no TAL effectors
C8	*Xanthomonas oryzae* pv *oryzae*	Chinese strain
KACC10331	*Xanthomonas oryzae* pv *oryzae*	Korean sequenced strain
MAFF311018	*Xanthomonas oryzae* pv *oryzae*	Japanese sequenced strain
BLS256	*Xanthomonas oryzae* pv *oryzicola*	Philippine sequenced strain
Plasmids		
pHM1		Cosmid vector, Broad-host range, derivative of pRI40, low copy
pKEB31		Addgene plasmid 31224, high copy

Table 3: MAGIC parents have differential levels of resistance to PXO99A-pHM1 and PXO99A-pHM1-*tal7b*. Reported LL are simple average lesion lengths in cm. * p-value <0.0001. [1] PSBRc158 was not inoculated with PXO99A due to poor germination.

	Preliminary Screen on MAGIC parents			**Screen with MAGIC population**		
	PXO99A LL (cm)	PXO99A-pHM1-*tal7b* LL (cm)	p-value	PXO99A LL (cm)	PXO99A-pHM1-*tal7b* LL (cm)	p-value
Fedearroz 50	13.6	13.2	0.682	12.0	18.4	**0.002**
IR45427-2B-2-2B-1-1	17.7	17.8	0.959	22.8	19.4	**0.098**
IR4630-22-2-5-	7.1	7.6	0.720	11.3	19.2	**0.000***
IR77298-14-1-2-10	11.6	13.5	**0.083**	11.8	16.2	**0.013**
PSBRc82	6.3	7.6	0.242	10.8	13.2	0.195
PSBRc158	15.8	15.5	0.837	ND[1]	11.5	ND
Sanhuangzhan-2	14.0	11.7	**0.069**	11.3	13.8	**0.022**
Samba Mahsuri-*sub1*	13.4	12.6	0.515	9.8	13.5	0.291

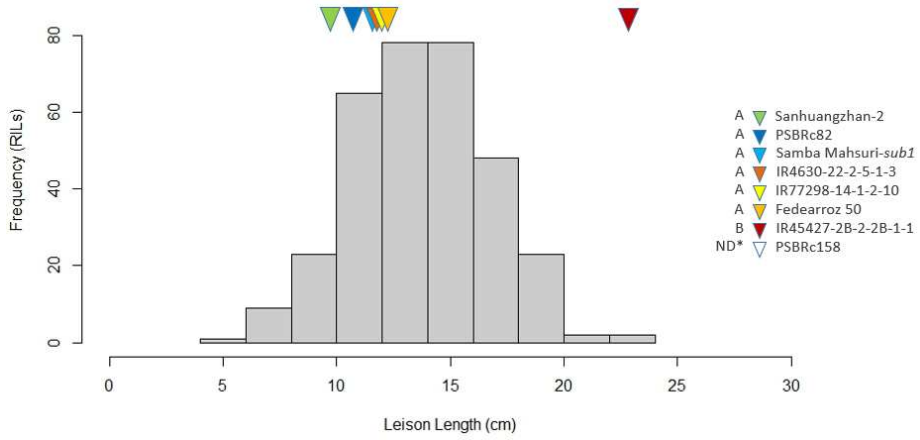
Table 4: Mixed model ANOVA for the MAGIC population response to PXO99A-pHM1 +/- *tal7b*. p-values of significant differences in variance show that the MAGIC population (including parents and AILs) are significantly different in resistance response. Additionally, the two strains, PXO99A and PXO99A-pHM1-*tal7b* are significantly different in virulence on the MAGIC lines. Finally, of importance to environmental effect, the 3 replications as well as the blocks with the complete random block design are significantly different.

Type 3 Analysis of Variance								
Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
MAGIC AILs	338	18693	55.31	Var(Residual) + Q(Line, Line*Strain)	MS(Residual)	1251	4.20	<.0001
Strain	1	5277.37	5277.37	Var(Residual) + Q(Strain, Line*Strain)	MS(Residual)	1251	400.40	<.0001
AILs * Strain	336	3995.45	11.89	Var(Residual) + Q(Line*Strain)	MS(Residual)	1251	0.90	0.8760
Rep	2	8566.14	4283.07	Var(Residual) + 206.62 Var(Block(Rep)) + 619.86 Var(Rep)	1.48 MS(Block(Rep)) - 0.48 MS(Residual)	5.73	15.94	0.0046
Block(Rep)	6	1117.18	186.12	Var(Residual) + 139.89 Var(Block(Rep))	MS(Residual)	1251	14.13	<.0001
Residual	1251	16488	13.18	Var(Residual)	.	.	.	.

