2009

Different Responses of Two Genes Associated with Disease Resistance Loci in Maize (Zea mays L.) to 3-allyloxy-1,2-benzothiazole 1,1-dioxide

Jiazheng Yuan
Jennifer Tedman
Liakat Ali
Jie Liu
Jeff Taylor

See next page for additional authors

Follow this and additional works at: http://opensiuc.lib.siu.edu/psas_articles
Published in Current Issues in Molecular Biology, (2009) 11 (Suppl. 1): i85-94

Recommended Citation
Authors
Jiazheng Yuan, Jennifer Tedman, Liakat Ali, Jie Liu, Jeff Taylor, David A. Lightfoot, Michiaki Iwata, and K. Peter Pauls

This article is available at OpenSIUC: http://opensiuc.lib.siu.edu/psas_articles/6
Different Responses of Two Genes Associated with Disease Resistance Loci in Maize (Zea mays L.) to 3-allyloxy-1,2-benzothiazole 1,1-dioxide

Jiazheng Yuan1,2, Jennifer Tedman1, Liakat Ali1, Jie Liu1, Jeff Taylor1, David Lightfoot2, Michiaki Iwata3, and K. Peter Pauls*1

1Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1 Canada
2Department of Plant, Soil and Agriculture Systems, Southern Illinois University at Carbondale, Carbondale, IL 62901, USA
3Agricultural & Veterinary Research Labs, Agricultural & Veterinary Division, Meiji Seika Kaisha, Ltd, 760 Morookacho, Kohokuku, Yokohama, 222-8567, Japan

Received 17 September 2008
Revised 18 October 2008
Accepted 20 October 2008

Abstract

Probenazole (3-allyloxy-1,2-benzothiazole 1,1-dioxide, PBZ) is a bactericide and fungicide that acts by inducing plant defense systems. It has been shown to induce the expression of NBS-LRR genes like RPR1 (rice probenazole-response gene) in rice (Oryza sativa L.) and systemic acquired resistance (SAR)-like disease resistance. Two maize (Zea mays L.) genes Znmbsslrr1 (a NBS-LRR gene, cloned from a disease resistance analog PIC11 based) and Zmgc1, (a putative guanylyl cyclase-like gene) have both been associated with quantitative resistance loci (QTL) for resistance to Fusarium graminearum. PIC11 was associated with Fusarium stalk rot and ZmGc1 showed resistance to Gibberella ear rot caused by F. graminearum. The objectives of the current study here were to characterize the Znmbsslrr1 gene and to determine whether it and Zmgc1 respond to the inducer PBZ. The transcript abundance of Znmbsslrr1 expression was significantly reduced in corn seedlings of the Gibberella ear rot resistant genotype CO387 48 h after PBZ treatment. In contrast, the transcript abundance of the maize Zmgc1 gene increased more than 10-fold 8 h after the treatment. Therefore, the two genes do not appear to be coordinately regulated by PBZ.

Keywords: maize, Znmbsslrr1, Zmgc1, probenazole

Introduction

Plants utilize chemical barriers, consisting of antimicrobial compounds, physical barriers such as reinforced cell walls, and sensing barriers, including arrays of resistance (R) genes, to prevent pathogen infection (Dangl and Jones, 2001). Pathogen infestation can cause accumulations or reductions in transcript levels (Iqbal et al., 2005). Plant disease resistance and response genes can be classified into many distinct groups based on the characteristics of the proteins that they encode and the regulators that control their expression. The largest group of such genes encodes proteins with a nucleotide binding site (NBS) motif in the amino-terminal domain followed by a variable number of leucine rich repeats (LRRs) domain in carboxy-terminal domain (Meyers et al., 2003; Belkhadir et al., 2004). The NBS domain may function in ATP hydrolysis and signal transduction while the LRR is hypothesized to be responsible for recognizing a pathogen derived signal (Belkhadir et al., 2004). NBS-LRR genes are wide-spread in plants and may also be involved in plant development and responses to abiotic stresses (Michelmore, 2000).

In some situations, a single resistance gene might provide protection against one or more strains of a particular pathogen when introduced into a susceptible plant of the same species. However, many disease resistance genes recognize only very limited number of pathogen isolates and therefore, they are the quickly defeated by co-evolving pathogens (Pink, 2002). The evolutionary tug of war, often called the “Red Queen Hypothesis” assumes that plant pathogens will constantly evolve to overcome plant defense barriers (Clay and Kover, 1996).

