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ORIGINAL PAPER

High-resolution mapping of *Rsn1*, a locus controlling sensitivity of rice to a necrosis-inducing phytotoxin from *Rhizoctonia solani* AG1-IA

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Abstract *Rhizoctonia solani* is a necrotrophic fungal pathogen that causes disease on many crop-plant species. Anastomosis group 1-IA is the causal agent of sheath blight of rice (*Oryza sativa* L.), one of the most important rice diseases worldwide. *R. solani* AG1-IA produces a necrosis-inducing phytotoxin and rice cultivar's sensitivity to the toxin correlates with disease susceptibility. Unlike genetic analyses of sheath blight resistance where resistance loci have been reported as quantitative trait loci, phytotoxin sensitivity is inherited as a Mendelian trait that permits high-resolution mapping of the sensitivity genes. An F_2 mapping population derived from parent cultivars 'Cypress' (toxin sensitive) and 'Jasmine 85' (toxin insensitive) was used to map *Rsn1*, the necrosis-inducing locus. Initial mapping based on 176 F_2 progeny and 69 simple sequence repeat (SSR) markers located *Rsn1* on the long arm of chromosome 7, with tight linkage to SSR marker RM418. A high-resolution genetic map of the region was subsequently developed using a total of 1,043 F_2 progeny, and *Rsn1* was mapped to a 0.7 cM interval flanked by markers NM590 and RM418. Analysis of the corresponding 29 Kb genomic sequences from reference cultivars 'Nipponbare' and '93-11' revealed the presence of four putative genes within the interval. Two are expressed cytokinin-*O*-glucosyltransferases, which fit an apoptotic pathway model of toxin activity, and are individually being investigated further as potential candidates for *Rsn1*.

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Introduction

Sheath blight disease of rice is caused by *Rhizoctonia solani* Kühn [teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk] anastomosis group (AG) 1, intraspecific group IA (Webster and Gunnell 1992). Sheath blight occurs wherever rice is grown and is considered one of the most important rice diseases worldwide (Webster and Gunnell 1992). Rice cultivars vary in levels of susceptibility to the disease, and moderate resistance is known in cultivated *O. sativa* and related wild species, but none are fully resistant to sheath blight (Pan et al. 1999a; Prasad and Eizenga 2008). These germplasm sources are useful for variety improvement, but the polygenic inheritance of partial resistance complicates resistance breeding for sheath blight.

Sheath blight resistance assessment is often confounded by extraneous factors such as plant height or maturity (Pan et al. 1999a; Pinson et al. 2005). In many reports, mapping of sheath blight resistance QTL has revealed confounding factors associated with the same genomic loci (e.g. heading date; Channamallikarjuna et al. 2010; Pan et al. 1999a; Sato et al. 2004; Zou et al. 2000). In these analyses, it was impossible to determine if the resistance accounted for at such loci was real, or an effect from the confounding factor. This has led to the dismissal of such loci as false positives, and research focus directed to resistance loci that had no association with potentially confounding traits.

Numerous genetic analyses of sheath blight resistance exist in the scientific literature (Channamallikarjuna et al. 2010; Che et al. 2003; Han et al. 2002; Kunihiro et al.

2002; Li et al. 1995; Liu et al. 2009; Pan et al. 1999ab; Pinson et al. 2005; Sato et al. 2004; Tan et al. 2005; Zou et al. 2000), and date back to as early as 1995 (Li et al. 1995). Since no source of complete resistance is known in *O. sativa* or its wild relative species (Pan et al. 1999a), most reports described QTL for partial resistance (Channamallikarjuna et al. 2010; Han et al. 2002; Kunihiro et al. 2002; Li et al. 1995; Liu et al. 2009; Pinson et al. 2005; Sato et al. 2004; Tan et al. 2005; Zou et al. 2000), although reports of major genes for partial resistance also exist (Che et al. 2003; Pan et al. 1999a). The greatest limitation to the precision of QTL mapping lies in the disease phenotype, which is confounded by environment, plant height, maturity, tillering, canopy density and fertilizer input (Webster and Gunnell 1992). In attempts to control these factors some researchers have resorted to using greenhouse assays (Liu et al. 2009; Channamallikarjuna et al. 2010). However, the greatest resolution obtained to-date was with a QTL on chromosome 11 in an interval of 0.85 Mb, which contained 154 predicted genes, 26 of which were putatively related to disease resistance and defense response (Channamallikarjuna et al. 2010). Therefore, even the most precise QTL mapping obtained over the last 15 years still lacked the precision necessary to readily identify sheath blight resistance genes.

The development of resistant rice cultivars for the United States and other rice-producing countries has promise for reducing disease severity and corresponding yield losses. However, current genetic analyses are inadequate for resolving the complex quantitative inheritance, and making progress toward development of resistant cultivars. To date, no genes have been identified for sheath blight resistance, and few reports exist describing marker-assisted selection (MAS) for sheath blight resistance (Tan et al. 2005; Srinivasachary et al. 2010; Zuo et al. 2008).

