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Mapping *Fusarium solani* and *Aphanomyces euteiches* Root Rot Resistance and Root Architecture Quantitative Trait Loci in Common Bean

C. H. Hagerty, A. Cuesta-Marcos, P. B. Cregan, Q. Song, P. McClean, S. Noffsinger, and J. R. Myers*

ABSTRACT

Root rot diseases of bean (*Phaseolus vulgaris* L.) are a constraint to dry and snap bean production. We developed the RR138 RI mapping population from the cross of OSU5446, a susceptible line that meets current snap bean processing industry standards, and RR6950, a root rot resistant dry bean with small brown seeds. We evaluated the RR138 RI population beginning in the F₆ generation for resistance to *Fusarium solani* f. sp. *phaseoli* (Burk.) root rot in Oregon and *Aphanomyces euteiches* (Drechsler) root rot in Wisconsin. The population was evaluated for a set of root architecture traits at the Oregon location. *Fusarium solani* root rot resistance was evaluated in three seasons, whereas *A. euteiches* resistance was evaluated in two seasons. For each disease, RR6950 was resistant and OSU 5446 was susceptible. The recombinant inbred (RI) population was normally distributed for reaction to both diseases. We assembled a high-density linkage map using 1689 single-nucleotide polymorphisms (SNPs) from an Illumina 6000-SNP BARCbean6K_3 Beadchip. The map spanned 1196 cM over 11 linkage groups at a density of one SNP per 1.4 cM. Three quantitative trait loci (QTL) associated with *A. euteiches* were identified, each accounting for 5 to 15% of the total genetic variation, and two QTL associated with *F. solani* resistance accounted for 9 and 22% of the total genetic variation. A QTL for taproot diameter (TD) and one QTL for basal root angle were identified. The QTL for resistance to the two diseases mapped to different genome locations indicating a different genetic control.

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Abbreviations: ARR, *Aphanomyces euteiches* resistance; CIM, composite interval mapping; CTAB, hexadecyltrimethyl ammonium bromide; FRR, *Fusarium solani* resistance; HARS, Hancock Agricultural Research Station; LOD, logarithm of the odds; LR, likelihood ratio; OSU, Oregon State University; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RCBD, randomized complete block design; RI, recombinant inbred; SNP, single-nucleotide polymorphism; TD, taproot diameter; VRF, Vegetable Research Farm.

ROOT DISEASES of bean limit yields wherever the crop is grown and constitute a major constraint to dry edible and snap bean production worldwide (Schneider et al., 2001). Schneider et al. (2001) reported root rot caused by *F. solani* in the United States could cause yield losses of up to 84%. Reported yield losses from bean root rot complex are even more devastating in the developing world. Disease pressure is likely worse in developing countries because of higher abiotic stress. As a result of increased stress, bean root rot complex has been attributed to yield losses of up to 100% in Uganda and up to 70% in Rwanda (Mukankusi and Obala, 2012).

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Root rot resistance is key to overall plant health and optimal growth and development because a poorly developed root system limits nutrient and water uptake. Root diseases are also a significant factor in the effort to maintain or increase yields in the face of climate change. If a plant lacks a robust and healthy root system, it is unable to deal with abiotic stress, particularly drought and heat, which are becoming more common with climate change.

Several organisms cause root rot in common bean, the most common in the United States being *Rhizoctonia solani* (Kuhn), *F. solani* (Martius), *F. oxysporum* (Schlecht), and *A. euteiches*. *Fusarium solani* and *R. solani* are often found in pathogen complexes whereas *F. oxysporum* and *A. euteiches* may be found in complexes or alone but most often occur where cropping has been most intensive.

Fusarium solani root rot on bean is widespread and occurs in most bean fields throughout the world (Hall et al., 2005). *Fusarium solani* root rot is the predominant root rot pathogen reducing yield in Oregon snap bean production. Symptoms on roots include dark brown or rusty reddish colored lesions, sunken lesions in the lower hypocotyls, rotting of lateral roots, and vascular discoloration of the upper taproot and even the lower stem in severe cases (Yang and Hagedorn, 1966; Pfender and Hagedorn, 1982; Navarro et al., 2009). If allowed to progress, lesions on the stem coalesce, affecting the entire subsoil root system (Abawi et al., 1985). In severe cases, root rot can kill plants completely, breaking off the crown foliage from the main lower stem. The widespread nature of *F. solani* as the predominant root rot pathogen in common bean emphasizes the need for effective control through the development of resistant cultivars (Boomstra et al., 1977; Schneider et al., 2001; Navarro et al., 2009).

Aphanomyces euteiches is the predominant root rot pathogen reducing yield in Wisconsin snap bean production, although it is found in complexes with *Pythium* spp. and *F. solani* (Hall et al., 2005). *Aphanomyces euteiches* root rot causes seedling damping off and root rot disease of many legumes. *Aphanomyces euteiches* root rot can affect germination in severe cases and plant vigor and seed yield in almost all cases. Symptoms may be visible at germination or in later stages of plant maturity depending on whether conditions favor one or more of the pathogens (Pfender and Hagedorn, 1982). Found commonly in irrigated sandy soils, *A. euteiches* oospores can persist in a dormant state in the soil for years. Lesions on roots are initially yellow-brown, rapidly coalescing to involve most of the roots, which become softer as the pathogen destroys the cortex (Hall et al., 2005). The pathogen infects the cortex of primary and lateral roots and oospores are formed within the root tissues (Gaulin et al., 2007). *Aphanomyces euteiches* can infect plants soon after emergence or late in the season, most of the root system may be destroyed, and plants may be severely stunted (Hall et al., 2005).