For a more sustainable plant pathogen management system, partial resistance combined with chemical control may be an option. Probenazole (3-allyloxy-1,2-benzisothiazole 1,1-oxide; PBZ) has been widely used with partially resistant rice (Oryza sativa) cultivars to control rice blast disease for decades in Asia (Watanabe et al., 1979). PBZ induces systemic acquired resistance (SAR)-like disease resistance in rice (Sakamoto et al., 1999). PBZ was registered for use in several other crops mainly against bacterial pathogens (Oostendorp et al., 2001). The main metabolites of probenazole in rice are 2-sulfamoylbenzoate, saccharin, and N-o-glucopyranosylsaccharin (Uchiyama et al., 1973). Part of the effect of PBZ against pathogens may be due to weak fungitoxicity against the rice pathogen Pyricularia oryzae.

However, similarities between probenazole induced SAR-like resistance and the SAR-like resistance induced by pathogen suggested that the protective effects of the chemical were mainly due to activation of host defense mechanism. Treated plants respond more quickly to infection (Langcake et al., 1983). Sakamoto et al., (1999) identified a gene, named the RPR1 (rice probenazole responsive) gene, which was induced by treatment
with probenazole, other chemical inducers of systemic acquired resistance (SAR) such as benzo(thio)diiazole (BTH) and salicylic acid (SA), as well as pathogens. The RPR1 protein contained NBS and LRR domains similar to several known R gene products such as RPM1 (Grant et al., 1995), Tobacco N (Whitham et al., 1994), and Mi (Rossi et al., 1998). PR1 has antifungal activities and its synthesis serves as a biomarker of plant responses to pathogens (Durner et al., 1998; Ryals et al., 1996). In addition, enhanced activity of cell membrane GTPase (EC number: 3.6.5.5), an enzyme involved in intracellular signal transduction pathways, has been observed in rice plants treated with probenazole (Kanoh et al., 1993).

Guanylyl cyclases (EC 4.6.1.2 also called guanylate cyclases) catalyze the formation of guanosine 3′,5′-cyclic monophosphate (cGMP) from guanosine 5′-triphosphate (GTP; Bowler et al., 1994). cGMP is a ubiquitous second messenger in plants (Bowler et al., 1994) and Durner et al. (1998) demonstrated that it functions as a second messenger in the induction of PAL (phenylalanine ammonia lyase) by exogenous nitric oxide (NO). Therefore, cGMP was associated with the synthesis of phenylpropanoid products and defense functions. In addition, reactive oxygen intermediates (ROI) induce hypersensitive cell death by raising cytosolic calcium (Levine et al., 1996) and NO can reduce the threshold for calcium signaling by modulating cGMP-gated ion channels (Berridge, 1993).

Fusarium graminearum, Schwabe infects a wide variety of cereal grains, including: rye, barley, wheat, oats, and maize. The pathogen causes Gibberella ear rot and Fusarium stalk rot in maize and wheat and barley head blight in temperate areas (Desjardins, 2003). Gibberella ear rot is characterized by the growth of reddish-white mold beginning at the tip of the ear that can cover the entire ear (Sutton, 1982). Infected grain contains mycotoxins, such as deoxynivalenol (DON, vomitoxin) and zearalenone (ZEN) (Greenhalgh et al., 1983; Molto et al., 1997). Consumption of contaminated food is dangerous to livestock and humans. Mycotoxin contaminated grain fed to livestock (especially swine) induces emesis characterized by vomiting, feed refusal, and decreased weight gain (Christensen and Meronuck, 1986).

Resistance to Gibberella ear rot in maize is quantitative (partial), specific to the mode of fungal entry (silk channels or kernel wounds) and is highly influenced by the environment (Sutton, 1982; Reid et al., 1996; Chungu et al., 1996; Ali et al., 2005). Partial resistance to the related Fusarium pathogens of legumes, including Nectria hematococca and Fusarium solani f. sp. glycines (now Fusarium virguliforme Roy [Aoki]), has been shown to prevent the loss of transcripts from genes like PAL, CHS, and PR1 in their host species during infection (Covert et al., 2001; Iqbal et al., 2005). The molecular mechanism of Gibberella ear rot resistance in maize is still not well characterized.

Maize NBS-LRR resistance gene analogue (RGA) PIC11 (Collins et al., 1998) was mapped to chromosome 5. It was located a genomic region that defined a QTL associated with corn Gibberella stalk rot resistance (Pe et al., 1993). A maize gene (Zmgc1) encoding a putative 267-amino acid guanylyl cyclase-like protein (ZmGC) was shown to be significantly associated with Gibberella ear rot across different environments. (Yuan et al., 2008). The Zmgc1 coding sequences were identical in a Gibberella ear rot-resistant line (CO387) and a susceptible line (CG62) but several differences in nucleotide sequences were identified in their UTRs and introns. Infection with *F. graminearum* increased the transcript abundance of Zmgc1 in both genotypes within hours (Yuan et al., 2008).

