Characterizing and Mapping Resistance in Synthetic-Derived Wheat to *Rhizoctonia* Root Rot in a Green Bridge Environment

A. K. Mahoney, E. M. Babiker, T. C. Paulitz, D. See, P. A. Okubara, and S. H. Hulbert

All authors: Department of Plant Pathology, Washington State University, Pullman 99164-6430; and third, fourth, and fifth authors: United States Department of Agriculture–Agricultural Research Service, Wheat Health, Genetics and Quality Research Unit, Pullman, WA 99164-6430.

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ABSTRACT

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Root rot caused by *Rhizoctonia* spp. is an economically important soil-borne disease of spring-planted wheat in growing regions of the Pacific Northwest (PNW). The main method of controlling the disease currently is through tillage, which deters farmers from adopting the benefits of minimal tillage. Genetic resistance to this disease would provide an economic and environmentally sustainable resource for farmers. In this study, a

collection of synthetic-derived genotypes was screened in high-inoculum and low-inoculum field environments. Six genotypes were found to have varying levels of resistance and tolerance to *Rhizoctonia* root rot. One of the lines, SPBC-3104 ('Vorbey'), exhibited good tolerance in the field and was crossed to susceptible PNW-adapted 'Louise' to examine the inheritance of the trait. A population of 190 BC₁-derived recombinant inbred lines was assessed in two field green bridge environments and in soils artificially infested with *Rhizoctonia solani* AG8. Genotyping by sequencing and composite interval mapping identified three quantitative trait loci (QTL) controlling tolerance. Beneficial alleles of all three QTL were contributed by the synthetic-derived genotype SPBC-3104.

Wheat-based cropping systems in the Pacific Northwest (PNW) typically include several tillage operations for residue management and weed control. However, multiple tillage operations have negative effects, including soil erosion, water loss through evaporation, and increased fuel and labor costs (Triplett and Dick 2008). To reduce these issues, many growers in the PNW are transitioning to minimal tillage practices, including no-till or direct-seeding. Higher incidences of root rot from soilborne pathogens in minimal or no-tillage fields has been reported (Cook 2001; Paulitz 2006). To manage weeds and volunteer plants in a direct-seeding system, growers typically rely on herbicide applications before seeding. Dying plants following herbicide application temporarily increase the inoculum levels of several soilborne pathogens that cause root and crown diseases, producing a "green bridge" that promotes these diseases (Bailey et al. 2009; Cook 2001; Paulitz 2006; Paulitz et al. 2003b).

The green bridge effect is associated with the several cereal pathogens, including *Rhizoctonia solani* J. G. Kühn AG8, *R. oryzae* Ryker & Gooch, and multiple *Pythium* spp. *Rhizoctonia* spp. are ubiquitous soil pathogens with a very broad host range (Goll et al. 2014; Hane et al. 2014). Root rot caused by *R. solani* is characterized by stunted seedlings, brown atrophic lesions of the root cortex, spear tipping, and chlorotic tissues (Weller et al. 1986). These symptoms cause yield and developmental delays, as well as reduced tolerance to drought stress and nutrient deficiencies (Mazzola et al. 1996; Weller et al. 1986).

Yield losses from soilborne pathogens in cereal production regions of the PNW can range from 10 to 15% depending on the

inoculum levels (Cook et al. 2002). In severe cases, as the levels of *Rhizoctonia* spp. increase, patches occur where the plants are severely stunted. These bare-patch symptoms can cause yield losses near 30% (Cook et al. 2002; Paulitz 2006). Unfortunately, there are very few control methods to prevent *Rhizoctonia* root rot in wheat production. Tillage is currently the best control method to reduce disease pressure, because it is thought to disrupt the hyphal networks (Gill et al. 2002) and can also alter soil microbiology (Al-Hazmi and Al-Nadary 2015; Liu et al. 2015; Yin et al. 2010). An interval of two or more weeks after spraying crop volunteers and weeds with herbicides and planting could reduce seedling disease in spring crops by decreasing inoculum levels in the soil (Babiker et al. 2011; Rovira and McDonald 1986; Smiley et al. 1992) but delayed seeding also delays maturity and can decrease yields. There are newer classes of fungicides such as sedaxane which may be effective against some species of *Rhizoctonia* (Goll et al. 2014; Zeun et al. 2013). Current control options have economic trade-offs or are not agriculturally sustainable. Therefore, farmers who plant into a green bridge or a high-inoculum environment would greatly benefit from a tolerant and/or resistant cultivar of wheat.