To address the perpetual problem of limited resolution in genetic mapping due to imprecise disease phenotyping, we focused on one component of the complex disease. *R. solani* is a necrotrophic pathogen that is known to produce host-selective toxins that may act as pathogenicity or virulence factors (Aoki et al. 1963; Danson et al. 1999; Lakshman et al. 2006; Mandava et al. 1980; Sherwood 1965; Vidhyasekaran et al. 1997). *R. solani* AG1-IA produces a phytotoxin that is genotype-specific, where sensitivity and insensitivity to the toxin were observed in cultivars of the host species (Brooks 2007). Brooks (2007) also showed that toxin sensitivity was positively correlated with disease susceptibility.

Previously, Brooks (2007) made a preliminary description of the heritability of *R. solani* phytotoxin sensitivity in rice. In that report, two unlinked loci were shown to control toxin sensitivity in a cross between the cultivars Cypress and Jasmine 85. Cypress was fully sensitive (necrosis of all leaf area infiltrated with the toxin) and Jasmine 85 was completely insensitive (no response) to the toxin. For illustrative purposes, 'N' (now *Rsn1*) was used to label the necrosis locus, and 'C' was used for the locus regulating tissue chlorosis. In an F_2 population from this cross (NN/CC × nn/cc) four phenotypic classes were observed and corresponding genotypes predicted: fully necrotic (N_/C_), intermediate necrotic (N_/cc), fully chlorotic (nn/C_), and completely insensitive (nn/cc).

Due to the Mendelian inheritance of the toxin sensitivity trait (9:3:3:1 segregation for each phenotypic class respectively), this population was used for high-resolution mapping of the genes controlling *R. solani* phytotoxin sensitivity in rice. In this report, a high-resolution genetic map is presented for the locus regulating tissue necrosis, designated *Rsn1*, for "*Rhizoctonia solani* necrosis gene number one". Our goal is to identify diagnostic DNA markers for efficient MAS, identify the molecular basis of *Rsn1* function, and explore the possibility of manipulating this locus for rice improvement.

Materials and methods

Plant materials

Rice cultivars Cypress (PI 561734) and Jasmine 85 (PI 595927) were obtained from the University of Arkansas Rice Research and Extension Center (Stuttgart, AR). Cypress is toxin sensitive (tox-S) and very susceptible to sheath blight (VS), and Jasmine 85 is toxin insensitive (tox-I) and moderately resistant to sheath blight (MR) (Brooks 2007). Single plant selections from each cultivar were maintained as pure lines and used as parents of the F_2 mapping population. A total of 1,043 F_2 plants were grown under greenhouse conditions for trait phenotyping, DNA extraction, and molecular marker analysis.

Fungal culture and toxin preparation

Rhizoctonia solani AG1-IA isolate B1 was collected as sclerotia from naturally infected rice plants in Stuttgart Arkansas, and confirmed using Koch's postulates and DNA-based molecular markers (Brooks 2007). The master isolate was sub-cultured annually onto potato dextrose agar (PDA), and was used as the source of sclerotia for all toxin-preparation cultures. The phytotoxin was prepared from isolate B1 as previously described (Brooks 2007). Briefly, liquid cultures were made by transferring a PDA plug of actively growing mycelia into an Erlenmeyer flask containing sterile rice leaf medium and incubating at 28°C for 10–14 days. The liquid fraction was recovered by vacuum filtration through Whatman 541 filter paper (Whatman

International Ltd., Maidstone, England) and concentrated to 5-times the starting volume (5X TOX) using a Rotavapor R-205 (BÜCHI Labortechnik AG, Postfach, Switzerland). Phytotoxin preparations were stored at −20°C until used.

Phytotoxin sensitivity trait evaluation

Following the protocol of Brooks (2007), 5–10 µl of 5X TOX was infiltrated into fully expanded rice leaves using a Hagborg device (Hagborg 1970). For each independent evaluation of 30- to 60-day-old plants a minimum of ten replications were conducted. Multiple infiltrations were possible on individual leaves and plants were tested repeatedly on newly formed leaves. Visual ratings of necrosis in the infiltrated leaf areas were conducted 6–7 days post-infiltration, and scored as fully necrotic, intermediate necrotic, fully chlorotic, and insensitive.

DNA extraction

Two methods of DNA extraction were used. First, the rapid DNA extraction method described by Xin et al. (2003) was used to facilitate high-throughput extraction of DNA from seedling leaf tissue. Second, the CTAB extraction method described in Hulbert and Bennetzen (1991) was used to purify DNA from selected recombinants for multiple marker assays.

Microsatellite markers

SSR markers developed by McCouch et al. (2002) were used for the initial phase of linkage mapping. Marker information ('RM' coded series) and corresponding primer sequences were obtained from the Gramene database (http://www.gramene.org). Following initial linkage detection of *Rsn1* with RM418, an additional 18 SSR markers on chromosome 7 were added to construct an initial framework map (RM295, RM5752, RM481, RM125, RM214, RM2, RM21605, RM21606, RM21612, RM21615, RM8257, RM21649, RM11, RM3404, RM455, RM505, RM234, RM1362).