A Comparison of MAGIC population response to *Xoo* PXO99A-pHM1 +/- *tabl7b*

Strain	Average Lesion Length (cm)	Minimum and maximum (cm)	# of resistant AILs (LL < 5.0 cm)	# of moderately resistant AILs (LL < 10.0 cm)	# of susceptible AILs (LL > 20.0 cm)
PXO99A-pHM1	13.8	4.4 - 22.9	1	34	4
PXO99A-pHM1-*tal7b*	17.1	3.2 – 26	1	10	81

B MAGIC population and parental response to *Xoo* PXO99A-pHM1



C MAGIC population and parental response to *Xoo* PXO99A-pHM1-*tal7b*

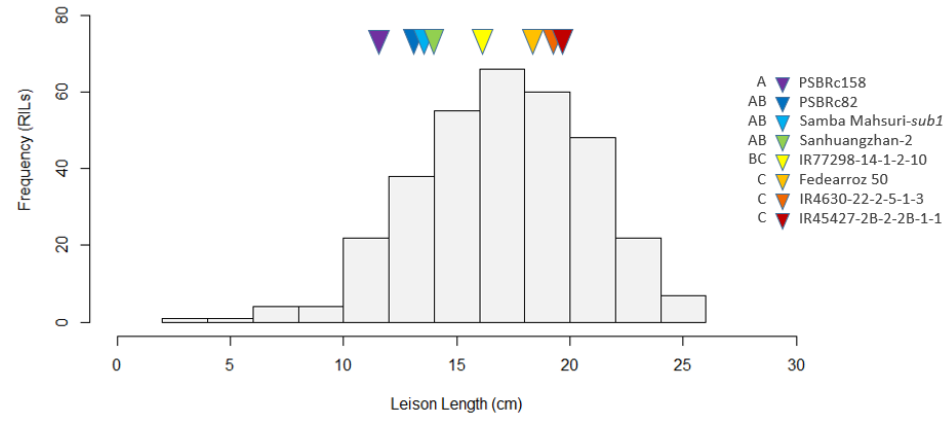


Figure 1: TAL7b is a virulence effector. (A) Disease, measured in lesion lengths (cm), increased with the addition *tal7b* to *Xoo* PXO99A. Average lesion length was calculated with LSMeans. (B, C) Distribution of lesion lengths in the MAGIC population with parental lesion lengths indicated with downward arrows. Connecting letters report of significantly different parental responses to the right of the downward arrows in legend

A

	# Markers	Chr size (Mb)	Marker/Mb	Average distance between markers (bp)
Chr 1	1878	43.3	43.4	23,056
Chr 2	1976	35.9	55.0	18,168
Chr 3	1079	36.4	29.6	33,735
Chr 4	1585	35.5	44.7	22,397
Chr 5	706	30.0	23.5	42,492
Chr 6	1035	31.2	33.2	30,144
Chr 7	1094	29.7	36.8	27,148
Chr 8	1178	28.4	41.5	24,109
Chr 9	728	23.0	31.7	35,593
Chr 10	787	23.2	33.9	29,479
Chr 11	988	29.0	34.1	29,352
Chr 12	1527	27.5	55.5	18,009
Overall	14,561	373.1	39.0	25,623