The development of cultivars resistant to *Rhizoctonia* spp. in the field has been problematic due to poor phenotyping methods and lack of resistant germplasm (Neate 1989; Okubara et al. 2009; Smith et al. 2003). Two wheat genotypes, a chromosome 4 addition line (Okubara and Jones 2011) and an ethyl methanesulfonate (EMS) mutagenesis-derived line, Scarlet-Rz1 (Okubara et al. 2009), showed tolerance to *Rhizoctonia* spp. in greenhouse assays. Scarlet-RZ1 was tested for resistance in the field and was not found to improve yield (P. A. Okubara and K. K. Kidwell, personal communication). There have been comprehensive efforts to improve the genetic diversity of wheat through the exploitation of wild wheat germplasm. The International Maize and Wheat Improvement Center (CIMMYT) has produced over 1,000 synthetic wheat lines to increase diversity while improving agronomic and disease resistance traits (Yang et al. 2009). Synthetic wheat is developed by crossing tetraploid

Corresponding author: S. H. Hulbert; E-mail address: scot_hulbert@wsu.edu

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wheat (*Triticum turgidum* L., $2n = 4x = 28$, AB genomes) with *Aegilops tauschii* Coss. ($2n = 2x = 14$, D genome), resulting in hybrids that contain the A, B, and D genomes. After chromosome doubling, these new lines are rich sources of genetic variation for introgression into breeding programs. However, many of these progenitors carry unfavorable alleles that can affect other important agronomic traits (Naz et al. 2014; Tanksley and Nelson 1996). To circumvent this issue, advanced backcrossing with an elite cultivar allows for introgression of beneficial alleles and reduces the proportion of the wild progenitor's genome. By pairing these breeding methods with molecular tools, a suite of possibilities for wheat improvement exists.

The increased affordability of next-generation sequencing (NGS) technologies continues to advance breeding capabilities. Genotyping by sequencing (GBS) is now more feasible for large genome species such as wheat and can be used to identify and map the loci controlling quantitative traits (Poland et al. 2012). With the recent sequencing of the wheat genome (Brenchley et al. 2012; Mayer et al. 2014), there is now computational information to create single-nucleotide polymorphic (SNP) markers, localize these markers to their respective genomes, and create dense genetic maps for large genomes. The objectives of this study were to (i) evaluate the resistance of different synthetic wheat genotypes to varying levels of *R. solani* AG8 and *R. oryzae* in the field and greenhouse and (ii) identify quantitative trait loci (QTL) using GBS in a backcrossed biparental mapping population to improve stunting to *Rhizoctonia* spp. in a green bridge environment.

MATERIALS AND METHODS

Screening of the CIMMYT wheat germplasm collection.

In total, 412 synthetic hexaploid wheat genotypes were provided by Dr. Arthur Klatt, Department of Plant and Soil Science, Oklahoma State University. These were planted for 2 years (2007 and 2008) in an area with a history of *R. solani* AG8 and *R. oryzae* (Babiker et al. 2011; Okubara et al. 2008; Schroeder et al. 2006) at the Palouse Conservation Field Station (PCFS) near Pullman, WA. Inoculum development (green bridge) was encouraged in the plots by allowing grassy weeds and volunteer cereals to grow over the winter. High and low levels of *Rhizoctonia* spp. inoculum were created by spraying grassy weeds and volunteer plants with glyphosate at a rate of 0.85 kg/ha (Alecto 41S; Ritter Chemical LLC) at 1 day (for high pathogen inoculum levels or green bridge) and 14 days (for low inoculum level) before planting. Seed were directly drilled into the soil to a depth of approximately 3 cm using a head row planter. Each of these genotypes was tested without replication as paired single rows with high and low levels of *Rhizoctonia* spp. inoculum. Four weeks after emergence, shoot length for each genotype was obtained from nine randomly selected plants. Field screening was started in the spring and seed was harvested in late summer.

Growth chamber screening of synthetic wheat accessions.

Greenhouse assays were conducted in the winter, between field screenings. For the greenhouse assays, two soils types, a silty loam (Pullman, WA) and a fine sandy loam (Ritzville, WA), were used. Inoculum was prepared from *R. solani* AG8 (isolate C1) (Weller et al. 1986) and *R. oryzae* (isolate 801387) (Paulitz et al. 2003a) on autoclaved oat seed, as described by Paulitz and Schroeder (2005). Each genotype was scored for resistance in three independent, completely randomized trials, with four replicates each. Seed of each genotype were disinfested for 5 min with a 10% bleach solution, rinsed with distilled water, and placed into plastic cones (Stuewe and Sons) with approximately 60 g of either soil type. Soils were infested with either *R. solani* AG8 or *R. oryzae* inoculum at 200 propagules per gram (ppg). The control (no inoculum) soils contained 1% (wt/wt) ground autoclaved oat seed. Seedlings were evaluated at 14 days after planting. Disease ratings were scored on a scale of 0 (no disease) to 8 (severe root rot) (Kim et al. 1997).