InDel (insertion/deletion) markers

An additional ten InDel-based molecular markers were developed to increase marker saturation within the targeted region on the long arm of chromosome 7 (Table 1). Putative gene sequences from rice cultivars Nipponbare (*O. sativa* ssp. *japonica*) and 93-11 (*O. sativa* ssp. *indica*) were aligned using nucleotide BLAST (Altschul et al. 1990; http://blast.ncbi.nlm.nih.gov/Blast.cgi) to detect InDels between the two reference sequences in the RM2–RM11 interval. Sequences were obtained from the MSU Rice Genome Annotation Project (Ouyang et al. 2007; Yuan et al. 2005; http://rice.plantbiology.msu.edu/), and Gramene (http://www.gramene.org/Oryza_sativa/). The specific primers for each InDel were designed using Primer 3 software (Rozen and Skaletsky 2000). InDel polymorphisms detected between the *japonica/indica* reference sequences were expected to be conserved with the parents of the mapping population, as Cypress is a *japonica*-type cultivar and Jasmine 85 is closely related to *indica*-type cultivars. Primer sequences, annealing temperatures, expected allele sizes and map positions for the InDel markers developed in this study are also presented in Table 1.

Polymerase chain reaction (PCR) and allele detection

PCR reactions were performed in 25 µl volumes containing 20 ng of template DNA, and final concentrations of: 10 mM Tris–HCl pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 300 nM each primer, and 1 unit of Taq DNA polymerase (Promega, Madison, WI, USA). For PCR reactions with template DNA prepared using the rapid extraction method, bovine serum albumin (0.1%) and PVP40 (1%) were added at concentrations relative to the final reaction volume. For each marker, one primer was labeled with a fluorescent dye (6FAM, NED, PET or HEX) purchased from Applied Biosystems (Foster City, CA, USA) or Integrated DNA Technologies (Coralville, IA, USA). PCR reactions were performed in MJ Research thermal cyclers (Waltham, MA, USA) under the following conditions: (1) initial denaturing at 94°C for 5 min; (2) 35 cycles of 94°C for 1 min, 55–67°C (marker dependent annealing temperature) for 1 min, 72°C for 2 min; and (3) 5 min final extension at 72°C. PCR products were pooled based upon dye color and size range of the amplified fragments (typically three markers per run including ROX or LIZ-labeled size standards), and the products denatured by heating at 94°C for 5 min. The products were resolved using an ABI Prism 3730 DNA Analyzer according to the manufacturer's protocols, and automated genotyping was performed using GeneMapper Version 3.7 software (Life Technologies Corp., Carlsbad, CA, USA).

Linkage analysis and mapping

One hundred and twenty SSR markers evenly distributed on the 12 rice chromosomes were selected to map the *Rsn1* gene. A parental survey was conducted with these markers to identify clear polymorphism between the two parental lines Cypress and Jasmine 85. Ultimately 69 SSR markers (5–6 markers per chromosome) were used for linkage detection with *Rsn1* on the initial set of 176 F_2 progeny. Following linkage detection, additional SSR markers were used to construct a low-resolution framework map spanning

Table 1 Summary of InDel markers and corresponding primer sequences developed by comparison of the Nipponbare (NB) and 93-11 reference sequences for marker saturation of the *Rsn1* locus on rice chromosome 7

Marker name	Primer sequence (5′–3′)	Annealing temp. (°C)	Expected fragment sizes (bp) NB/93-11	Physical position (Mb)
NM28160F	GGTGGGAGCACGCATGAACTGT	55	120/114	16.44
NM28160R	TCATATCGCGCACACCTTCCTGA			
NM750F	TCTCGTGACTACACCAACAAGTGG	58	260/266	17.49
NM750R	TCAAATGACCCACATGGATTGCCC			
NM150F	AGGAGTTGGCCCACACCGACA	56	208/213	17.79
NM150R	TCTCCGGGCCAGCAATAGCCA			
NM210F	TTGATGGATGGATCATGGAGGCGT	56	240/242	17.84
NM210R	TGGAGCTGTACCAAACATGCCCTA			
NM300F	GGACCTCGAGCACGCCACAC	56	348/354	17.90
NM300R	CCATGGAATAGTAGCAGTAG			
NM510F	CATTGCACAGTGACCGACACACTA	55	283/286	18.05
NM510R	GACACGTTGTTGCAGGCCTATTCT			
NM590F	GTTCCCAAGAAGCATTTGCCCTGT	58	162/172	18.10
NM590R	ACGGTAAGGTAAGGCACCCAACAA			
NM691F	TTAAGAGCAGTAGGCATCGTGGCA	56	393/420	18.20
NM691R	AGTATGGCACCGCTCTTTATCGCA			
NM190F	TCATCGGGCGTCCGTGCAG	55	436/410	18.40
NM190R	TGGTGGGGGTGGTGGCAGAT			
NM31550F	TGATTAGCAGGTGTAGCACC	55	280/290	18.70
NM31550R	CAAAGAATAGCATTGGTGAC			

the *Rsn1* locus. From this map flanking SSR markers (RM2 and RM11) were selected to identify additional recombinant F_2 progeny (as seedlings). All recombinants were grown to maturity, phenotyped for toxin sensitivity, and extensively genotyped to increase map resolution. The final population size totaled 1,043 F_2s with 28 chromosome 7 markers (18 SSRs and 10 InDels).