B

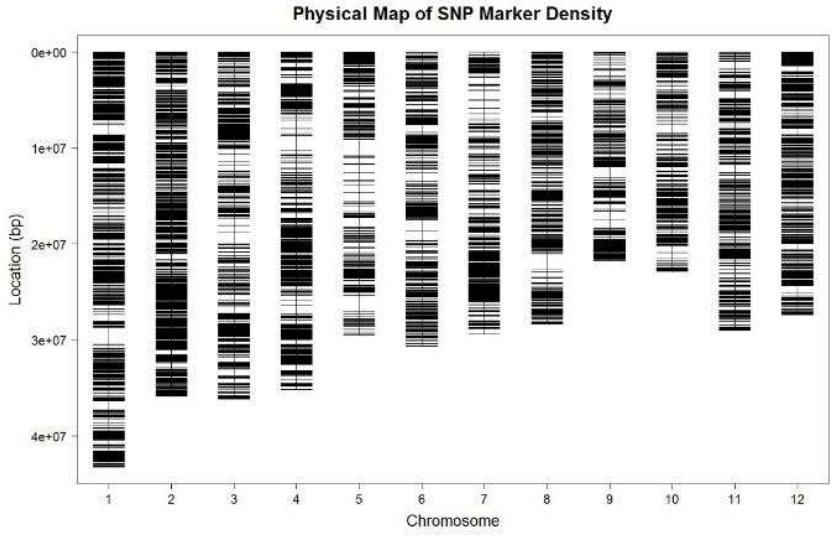
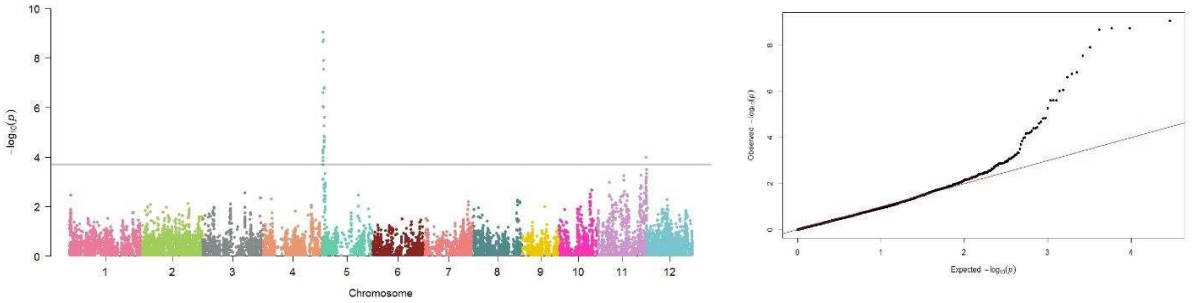
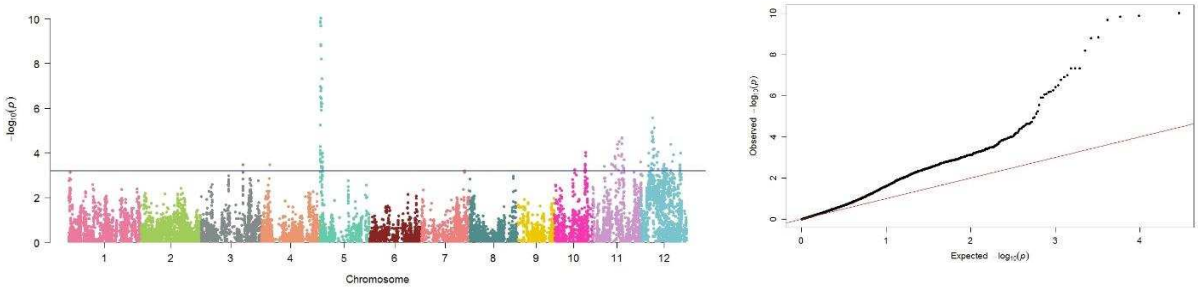


Figure 2: SNP Marker Density. A total of 14,561 SNP markers were retained after filtering. This SNP density averaged to approximately 1 SNP per 25.6 kb across the rice genome. With the overall estimated linkage disequilibrium for the MAGIC population at 250 kb (Bandillo et al. 2013), we expect nearly 10 SNPs per linkage block. Chromosome size estimates are from MSU Rice Genome Release 7. Gaps in reference genome account for missing genome size.

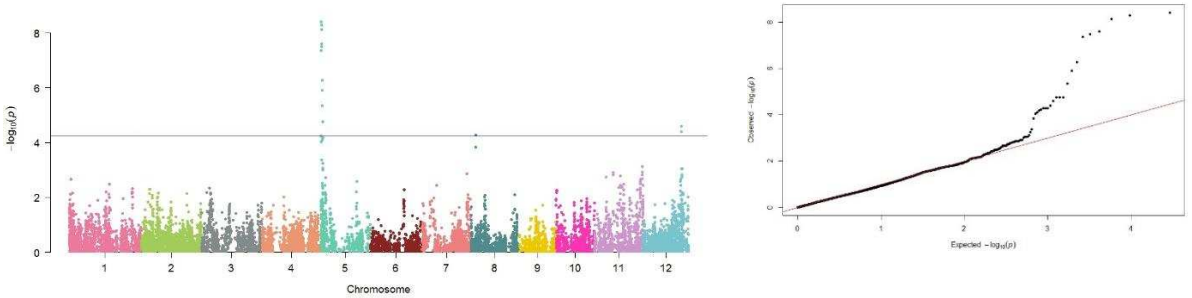
A PXO99A-pHM1 (MLM)