Mapping population development. In total, 190 BC₁F₆ recombinant inbred lines (RIL) were constructed by crossing spring wheat 'Louise' (Kidwell et al. 2006) and SPCB-3014, and backcrossing the F₁ to Louise. The population was advanced to the BC₁F₆ generation via single-seed descent. SPCB-3104 'Vorobey' is a synthetic-derived hexaploid wheat created at CIMMYT (Table 1). This genotype was chosen from preliminary and advanced field screening. SPCB-3104 exhibited high levels of tolerance to natural inoculum of *R. solani* AG8 and *R. oryzae* in a green bridge environment. This genotype is well adapted for multiple environments, with increased root mass under drought-like field conditions (Lopes and Reynolds 2011).

Evaluation of the mapping population. Field nurseries at the PCFS were used to evaluate the mapping population for resistance to root rot pathogens in a green bridge environment in 2012, 2013 (two different fields), and 2014. However, in the 2012 site and one of the 2013 sites, wireworm (*Agroites mancus*) infestations generated discrepancies in the phenotyping data; therefore, these data were excluded. High and low *Rhizoctonia* spp. inoculum levels at each site were as described as for screening of the synthetic wheat germplasm collection above. Seed were treated with imidacloprid (Bayer) to reduce root damage from soilborne insects, following the manufacturer's recommended rate. Approximately 5 g of seed of each RIL and parental line was seeded in 90-cm rows. Each row was replicated three times in a randomized complete block. Five representative seedlings from each row were removed below the root crown at 4 weeks, placed into sealable bags, and refrigerated until they were measured. *Rhizoctonia* root rot was assessed for each treatment by measuring shoot lengths for each RIL and parental line and calculating their percent shoot length reduction (SLR) in the high-inoculum rows compared with the low-inoculum rows.

The mapping population was scored in three separate completely randomized trials, each with four replicates (seedlings). Seed of each genotype were cold stratified on germination paper to synchronize germination at 4°C for 24 h and incubated at 22°C for another 24 h before planting. In each replicate, a seed of Louise, SPCB-3014, and each RIL was placed onto 60 to 70 g of sieved pasteurized Ritzville soil completely mixed with either sterile autoclaved ground oat (1% wt/wt) or ground oat infested with *R. solani* AG8 at 200 ppg. In each cone, 5 g of noninfested soil covered the seed. All cones were watered and placed into a 15°C growth chamber with a 12-h photoperiod and watered as needed. Seedlings were evaluated at 14 days after planting and scored for PSRL in the cones with inoculum compared with the control cones.

GBS library development and SNP marker discovery.

Genomic DNA of Louise, SPCB3104, and their RIL were harvested from lyophilized tissue of 14-day-old seedlings. DNA was isolated from ground tissue using a BioSprint 96 robot using BioSprint 96 DNA plant kits (Qiagen). GBS libraries were constructed as described by Poland et al. (2012). Briefly, samples were digested with restriction enzymes *Pst*I and *Msp*I (New England BioLabs Inc.) and barcoded adapters were ligated onto the samples using

TABLE 1. Pedigrees of synthetic-derived and control wheat genotypes that were tested for resistance to *Rhizoctonia solani* AG8 and *R. oryzae*

Genotype	Pedigree
SYN-30	CROC_1/ <i>Aegilops tauschii</i> (210)
SYN-172	SNIPE/YAV79//DACK/TEAL/3/ <i>A. tauschii</i> (904)
SYN-182	CROC_1/ <i>A. tauschii</i> (518)
SYN-201	68112/WARD// <i>A. tauschii</i> (369)
SPCB-3104 (Vorobey)	CROC_1/ <i>A. tauschii</i> (224)//OPATA/3/PASTOR
SPCB-3220	ALTAR 84/ <i>A. tauschii</i> (BANGOR)//ESDA
Louise	Wakanz/PI 506352//Wawawai/PI 574538)
Scarlet	HF820049/WA007301//Tecumseh/K8405055
Scarlet-Rz1	EMS mutagenized Scarlet genotype (Okubara et al. 2009)

T4 DNA ligase (New England BioLabs, Inc.). Samples were purified, pooled, and sequenced on an Ion Proton system (Life Technologies Inc.).

The SNP marker pipeline created by the United States Department of Agriculture–Agricultural Research Service Western Regional Small Grains Genotyping Center was used to analyze the sequencing reads (D. Z. Skinner, unpublished). The pipeline uses Unix, Python, and R scripts. From this pipeline, unique sequence tags with three or fewer mismatches between parents were aligned and clustered to identify the nucleotide position of the SNP. Shared sequences between the parents with putative SNP were surveyed across the population and were retained if they were present in the population at specific thresholds. GBS markers with >10% heterozygosity and missing >20% data were discarded from genetic map construction.