A 1674 bp genomic DNA fragment encoding an open reading frame (ORF, Zmnsb1rl) was isolated in our current work based on the sequence information of the RGA PIC11 to investigate the role of this gene in Gibberella ear rot resistance and to test its inducibility by PBZ. The Blast results indicate that the ORF is an NBS-LRR gene shared the most significant similarity with genes that possess motifs of nucleotide binding sites (NBS) and leucine-rich repeats (LRR) including RPR1 (protein induced in rice treated with probenazole) (Sakamoto et al., 1999), LRR19 (Madsen et al., 2003), and RXO1 (Zhao et al., 2005). The overall objective of the current study was to determine whether probenazole treatment could affect the activity of two genes that were associated with resistance to *F. graminearum*. We found that the Zmnsb1rl and Zmgc1 genes responded to probenazole differently, suggesting that the two genes were not regulated in the same way by PBZ.

**Materials and methods**

**Plant materials**

Maize seeds were from a F₅ recombinant inbred (RI) population (n=144) was derived by single seed descent from a cross between the inbred lines, CG62 and CO387 (Ali et al., 2005). CG62 was developed at the University of Guelph from Pioneer 3902 (Lee et al., 2001) and is susceptible to *F. graminearum* after silk or kernel inoculation (Ali et al., 2005), CO387, developed at Agriculture and Agri-Food Canada (AAFC, Ottawa, ON), has resistance to Gibberella ear rot following silk or kernel inoculation (Reid et al., 1994; Reid and Hamilton, 1996). This F₅ population was previously genotyped with 162 molecular markers and phenotyped for Gibberella ear rot disease in 4 environments, resulting in the identification of 18 QTL for resistance to *F. graminearum* (Ali et al., 2005).

**Nucleic acid isolation**

DNA for cloning the Zmnsb1rl gene and gene mapping was obtained from maize (*Zea mays* L.) leaf samples. The leaves were harvested from field-grown plants (F₀), freeze-dried, and ground to fine powder. DNA was extracted from the powdered leaf material using the CTAB (Cetyltrimethyl ammonium bromide) method (Hoisington et al., 1994). The DNA was treated with RNase and stored in Tris-EDTA buffer.

To collect RNA samples, seeds of inbred CO387 were germinated in a commercial soil mixture (Terra-lite Redi-earth, WR Grace & Co., Canada Ltd., Ajax, ON). The pots (30 x 30 x 5cm) were maintained in a greenhouse kept at 24°C with 800 microeinsteins/m²/sec of light on a 16-h photoperiod. Two-week-old seedlings were arranged in...
a completely randomized design and sprayed with 2% (w/w) probenazole (Meijiseika Co., Tokyo, Japan) to foliar runoff. At intervals starting before the chemical spray and ending 6 days after the treatment, plants were harvested to obtain leaf samples. Three plants were harvested for the treatment at each time point. The plant tissues were immediately frozen in liquid nitrogen and kept at –80ºC. Total RNA was isolated using the RNeasy plant mini kit [Qiagen, Mississauga, ON] in combination with DNase treatment using RNase-free DNase kit [Qiagen GmbH, Hilden, Germany], according to the manufacturer’s instructions.

**Genome walking and sequence analyses**

Primer sets were synthesized by Sigma-Aldrich (Sigma-Aldrich, Mississauga, ON). The universal GenomeWalker™ kit (BD Biosciences, Palo Alto, CA) was used to obtain sequences adjacent to the previously characterized 420 bp PIC11 (RGA) fragment [Collins et al., 1998]. In short, DNA libraries were constructed by digesting genomic DNA with four different restriction enzymes (in 100 μl mixtures containing 3 μg of genomic DNA, 80 units of restriction enzyme and 1× restriction buffer) and adaptors were ligated to the ends of the fragments according to manufacturer’s instructions. Several GC rich (>40%) gene specific primers (gsp’s, 26–30bp) were designed for Genome-walking on both directions based on sequence information for the 420 bp PIC11 RGA fragment [Collins et al., 1998]. The outer adaptor primer (AP1) provided in the kit, a gene-specific primer gsp1 (5′-TGACATGCTTGCTCTCTGATCTATTAGCT-3′) and DNA of each of the libraries were used for the primary PCR reaction. A single, major PCR product from at least one of the four libraries was gel purified using a gel purification kit (Qiagen, Mississauga, ON), cloned into TOPO TA using the TOPO cloning kit (Invitrogen, Burlington, ON) and introduced into Escherichia coli One-Shot competent cells according to the manufacturer’s instructions (Invitrogen, Burlington, ON). Several clones were picked and plasmid DNA was isolated with a Qiagen Miniprep Kit (Qiagen, Mississauga, ON). Cloned fragments were sequenced with a CEQ2000 sequencer (Beckman-Coulter, Fullerton, CA) after dye-deoxy nucleotide sequencing reaction using DTCS Quick Start sequencing kit (Beckman-Coulter, Fullerton, CA). All of the DNA fragments began in a known sequence in GSP1 and extended into the unknown adjacent genomic DNA. The new sequence information was used to design new gene specific primers for further walking.