B PXO99A-pHM1 (GLM)



C PXO99A-pHM1-*tal7b* (MLM)



D PXO99A-pHM1-*tal7b* (GLM)

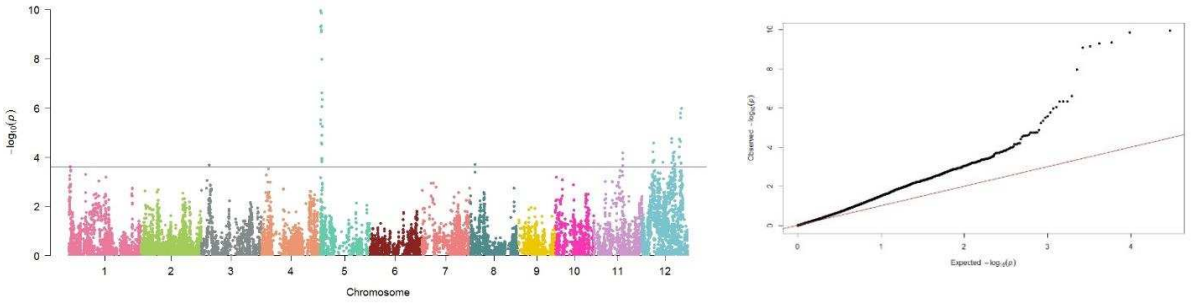


Figure 3: GWAS results of SNPs associated with *Xoo* strains PXO99A-pHM1 and PXO99A-pHM1-*tal7b* disease. Horizontal line represents significance cutoff of q-value < 0.05. Manhattan plots (right) and corresponding QQ-plots (left) for Mixed Linear Models (kinship matrix) and General Linear Models (no population structure control). QQ-plots, representing fitness of the models, indicate that GLMs overestimate significant associations.

Table 5: GWAS markers significantly associated with *Xoo* PXO99A-pHM1 resistance. The R^2 estimates the percent of variance in PXO99A-pHM1 disease due to the given marker. Effect Estimate compares average lesion lengths of AILs with the contrasting SNP allele; negative effects correspond to a decrease in lesion length and therefore resistance. Effect Allele/Null gives the SNP allele associated with the effect estimate. AILs with Effect allele/Null gives the number or AILs for each allele. The most significant SNP in a region is in bold.