Four SNP loci were selected to test the origin of the tolerance from the durum wheat ‘Croc_1’ in the synthetic and synthetic-derived (SPCB) wheat genotypes and Louise (Table 1). Genomic DNA was isolated from these genotypes using the PowerPlant DNA isolation kit (MO BIO Laboratories) following manufacturer’s directions. To amplify the region containing the SNP, polymerase chain reaction (PCR) primers (Supplementary Table S1) were designed to flank the sequence containing the SNP. PCR contained designated primers, Q5 high-fidelity DNA polymerase, and the manufacturer’s recommend components (NEB). PCR amplifications were performed as follows: 95°C for 5 min followed by 35 cycles of 95°C for 30 s, a gradient of different annealing temperatures of 56 to 62°C (primer-dependent), and 72°C for 30 s. A final extension step was performed at 72°C for 1 min; samples were held at 4°C. Samples were electrophoresed on 2% agarose Tris-acetate EDTA gel with ethidium bromide. Bands were size selected, removed from the gel, and purified with the QIAquick gel extraction kit (Qiagen). Fragments were a-tailed using Taq DNA polymerase and ThermoPol buffer, following the manufacturer’s protocol (NEB). Fragments were cloned into a pGem vector (Promega Corp.) and transformed into DH5α cells using the heat shock method. Plasmids were purified from these cells using a QIAprep spin miniprep kit (Qiagen). Plasmids were sequenced using the M13F primer on an ABI3730xl DNA sequencer (Eurofins Genomics). Sequences were aligned, trimmed, and compared using Geneious software (v8.1.1; Biomatters).

Linkage analysis and QTL identification. A linkage map was developed from the GBS SNP markers using MapDisto, version 1.8.1 (Lorieux 2012). Linkage groups were established using a log of the odds (LOD) score of 5 and general default settings. The “order” and “ripple” function for each sequence was used to determine the best arrangement for each marker. Each linkage group was individually surveyed for the correct order. Recombination

distances were converted into centimorgans using the Kosambi function (Kosambi 1943). Linkage groups were assigned to a physical wheat chromosome by using the BLASTn (Altschul et al. 1997) results from the ‘Chinese Spring’ database (Mayer et al. 2014).

The BCsFt function (Shannon et al. 2013) and composite interval mapping available in R/QTL (Broman et al. 2003) identified significant QTL. The “convert2bcsft” function, with a BC.gen = 1, F.gen = 6 setting, was used for the scan. A 2-centimorgan (cM) marker regression interval was used for the genome-wide analysis. The RIL mean phenotype for PSLR for each year was used in the QTL analysis. A significant LOD threshold of 2.5 ($P \leq 0.05$) was determined using 1,000 permutations. The function FitQTL in R/QTL estimated QTL phenotypic variance (R^2) and additive effects. A QTL interval was estimated by a one-LOD drop off from the peak position. QTL graphs were constructed using Map Chart, version 2.3 (Voorrips 2002).

Statistical analysis. The growth chamber experiments for screening the six synthetic wheat genotypes were arranged in a split-split-plot design, with soil as the main plot treatment, soil infestation as subplot treatment, and wheat genotype as sub-subplot treatment. An analysis of variance was conducted to test for significance of soil, treatments, genotype, environment, and all interactions. Tukey’s honestly significant difference was used to determine the significant differences between the two soils, soil infestation treatments, shoot and root measurements, and mean disease ratings. Paired genotype differences to high and low inoculum were compared using a Student’s *t* test. A Pearson’s correlation for mean disease ratings was used to determine the associations between Rhizoctonia root rot rating for the PSLR and seedling fresh weight reduction. All statistical analyses were conducted using JMP11 (SAS Institute) and add-in SAS scripts.

The narrow-sense heritability (h^2) for shoot length reduction as a percentage was estimated from the formula $h^2 = 1 - [\text{mean square}(\text{genotype} \times \text{environment}) / \text{mean square}(\text{genotype})]$ (Knapp et al. 1985; Zhu et al. 2016).