All genetic linkage maps were assembled using JoinMap 4.0 software (Van Ooijen 2006). The critical $\log_{10}$ of odds ratio (LOD) value of 2.0 or more was used to assign marker loci to linkage groups. The Kosambi mapping function (Kosambi 1944) was used to convert recombination frequencies to map distances in centiMorgans. Linkage groups were identified to represent specific chromosomes on the basis of the position of known SSR markers used in previously published rice genome maps. The analytical approach used to identify and validate putative regions associated with the necrosis-inducing locus, segregating in the in the 176 F_2 initial population, used the established linkage map and the observed phenotypic traits implementing the non-parametric Kruskal–Wallis *K* test (Hollander and Wolfe 1973; Lehmann and D'Abrera 1975). For the *K* test, an association was indicated when the mean values of the marker classes were significantly different at $P < 0.01$.

Results

The first 176 F_2 plants were all phenotypically classified as parent-A (Cypress) or heterozygous (H) type when any portion of the infiltrated leaf area became necrotic. This rating included both fully necrotic and intermediate necrotic types, as both phenotypes indicated the presence of a dominant allele at *Rsn1*. The expected phenotypic ratios were 9/16 and 3/16 respectively, which was equivalent to 3/4 of all F_2 plants. Plants that were chlorotic or insensitive to toxin infiltration were scored as parent B type, as these phenotypes indicated homozygous recessive alleles at *rsn1*. The expected phenotypic ratios were 3/16 and 1/16 respectively, which was equivalent to 1/4 of all F_2 plants. Although two genes were segregating for toxin sensitivity in the F_2 population, the unique phenotypes permitted independent scoring of the *Rsn1* locus as 3/4 *Rsn1*, _ and 1/4 *rsn1, rsn1*. The critical value of χ^2 ($df = 1$, alpha = 0.05) is 3.841. The calculated χ^2 of 0.758 failed to reject the null hypothesis for a single dominant locus conferring necrosis in the population.

Preliminary *Rsn1*-marker linkage detection was performed using 69 polymorphic SSRs with genome-wide coverage on the initial set of 176 F_2 progeny. One of five markers on the long arm of chromosome 7 (RM418) was tightly linked with *Rsn1*. After establishing linkage

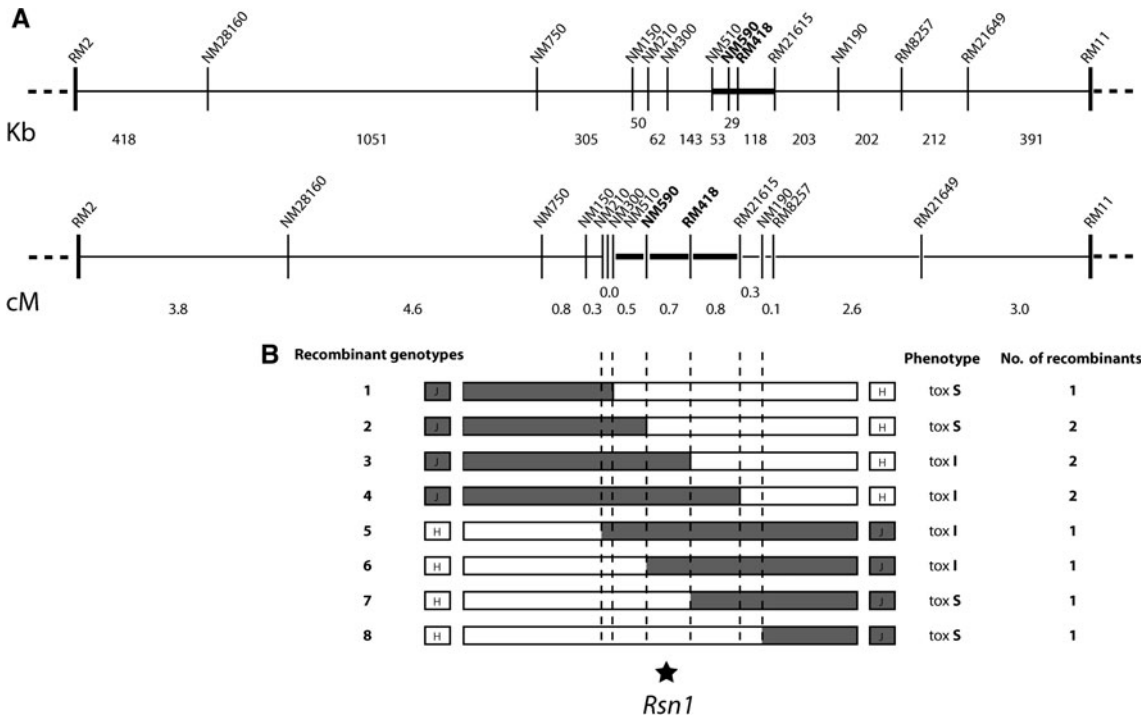

Fig. 1 Schematic representation of the RM2–RM11 interval on rice chromosome 7 that spanned the *Rsn1* locus. Map orientation for the long arm was proximal (*left*) to distal (*right*). **a** Comparison of the physical and genetic linkage maps. Map scales were in kilobases (Kb; *top*) and centiMorgans (cM; *bottom*). Marker names were indicated on *top* and distances below each map. Markers in *bold* were the closest flanking markers for *Rsn1*. **b** Eight recombinant genotypes and the corresponding toxin phenotype. Phenotypes were tox-S (sensitive Cypress-type) and tox-I (insensitive Jasmine 85-type). Homozygous *rsn1/rsn1* regions were indicated with *shaded bars* and 'J' for Jasmine 85-type. *Open bars* indicated *Rsn1/rsn1* heterozygous regions and were labeled 'H'. The number of recombinants comprising each haplotype out of 1,043 F_2 progeny are indicated at *right*

