QTL ANALYSIS AND CANDIDATE GENES IDENTIFICATION ASSOCIATED WITH FUSARIUM ROOT ROT RESISTANCE IN COMMON BEANS (*PHASEOLUS VULGARIS*)

By

Weijia Wang

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ABSTRACT

QTL ANALYSIS AND CANDIDATE GENES IDENTIFICATION ASSOCIATED WITH FUSARIUM ROOT ROT RESISTANCE IN COMMON BEANS (*PHASEOLUS VULGARIS*)

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Common bean (*Phaseolus vulgaris L.*) is an important grain legume that is high in protein, dietary fiber, and micronutrients. Common beans are diverse in seed types and are grown worldwide, especially in Americas, Africa and Asia. However, bean diseases have severely constrained bean production and farmers have experienced significant crop losses especially those with low input small-scale farms in developing countries. Fusarium root rot (FRR) is a commonly recognized soil-borne disease to cause lesion and necrosis on roots of common beans. In the first part of this study, different *Fusarium* species isolated from infected bean roots were screened for virulence. Variations of virulence among tested isolates and genotypic variation of plant responses were found. A *Fusarium brasilense* isolate was selected from tested isolates and used in greenhouse phenotyping of a recombinant inbred line (RIL) population of MLB-49-89A x CAL96 for FRR resistance in the second part of this study. QTL mapping was conducted with field and greenhouse generated data. Eight QTL related to FRR resistance for disease severity score, root biomass, and root/shoot biomass reduction were identified. Disease resistance (R) genes were found in/near three of the QTL regions. In the last part of this study, R genes identified through the transcriptome profiles of two navy beans were characterized and 24 of these R genes were found to contain SNPs. A map was created to overlay genomic regions of R genes and QTL for FRR resistance. The results of this study provide useful information that can be applied to the development of FRR resistant common bean cultivars.

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Agricultural Importance

Common bean (*Phaseolus vulgaris L.*), also called dry bean, is an important food legume for human consumption as a seed or vegetable and is considered to be a perfect food resource based on high content of protein, dietary fiber and micronutrients (Siddiq & Uebersax, 2012). The origin of common bean can be dated back to as early as 7,000 years B.C. in Mexico and Central America where the natives started to use common beans in their diets (Salinas, 1988). Nowadays, common beans are distributed worldwide and are very diverse in seed types, cultivation methods, usage, and the range of environments to which they have adapted. Beans play an essential role in the daily diet in tropical America, eastern and southern Africa, as well as many other regions in the world (Beebe, 2012). In Africa, common bean is the second most important source of dietary protein and third most important source of calories for low income families (Asfaw et al., 2009), and beans are also gaining an important place in the export market and providng income for households with small-scale farms (Beebe, 2012).

Genetic Background

Common bean ($2n = 2X = 22$), is a self-pollinated diploid crop with a relatively small genome size of about 600 Mb and 11 chromosomes (Broughton et al. 2003; Kelly & Cichy, 2012). Common bean is composed of two gene pools: The Middle American gene pool, which originates from northern Mexico to Colombia, and the Andean gene pool, which originates from southern Peru to northwestern Argentina (Gepts, 1998). The Middle American gene pool is divided into three races: the medium-seeded, semi-climbing Durango, the medium-seeded, climbing Jalisco, and the small seeded, climbing Mesoamerica. The Andean gene pool is divided

into three large-seeded races: Chile, Nueva Granada, and Peru (Singh, 1991). Each of those races has distinct ecological characteristics and agricultural traits. According to a population structure study with microsatellite markers (Kwak & Gepts, 2009), a mixed sample of Andean and Middle American beans had a high linkage disequilibrium (LD) level, but the LD level was significantly lower when the sample was subdivided into groups with only Andean or Middle American cultivars. This result confirmed the divergence between Andean and Middle American gene pools and their differentiated racial structures (Kwak & Gepts, 2009). The diverged Andean and Middle American gene pools can be a challenge for breeders because of their partial reproductive isolation (Koinange & Gepts, 1992), but this divergence also offers the opportunity to develop new gene combinations.