RESULTS

Field assessment of selected genotypes in a green bridge environment. A green bridge environment was used to select for root rot tolerance or resistance of 412 synthetic and synthetic-derived wheat genotypes. The green bridge (high inoculum levels) environment was made by spraying wheat seedlings and volunteer weeds with glyphosate the day before planting so that inoculum from dying plants could be increased and infect adjacent wheat seedlings as they germinated and emerged. For comparison, rows were planted on adjacent strips by more standard no-till practices, in which the strips were sprayed with glyphosate 2 weeks before planting so that the weeds and volunteers had time to die and decompose and inoculum levels would be lower. The fields used were on a region of the PCFS farm that had a history of *R. solani* and *R. oryzae* infestation. Six lines (SYN-30, SYN-172, SYN-182, SYN-201, SPCB-3104, and SPCB-3220) that showed little or no stunting in the green bridge environment were selected for further tests by the same assay. Three wheat lines were included for comparison: Louise, ‘Scarlet’ (Kidwell et al. 1999), and the EMS-induced mutant line Scarlet-Rz1. Shoot length, first leaf length, and root fresh weight were measured to determine the degree of growth suppression in the high-inoculum level treatment. Because the germplasm examined was very diverse, the lines varied in the growth rates of their seedling shoots and roots; therefore, resistance or tolerance was estimated by the percent reduction in the high-inoculum environment (Table 2). In general, stunting was observed in the high-inoculum level environment, as expected, especially in the control lines. Only one of the synthetic-derived lines, SYN-30, showed a significant reduction in shoot length, and the percent reduction was less than half that of the control lines. Similarly, only

TABLE 2. Extent of growth reduction in high- and low-inoculum level treatments for different wheat genotypes in field experiments^z

Parameters	Shoot length		First leaf length		Root fresh weight	
	Reduction (%)	<i>P</i> value	Reduction (%)	<i>P</i> value	Reduction (%)	<i>P</i> value
Genotypes						
SYN-30	10.2	0.01	8.3	0.109	9.0	0.348
SYN-172	0.0	0.965	−1.2	0.388	−0.4	0.214
SYN-182	0.6	0.823	−13.1	0.001	−25.4	0.001
SYN-201	−4.3	0.412	0.0	0.776	5.9	0.439
SPCB-3104	5.6	0.454	4.5	0.042	2.8	0.392
SPCB-3220	−4.3	0.191	−4.8	0.112	18.3	0.001
Louise	21.4	0.003	8.6	0.012	18.4	0.001
Scarlet	23.9	0.001	9.7	0.002	38.5	0.013
Scarlet-Rz1	35.1	0.001	4.1	0.001	43.2	0.001

^z Probability values are listed for a paired *t* test between the high-inoculum (green bridge) and low-inoculum environments for each genotype. Probabilities are in bold when traits are statistically reduced (positive value) from the high inoculum. Values are means of three replications per genotype for each treatment.

one of the synthetic-derived lines had significant reductions in each of the first-leaf-length and root-fresh-weight traits: SPCB-3104 and SPCB-3220, respectively. SYN-201, SYN-182, and SYN-172 showed little if any reduction in any of the three traits (Table 2) in the high-inoculum environment; SYN-182 actually averaged longer first-leaf-lengths and root weights. When considering all three traits, all of the six synthetic-derived lines exhibited good tolerance in the presence of the green-bridge-enhanced inoculum levels in the field.

Growth chamber germplasm assays. To examine the nature of resistance or tolerance of the lines showing promise in the field tests to specific pathogens, they were inoculated with *R. solani* AG8 and *R. oryzae* in growth chamber experiments and the roots were examined for the extent of colonization and disease. Two different pasteurized soils were infested with the inoculum with the two *Rhizoctonia* spp. for four treatments in total. Disease levels varied with soil type and *Rhizoctonia* spp. (Table 3). The genotypes SYN-30, SYN-172, and SYN-182 had the lowest disease score for *R. solani* AG8 in the Pullman soils while these three along with SPCB-3220 and Scarlet-Rz1 had lower levels of disease for *R. solani* in the Pullman soil. Higher disease scores were typically observed in the sandier Ritzville soils, and some of the lines that appeared moderately resistant in the Pullman soil appeared more susceptible in the Ritzville soil. SYN-172 had the lowest disease scores in both soil types infested with either species, indicating higher levels of resistance for this genotype. Alternatively, SPCB-3104 and SPCB-3220 only had significantly better disease ratings than Louise in the Pullman soils infested with *R. oryzae*. The results indicate that the performance of these latter two lines in field assays would be better described as tolerance rather than resistance.

Pearson correlation tests were performed to determine how the different traits (growth variables) measured in the field assays of *Rhizoctonia* root disease compared with similar assays in the growth chamber with pasteurized soils containing inoculum. Disease ratings of the different lines in the growth chamber showed a better correlation with shoot length reductions ($R^2 = 0.49$, $P = 0.03$) than they did with reductions in the first leaf length ($R^2 = 0.27$) or fresh weight of the roots ($R^2 = 0.11$) in the field experiments. Therefore, we used PSLR as our main trait for evaluating seedlings of the RIL populations in field assays on the basis that this may best reflect root disease levels.