Cultural and chemical disease control methods are of limited value against root rots. Seed or soil treatments with selective fungicides, crop rotations, cover crops, seedbed preparations, and other measures have, in some cases, improved yield in the presence of *A. euteiches* and *F. solani* root rot disease. However, none of these measures have been consistently economical or effective against root rot (Abawi et al., 1985). Crop rotation is the most effective way to reduce both *F. solani* and *A. euteiches* root rot infection, but the length of rotation may be impractical for most cropping systems because it requires avoiding cultivation of legumes in infected fields for up to 10 yr (Gaulin et al., 2007; Navarro et al., 2009). Decreasing soil compaction through deep ripping is also an effective method for reducing root rot disease pressure (Burke and Miller, 1983); however, no single cultural practice is completely effective by itself, therefore, they must be combined and deployed as part of a package.

Genetic variation for root rot resistance exists, but there are few commercial varieties with high levels of resistance. Resistance has often been found in unadapted backgrounds, and that issue, along with the quantitative nature of resistance, has made transfer into elite lines difficult. Understanding the architecture of resistance and identifying markers for marker-assisted selection would facilitate genetic improvement. N203 (PI203958) has been the *F. solani* resistance source most widely used by bean breeders (Wallace and Wilkinson, 1965, Silbernagel and Hannan, 1992). Silbernagel (1987) released the germplasm line FR266, which used the N203 source of resistance, and other sources of *F. solani* root rot resistance were identified in *P. coccineus*, but introgression was discontinued in favor of N203 (Baggett et al., 1965).

Economic losses from *A. euteiches* root rot have generally been confined to snap beans with little effort having been made to breed for resistance in dry beans. Some germplasm with slight to moderate levels of resistance to *A. euteiches* root rot have been identified (Pfender and Hagedorn, 1982) and have been incorporated into some commercial snap bean and red kidney cultivars (K. Kmiecik, Monsanto Corp., personal communication, 2013). ‘Pueblo 152’ has also been identified as a potential source of *A. euteiches* root rot resistance and a random amplified polymorphic DNA (RAPD) marker associated with resistance has been identified (Navarro et al., 2008, 2009).

The objectives of this research were (i) to characterize resistance to two root rot pathogens (*F. solani* and *A. euteiches*) in the RR6950 × OSU5446 RI population, (ii) to map QTL for resistance and determine whether the same or different QTL were responsible for resistance for each of the pathogens, and (iii) to investigate mechanisms underlying resistance to root rot in snap beans.

MATERIALS AND METHODS

Plant Materials and Character Evaluation

The parents used to create a RI population to study resistance to root rot were RR6950 (small brown-seeded accession with type IIIA growth habit, received from J.R. Baggett, Oregon State University, but ultimate origin is unknown) and OSU5446 (type I bush blue lake four-sieve green bean breeding line). While RR6950 is of Mesoamerican origin, OSU5446 was derived from the cross of 'Smilo' × 'OR91G', and, as a result, is probably of mixed Mesoamerican and Andean origin. We had previously screened both of these lines in our *Fusarium* root rot nursery in Oregon and *Aphanomyces* root rot nursery in Wisconsin and determined that they represented the extremes in terms of resistance (RR6950 resistant, OSU5446 susceptible) in both nurseries. In 2003, RR6950 was crossed as the male to OSU5446 to produce the RR138 RI mapping population. The F₂ single plants of the RR138 population were advanced without selection to produce F₃ families. In the F₃ generation, single plant families were homozygous for *Fin* (indeterminate vine habit), segregating for *Fin*, or homozygous for *fin* (determinate bush habit). Families that were homozygous for *Fin* were discarded. Indeterminate growth habit is not acceptable in snap beans and we had no prior evidence to suggest that growth habit was associated with variation in root rot resistance. A single determinate plant from each segregating and homozygous *fin* family was retained. The F_{4.5} generations were advanced by randomly selecting a single plant from each family. In 2008, plants within each F₅ family were bulked. A total of 177 families were retained in the F₅ and subsequent generations. Phenotypic evaluations were conducted in 2010 and 2011 on F₆ generation materials and DNA was extracted at the F₆ generation in 2011. In 2012, disease and root morphology evaluations were performed on the F₇ generation after seed increase.