Chr	Marker (Chr_Pos)	p-value	q-value	R^2	Effect Estimate (cm)	Effect Allele/Null	AILs with Effect allele/Null
5	S5_37878	6.40×10^{-5}	3.59×10^{-2}	5.84	-1.75060	A/G	211/70
	S5_69302	5.20×10^{-5}	3.29×10^{-2}	5.55	-1.75400	C/T	228/70
	S5_103237	2.42×10^{-7}	3.92×10^{-4}	9.41	-2.37150	C/T	59/238
	S5_179519	5.79×10^{-5}	3.51×10^{-2}	5.57	1.99713	A/C	46/255
	S5_196176	6.36×10^{-5}	3.59×10^{-2}	5.53	1.96248	A/G	47/255
	S5_219803	1.04×10^{-4}	5.24×10^{-2}	5.21	1.91462	G/T	47/253
	S5_227187	**8.76×10^{-10}**	**7.78×10^{-6}**	**13.12**	**-3.63670**	**A/C**	**33/270**
	S5_269480	2.01×10^{-4}	9.46×10^{-2}	5.32	-1.94530	A/G	222/41
	S5_285834	2.14×10^{-9}	7.78×10^{-6}	12.47	-3.68220	A/G	30/277
	S5_312457	1.40×10^{-4}	6.81×10^{-2}	5.01	-1.85890	A/C	252/48
	S5_340482	6.82×10^{-5}	3.68×10^{-2}	5.76	-1.60470	C/T	80/194
	S5_347328	6.82×10^{-5}	6.82×10^{-5}	8.28	2.25709	A/G	239/60
	S5_353165	1.83×10^{-9}	7.78×10^{-6}	12.48	3.46202	C/T	269/35
	S5_365871	9.48×10^{-7}	1.30×10^{-3}	9.28	-2.35270	A/G	52/218
	S5_440644	2.78×10^{-8}	6.74×10^{-5}	10.88	-3.32040	A/G	33/260
	S5_453169	1.83×10^{-9}	7.78×10^{-6}	12.41	-3.64740	A/G	32/276
	S5_574926	1.23×10^{-8}	3.59×10^{-5}	11.36	3.30109	C/T	264/35
	S5_704336	2.11×10^{-5}	1.71×10^{-2}	6.33	1.93800	A/G	230/67
	S5_759048	1.45×10^{-5}	1.29×10^{-2}	6.44	-1.97890	C/T	65/227
	S5_761061	3.61×10^{-5}	2.63×10^{-2}	5.90	1.64879	C/T	191/102
	S5_761063	4.03×10^{-5}	2.67×10^{-2}	5.82	-1.63510	C/T	103/191
	S5_761076	4.03×10^{-5}	2.67×10^{-2}	5.82	1.63509	C/T	191/103
	S5_849335	5.42×10^{-6}	5.26×10^{-3}	7.27	2.95058	C/T	280/25
	S5_850180	1.52×10^{-7}	3.16×10^{-4}	9.47	-3.14340	C/G	32/272
	S5_904372	2.43×10^{-5}	1.86×10^{-2}	6.26	2.01421	G/T	242/50
	S5_934093	1.74×10^{-7}	3.16×10^{-4}	11.55	-3.43520	A/T	26/228
	S5_1200961	2.42×10^{-7}	3.92×10^{-4}	7.44	-2.72030	A/G	34/274
	S5_1200964	2.49×10^{-6}	2.59×10^{-3}	7.44	-2.72030	C/G	34/274
	S5_1200971	2.49×10^{-6}	2.59×10^{-3}	7.44	2.72033	G/T	274/34
	S5_1224178	2.49×10^{-6}	2.59×10^{-3}	6.42	-2.46180	A/G	33/275
11	**S11_28483987**	**1.02×10^{-4}**	**5.42×10^{-2}**	**5.61**	**-3.04360**	**C/T**	**267/16**

Table 6: QTL mapping intervals (95% Confidence Intervals) significantly associated with *Xoo* PXO99A-pHM1 resistance. Parental sources of resistance report the parent with the largest resistance effect for the given QTL. Corroboration compares the given QTL to the GWAS analysis or previous mapping studies.

Chr	QTL position	Markers flanking the QTL (size)	p-value	Parental source of resistance	Corroboration
1	S1_38949958	S1_37958733 - S1_40897952 (2.9 Mb)	5.97 e-04	Samba Mahsuri-*sub1*, PSBRc82 and Sanhuangzhan-2	Almost significant in GWAS MLM (p-value 0.08)
5	S5_361080	S5_347328 - S5_542193 (194 kb)	1.83 e-10	PSBRc82	Highly significant in GWAS MLM (p-value 8.76×10^{-10})
7	S7_28335793	S7_28223058 - S7_28741818 (519 kb)	7.30 e-04	Samba Mahsuri-*sub1* and PSBRc82	Almost significant in GWAS MLM (p-value 0.07) and significant in GWAS GLM (p-value <0.001). Blast resistance association at 27Mb (Bandillo et al. 2013)
10	S10_19832491	S10_19621750 - S10_19916740 (295 kb)	1.41 e-05	Fedearroz 50	Almost significant in GWAS MLM (p-value 0.07) and significant in GWAS GLM (p-value <0.0001).
11	S11_17316445	S11_17230702 - S11_17352369 (122 kb)	2.06 e-05	Sanhuangzhan-2	Significant at the 0.005 p-value level for GWAS MLM.
12	S12_6733540	S12_5033870 - S12_6913707 (1.9 Mb)	8.44 e-05	IR.4630.22.2.5.1.3	Almost significant in GWAS MLM (p-value 0.05) and significant in GWAS GLM (p-value <0.0001). Bandillo et al. 2013 detected 29 markers on Chr 12 between 5-14 Mb.

Table 7: Candidate resistance genes near GWAS marker for *Xoo* PXO99A-pHM1 resistance.

Marker	**Candidate genes** Gene name (locus identity, if applicable),	Distance from Marker	Citation
S11_28483987	*Xa40(t)* (LOC_Os11g46900)	303 kb	Kim et al. 2015
	Xa3/Xa26/Xa6/Xa9/Xaw (LOC_Os11g47210)	81 kb	Niño-Liu et al. 2006
	Xa4 and Xa22(t) are linked to *Xa3*		Niño-Liu et al. 2006; Kim et al. 2015

Table 8: Candidate genes within or near interval mapping QTL for *Xoo* PXO99A-pHM1 resistance.