Root rot assays of the genetic mapping population. Parents and the 190 BC₁F₆ RIL derived from the Louise × SPCB-3104 cross were compared in Pullman in 2013 and 2014 in the high-inoculum (green bridge) and low-inoculum environments. Reduction in plant height was readily observable for most lines at approximately 3 weeks postgermination. In 2013 and 2014, SPCB-3104 exhibited shoot length reductions of 6.4 and 7.9%, respectively. Louise was highly susceptible in these environments and exhibited shoot length reductions of 22.1 and 18.9%, respectively (Fig. 1). There was significant variation among RIL for shoot length reduction in these environments (Table 4). Percent reduction for shoot length ranged

from 0 to approximately 30% across the two field environments, with a mean of 13.8% (Fig. 1).

Screening the population in the growth chamber with *R. solani* AG8-infested soils showed similar variation to the field nurseries. Louise and SPCB-3104 averaged shoot length reductions of 28 and 3% reduction, respectively. The population had a shoot length reduction of 9%, with a range of 3 to 35% (Fig. 1C).

An analysis of variance for PSLR for the mapping population revealed that both genotype ($P = 0.041$) and environment ($P < 0.002$) were statistically significant across the three environments (two fields and one growth chamber) (Table 4). However, the genotype–environment interaction was not statistically significant ($P = 0.164$). The narrow-sense heritability for PSLR was estimated at 0.68.

Fig. 1. Phenotype distribution of the 190 Louise × SPCB-3104 recombinant inbred lines (RIL) for **A**, Pullman 2013; **B**, Pullman 2014; and **C**, growth chamber. Percentages are the mean shoot length reduction for three replications of each RIL in their respective environments.

TABLE 3. Mean disease rating of wheat genotypes in different soils evaluated for resistance to *Rhizoctonia solani* AG8 and *R. oryzae*^a

Genotype	Pullman soil		Ritzville soil	
	<i>R. solani</i>	<i>R. oryzae</i>	<i>R. solani</i>	<i>R. oryzae</i>
SYN-30	3.0 c	2.9 c	4.3 c	4.0 cde
SYN-172	3.0 c	2.1 d	3.2 d	2.5 f
SYN-182	3.0 c	3.0 c	4.5 c	2.5 f
SYN-201	3.4 b	2.9 c	4.0 cd	4.8 abc
SPCB-3104	4.0 ab	4.0 b	4.3 c	3.7 de
SPCB-3220	4.1 ab	2.6 d	5.7 ab	5.5 a
Louise	4.3 a	4.7 a	4.8 bc	3.2 ef
Scarlet	3.9 ab	3.7 b	6.5 a	4.3 bcd
Scarlet-Rz1	3.9 ab	2.7 cd	4.7 c	4.8 abc

^a Ratings were scored on a scale of 0 (no disease) to 8. Ratings followed by different letters within a column are significantly different at 5% level of probability according to Tukey's honestly significant difference test.

TABLE 4. Analysis of variance for the recombinant inbred lines (RIL) of Louise × SPCB-3104 for percent shoot length reduction in paired high- and low-inoculum treatments in three environments^a

Source	df	Mean square	F value	Pr > F
Genotype	188	79.65	9.87	0.041
Environment	2	696.50	86.28	<0.002
Genotype × environment	376	25.16	3.12	0.164

^a Environments included field assays in 2013 and 2014 and a growth chamber experiment in pasteurized soil.

Genetic mapping and QTL detection. In total, 5,570 GBS markers were identified as being polymorphic between Louise and SPCB-3104. From these markers, 412 were used for map construction. In total, 36 linkage groups covering all 21 wheat chromosomes were created, which had a total map distance of 2,290 cM. On average, the spacing between markers was 5.5 cM.

In the Louise × SPCB-3104 mapping population, two QTL (*QSlr wsu-2AS* and *QSlr wsu-2DL*) were detected for PSLR for *Rhizoctonia*

TABLE 5. Quantitative trait loci (QTL) for percent shoot length reduction (Slr) for green bridge and *Rhizoctonia* tolerance in the backcrossed Louise × SPCB-3104 mapping population^x

Environment (year), QTL ^y	Interval (cM)	LOD	PVE (%)	Add ^z
Pullman (2013)				
<i>QSlr wsu-2AS</i>	6.2	2.7	4.6	-2.9
<i>QSlr wsu-2DL</i>	15.8	2.9	4.7	-2.9
Total	...	5.0	11.2	...
Pullman (2014)				
<i>QSlr wsu-1BL</i>	9.3	3.8	5.2	-2.8
<i>QSlr wsu-2AS</i>	6.2	3.4	5.9	-2.9
<i>QSlr wsu-2DL</i>	15.8	3.3	4.8	-2.1
Total	...	9.2	19.5	...
Greenhouse				
<i>QSlr wsu-1BL</i>	5.2	3.0	3.8	-2.6
<i>QSlr wsu-2AS</i>	6.2	6.8	12.4	-4.5
Total	...	11.5	19.4	...