Study Sites and Experimental Design

Vegetable Research Farm, Corvallis, Oregon

The *Fusarium* root rot evaluation site was located at the Oregon State University (OSU) Vegetable Research Farm (VRF) on Chehalis silty clay loam soil (fine-silty, mixed, superactive, mesic Cumulic Ultic Haploxerolls; 44.571209° N, 123.243261° W). The study site plot had been in continuous bean production for at least 20 yr, resulting in high, uniform *F. solani* disease pressure throughout the field. A randomized complete block design (RCBD) with three replicates was used in all years. Phenotypic screening was conducted and optimized in 2010. The 2010 data were not used because of a high level of statistical noise, but the 2010 field season served to identify the best phenotypic screening procedure. Plots consisted of single rows spaced at 76 cm between rows and 2 m in length. One hundred seeds per plot were planted to maintain a within-row spacing of one seed per 3 cm. A single border row on the sides and 1.5-m end plots of OSU5446 was used to minimize edge effects. *Fusarium solani* root rot was screened at a consistent physiological maturity when pods in a plot were at 50% buckskin. Fifty percent buckskin stage occurs when half of the pods per bush have lost their chlorophyll and have taken on a leathery texture. Untreated seed was used in 2010 and 2011. In 2012, seed was treated with Captan (Bonide) to minimize germination

and emergence problems caused by *Pythium* spp. Overhead irrigation early in the season followed by under irrigation after pod set was used to promote *Fusarium* root rot disease pressure. Overhead irrigation consisted of applying approximately 25 mm wk⁻¹ compared with a normal application of 7.6 mm wk⁻¹. After pod set, irrigation amounts were reduced to normal and the interval was increased to 10 d. The experiment was fertilized with 168 kg ha⁻¹ of 12-29-10 (N-P-K), sprayed with 1.1 kg ha⁻¹ of Dual (Syngenta) S-metolachlor herbicide at planting and treated with 2.33 L ha⁻¹ of Sevin (Bayer) 1-naphthyl N-methylcarbamate insecticide for cucumber beetle control as needed.

Hancock Agricultural Research Station, Hancock, Wisconsin

The RI population was screened for resistance to *A. euteiches* at the University of Wisconsin Hancock Agricultural Research Station (HARS), in the summers of 2011 and 2012. The Wisconsin site (44.120804° N, -89.534250° W) had a mixture of Plainfield sandy (mixed, mesic Typic Udipsamments) and Sparta loamy (sandy, mixed, mesic Entic Hapludolls) soil. The Wisconsin site had been in continuous snap bean production for the last 25 yr at the time of this study, again ensuring high and uniform *A. euteiches* disease pressure. Wisconsin fields were heavily irrigated with overhead irrigation to promote *A. euteiches* root rot. The Wisconsin site was planted in a RCBD with two replicates. Replicates were blocked to avoid any north to south variation within the field. The northern 15.24 m were avoided, as they traditionally have less severe *A. euteiches* disease pressure. Wisconsin fields were heavily irrigated with overhead irrigation to promote *A. euteiches* root rot. The Wisconsin site was planted in a RCBD with two replicates. Replicates were blocked to avoid any north to south variation within the field. The northern 15.24 m were avoided, as they traditionally have less severe *A. euteiches* disease pressure than the rest of the field. One hundred twelve kg ha⁻¹ of 6-24-24 (N-P-K) with Platinum starter fertilizer was applied in row, followed by 168 kg ha⁻¹ of 21-0-0-24 (N-P-K-S) applied at the second trifoliolate growth stage. Three hundred fifteen g ha⁻¹ of Brigade (FMC Agricultural Solutions) bifenthrin insecticide was applied for cucumber beetle control as needed.

Root Rot Evaluation

During summer 2010 and 2011 at VRF, five plant samples from each plot were extracted from the soil when the plants were at 50% buckskin stage. After excess soil was removed, the stem, hypocotyl, and taproot were bisected and were visually evaluated using a 1-to-5 rating scale (1 = no disease, 5 = severe disease). Disease severity was based on discoloration of the inner pith of the taproot. Orange inner pith transitioning to necrotic black was indicative of disease. Root rot score was averaged over the five plants uprooted from each plot. Also during summer 2010, seed coat color and flower color of each genotype were noted. During summer 2012 at the VRF, a Shovelomics protocol (Lynch and Brown, 2013) was used. Two plants per plot were dug with a 30-cm border around each plant to avoid damage to the roots. Root samples were left to soak in a fresh water basin for 4 h, and excess soil was rinsed off. Root rot scores in 2012 were based on total root health (1 = clean, 5 = severe disease). After disease ratings were completed, root angles were measured with a protractor board and TD was measured with a digital caliper. Taproot diameter was measured 1 cm below where the hypocotyl transitions to the taproot.

In 2011 at HARS, *A. euteiches* resistance was evaluated using aboveground plant vigor and productivity without examination of the roots. Scores were based on a 5-point scale (1 =

healthy normal foliage, 3 = chlorotic and stunted, 5 = nearly dead). In summer 2012, two plants per plot were dug, excess sand was shaken off, and roots were visually rated for disease. Scores were based on a 5-point scale (1 = healthy or clean roots, 3 = necrosis and root pruning, 5 = nearly dead). Variability within samples collected was low. We used SAS version 9.3 (SAS Institute, 2011) to obtain Pearson's correlation coefficients for all traits in this study and to perform correlation analyses between years and locations of the study.