**Primers**

PIC11-F 5′-ATTCCAGACATGGCATCCCC-3′, PIC11-R 5′-GTTCCCAGGATTCAGCATATTG-3′, and PIC11-T 5′-GATGCCCTATTTGTA-3′ were used to obtain a PCR fragment encompassing the complete open reading frame of the gene from CO387 genomic DNA after three steps of GenomeWalking. The final amounts in the PCR mixture used to clone the entire gene were: 10–20 ng template, 0.4 M of each primer, 1× PCR buffer, 2 mM MgSO4, 0.2 mM dNTP and 1 unit high fidelity Platinum Taq (Invitrogen, Burlington, ON). The reaction was performed under the following conditions: denaturation at 94ºC for 3 min, 35 cycles with denaturation at 94ºC for 30 s, annealing at 55–65ºC for 45 s, extension at 68ºC for 3–6 min and a final hold at 10ºC. The PCR samples were electrophoresed through 0.8 % (w/v) agarose gels, stained with 0.5 μg/ml ethidium bromide and visualized under UV light. The DNA fragment was cut from the gel, purified, cloned, and sequenced as described in the preceding section.

**RFLP and SCAR analyses**

Genomic DNA samples (20 μg) from lines in the population were digested with restriction enzymes (30 units of EcoRV), and fractionated by electrophoresis through 0.8 % agarose gels. The gels were stained with ethidium bromide, visualized under UV light and the DNA was transferred onto nylon membranes (Hybond-N+, Amersham Biosciences, Arlington Heights, IL) using protocols recommended by the manufacturer. The forward primer (RFLP-PIC11-F 5′-CTAATTAGATCAGAGACAA GCA-3′) and the reverse primer (RFLP-PIC11-R 5′-GTTCGCCAGGATTCAGCATATTG-3′) for the 420 bp PIC11 fragment were used with genomic DNA to PCR amplify a probe for hybridization. The RFLP probe was labeled Digoxigenin-11-dUTP into the PCR reaction. The membranes were pre-hybridized overnight at 37–42ºC in DIG Easy-Hyb solution and hybridized in DIG Easy Hyb solution containing the PIC11 probe for at least 24 hours at 37–42ºC according to the instructions of manufacturer (Boehringer Mannheim, Germany). After several washings with 2X SSC in room temperature and 0.5X SSC at 65ºC, the hybridization pattern was visualized by chemiluminescence (with CDP-star as the substrate, Roche Diagnostics, Laval, PQ) and recorded by exposure of X-ray film (Kodak Inc., Rochester, NY).

A SCAR (sequence-characterized amplified region) marker, developed from the total Zmnbslrr1 sequence fragment, was used to screen entire mapping population (data not shown). The 2100 bp fragment was PCR amplified using primers SCAR-PIC11-F 5′- ACTGGGCTAGAAAAAGTACTAG-3′ and SCAR- PIC11-R 5′- CTTAATCCGGTCTTGTATTTAGT-3′. The PCR conditions were as following: denaturation at 94ºC for 3 min, 35 cycles with denaturation at 94ºC for 30 s, annealing at 60–65ºC for 45 s, extension at 72ºC for 3 min and final hold at 10ºC. Following amplification, the samples were electrophoresed through 0.8 % (w/v) agarose gels, stained with 0.5 μg/ml ethidium bromide and visualized under UV light.

**Mapping population and statistic analysis**

PIC11 (Zmnbslrr1 anchored) was mapped by adding the RFLP and SCAR F5 segregation data was to the previous scores for RAPD, RFLP, SSR and SCAR markers for the individuals in the CG62 x CO387 RIL population [Ali et al., 2005] and reanalyzing linkages with MAPMAKER/EXP version 3.0b (Lander et al., 1987) with the Kosambi mapping function (Kosambi, 1944). Linkage groups were identified using the “Group” command with a LOD score of 4.0 – 6.0 and a recombination fraction of 0.4 [Ali et al., 2005]. The Zmnbslrr1 (PIC11) derived RFLP markers were added to existing linkage groups with the “Try” command. To determine the locations of the QTL on the map and estimate their phenotypic effects, composite interval mapping (CIM) was performed using QTL
Cartographer V2.0 (Wang et al., 2006; Ali et al., 2005). The chromosome orientations followed the Intermediated B73 x Mo17 (IBM) maize map (Davis et al., 1999; http://www.maizegdb.org) with the lowest bin positions at the top. The PIC11-RFLP and SCAR marker were evaluated individually by χ² analyses for goodness of fit against a 1:1 segregation ratio at a 0.01 probability level. The RFLP and SCAR associations with F. graminearum resistance were tested by a single marker analysis using an ANOVA (V8.2, SAS Institute, Cary, NC, USA).