QTL	**Candidate genes** Locus identity (gene name, if applicable), annotation	Distance from QTL	Importance	Citation
S1_38949958	LOC_Os01g68740, keratin, type I cytoskeletal 9, putative, expressed	Within	Putative target of PXO99A TAL7a (homolog of TAL7b)	Moscou & Bogdanove 2009
	LOC_Os01g65880 (*OsSWEET1a)* nodulin MtN3 family protein, putative, expressed	Within	Similar to known susceptibility genes, but not shown to be a target	Streubel et al. 2013
	LOC_Os01g68770 (*OsSBP*) selenium-binding protein, putative, expressed	Within	Rice *OsSBP* overexpression lines show increased resistance to *Xoo*	Sawada et al. 2004
S5_361080	*Xa5/xa5*	Within	Known *Xoo* resistance gene	Iyer & McCouch 2004

S7_28335793	LOC_Os07g47790 AP2 domain containing protein	Within	Putative target of TAL3c and TAL6 of *Xoc* strain BLS256	Moscou & Bogdanove 2009; Pérez-Quintero et al. 2013
	LOC_Os07g46450 (*SPIN6*) pleckstrin homology domain-containing protein, putative, expressed	1 Mb	Works with SPL11 to negatively regulate innate immunity in rice	Liu et al. 2015
S10_19832491	LOC_Os10g38590 and LOC_Os10g38640 glutathione S-transferase, N-terminal domain containing protein, expressed	700 kb	Implicated in redox control by BLS256, but was not shown to be induced by PXO99A	Cernadas et al. 2014
S11_17316445	LOC_Os11g31190 (*OsSWEET14/xa41)* nodulin MtN3 family protein, putative, expressed	850 kb	Target of TAL effectors AvrXa7 of PXO86 and TalC of BAI3	Römer et al. 2010; Yu et al. 2011
	LOC_Os11g31540 BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative, expressed	1 Mb	Differentially expressed in response to PXO99A inoculation Homologous to *SbLRR2*, a lead transporter in sorghum	Zhu et al. 2013; Cernadas et al. 2014

Table 9: GWAS markers significantly associated with *Xoo* PXO99A-pHM1-*tal7b* resistance. The R^2 estimates the percent of variance in *Xoo* PXO99A-pHM1-*tal7b* disease due to the given marker. Effect Estimate compares average lesion lengths of AILs with the contrasting SNP allele; negative effects correspond to a decrease in lesion length and therefore resistance. Effect Allele/Null gives the SNP allele associated with the effect estimate. AILs with Effect allele/Null gives the number or AILs for each allele. The most significant SNP in a region is in bold.

Chr	Marker (Chr_Pos)	p-value	q-value	R^2	Effect Estimate (cm)	Effect Allele/Null	AILs with Effect allele/Null
	S5_103237	5.86×10^{-5}	4.74×10^{-3}	5.76	-2.2181	C/T	59/236
	S5_227187	**3.89×10^{-9}**	**3.60×10^{-5}**	**12.26**	**-4.3477**	**A/C**	**33/268**
	S5_285834	4.37×10^{-8}	1.06×10^{-4}	10.51	-4.1760	A/G	30/275
	S5_353165	7.42×10^{-9}	3.60×10^{-5}	11.65	4.1393	C/T	267/35
	S5_440644	3.22×10^{-8}	9.39×10^{-5}	11.29	-4.1221	A/G	33/258
	S5_453169	5.17×10^{-9}	3.60×10^{-5}	11.91	-4.4172	A/G	32/274
	S5_574926	2.46×10^{-8}	8.97×10^{-5}	11.18	4.0330	C/T	262/35
	S5_849335	4.55×10^{-6}	7.36×10^{-3}	7.63	3.6595	C/T	278/25
	S5_934093	1.24×10^{-6}	2.26×10^{-3}	9.51	-3.9703	A/T	26/226
	S5_1200961	1.72×10^{-5}	2.08×10^{-2}	6.23	-3.0855	A/G	34/272
	S5_1200964	1.72×10^{-5}	2.08×10^{-2}	6.23	-3.0855	C/G	34/272
	S5_1200971	1.72×10^{-5}	2.08×10^{-2}	6.23	3.0855	G/T	272/34
	S8_2778495	5.22×10^{-5}	4.46×10^{-2}	5.53	-3.7745	C/T	16/288
	S8_2778496	5.22×10^{-5}	4.46×10^{-2}	5.53	3.7745	C/T	288/16
	S8_2778497	5.22×10^{-5}	4.46×10^{-2}	5.53	-3.7745	G/T	16/288
	S12_23092043	**4.04×10^{-5}**	**4.21×10^{-2}**	**6.12**	**-2.2773**	**C/T**	**196/93**
	S12_23120151	2.57×10^{-5}	2.88×10^{-2}	6.43	2.3934	A/G	91/197