DNA Isolation and Genotyping

DNA was extracted from young trifoliolate leaves using a hexadecyltrimethyl ammonium bromide (CTAB) protocol modified from Miklas et al. (1993). This protocol involves grinding leaf samples in CTAB buffer solution, chloroform–isoamyl alcohol extraction, ammonium acetate solution wash, RNase treatment, and final DNA storage in TE buffer for molecular marker analysis. DNA was quantified using a NanoDrop ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc.) and diluted to a final concentration of 100 ng μL^{-1} . Fifty microliters of DNA of each genotype was sent to the USDA–ARS Beltsville Agricultural Research Center laboratory, Beltsville, MD, where SNP analysis was run. The RR138 population was genotyped using the Illumina 6000 SNP BARCbean6K_3 Beadchip. Access to the Illumina chip was provided through the Bean Coordinated Agriculture Project (BeanCAP) project (<http://www.beancap.org>). Single-nucleotide polymorphism genotyping was conducted at the USDA–ARS Soybean Genomics and Improvement Laboratory, Beltsville, MD, on the Illumina platform following the Infinium HD Assay Ultra Protocol (Illumina, Inc.). Single-nucleotide polymorphism allele calling was done using the GenomeStudio Module v1.8.4 (Illumina, Inc.).

Linkage Map Construction and Quantitative Trait Loci Analysis

A linkage map was constructed using Joinmap 4 (Van Ooijen, 2006). Raw SNP data was manually inspected in Microsoft Excel 2007. All SNP data with no call and SNPs monomorphic between parents were discarded. The criteria to assess the quality of SNPs for linkage map construction were number of missing allele calls for polymorphic loci and segregation distortion greater than 10%. Linkage map graphics were created using MapChart 2.0 (Voorrips, 2002).

Linkage groups were established using a logarithm of the odds (LOD) threshold of 4.0 for significant pairwise marker linkages and assigned to chromosomes by comparison with previous SNP maps. Recombination frequencies were converted to centimorgans (cM) using Haldane's mapping function and the maximum likelihood algorithm.

Quantitative trait loci analysis was performed using the composite interval mapping (CIM) procedure (Zeng, 1994) implemented in Windows QTL Cartographer 2.5 (Wang et al., 2007). Up to seven cofactors for CIM were chosen using a stepwise forward selection and backward elimination procedure with a significance threshold of 0.1. A scan window to 30 cM beyond the markers flanking the interval tested was used. Genome-wide significance ($\alpha = 0.05$) likelihood ratio (LR) test thresholds for QTL identification were determined with 300

permutations and expressed as LOD (LOD = 0.217 LR). For QTL mapping purposes, cosegregating markers were removed from the original linkage map; therefore, a single marker was retained at each unique map position.

Narrow-sense heritabilities were calculated for each trait of interest in this study to understand the degree to which each trait is under genetic control and if gain from selection of the trait is feasible. We used a formula described by Hallauer et al. (2010; section 4.2 p. 90) to calculate narrow-sense heritability:

$$\hat{h}^2 = \frac{\sigma_g^2}{\frac{\sigma^2}{r} + \frac{\sigma_{ge}^2}{e} + \sigma_g^2}$$

where σ_g^2 is the genotypic variance, σ^2 is the pooled error variance, σ_{ge}^2 is the genotype \times environment interaction variance, r is number of replications, and e is number of environments. Because of the level of inbreeding in this population, σ_g^2 is assumed to be essentially additive genetic variance.

RESULTS

Phenotyping

Oregon *F. solani* nursery results were significantly correlated across years 2010, 2011, and 2012. Mean *F. solani* root rot score across all 3 yr of the study was 2.83 on a scale of 1 to 5 (Fig. 1). Oregon 2011 and 2012 trials produced more robust data. Wisconsin *A. euteiches* nursery data were significantly correlated in 2011 and 2012. Mean *A. euteiches* root rot score across both years of the study was 3.19 on a scale of 1 to 5 (Fig. 1).

Generally, *A. euteiches* resistance has higher heritability than *F. solani* resistance (Table 1). *Fusarium solani* resistance heritability average for 2011 and 2012 was 0.203, whereas, *A. euteiches* average for 2011 and 2012 was 0.256. Root architecture traits measured in this study had lower heritabilities than disease resistance. Taproot diameter had the lowest heritability, followed by shallow basal root angle (Table 1).

Genotyping

Of the 5398 bead types, 2077 SNPs were polymorphic between RR6950 and OSU5446 parents. The SNP markers with segregation distortion at probability level below 0.05 were excluded, resulting in 1689 mapped SNP markers in the RR6950/OSU5446 RI population. The resulting linkage map was 1196 cM in length with an average marker density of one SNP for every 1.4 cM spanning across all 11 chromosomes. More than one linkage group was obtained for chromosomes Pv01 and Pv11. Large genomic regions lacking polymorphic markers in these chromosomes prevented the consolidation of their markers into a single linkage group. As such, these groups were assigned and aligned using information on SNP location and position from the 'Stampede'/'Redhawk' common bean map (Schmutz et al., 2014). There were also gaps as