BLAST searches were performed with default settings using the National Center for Biotechnology (NCBI) search engine (http://www.ncbi.nlm.nih.gov/BLAST). Sequence alignments and phylogenetic analyses were performed by using Clustal W (Thompson et al., 1994) for nucleotide and Clustal X (Thompson et al., 1997) for amino acid with default settings at site of the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw).

Real-time PCR quantification
A Ribogreen RNA quantitation kit (Molecular Probes, OR) and a Wallace Victor microplate reader (Perkin-Elmer Life Sciences, St. Laurent, PQ) with the lamp filter F485nm and the emission filter F530nm were used to quantify the RNA. RNA quality (ribosomal band integrity) was determined by separating 2 μg of total RNA on 1% TBE of 0.8% (w/v) agarose gel and staining with 0.5 μg/ml ethidium bromide. Reverse transcription was carried out using 1 μg of total RNA for all samples with the Restroscript kit at 50ºC for one hour and 92ºC for 5 min, following manufacturer’s instruction (Ambion, Austin, TX).

Quantitative real-time PCR was carried out with an iCycler (BIO-RAD Laboratories, Mississauga, ON) with the Real-time Optical Module (BIO-RAD Laboratories, Mississauga, ON) and a SYBR Green Supermix kit (BIO-RAD). The real-time PCR primers that were used were: Zmgc1-Realtime-F 5′-ACAACAGACAACAGTGAGCCTGACT-3′ and Zmgc1-Realtime-R 5′-ATTTCGCGGCTCCTCGTCAACGCTCTTATTTG-3′, which were based on the sequences obtained for exon 6 and exon 8 of the Zmgc1 gene, respectively, and Zmbslr1-Realtime-F 5′-AGCTAATAGATCAAGAGCAAGCATGCA-3′ and Zmbslr1-Realtime-R 5′-TCTTTTCTCCAGGTAGTTATACGACTTTTG-3′. Both real time PCR products were approximately 150bp. The final 20 μL PCR reaction mixture contained: 2 μl of cDNA, 4nM primers and 10 μl 2X SYBR green supermix (BIO-RAD, Hercules, CA). Gene copy number was calculated using a standard curve method with the real-time PCR products as standards. The standard curve and the no template controls. Analysis of variance (ANOVA) with Tukey’s HSD test was employed to determine the differences between treatment intervals.

Results

Gene structure of Zmnbslrr1 and Zmgc1 gene
In total 2300 bp of genomic DNA from the downstream and upstream of the 420 bp PIC11 RGA fragment (Collins et al., 1998) was cloned and sequenced by performing genome walking steps with genomic DNA from genotype CO387 (data not shown). A 1674 bp open reading frame (ORF, Zmnbslrr1) was identified in the sequence (Fig. 1). The region upstream of the gene was GC-rich and appears to be a TATA-less promoter (Smale, 1997). The ORF was not interrupted by introns. EcoRV digested CO387 and CG62 genomic DNA samples were probed with the DIG labeled 420 bp PIC11 fragment (Collins et al., 1998) that gave a single band of different sizes. Parent CO387 sample had upper band whilst parent CG62 samples had lower band (Fig. 2A). The RILs derived from a cross between these inbred lines had either the parental patterns or heterozygote for the two patterns (Fig. 2A).

A SCAR marker was developed for the Zmnbslrr1 sequence that showed a 2100 bp band in CO387 and no band in CG62 (Fig. 2B).

An analysis of the predicted amino acid sequence for the ZmBNSLRR1 protein indicated that it shared a large number of homologies with NBS-LRR-like disease resistance genes found in rice, barley, wheat, and other crops. Alignment of conserved regions among resistance gene products revealed that the putative amino acid residues of ZmBNSLRR1 matched the consensus sequence found among resistance genes. Fig. 3A showed the significant similarity between the ZmBNSLRR1 amino acid sequence and the products of NBS-LRRs resistance genes such as RPR1 (BA7A5812; Sakamoto et al., 1999), LRR19 (AAK20736; Feuillet et al., 2001), RXO1 (AAX31149; Zhao et al., 2005), OS-NB-ARC (ABA92222; Buell et al., 2005) and HV-NBS-LRR (CAD45036; Madsen et al., 2003). The ZmBNSLRR1 possessed an ATP/GTP-binding motif (Traut, 1994) in the N-terminus that was composed of a P-loop domain (Saraste et al., 1990) at residues 201–211, a kinase 2 domain at 278–290 and followed by PLGL domain at 371–383. However, the Zmbslr1 showed a reduced domain in the C-terminus and imperfect LRRs (Fig. 3B). Those similar genes all belonged to the CC-NBS-LRR gene family. Clustal analysis demonstrated that Zmbslr1 was not most closely related with RPR1. Since RPR1 was induced by probenazole (Fig. 4), Zmbslr1 may be more functionally close to barley NBS-LRR (Madsen et al., 2003).