Table 2 List of candidate genes predicted within the 29 Kb genomic interval between *Rsn1* flanking markers NM590 and RM418 from cultivar 'Nipponbare' (Ouyang et al. 2007; Yuan et al. 2005)

Gene identifier	Predicted function	Predicted length (aa)	Rice FL-cDNA
LOC_Os07g30600	Male sterility protein, putative	476	No
LOC_Os07g30610	Cytokinin-*O*-glucosyltransferase 2, putative	483	Yes
LOC_Os07g30620	Cytokinin-*O*-glucosyltransferase 2, putative	499	Yes
LOC_Os07g30630	Hypothetical protein	164	No

aa amino acid polypeptide length, *FL-cDNA* full-length cDNA reported?

between *Rsn1* and RM418, seven more chromosome 7 SSR markers were tested on the initial 176 F_2 progeny. This chromosome 7 linkage map included 12 RM-SSR markers spanning 124.8 cM. The *Rsn1* locus was flanked by SSRs RM2 and RM11 within a 14.6 cM interval. These markers were used to identify 116 recombinants in the RM2–RM11 interval out of 867 additional F_2 progeny, which made the effective population size 1,043 F_2 individuals.

A high-resolution genetic map (Fig. 1) was constructed using all RM2–RM11 recombinants, 12 additional markers in the interval, and the phenotypic results of each line. *Rsn1* mapped to a 0.7 cM interval flanked by markers NM590 and RM418. A comparison of the genetic map with the Nipponbare physical map showed colinearity of the molecular markers, and the NM590-RM418 0.7 cM interval corresponded to a 29 Kb physical distance. Four genes were predicted within the interval and are candidates for *Rsn1* (Table 2), including two putative cytokinin-*O*-glucosyltransferases (LOC_Os07g30610 and LOC_Os07g30620), a putative male sterility protein (LOC_Os07g30600) and a hypothetical protein (LOC_Os07g30630). Full-length cDNA transcripts existed for both putative cytokinin-*O*-glucosyltransferases, while expression evidence for the remaining two genes is uncertain (Table 2).

Sequence analyses from Nipponbare and 93-11 showed that the two putative cytokinin-*O*-glucosyltransferases were

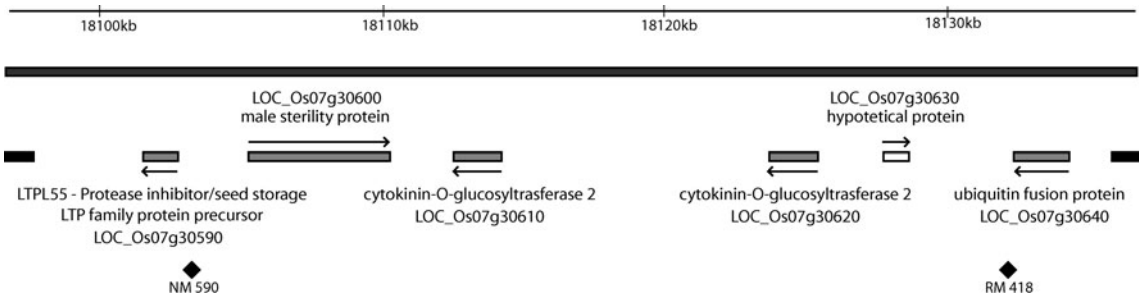

Fig. 2 Diagram of the Nipponbare chromosome 7 genomic interval between molecular markers NM590 and RM418. The predicted gene models are represented in *boxes*: *black colored boxes* represent predicted transposable elements, *gray boxes* indicate genes with putative function, *white boxes* are hypothetical proteins. *Arrows* indicate the 5′–3′ ORF orientation

tandemly arranged on the minus strand of the chromosome 7 pseudomolecule (Fig. 2). No genes were predicted between the two homologs in the 9.2 Kb intergenic region in Nipponbare, or the 7.8 Kb intergenic region in 93-11. The difference in size of the intergenic regions is due to a 1.3 Kb InDel (present in Nipponbare) located approximately 600 bp downstream from the 3′ end of LOC_Os07g30620. A comparison of nucleotide and amino acid sequences (Nipponbare vs. 93-11) revealed 99% similarity for each gene between the cultivars. The gene models predict single open reading frames (ORF) of nearly identical size for LOC_Os07g30610 and LOC_Os07g30620 (1,449 and 1,497 bp respectively). Four amino acid substitutions occur between the LOC_Os07g30610 homologs, while only one substitution was found between LOC_Os07g30620 homologs. An additional comparison of predicted amino acid sequences of the two genes within each cultivar revealed 72% amino acid identity.

Discussion

Phytotoxins are key components of pathogenicity and virulence in necrotrophic fungi, but have had limited description thus far in *R. solani* (Aoki et al. 1963; Brooks 2007; Danson et al. 1999; Lakshman et al. 2006; Mandava et al. 1980; Sherwood 1965; Vidhyasekaran et al. 1997). Although the phytotoxin produced by *R. solani* AG1-IA has not been fully characterized, host sensitivity has been described to toxin-containing culture filtrates and partially purified fractions (Brooks 2007; Vidhyasekaran et al. 1997). By focusing on this component of the pathosystem we were able to fine map a gene conferring host-plant sensitivity to the phytotoxin.