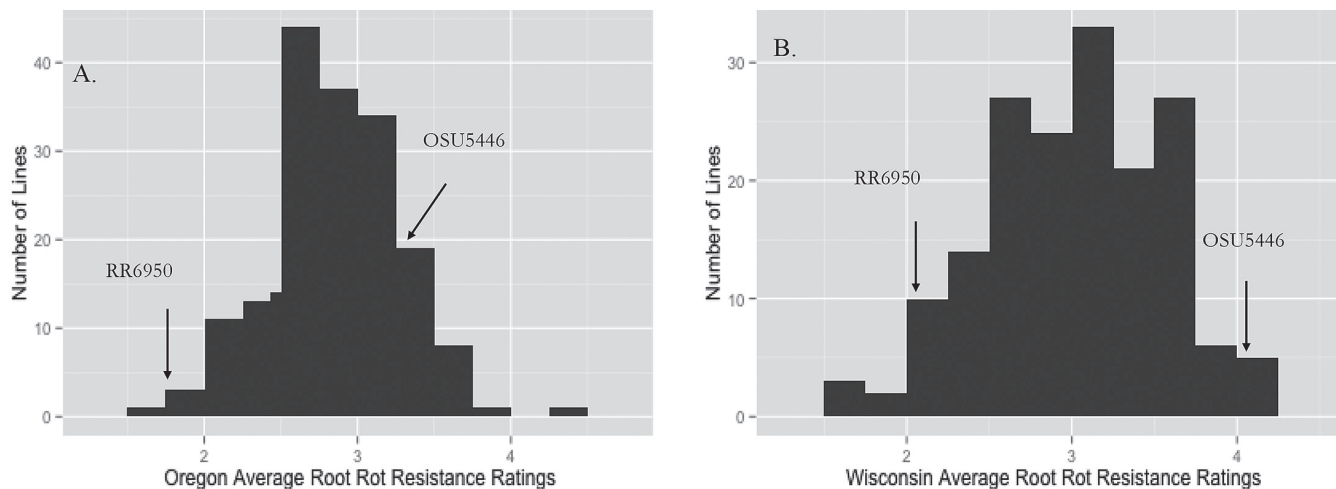


Figure 1. Distribution of average root rot resistance in *P. vulgaris* ratings in Oregon and Wisconsin (1 = resistant, 5 = susceptible). (A) Oregon averages from 2010, 2011, and 2012. Resistant parent RR6950 scored 1.5 average resistance, susceptible parent OSU5446 scored 3.3 average resistance. (B) Wisconsin averages from 2011 and 2012. Resistant parent RR6950 scored 2.1 average resistance, susceptible parent OSU5446 scored 4.1 average resistance.

Table 1. Narrow-sense heritability (h^2) and standard error (SE) of (h^2) calculations for root rot resistance and root architecture traits on a *P. vulgaris* recombinant inbred line population derived from a cross between RR6950 and OSU5446.

Trait [†]	h^2	SE of h^2
FRR 2011	0.198	0.001
FRR 2012	0.208	0.001
ARR 2011	0.237	0.009
ARR 2012	0.278	0.007
Taproot diameter	0.108	0.001
Shallow basal root angle	0.095	0.109

[†]FRR, *F. solani* root rot resistance; ARR, *A. euteiches* root rot resistance.

a result of areas of low recombination ranging from 7 to almost 50 cM in length on almost every linkage group.

There were numerous apparently cosegregating SNPs mapping to the same position. With the exception of breaks on Pv01 and Pv11, the SNP marker order and location in the map generally agreed with order and chromosome assignment in the Stampede/Redhawk common bean map (Schmutz et al., 2014).

Quantitative Trait Loci

Three QTL for *A. euteiches* resistance (ARR) were found in 2011 and 2012 on Pv02, Pv04, and Pv06, explaining 15, 18, and 5% of total genetic variation (Table 2). Two QTL for *F. solani* resistance (FRR) were found in 2011 on Pv03 and Pv07, explaining 9 and 22% of total genetic variation. One QTL was found in 2012 for TD on Pv02, explaining 10% of genetic variation. Finally, one QTL was found in 2012 for shallow basal root angle on Pv05, explaining 19% of genetic variation (Table 2; Fig. 2).

Quantitative trait loci ARR2.1 and TD2.1 cluster in the same region of Pv02 with overlapping 1 LOD intervals (Fig. 2). Pearson correlation coefficients for TD and

root rot resistance data revealed a correlation of -0.33 and -0.22 in Oregon and Wisconsin, respectively (Table 3). As TD increases, both *F. solani* and *A. euteiches* root rot becomes less severe (Table 2). Oregon *F. solani* and Wisconsin *A. euteiches* data were not correlated.

The *P* gene controls color in the seed coat and flower. The *P* gene allows other genes affecting color to be expressed; *p* suppresses color to produce white flowers and seeds. The *P* gene was located in close proximity to the FRR7.1 (Fig. 2), although the gene does not underlie the peak of the QTL, rather it is found the shoulder of the two LOD region of the peak.

DISCUSSION

Field Evaluation

The two field screening environments in Oregon and Wisconsin were substantially different as revealed by the phenotypic characterization of the mapping population and the separate QTL for resistance found in each region. However, parents of the RR138 population exhibited similar phenotypic patterns in each location, with RR6950 showing resistance to both *A. euteiches* and *F. solani* and OSU5446 exhibiting susceptibility. Koch's postulates were performed for *F. solani* in Oregon (Hagerty, 2013). Koch's postulates was not performed for *A. euteiches*, however the fields at the Hancock research station have a long history of *A. euteiches* root rot of beans and peas dating back to the 1950s (Sherwood and Hagedorn, 1958).

Root rot organisms are never found in isolation in the field, and while *F. solani* may have dominated the pathogen complex in Oregon, *R. solani* may have also been present. *Pythium* spp. symptoms are rarely ever observed under field conditions in Oregon and *A. euteiches* pathogenic on beans has never been reported in the state.