The common bean reference genome was recently released by sequencing the Andean inbred landrace line G19833 using a whole-genome shotgun sequencing strategy (Schmutz et al., 2014). The reference genome will facilitate the comparison of genome differences among related species, the understanding of domestication events, and the study of functional genes in common beans. Two independent domestication events were confirmed for Andean and Middle American gene pools in the study and significant effects of the domestication events have been found in selection of genes related to flowering, seed size and disease resistance (Schmutz et al., 2014).

Fusarium Root Rot and Management

Bean root rot is caused by a complex of soil-borne fungal pathogens and has been considered a major disease that reduces bean yield and constrains production (Abawi, 1989). Commonly recognized bean root rot diseases include: Fusarium root rot caused by *Fusarium*

solani f. sp. phaseoli; Rhizoctonia root rot caused by *Rhizoctonia solani*; Pythium root rot caused by *Pythium spp.*; and Sclerotium root rot (southern blight) caused by *Sclerotium rolfsii* (Abawi & Corrales, 1990). Although the causal pathogen of Fusarium root rot (FRR) was originally reported as *F. solani f. sp. phaseoli* (Hall, 1991), *F. cuneirostrum* was also reported to cause FRR (Aoki et al. 2005).

In fact, *F. solani* has now been known as the *F. solani* species complex (FSSC) which consists of over 45 phylogenetic species that are classified into three major groups named Clade 1, 2, 3 (O'Donnell 2000, Zhang et al. 2006) and many undescribed species considered as *Fusarium solani* f. sp. *phaseoli* (Nalim, 2011). *F. phaseoli* and *F. cuneirostrum* are both belong to the FSSC. According to O'Donnell et al. (2010), four closely related soybean sudden death syndrome (SDS) causal pathogen (*F. virguliforme, F. tucumaniae, F. brasilien*se, and undescribed *Fusarium sp*.) are paraphyleticly related with these two FRR causal pathogen (*F. phaseoli* and *F. cuneirostrum*), and these six species form a exclusive sub-clade in Clade 2 of the FSSC.

Additionally, some other *Fusarium* species that were not commonly known as FRR causal pathogens were also found to cause root rot in beans. The examples include *F. oxysporum* f. sp. p*haseoli* reported in northeast Brazil (Abawi, 1989), *F. graminearum* reported in North Dakota, USA (Bilgi et al., 2011), and *F. brasilense* and *F. virguliforme* in Michigan, USA (Chilvers, personal communication).

The morphological characters shared among *Fusarium* species include septate, hyaline mycelia, three or four septate macroconidia, globose chlamydospores, conidia produced in sporodochia, and microconidia rarely present during their lifecycle (Hall, 1991). Macroconidia are the most important cultural characteristic to identify *Fusarium* species (Leslie & Summerell,

2006), based on their variations in size, shape, and the two ends of the macroconidia (Figure L.1). In soil, *Fusarium* species usually persist as thick-walled chlamydospores (tough survival spores) (Figure L.2), which germinate when stimulated by nutrients exuded by germinating seeds. Hyphae are generated and penetrate the plant through stomata, wounds, and intercellular spaces of the cortex (Christou & Snyder, 1962). As infected tissues degenerate, conidia and hyphae develop into chlamydospores to complete the life cycle (Figure L.3). The chlamydospores can germinate and reproduce in soil near organic matter, and near seeds and roots of many non-host plants, thus, the fungus may survive in infested fields indefinitely (Schroth & Hendrix, 1962).