Our previous study (Yuan et al., 2008) showed that genomic DNA of Zmgc1 gene spanned 3.3 kb while cDNA clones were 1.3 kb and contained a predicted protein of 267 amino acids (Yuan et al., 2008). In the upstream of the gene coding sequence, the gene contained GC-rich sequences and no obvious TATA box was identified. The complete nucleotide sequence consisted of nine exons
interrupted by eight introns evenly distributed within the gene. The ortholog in Arabidopsis AtGC1 (GenBank accession number AAM51559) was the most similar GC known to ZmGC1 at 53% identical and 65% similar to ZmGC1. Deduced amino acid sequence of ZmGC1 was aligned with those of GCs that had been used to search common motifs (Lukiki and Gehring, 2003; Yuan et al., 2008). However, Zmnbslrr1 was not assigned any Fusarium resistance in our mapping population.

Genetic mapping and disease resistance of the Zmnbslrr1 and Zmgc1

PIC11 marker was positioned on the linkage group 5 of developed previously for genetic map of the population and 9 cM and 16 cM from molecular markers umc1056 and BC399_1300 respectively based on RFLP data (Fig. 5). The SCAR (sequence characterized amplified region) marker (Fig. 2B) derived from total GenomeWalking sequence containing Zmnbslrr1 gene sequence also

Different Responses of Two Genes to 3-allyloxy-1,2-benzothiazole 1,1-dioxide i89 Fig. 1. ORF of Zmnbslrr1 and the deduced amino acid sequences from genotype CO387. The amino acid coding sequence was shown by the one-letter code. The positions of the primers (Zmnbslrr1-Realtime-F and Zmnbslrr1-Realtime-R) for real-time PCR were marked by black arrows. The alignment was performed using ClustalX (Thompson et al., 1997).
Fig. 2. Two molecular mapping approaches to position the Zmnbslrr1 gene on the linkage map derived from F5 RIs crossed from CG62 x CO387. (A) X-ray films exposed to membranes of the F5 population genomic DNA hybridized with a probe derived from sequence information of Zmnbslrr1 gene. The probe was amplified using primers RFLP-PIC11-F 5′-CTAATAGATCAGAGAGCAAGCA-3′ and RFLP-PIC11-R 5′-GTTCCCAGGATTCAGCATATTG-3′ labeled with DIG method (Boehringer Mannheim, Germany). Recombinant inbred (RI) lines were digested with restriction enzyme EcoRV and one RFLP co-dominant marker was scored. A: genotype same as CG62, B: genotype same as CO387, H: heterozygous. (B) Sequence-characterized amplified region (SCAR) marker of PIC11. The fragments were amplified from parental DNA CO387 and CG62 and RI lines using primers (primer SCAR-PIC11-F 5′-ACTGGGCATGAAGGGTACTAG-3′ and SCAR-PIC11-R 5′-CTTAATCAGGTCCCTGGTTAGT-3′). The map position of the SCAR marker is the same as that of RFLP. A: genotype same as CG62, B: genotype same as CO387.

Fig. 3. Alignment of the deduced amino acid of ZmNBSLRR1 with other NBS-LRR genes. A Similarity between the ZmNBSLRR1 amino acid sequence and the products of NBS-LRRs resistance genes such as the rice RPR1 (BAA75812; Sakamoto et al., 1999), LRR19 (AAK20736; Feuillet et al., 2001), RXO1 (AAX31149; Zhao et al., 2005), OS-NB-ARC (ABA92222; Buell et al., 2005) and HV-NBS-LRR (CAD45036; Madsen et al., 2003). The ZmNBSLRR1 possesses an ATP/GTP-binding motif in the N-terminus that was composed of a P-loop domain, a kinase 2 domain, and followed by PLGL domain. B ZmNBSLRR1 showed obviously a reduced size domain in the C-terminus and it contained imperfect LRRs.
confirmed the gene position on the genetic map. The mapping position matches that of others (Collins et al., 1998).

Six polymorphic markers derived from Zmgc1 gene were identified in the membranes containing the individuals digested with restriction enzyme EcoRI (ER1_1 to ER1_5) and EcoRV (ER5_1). Two RFLP bands, ER1_1 and ER5_1, were significantly associated with gibberella ear rot resistance in maize across different environments in a recombinant inbred (RI) population (Yuan et al., 2008).