Table 2. Quantitative trait loci (QTL) for root rot resistance and root architecture-related traits detected in *P. vulgaris* recombinant inbred line population derived from a cross between RR6950 and OSU5446.

Trait	QTL name [†]	Chromosome	Position cM	LOD [‡]	LOD threshold	Additive effect	R ²	Closest SNP [§]
<i>A. euteiches</i> resistance	ARR.1	Pv02	96.5	9.3	4.0	0.26	0.15	ss715647851
<i>A. euteiches</i> resistance	ARR.4.1	Pv04	4.6	6.7	4.0	0.18	0.10	ss715647818
<i>A. euteiches</i> resistance	ARR.6.1	Pv06	12.3	7.7	3.9	-0.19	0.05	ss715649329
<i>F. solani</i> resistance	FRR.3.1	Pv03	22.8	6.2	4.0	0.23	0.09	ss715641537
<i>F. solani</i> resistance	FRR.7.1	Pv07	47.8	11.5	4.0	0.32	0.22	ss715649511
Taproot diameter	TD.1	Pv02	93.6	5.9	4.1	-0.09	0.10	ss715646264
Shallow basal root angle	SBRA.5.1	Pv05	80.0	7.9	4.0	-3.28	0.19	ss715645443

[†] ARR, *A. euteiches* root rot resistance (Wisconsin); FRR, *F. solani* root rot resistance (Oregon); TD, taproot diameter; SBRA, shallow basal root angle.

[‡] LOD, logarithm of odds.

[§] SNP, single nucleotide polymorphism.

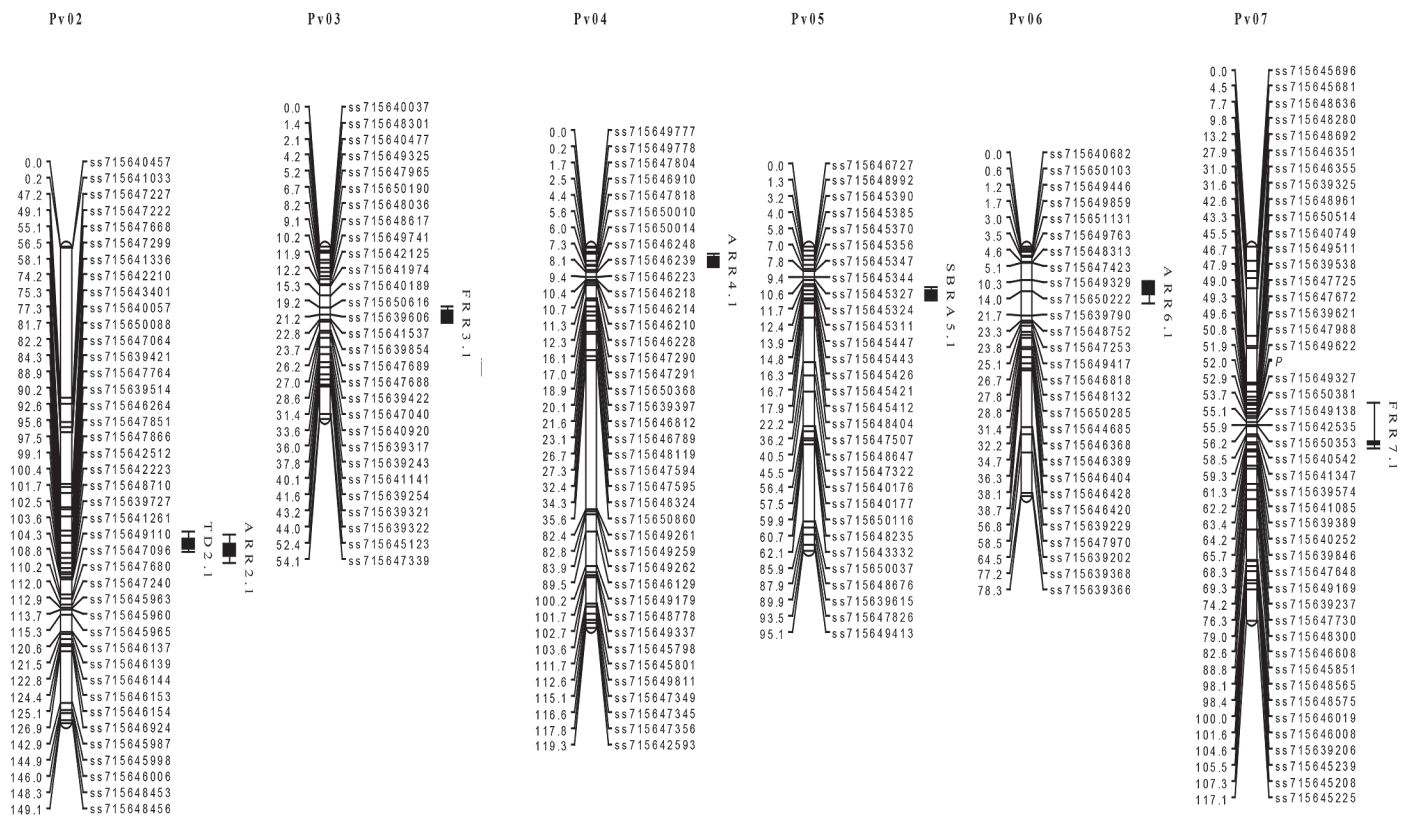


Figure 2. Six linkage groups harboring significant quantitative trait loci (QTL) for root rot resistance and root architecture-related traits in a *P. vulgaris* recombinant inbred line population derived from a cross between RR6950 and OSU5446. The box delineates one-logarithm of odds (LOD) support interval, and the whiskers of the box delineates two-LOD support interval. Because of space constraints, QTL are oriented in relation to the cross bars on the chromosome, not the text. TD, taproot diameter; ARR, *Aphanomyces euteiches* root rot resistance; FRR, *Fusarium solani* root rot resistance; SBRA, shallow basal root angle.