Rsn1, which induces necrosis of host tissue in response to toxin infiltration, maps to the long arm of chromosome 7. Interestingly, Pan et al. (1999b) described a QTL for sheath blight resistance (qSB-7) in the same region as *Rsn1* and from the same resistant-source used herein (Jasmine 85). The cross included the susceptible cultivar Lemont, which is also in the pedigree of the susceptible cultivar Cypress. However, Zou et al. (2000) dismissed qSB-7 as a false locus due to detection of a heading date QTL in the same region of chromosome 7. Independently, Liu et al. (2009) also performed QTL mapping of sheath blight resistance in a population derived from Lemont/Jasmine85. This group reported QTL detected using greenhouse disease assays, and validated those QTL using field assays. However, due to the focus of the paper the authors did not report a significant QTL they detected on chromosome 7, in the same region as *Rsn1*, because it was detected under field conditions only (G. Liu personal communication). This QTL (qShB7) was detected in field studies conducted in both Arkansas and Louisiana, and also had the highest LOD scores for the study. Whether or not *Rsn1* is the gene responsible for qSB-7 and qShB7 is unknown. However, it is possible that they are one in the same, since the two populations share identical sources of resistance with R-loci mapping to the same position. The InDel markers developed in the present work, which are tightly linked to the gene, will be valuable for future work to determine if *Rsn1* is the gene previously detected as a sheath blight resistance QTL.

The two most promising candidate genes identified for *Rsn1* are the cytokinin-*O*-glucosyltransferases. Within a single cultivar (e.g. Nipponbare) there is 72% amino acid identity between the two predicted polypeptide sequences (LOC_Os07g30610 vs. LOC_Os07g30620). This degree of sequence conservation has sufficient polymorphism for independent gene silencing by RNA interference, enabling us to use this technique to identify *Rsn1* without the risk of knocking down both genes simultaneously. Furthermore, there is very little polymorphism within each gene between the Nipponbare and 93-11 reference sequences. Only four amino acid differences occur between the LOC_Os07g30610 predicted polypeptides, and one amino acid difference between the LOC_Os07g30620 predicted polypeptides. This high degree of sequence conservation

will enable quick identification of the functional polymorphism(s) if one of these genes is confirmed as *Rsn1*.

The cytokinin-*O*-glucosyltransferases are the most likely *Rsn1* candidates based upon putative gene function. Glucosyltransferases comprise a large family of enzymes, some of which have been shown to detoxify mycotoxins produced by *Alternaria brassicae, Fusarium* sp., and *Sclerotinia sclerotiorum* (Pedras et al. 2002; Poppenberger et al. 2003; Schweiger et al. 2010; Sexton et al. 2009). Cytokinin-*O*-glucosyltransferases recognize cytokinins as acceptor molecules for glucosylation, forming *O*-glucosides that are thought to play a role in hormone homeostasis (Hou et al. 2004). The enzymes also transfer the sugar group from a donor molecule (do not require a free sugar) to the acceptor, so they are involved directly in the degradation of the carbohydrate donor (Tai et al. 2008). The cytokinin-glucosides are known to be inactive forms of the hormone, presumably formed to regulate the amount of hormone present in plant tissues (Mok and Mok 2001). However, cytokinin-ribosides have been shown to inhibit cell growth and are capable of inducing apoptosis (Ishii et al. 2002). Others (Carimi et al. 2003; Gahan et al. 2003) have also shown the involvement of cytokinins in apoptosis, which is contrary to the widely accepted functions of these hormones. Therefore, glycosylation of cytokinins can either nullify the hormone, or reverse their "normal" function to induce cell death.

The potential role of cytokinin-glucosides as inactivated hormones or inducers of cellular apoptosis is an important feature for phytotoxin sensitivity. Phytotoxins are known inducers of apoptosis (Wolpert et al. 2002), and an enzyme that nullifies the anti-senescence function of cytokinins or alters the function to induce cell death, fits the model of toxin induction of tissue necrosis via cellular apoptosis. Interestingly, Vidhyasekaran et al. (1997) showed that RS-toxin was a carbohydrate, and the partial purification and analysis showed that the toxin was composed primarily of glucose moieties. Later, Shanmugam et al. (2001) showed that an α-glucosidase derived from *Trichoderma viridae* was capable of degrading RS-toxin, which would catalyze the release of free α-glucose during degradation of the toxin. Based upon this evidence we speculate that *Rsn1* is a cytokinin-*O*-glucosyltransferase that may interact directly with the *R. solani* carbohydrate-based phytotoxin (i.e. RS-toxin). In doing so, glucose moieties would be bound to cytokinin acceptor molecules, producing cytokinin-glucosides. (Whether or not the glucose moieties could be derived from the toxin itself is of particular interest.) In this process, the cytokinin(s) would be inactivated and their inhibition of apoptosis stopped, which would allow tissue necrosis to progress via programmed cell death. However, a second chlorosis-inducing gene is known to be involved in sensitivity to this toxin (Brooks 2007). Therefore, this tentative model can serve only as the basis for hypotheses in future work to elucidate the toxin sensitivity pathway(s).