Zmnbslrr1 and Zmgc1 gene expression
In order to examine the function of Zmnbslrr1 and Zmgc1 gene in maize, the response of the transcript abundances to probenazole was measured. The pattern of mRNA expression of the Zmgc1 gene showed clear differences compared to that of the Zmnbslrr1 gene in corn seedling tissue after the probenazole treatment (Fig. 6).

Fig. 6 showed that the basal expression level of Zmgc1 gene was low in untreated plants and the transcript abundance rapidly reached a peak within 2h after pathogen challenge. In contrast, the reduction of Zmnbslrr1 gene expression was observed at this early stage. Significantly increased Zmgc1 transcript abundance (more than 10 folds) was detected 8hours after treatment with probenazole. The transcription abundance remained higher throughout the time-course study. For six days after the probenazole applied, the abundance of the Zmgc1 mRNA was still notably higher than that of basal level. However, transcript abundance of Zmnbslrr1 in the seedling treated with probenazole was reduced substantially by PBZ treatment throughout the time course. The transcript abundance of Zmnbslrr1 gene was notably lower than that of Zmgc1 gene.

Discussion
RGAs can be cloned by PCR-based approaches and/or by using genomic information and then genetically mapped. Kojima et al., (2002) demonstrated a successful example using known gene sequences to isolate related genes, in which the QTL had been confirmed as candidate genes whose function was known from the researches of Arabidopsis. Collins et al., (1998) presented one of the molecular markers named PIC11 derived from a RGA. PIC11 RGA had been mapped to a locus that was a previously defined resistance locus. The locus was associated with Gibberella stalk rot that was caused by Gibberella zeae (petch), which is the sexual form of F. graminearum (Pe et al., 1993). If functionality of the Zmnbslrr1 gene can be tested, then it will be easy to determine whether the candidate gene confers resistance when it is expressed as a transgene in a susceptible line. The putative amino acid sequence of ZmnBSLRR1 showed the similarity with NBS-LRR genes including RPR1 (BAA75812; Sakamoto et al., 1999), LRR19 (AAK20736; Feuillet et al., 2001), RXO1 (AAX31149; Zhao et al., 2005), OS-NB-ARC (ABA92222; Buell et al., 2005) and HV-NBS-LRR (CAD45036; Madsen et al., 2003). Sakamoto et al., (1999) demonstrated that the gene product, RPR1, contained motifs of a nucleotide binding site (NBS) and a leucine-rich repeats (LRR) domain and RPR1 gene expression was up-regulated by treated with probenazole, chemical inducers of systemic acquired
resistance (SAR) such as BTH and SA, and pathogens. However, PIC11 was mapped to the same locus as that of other’s study but it was not assigned any resistance in our mapping population. Hubert et al., (2001) suggested that resistance loci are often comprised of complex multicyclic clusters of genes. The duplication events may play an important role in generating new resistance gene specificities in R-gene and RGA clusters. Duplicated RGA sequences result from transposition, unequal crossing-over, gene conversion, and point mutation in several crops (Meyers et al., 1998; Collins et al., 1999; Ellis et al., 1999). However, the reason for the reduced size of Zmnbslrr1 gene is not known and only a single copy of Zmnbslrr1 was identified. Zmnbslrr1 might be a pseudogene due to the size of the gene but the detected transcript suggested that it was expressed.  

Probenazole induces systemic acquired resistance (SAR)-like disease resistance in rice (Oryza sativa) has been widely used against rice blast disease for many years in Asia (Sakamoto et al., 1999). For plant sustainable disease management, chemical activated disease resistance may be an option for growers to protect their crops from plant diseases if the genetic resources could not provide sufficient resistance. It is necessary to understand about the various defense signal pathways in plants previous application of commercial chemical activators. The differential expression of Zmnbslrr1 and Zmgc1 gene before and after probenazole treated suggested that these genes might act different metabolic pathways corresponding to same signal. Induction of Zmgc1 transcript was the response expected for a gene involved in partial resistance. Reduction in Zmnbslrr1 transcript abundance may suggest that it is not involved in resistance to F. graminearum, but represents a competing pathway or contributes to susceptibility.  

In plants, cGMP is involved in modulating gene expression of many defense and stress related pathways either functioning alone or combining with calcium (Bowler et al., 1994). Whether the guanylyl cyclase plays direct roles in perception of host stimuli or has roles in signal processing after probenazole remains to be determined. Gibberella ear rot infections usually occur when the cool weather precedes wet and warm condition during flowering. If the abiotic stress (cold) affects the abundance of cGMP and transcription of the GC gene(s) remains to be addressed. Enhancing activity of cell membrane GTPase, an enzyme involved in intracellular signal transduction pathway had been identified after the rice plant treated with probenazole (Kanoh et al., 1993). Although the relationship of probenazole induced resistance and SAR-like resistance induced by pathogen is still fragmentary, it suggested that the protective effects of the chemical mainly due to activation of host defense mechanism (Sakamoto et al., 1999). If there is any correlation between GTP metabolism and disease resistance or probenazole induction is still unknown. How the Zmgc1 gene complement interacts with other genes is also not clear, but understanding these processes may lead to novel strategies to interfere in recognition host-pathogen.  