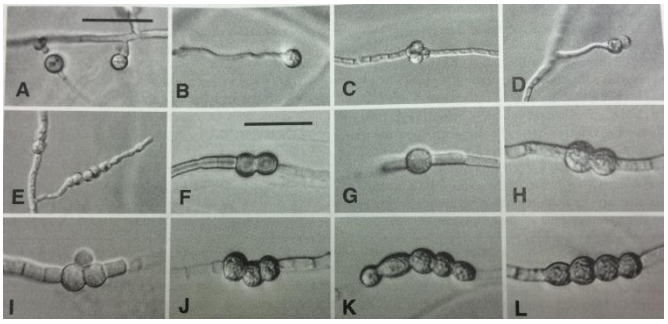


Figure L.1 Different types of chlamydospores of *Fusarium* species. Source: Leslie & Summerell, 2006.

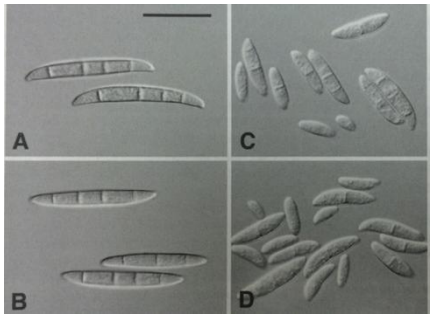


Figure L.2 Macroconidia (A-B) and microconidia (C-D) of *Fusarium solani*. Source: Leslie & Summerell, 2006.

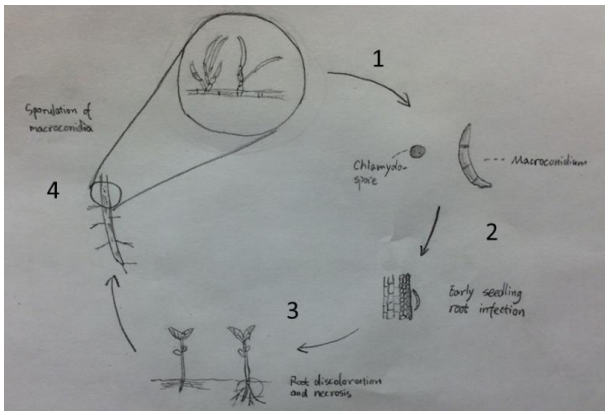


Figure L.3 Disease cycle of Fusarium root rot.
(1) Chlamydospores or macroconidia formed and released from decaying roots, and transported by wind or in soil; (2) they germinate when in contact with seedling roots; (3) Infected roots may produce adventitious roots; (4) The fungus sporulates on infected roots and forms chlamydospores to survive in soil for long periods of time.

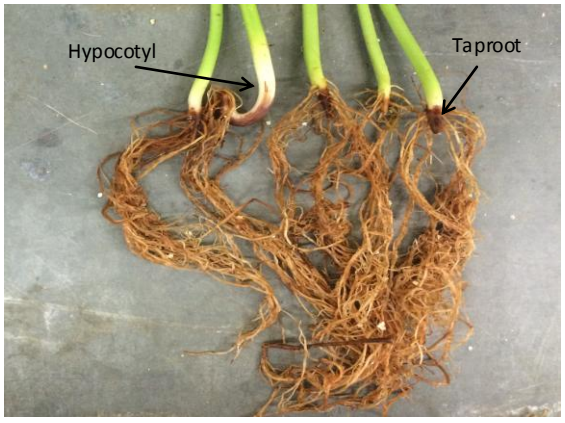


Figure L.4 Dry bean roots infected by a *Fusarium brasilense* isolate in greenhouse.

Usually, the first symptoms of *Fusarium* infection on roots appear about 7 – 10 days after germination with narrow, longitudinal, red to brown streaks on hypocotyls and taproots (Hall, 1991). Affected areas generally enlarge and progress down the main taproot, and turn necrotic. In some cases, disease symptoms extend up to the hypocotyl near the soil surface (Román-Avilés et al., 2003) (Figure L.4). In response to infection, horizontal adventitious roots from the hypocotyl are produced to help the plant survive (Román-Avilés & Kelly, 2005). Infected plants

are usually stunted, grow slower than healthy plants, and their weak root system cannot absorb nutrients and water efficiently (Román-Avilés et al., 2003).