Cytokinins are well-known plant growth hormones that have numerous functions in plant growth and development. However, their role in plant defense responses is poorly understood. In a model proposed by Robert-Seilaniantz et al. (2007) cytokinins were involved in cross-talk between the jasmonic acid/ethylene (JA/ET) and salicylic acid (SA) resistance pathways. In their model cytokinins positively influenced JA/ET mediated resistance to necrotrophs where cell death was inhibited. Conversely, negative influences on cytokinins reversed this effect via the SA pathway leading to necrotroph susceptibility. Cloning of *Rsn1* may ultimately lead to a better understanding of the role of cytokinin in host plant defense responses. Currently, we have preliminary evidence that *Rsn1* is a cytokinin-*O*-glucosyltransferase and have begun plant transformation experiments using the two genes identified herein to provide conclusive proof of gene identity. In parallel, we are in the process of map-based cloning of the "chlorosis" sensitivity gene that is known to act epistatically with *Rsn1* (Brooks 2007). Cloning the chlorosis gene will provide the means to begin to assemble the pathway in which the toxin induces tissue necrosis and increases susceptibility to *R. solani*. This work not only advances studies in the rice-sheath blight system, but may also be useful for understanding *R. solani* phytotoxin sensitivity and disease susceptibility in other hosts.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Aoki H, Sassa T, Tamura T (1963) Phytotoxic metabolites of *Rhizoctonia solani*. Nature 200:575

Brooks SA (2007) Sensitivity to a phytotoxin from *Rhizoctonia solani* correlates with sheath blight susceptibility in rice. Phytopathology 97:1207–1212

Carimi F, Zottini M, Formentin E, Terzi M, Schiavo FL (2003) Cytokinins: new apoptotic inducers in plants. Planta 216:413–421

Channamallikarjuna V, Sonah H, Prasad M, Rao GJN, Chand S, Upreti HC, Singh NK, Sharma TR (2010) Identification of major quantitative trait loci qSBR11–1 for sheath blight resistance in rice. Mol Breed 25:155–166

Che KP, Zhan QC, Xing QH, Wang ZP, Jin DM, He DJ, Wang B (2003) Tagging and mapping of rice sheath blight resistance gene. Theor Appl Genet 106:293–297

Danson J, Wasano K, Nose A (1999) Effect of culture nutrients on the production of *Rhizoctonia solani* toxins. IRRN 24:16

Gahan PB, Wang L, Bowen ID, Winters C (2003) Cytokinin-induced apoptotic nuclear changes in cotyledons of *Solanum aviculare* and *Lycopersicum esculentum*. Plant Cell Tissue Org 72:237–245

Hagborg WAF (1970) A device for injecting solutions and suspensions into thin leaves of plants. Can J Bot 48:1135–1136

Han YP, Xing YZ, Chen ZX, Gu SL, Pan XB, Chen XL, Zhang QF (2002) Mapping QTL for horizontal resistance to sheath blight in an elite restorer line Minghui 63. Chin J Genet 29:622–626

Hollander M, Wolfe D (1973) Nonparametric statistical methods. Wiley series in probability and mathematical statistics. Wiley, New York

Hou B, Lim E-K, Higgins G, Bowles DJ (2004) *N*-glucosylation of cytokinins by glycosyltransferases of *Arabidopsis thaliana*. J Biol Chem 279:47822–47832

Hulbert SH, Bennetzen JL (1991) Recombination at the Rp1 locus of maize. Mol Gen Genet 226:377–382

Ishii Y, Hori Y, Sakai S, Honma Y (2002) Control of differentiation and apoptosis of human myeloid leukemia cells by cytokinins and cytokinin nucleosides, plant redifferentiation-inducing hormones. Cell Growth Differ 13:19–26

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kunihiro Y, Qian Q, Sato H, Teng S, Zeng DL, Fujimoto K, Zhu LH (2002) QTL analysis of sheath blight resistance in rice (*Oryza sativa* L.). Acta Genet Sin 29:50–55

Lakshman DK, Liu C, Mishra PK, Tavantzis S (2006) Characterization of the *arom* gene in *Rhizoctonia solani*, and transcription patterns under stable and induced hypovirulence conditions. Curr Genet 49:166–177

Lehmann EL, D'Abrera HJM (1975) Nonparametrics: statistical methods based on ranks. Holden-Day series in probability and statistics. Holden-Day, San Francisco

Li ZK, Pinson SRM, Marchetti MA, Stansel JW, Park WD (1995) Characterization of quantitative trait loci (QTL) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theor Appl Genet 91:382–388

Liu G, Jia Y, Correa-Victoria FJ, Prado GA, Yeater KM, McClung AM, Correll JC (2009) Mapping quantitative trait loci responsible for resistance to sheath blight in rice. Phytopathology 99:1078–1084

Mandava NB, Orellana RG, Warthen JD Jr, Worley JF, Dutky SR, Finegold H, Weathington BC (1980) Phytotoxins in *Rhizoctonia solani*: isolation and biological activity of *m*-hydroxy- and *m*-methoxyphenylacetic acids. J Agric Food Chem 28:71–75

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

Mok DWS, Mok MC (2001) Cytokinin metabolism and action. Annu Rev Plant Physiol Plant Mol Biol 52:89–118