Table 10: QTL mapping intervals (95% Confidence Intervals) significantly associated (p-value < 0.0001) with *Xoo* PXO99A-pHM1-*tal7b* resistance. Parental sources of resistance report the parent with the largest resistance effect for the given QTL. Corroboration compares the given QTL to the GWAS analysis or previous mapping studies.

Chr	QTL position	Markers flanking the QTL (size)	p-value	Parental source of resistance	Corroboration
3	S3_4771783	S3_3639639 - S3_6052491 (2.4 Mb)	9.53 e-05	Samba Mahsuri-*sub1*	Almost significant in GWAS MLM (p-value 0.03) and significant in GWAS GLM (p-value 0.008).
5	S5_353165	S5_37878 - S5_685714 (647.8 kb)	6.09 e-10	PSBRc82	Also in PXO99A. Also in GWAS, p-value = 3.89×10^{-9}
8	S8_2778548	S8_2208918 - S8_2913918 (705 kb)	7.47 e-04	Sanhuangzhan-2	Also detected in GWAS, p-value = 5.22×10^{-5}
10	S10_19598933	S10_19229371 - S10_20082337 (853 kb)	5.22 e-04	Fedearroz-50	Also in PXO99A. Almost significant in GWAS MLM (p-value 0.07) and significant in GWAS GLM (p-value < 0.0001).
11	S11_14981109	S11_14605845 - S11_15708610 (1.1 Mb)	3.22 e-04	IR.77298.14.1.2.10	Almost significant in GWAS MLM (p-value 0.07) and significant in GWAS GLM (p-value < 0.0001).
12	S12_19676337	S12_19452481- S12_19836912 (384.3 kb)	7.74 e-06	Fedearroz-50	Almost significant in GWAS MLM (p-value 0.04) and significant in GWAS GLM (p-value < 0.0001).

Table 11: Candidate genes near GWAS markers for *Xoo* PXO99A-pHM1-*tal7b* resistance.

Marker	**Candidate genes** Gene name (locus identity, if applicable), annotation	Distance from Marker	Importance	Citation
S12_23092043	(LOC_Os12g36920), calmodulin binding protein, putative, expressed	456 kb	Upregulated during PXO99A infection	Cernadas et al. 2014
S12_23120151	*SPL11* (LOC_Os12g38210), spotted leaf 11, putative, expressed	348 kb	Negative regulator of rice immunity	Zeng et al. 2004

Table 12: Candidate genes under or near the unique QTL identified through interval mapping for resistance to *Xoo* PXO99A-pHM1-*tal7b*.

QTL	**Candidate genes** Locus identity (gene name, if applicable), Annotation	Distance from QTL	Importance	Citation
S3_4771783	LOC_Os03g07540, bHLH family protein, putative, expressed	Within	Putative target of BLS256 TAL3c	Cernadas et al. 2014
S8_2778548	LOC_Os08g07760 (*OsSerk1)*, BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor, putative, expressed	1.4 Mb	Predicted target TAL7b homologs in C8 and PXO99A and a similar TAL in KACC10331	Pérez-Quintero et al. 2013
S11_14981109	LOC_Os11g26790, putative dehydrin	Within	Putative target of TALs in *Xoo* strains MAFF311018 and PXO99A Putative target of 9 *Xoc* TALs similar to BLS256 TAL2g	Pérez-Quintero et al. 2013 Wilkins et al. & Bogdanove, 2015
S12_19676337	LOC_Os12g29220 (*xa25/ OsSWEET13*) nodulin MtN3 family protein, putative, expressed	2 Mb	Upregulated by *Xoo* strains PXO339, KACC10331, MAFF311018, but not PXO99A. Suspected TAL effector target.	Liu et al. 2011; Pérez-Quintero et al. 2013

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