Under environmental conditions that restrict root growth, such as drought, flooding, soil compaction, and low soil fertility, the root rot symptoms become more severe and entire root systems can be infected and die (Hall, 1994). It has been suggested that disease incidence may increase under drought conditions when the pathogens have the ability to survive at low water potentials while the growth of antagonistic organisms is inhibited (Cook, 1973; Schoeneweiss, 1975). Under excess water and flooding conditions, root growth is restricted due to oxygen-deficiency which also causes toxic metabolites to accumulate in roots, and thus defense reactions of the host plant is suppressed (Stolzy et al., 1965). Reduced soil aeration also inhibits water and nutrient uptake and increases disease susceptibility of the plants (Cook, 1972; Schoeneweiss, 1975). Soil compaction can occur naturally depending on soil types, or can be created by heavy traffic. In compacted soil, porosity is decreased, soil structure is degraded, and thus water movement and root growth are constrained (Allmaras et al., 1988). Fusarium root rot becomes more severe in compacted soil, because less water and nutrients are accessed by infected and rotted roots (Burke et al., 1972; Harveson et al., 2005).

From a cultural management perspective, the use of species with resistance, minimization of soil compaction, good drainage, and high soil fertility can contribute to effective disease control of FRR (Abawi, 1990). Rotation with resistant crops such as alfalfa, barley, wheat, oats and corn can reduce residual populations of root rot organisms in soil, and a long rotation (three to five years) is recommended (Román-Avilés et al., 2003; Schwartz, 2011). Other recommended cultural management approaches include reduced plant density, planting when soil is warm (55℉), careful cultivation to avoid root damage, and appropriate irrigation (Román-Avilés et al.,

2003). Besides appropriate cultural management, root structure is also related to root rot disease tolerance. Cultivars that produce more adventitious roots and larger basal roots tend to be more resistant to root rot (Snapp et al., 2003; Cichy et al., 2007), and larger taproot diameters of roots are also found to be related to root rot resistance (Hagerty et al., 2015).

The use of biocontrol agents to manage bean root rot disease has also been investigated based on the successful examples of crop disease management with biological control (Narayanan et al., 2002). The application of *Bacillus subtilis* and *Trichoderma harzianum* (a biofungicide) or *Rhizobium* (soil bacteria that fix nitrogen) on common bean seeds was found effective for controlling root rot and increasing bean yield, compared with the standard seed treatment (SST) (Captan 400 + Streptomycin + Lorsban 50) (Abeysinghe, 2012; Estevez de Jensen et al., 2002). The vesicular arbuscular (VA) mycorrhiza *Glomus mosseae* (Gm) plays an important role in protecting beans from invasion by soil-borne pathogens, and the application of Gm combined with *Rhizobium leguminosarum* (Rl) increases plant biomass and root nodulation (Dar et al., 1997). The mixture of arbuscular mycorrhizal (AM) fungi *Glomus* and *Gigaspora* species as biocontrol agents was also found to reduce disease severity of infected beans and promote their growth (Al-Askar & Rashad, 2010). *Trichoderma harzianum* and *Trichoderma viride* are two fungal species that are registered commercial biological control agents (Whipps & Lumsden, 2001). Teixeira et al. (2012) in Brazil, tested six *Trichoderma*-based commercial products (TCP) and compared to the fungicide fludioxonil for their effectiveness in controlling FRR of common bean, and the results showed that these TCP were effective in reducing disease severity, but had no effect in reducing disease incidence.

Disease Resistance Genes

Resistance and tolerance are the two defense strategies that a host plant protects itself from infections (Mauricio, 1997). Resistance is the strategy that the host reduces pathogen burden before/after the infection is established. Tolerance allows the host to reduce the negative impact of an infection and decrease the damage caused by pathogens, and the ability of plants to tolerate presence of pathogens varies because of the genetic variation (Medzhitov et al., 2012). In general, the mechanisms of the plant innate immune system include two levels: 1) the structural and biochemical barriers to prevent the infection of pathogens or other pests; 2) the defense response on the molecular level that is triggered by the interaction between pathogen and plant (Michelmore et al., 2013).