Ouyang S, Zhu W, Hamilton J, Lin H, Campbell M, Childs K, Thibaud-Nissen F, Malek RL, Lee Y, Zheng L, Orvis J, Haas B, Wortman J, Buell CR (2007) The TIGR Rice Genome Annotation Resource: improvements and new features. Nucleic Acids Res 35:D883–D887

Pan XB, Rush MC, Sha XY, Xie QJ, Linscombe SD, Stetina SR, Oard JH (1999a) Major gene, nonallelic sheath blight resistance from the rice cultivars Jasmine 85 and Teqing. Crop Sci 39:338–346

Pan XB, Zou JH, Chen ZX, Lu JF, Zhu LH (1999b) Tagging major quantitative trait loci for sheath blight resistance in a rice variety, Jasmine 85. Chin Sci Bull 44:1783–1789

Pedras MS, Zaharia IL, Ward DE (2002) The destruxins: Synthesis, biosynthesis, biotransformation, and biological activity. Phytochemistry 59:579–596

Pinson SRM, Capdevielle FM, Oard JH (2005) Confirming QTL and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. Crop Sci 45:503–510

Poppenberger B, Berthiller F, Lucyshyn D, Sieberer T, Schuhmacher R, Krska R, Kuchler K, Glössl J, Luschnig C, Adam G (2003) Detoxification of the *Fusarium* Mycotoxin Deoxynivalenol by a UDP-glucosyltransferase from *Arabidopsis thaliana*. J Biol Chem 278:47905–47914

Prasad B, Eizenga GC (2008) Rice sheath blight disease resistance identified in *Oryza* spp. accessions. Plant Dis 92:1503–1509

Robert-Seilaniantz A, Navarro L, Bari R, Jones JD (2007) Pathological hormone imbalances. Curr Opin Plant Biol 10:372–379

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Sato H, Ideta O, Ando I, Kunihiro Y, Hirabayashi H, Iwano M, Miyasaka A, Nemoto H, Imbe T (2004) Mapping QTLs for sheath blight resistance in the rice line WSS2. Breed Sci 54:265–271

Schweiger W, Boddu J, Shin S, Poppenberger B, Berthiller F, Lemmens M, Muehlbauer GJ, Adam G (2010) Validation of a candidate deoxynivalenol-lnactivating UDP- glucosyltransferase from barley by heterologous expression in yeast. Mol Plant Microbe Interact 23:977–986

Sexton AC, Minic Z, Cozijnsen AJ, Pedras MS, Howlett BJ (2009) Cloning, purification and characterisation of brassinin glucosyltransferase, a phytoalexin-detoxifying enzyme from the plant pathogen *Sclerotinia sclerotiorum*. Fungal Genet Biol 46:201–209

Shanmugam V, Sriram S, Babu S, Nandakumar R, Raguchander T, Balasubramanian P, Samiyappan R (2001) Purification and characterization of an extracellular α-glucosidase protein from *Trichoderma viridae* which degrades a phytotoxin associated with sheath blight disease in rice. J Appl Microbiol 90:320–329

Sherwood RT (1965) Method of producing a phytotoxin. U.S. patent 3179653

Srinivasachary, Willocquet L, Savary S (2010) Resistance to rice sheath blight (*Rhizoctonia solani* Kühn) [(teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk.] disease: current status and perspectives. Euphytica 1–22 (article in press)

Tai F-J, Wang X-L, Xu W-L, Li X-B (2008) Characterization and expression analysis of two cotton genes encoding putative UDP-Glycosyltransferases. Mol Biol 42:44–51

Tan CX, Ji XM, Yang Y, Pan XY, Zuo SM, Zhang YF, Zou JH, Chen ZX, Zhu LH, Pan XB (2005) Identification and marker-assisted selection of two major quantitative genes controlling rice sheath blight resistance in backcross generations. Chin J Genet 32:399–405

Van Ooijen JW (2006) JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen

Vidhyasekaran P, Ponmalar TR, Samiyappan R, Velazhahan R, Vimala R, Ramanathan A, Paranidharan V, Muthukrishnan S (1997) Host-specific toxin production by *Rhizoctonia solani*, the rice sheath blight pathogen. Phytopathology 87:1258–1263

Webster RK, Gunnell PS (1992) Compendium of rice diseases. American Phytopathological Society, St. Paul

Wolpert TJ, Dunkle LD, Ciuffetti LM (2002) Host-selective toxins and avirulence determinants: what's in a name? Annu Rev Phytopathol 40:251–285

Xin Z, Velten JP, Oliver MJ, Burke JJ (2003) High-throughput DNA extraction method suitable for PCR. BioTechniques 34:802–826

Yuan Q, Ouyang S, Wang A, Zhu W, Maiti R, Lin H, Hamilton J, Haas B, Sultana R, Cheung F, Wortman J, Buell CR (2005) The institute

for genomic research osa1 rice genome annotation database. Plant Physiol 138:18–26

Zou JH, Pan XB, Chen ZX, Xu JY, Lu JF, Zhai WX, Zhu LH (2000) Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). Theor Appl Genet 101:569–573

Zuo S, Zhang L, Wang H, Yin Y, Zhang Y, Chen Z, Ma Y, Pan X (2008) Prospect of the QTL-qSB-9^{Tq} utilized in molecular breeding program of *japonica* rice against sheath blight. J Genet Genomics 35:499–505