^x Abbreviations: cM = centimorgans, LOD = log of the odds, and PVE = phenotypic variation explained.

^y Total = estimate of total PVE for the population using a multiple QTL model.

^z Add = estimated additive effect of QTL. A negative value indicates that the resistant allele was contributed by SPCB-3104.

spp. using the 2013 field data (Table 5). The LOD value for *QSlr wsu-2AS* and *QSlr wsu-2DL* was 2.73 and 2.97, respectively. These QTL together explained 11.2% of the phenotypic variation (R^2). Three QTL (*QSlr wsu-1BL*, *QSlr wsu-2DL*, and *QSlr wsu-2AS*) were identified using the 2014 field data for PSLR. These QTL had LOD scores ranging between 3.83 and 3.32, which explained 4.8 to 5.9% of the phenotypic variation, respectively. Two of these QTL (*QSlr wsu-1BL*, and *QSlr wsu-2AS*) were detected in the growth chamber screen where they had LOD of 3.0 and 6.8 and explained 3.8 and 12.4% of the phenotypic variation, respectively. *QSlr wsu-2AS* was detected on the short arm of chromosome 2A in a 6.2-cM interval and flanked between markers GBSA13723B6800 and GBSA95002B44076. *QSlr wsu-1BL* mapped to a 9.3-cM interval between markers GBSA54298B24271 and GBSA94995B44073, and *QSlr wsu-2DL* mapped to a 15.8-cM interval between markers GBSA82037B37700 and GBSA6334B2934 (Fig. 2).

Analysis of the RIL population in the three different environments detected three QTL with favorable alleles originating from SPCB-3104. *QSlr wsu-2AS* was statistically significant in all three environments. *QSlr wsu-2DL* was only statistically significant in the two field environments but appeared to have a small effect in the growth chamber experiments (LOD = 2.6, $P = 0.075$). Similarly, *QSlr wsu-1BL* was only statistically significant in the field in 2014 and growth chamber environments but appeared to have a small effect on the 2013 field data as well (LOD = 2.3, $P = 0.136$). Overall, the three QTL showed similar effects on shoot length reduction from high inoculum levels in the field. *QSlr wsu-2AS* showed the strongest effect in the growth chamber environment.

Three of the six genotypes identified in the tolerance screens (SYN-30, SYN-182, and SPCB-3104) have a shared parent (Croc-1, AB genome), in their pedigrees. Therefore, we wanted to determine

Fig. 2. Selected chromosome locations for three significant quantitative trait loci (QTL) (log of the odds [LOD] ≥ 2.5) on 1BL, 2AS, and 2DL detected for a mean of the shoot length reduction (%) in the Louise × SPCB-3104 mapping population. Positions are shown in genetic distances (centimorgans) on the left and their corresponding genotyping by sequencing (GBS) markers on the right. The interval for each QTL is defined by the ends of each bar. Black boxes are one-LOD drop-off from the peak of the QTL. Environments are listed in parenthesis after the QTL name.

whether SNP markers linked to the A and B genome QTL in SPCB-3104 were also present in the synthetic wheat genotypes. To compare the SNP, fragments carrying the GBS markers were amplified by PCR and the fragments were Sanger sequenced to determine shared SNP homology. Sequences for Louise, SPCB-3104, SYN-30, and SYN-182 were aligned for markers GBSA94995B44073 (chromosome 1B) and GBSA13723B6800 (chromosome 2A). For the 1B alignment, SYN-30 or SYN-182 did not carry the same SPCB-3104 allele as characterized by the 104-bp region. Similarly, in the 2A sequence alignment, we found that SYN-30 and SYN-182 carried a different allele than SPCB-3104 for marker GBSA13723B6800. Therefore, it seems likely that the tolerance in the SYN-30 and SYN-182 lines is not caused by the same QTL as tolerance identified in the A and B genomes of SPCB-3104, which is likely derived from other parents in its more complex pedigree (Table 1).

DISCUSSION

Recommendations for planting spring cereals without tillage include killing weeds well before planting so that pathogens from the dying plants do not infect emerging seedlings (Babiker et al. 2011; Rovira and McDonald 1986; Smiley et al. 1992). By planting into such a green bridge environment, six synthetic-derived CIMMYT genotypes were identified that consistently showed good tolerance to the green bridge environment. Examination of these lines in pasteurized soils infested with either *R. solani* AG8 or *R. oryzae* demonstrated that some of the lines such as SYN-172 had reduced levels of disease symptoms such as lesions and spear tipping on their roots. This indicated that the partial resistance to *Rhizoctonia* spp. was a component of their enhanced root performance in the green bridge environment. For other lines, the levels of partial resistance in the growth chamber assays depended on the soil type or *Rhizoctonia* spp. SPCB-3104 showed relatively poor resistance in these assays, indicating that its performance in the field may be due more to tolerance to *Rhizoctonia* spp. and, possibly, other soilborne pathogens.