Likewise, in Wisconsin, a complex of root rot pathogens has been found, with *A. euteiches* being the most important pathogen with *P. ultimum* and *R. solani* also present (Kobriger and Hagedorn, 1983). Because of the differences in prevalent pathogenic organisms and the differences in symptomatology, we feel that the subsequent linkage map construction and QTL analysis evaluated the genetics of resistance to different sets of organisms.

Phenotyping

Our results confirm what others have found in bean, in that resistance to both root rot complexes is under quantitative genetic control as shown by the unimodal distributions approximating a normal distribution for both diseases (Fig. 1). In Oregon, possible transgressive segregation, especially for RI population individuals more susceptible than the susceptible parent, were observed. These were seen in all years of the study. In 2010 and 2011 (but not 2012) there were a few transgressive segregates more resistant than

Table 3. Pearson correlation coefficients analysis for root rot resistance with taproot diameter. Root rot evaluations are broken out by year and location (2011, Oregon and Wisconsin). Only selected comparisons in environments where statistically significance correlations were detected are shown.

Environment	Taproot diameter
OR 2011	-0.09
OR 2012	-0.33***
WI 2011	-0.22**

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

RR6950. Wisconsin root rot data also showed transgressive segregants more evenly distributed to both ends of the distribution than found with the Oregon data (Fig. 1).

Genotyping

At 1196 cM with 1689 markers, the current linkage map has higher marker density but similar map length to all but the most recently constructed linkage map (Mukeshimana et al., 2014). Other studies with *P. vulgaris* have been published to date based on SNPs developed with the Illumina SNP BARCbean6K_3 Beadchip. In a study on drought tolerance in dry bean, Mukeshimana et al. (2014) constructed a linkage map of 1351 cM in length using 2122 SNP markers. Brisco et al. (2014) used SNP markers in common bean to detect QTL for *Empoasca* resistance. Felicetti et al. (2012) used SNPs to localize the slow darkening (*sd*) gene on Pv07. Our map may have fewer markers because the BeanCAP beadchip was optimized for dry bean and not for snap bean. The present map may be shorter in length because we found three regions with no polymorphic markers that were probably each greater than 50 cM in length.

Fin, the gene for determinant vs. indeterminate growth habit, is located on the short arm of Pv01. All families determined to be homozygous for *Fin* (indeterminate) were eliminated from the population in the F₃ generation. Because of linkage disequilibrium around this locus, it is highly likely that a substantial region on Pv01 lacked polymorphism among the RI progeny and that this created one of the gaps observed in the present study for this linkage group. Another gap observed on Pv01, and an additional one on Pv11, may be due to a lack of polymorphism between the parents. A low level of polymorphism might, in turn, be caused by degree of relatedness of the two parents. While RR6950 is race Mesoamerican, OSU5446 would be predicted to possess about 75% Mesoamerican alleles based on pedigree. It was also possible that the beadchip did not have markers located in these regions. However, we examined the SNP based map based on the Redhawk/Stampede population but found its linkage map not to contain any gaps larger than 30 cM (Cregan, 2011; McClean et al., 2011). Therefore, we conclude that the gaps in the RR138 linkage map are most

likely due to lack of polymorphic SNPs on Pv01 and Pv11. Because the physical position of SNPs is known, our linkage map can be aligned to the physical map to obtain an estimate of the size of the gaps.

We observed a number of SNPs located at the same linkage map position in our linkage map and these were thinned before conducting QTL analysis. When compared with the physical map, we determined that most of these do have different physical locations (data not shown). Our conclusion is that there was insufficient recombination (probably because of the size of the population) to resolve distances of tightly linked SNPs.

One QTL for disease resistance (ARR2.1) was in very close proximity to QTL for TD2.1 (taproot diameter) with overlapping confidence intervals. It is unclear whether this is the result of linked genes or the pleiotropic effect of a gene or cluster of genes. The QTLs ARR2.1 and TD2.1 were correlated, where increased TD was associated with resistance. A larger taproot may provide increased durability for nutrient and water acquisition in the presence of high disease pressure. Taproot diameter is a much easier, nonsubjective trait to score than root rot resistance and may provide a way for a breeder to conduct indirect selection for resistance, although, on the other hand, heritabilities were lower for TD2.1 than ARR2.1 (Table 1). Shallow basal root angle (SBRA5.1) on Pv05 was not found in proximity to other QTL for root rot resistance. Shallow basal root angles do have implications for root rot avoidance architecture; basal roots directed downward below the plow pan can serve to grow outside the zone of most severe disease pressure. While these relationships between root architecture traits and root rot resistance merit further investigation, they indicate that root architecture traits can in some situations be used as an indicator of robust resistance to root rot in breeding efforts.