Acknowledgments  
This work was supported by the Ontario Corn Producers, the Ontario Corn Consortium (including the Ontario Pork Producers, Pioneer Hi-Bred, Syngenta Seeds Canada, Inc., Agreo, King Agro, Pickseed Canada, W.G. Thompson, and Zeneca), the Ontario Ministry for Food and Agriculture, Ontario Research Enhancement Program, Canadapt, Federation des Producteurs de Cultures Commerciales du Quebec, the Natural Sciences and Engineering Research Council of Canada and Agriculture and Agri-Food Canada.  
Angela Hill is gratefully acknowledged for assistance in preparing the manuscript.  

References  


Uchiyama, M., Abe, H., Sato, R., Shimura, M., and Watanabe, T. (1973) Fate of 3-allyloxy-1,2-benzisothiazole 1,1-dioxide (Oryzemater) in rice plants. Agricultural and Biological Chemistry. 37, 737–745.


New Books

Caliciviruses
Molecular and Cellular Virology
Edited by: G.S. Hansman, J. Jiang, K.Y. Green
vii + 290 pp., April 2009
ISBN: 978-1-904455-43-1 $310 / £150
The recent advances.

Influenza
Molecular Virology
Edited by: Qinghua Wang and Yizhi Jane Tao
vii + 342 pp., February 2010
ISBN: 978-1-904455-57-8 $310 / £159
NS1, hemagglutinin, nucleoprotein, glycoproteins, M2 channel, virulence, polymerase, microarrays, vaccine design.

Neisseria
Molecular Mechanisms of Pathogenesis
Edited by: Caroline Genco and Lee Wetzler
x + 270 pp., January 2010
ISBN: 978-1-904455-51-4 $310 / £150
Genomics, biofilms, adhesion, invasion, immunity, complement, apoptosis, vaccine, epidemiology, antibiotic resistance.

Epstein-Barr Virus
Latency and Transformation
Edited by: Eric S. Robertson
c. 220 pp., April 2010
Expert virologists comprehensively review this important subject from a genetic, biochemical, immunological, and cell biological perspective. Essential reading.

RNA Interference and Viruses
Current Innovations and Future Trends
Edited by: Miguel Angel Martinez
c. 280 pp., February 2010
ISBN: 978-1-904455-56-1 $310 / £159
Expert RNAi specialists from around the world have teamed up to produce a timely and thought-provoking review of the area.

Frontiers in Dengue Virus Research
Edited by: K.A. Hanley and S.C. Weaver
viii + 304 pp., January 2010
Evolution, epidemiology, translation, replication, pathogenesis, host, animal models, mosquito interactions, transmission, vaccine, drugs, immunotherapy.

Metagenomics
Theory, Methods and Applications
Edited by: Diana Marco
x + 212 pp., January 2010
ISBN: 978-1-904455-54-7 $310 / £159
Essential reading for all researchers performing metagenomics studies. Highly recommended.

Lab-on-a-Chip Technology
Vol 1: Fabrication and Microfluidics
Edited by: K. E. Herold and A. Rasooly
xii + 260 pp., April 2009
Vol 2: Biomolecular Separation and Analysis
ISBN: 978-1-904455-49-3 $310 / £150

Bacterial Polysaccharides
Current Research and Future Trends
Edited by: Matthias Ullrich
xii + 358 pp., June 2009

Microbial Toxins:
Pili and Flagella
Current Research and Future Trends
Edited by: Ken Jarrell
x + 238 pp., August 2009
ISBN: 978-1-904455-45-5 $310 / £150

Microbial Toxins:
Current Research and Future Trends
Edited by: Thomas Proft
xii + 260 pp., March 2010
Details the major current advances in microbial population genetics and genomics.

Microbial Population Genetics
Edited by: Jianping Xu
c. 230 pp., May 2010
Details the major current advances in microbial population genetics and genomics.

Aspergillus
Molecular Biology and Genomics
Edited by: M. Machida and K. Gomi
x + 230 pp., May 2010
ISBN: 978-1-904455-52-3 $310 / £159
Systematics, bioinformatics, systems biology, regulation, genetics, genomics, metabolism, ecology, development.

Bacterial Secreted Proteins:
Secretory Mechanisms and Role in Pathogenesis
Edited by: Karl Wooldridge
xii + 512 pp., April 2009

Caister Academic Press
www.caister.com