In contrast, Scarlet-Rz1, which has been shown to be tolerant to *R. solani* AG8 in the growth chamber (Okubara et al. 2009), had increased susceptibility to the green bridge environment, indicating that environment has a major effect on resistance in this genotype. It cannot be ruled out that the decrease in tolerance in the field may also be attributed to susceptibility to other pathogens. Abiotic interactions may also have imposed a greater influence on plant performance in the field than in a controlled environment (Neate 1989; Smith et al. 2003).

SPCB-3104 (Vorobey) has been previously described as having thick and deep (long) roots (Lopes and Reynolds 2011). Root morphological traits such as thicker root systems may be correlated to tolerance and have a direct role in tolerance to *Rhizoctonia* root rot (Okubara et al. 2009). SPCB-3104 does not have shorter roots but has been shown to have an increased root mass under drought conditions (Lopes and Reynolds 2011). The extra root mass may play a role in the resistance mechanism. Root fresh weight for SPCB-3104 did not differ significantly between the green bridge and low-inoculum environments (Table 2). Variation in resistance to *R. solani* AG8 has been identified in *Brachypodium distachyon* accessions that could be attributed to longer root length (Schneebeli et al. 2016), providing further evidence for root mass and length as a tolerance mechanism. More recently, SYN-201 and SYN-172 were shown to have increased drought performance in a greenhouse setting (Becker et al. 2016). Specifically, SYN-201 had increased root biomass and root length under drought stress and was able to compensate for this stress by transitioning root production to areas of higher moisture content in the tube. This form of plasticity could possibly also allow for root development to shift from an area of high pathogen levels to a lower pressure area.

The three QTL detected from the SPCB-3104 × Louise population affected performance in both the green bridge environment and pasteurized soil infested with *R. solani* AG8, although one was not statistically significant in the latter environment. The fact that

the same QTL largely controlled tolerance in both types of environment is further evidence that *R. solani* AG8 is a major pathogen in a green bridge environment (Cook 2001; Paulitz 2006). The tolerance to root disease in both environments appeared to be controlled by genes with small effects. Although resistance was highly heritable ($h^2 = 0.68$), we were only able to map three QTL and they accounted for a relatively small amount of the genetic variation (Table 5). Furthermore, transgressive segregation for tolerance was observed in the field environments, indicating that alleles from the Louise parent contributed but no QTL from Louise were detected. It is likely that there were multiple other loci affecting tolerance to green-bridge-associated pathogens, including *Rhizoctonia* spp., in this cross but their effects were too small to detect in our analysis.

In previous studies, a significant challenge with *R. solani* AG8 root rot in a field environment has been low concentrations of inoculum and poor distribution of the pathogen (Neate 1989; Smith et al. 2003). In this study, high-inoculum (green bridge) field nurseries were created by bulk seeding volunteer wheat and spraying herbicide right before seeding. This method provided an adequate level of inoculum and provided a tractable phenotype to screen for germplasm tolerance and map QTL. This study also provides further evidence that virulence of *R. solani* AG8 and *R. oryzae* varies between soil types. These results suggest that virulence of *R. solani* AG8 is higher in sandy soils compared with silty loam soils. Previous reports have shown a similar correlation between higher incidences of *R. solani* AG8 root rot disease and sandier soils (Gill et al. 2000).

The use of NGS and GBS is becoming a cost-effective alternative to simple-sequence repeat markers and SNP array-based platforms (Poland et al. 2012). GBS markers used in the study were able to anchor to all 21 wheat chromosomes as identified by BLASTn results using the Chinese Spring reference sequence. The number of markers, linkage groups, and marker distribution was very similar to other reported QTL studies using similar GBS methods and mapping software (Bajgain et al. 2015; Kumssa et al. 2015).

Given the difficult nature of assaying resistance or tolerance to *Rhizoctonia* root rot and other green-bridge-associated diseases, marker-assisted selection would be an asset to breeding programs attempting to improve this trait. The tolerance from the SPCB-3104 line appeared to be controlled by many genes with small effects, however, and would likely require many markers and additional mapping efforts. Some of the other genotypes identified in this study showed very promising performance under disease pressure which appeared to be more associated with resistance than tolerance. It is quite possible that genetic analyses of this germplasm could reveal genes with larger effects, making the traits easier to transfer into adapted germplasm. The green bridge environment used to select the six lines identified in this study certainly included disease pressures other than *Rhizoctonia* spp. These lines could also be tested for other soilborne pathogens such as *Pythium* spp. and in other environments for similar disease traits.

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