In addition to TD located near to resistance QTL, we also observed that a locus controlling flower and seed color mapped near to FRR7.1. The *P* gene at this locus serves as a major switch for flavonoid biosynthesis in bean where the dominant allele allows expression of other color genes, but pigment is suppressed and flowers and seeds are white in color when recessive. It is unclear whether the resistance QTL and flavonoid biosynthetic gene are located in proximity by happenstance or as a pleiotropic effect. It is known that flavonoids and phenolics possess antimicrobial properties (Cowan, 1999) and it is certainly conceivable that the *P* locus conditions resistance to *F. solani*. If this is the case, then it is puzzling why this locus did not condition resistance to *A. euteiches*. It is important to determine if the resistance QTL is independent of *P*, since all contemporary snap bean cultivars intended for processing have the recessive allele because water-soluble anthocyanins may be present when the dominant allele is expressed, which adversely affect the quality of the

processed product. If tightly linked loci are involved, then it may be possible to break the linkage and recombine the *F. solani* resistance QTL with white seed color, otherwise this resistance QTL will not be usable in breeding contemporary processing type snap bean cultivars.

While three QTL were detected for *A. euteiches* resistance and two for *F. solani* resistance, in both cases the QTL accounted for approximately 30% of the total genetic variation. The QTL used in combination would increase root rot resistance significantly in a susceptible line. There may be other QTL for resistance to either pathogen that were not identified. In particular, a QTL linked to *Fin* might have been missed when plants with the indeterminate form of the allele were discarded.

With similar resistance patterns for parents when screened at the two locations, we expected to discover an overlap in resistance factors to the two root rot complexes. Likewise, one would expect that some of the same mechanisms conditioning resistance to root rots would be found (for example, the ability to withstand root pruning and rapid replacement of roots), but this is not the case. A major finding of this work is that QTL discovered for *F. solani* resistance do not cluster with QTL discovered for *A. euteiches* root rot resistance. This suggests that different genes control resistance to the different pathogens and that to have resistance to both pathogens, separate breeding efforts will have to be maintained.

Over 30 QTL with minor effect associated with *Fusarium* root rot resistance have been reported in RI populations derived from four resistance sources (Schneider et al., 2001; Chowdhury et al., 2002; Román-Avilés and Kelly, 2005). These researchers all used RAPD markers to map QTL. Sixteen QTL for *Fusarium* root rot resistance were identified in a RI population derived from the susceptible cultivar ‘Montcalm’ crossed with resistant line FR266 (Schneider et al., 2001). Two QTL were identified in a RI population derived from the susceptible cultivar ‘AC Compass’ crossed to resistant line NY2114-12 (Chowdhury et al., 2002), and 10 QTL were identified in two inbred backcross line populations derived from the susceptible cultivars Red Hawk and C97407 crossed to resistant line Negro San Luis (Román-Avilés and Kelly, 2005). The nine QTL significantly associated with *Fusarium* root rot resistance that Román-Avilés and Kelly (2005) found explained 7.3 to 53% of total phenotypic variation. Quantitative trait loci were found on Pv02, Pv05, Pv07, Pv08, and Pv09. High levels of resistance were also observed in several lines of the inbred backcross populations. A second QTL on Pv05 that explained up to 30% of the variation for resistance was linked to a marker previously identified as associated with root rot resistance (Schneider et al., 2001). Most QTL located on linkage groups Pv02 and Pv03 of the integrated bean map (Freyre et al., 1998) were close to a region where defense-response

genes, polygalacturonase-inhibiting protein and chalcone synthase, and pathogenesis-related proteins have been identified (Schneider et al., 2001). Because of the use of different types of markers, it is difficult to know whether any of the QTL we detected for *F. solani* resistance are located in similar regions to previously described QTL. Previous studies have found QTL on Pv03 and Pv07, so it is possible that the QTL we discovered are not new.

Quantitative trait loci for *A. euteiches* resistance are less studied than QTL for *F. solani* resistance. Six QTL for *A. euteiches* resistance linked to RAPD markers were identified in a RI population derived from susceptible snap bean cultivar Eagle crossed with resistant line Puebla 152. Navarro et al. (2008) evaluated an Eagle × Puebla 152 recombinant inbred line population and two inbred backcross populations derived from a cross of (Eagle × Puebla) to ‘Hystyle’ and discovered one QTL on Pv06 associated with *A. euteiches* resistance. One of our QTL for *A. euteiches* resistance was also located on Pv06.

The most promising QTL identified from this study include the three *A. euteiches* QTL that were consistent from year to year. Although QTL for *F. solani* explained a greater percentage of total variation than *A. euteiches*, QTL for *F. solani* were less consistent year to year.

The QTL discovered in this study help move bean resistance breeding toward a more efficient marker-assisted selection. Broadly, our results indicate that resistance to both root rot diseases are traits that could be improved through selection and breeding efforts. If breeders are able to release lines and improve existing varieties with the addition of root rot resistance, then common bean yield will improve. This has implications for common bean grown in the United States and in developing countries where bean comprise a greater proportion of the diet.

The full map with the NCBI Assay SNP ID and corresponding SNP position (cM) is available in a Supplemental Table S1.

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