In the gene-for-gene hypothesis, incompatibility occurs when a dominant resistance gene (*R* gene) in the host plant matches a dominant avirulence gene (*Avr* gene) in the pathogen, resulting in host plant showing resistance (Hammond-Kosack, 1997). The absence of one or both of the genes makes the plant susceptible to the disease. The term "avirulence gene" was created by Flor (1956), to indicate the pathogen genes that encode products (called effectors) being recognized by proteins of *R* genes (called receptors) directly or indirectly. In other words, when an *Avr* gene is expressed and detected by host plant R genes the lack of virulence of the pathogen is conferred (McDowell & Woffenden, 2003).

According to previous studies, receptor proteins are classified into two major groups based on their function in plant immunity (Michelmore et al., 2013). The first group contains pattern recognition receptors (PRRs) that are membrane-localized. PRRs trigger basal defense response by detecting extracellular pathogen-associated molecular patterns (PAMPs), which is called PAMP-triggered immunity (PTI). The second group includes the nucleotide-binding-site-

leucine-rich-repeat (NB-LRR) receptors, which detect pathogen effectors inside cells. NB-LRR receptors trigger effector-triggered immunity (ETI) that combines with PTI to cause a hypersensitive response (HR) which results in localized programmed cell death.

At the initial stage of infection, a pathogen delivers effectors into plant cells to either help the colonization of the pathogen or interrupt the defense of host plants, which triggers the plant to produce resistance proteins (*R* proteins) if detected by the plant (Gururani et al., 2012). However, experimental examples of direct interaction between an *R* protein and a corresponding *Avr* protein are very limited, which stimulated the formation of the "guard hypothesis" (Van der Biezen & Jones, 1998; McDowell & Woffenden, 2003). Besides the simplest model of direct interaction between *R* protein and *Avr* protein, two other types of interactions were discussed in previous studies: (a) *Avr* protein interacts with one or more host proteins (HP) that are associated with *R* protein, then the *R* protein recognize the Avr-HP interaction which triggers resistance; (b) *Avr* protein modifies a host protein and *R* protein recognizes the altered host protein, therefore triggering the resistance (Ellis et al., 2000).

Based on organization of amino acid motifs and membrane spanning domains, Gururani et al. (2012) divided plant resistance genes into eight groups (Table L.1). Most *R* genes encode leucine-rich repeats (LRRs) among which nucleotide-binding-site-leucine-rich-repeat (NB-LRR) is the most common structure. NB-LRRs consist of two sub-groups: TIR-containing NBS-LRR proteins (TNLs) and coiled-coil NB-LRR (CNLs), where TNLs has Toll/interleukin-1 receptor (TIR)-like motif in their amino-terminal domain and CNLs do not contain this motif. In some cases, a pair of NBS-LRR genes is required to function together or overexpress one of the partners for plants to obtain resistance (Eita and Dangl, 2010).

Table L.1 Major groups of plant resistance genes base on their functional domains.

No.	Major R-gene classes	Example
I	NBS–LRR–TIR	N, L6, RPP5
II	NBS–LRR–CC	I2, RPS2, RPM1
III	LRR–TrD	Cf-9, Cf-4, Cf-2
IV	LRR–TrD–Kinase	Xa21
V	TrD–CC	RPW8
VI	TIR–NBS–LRR–NLS-WRKY	RRS1R
VII	LRR–TrD–PEST–ECS	Ve1, Ve2
VIII	Enzymatic R-genes	Pto, Rpg1, Hm1

LRR: Leucine rich repeats; NBS: Nucleotide-binding site; TIR: Toll/Interleukin-1- receptors; CC: Coiled coil; TrD: Transmembrane domain; PEST: Amino acid domain; ECS: Endocytosis cell signaling domain; NLS: Nuclear localization signal; WRKY: Amino acid domain. Source: Gururani et al. (2012)

R genes are usually monogenic or major genes that control resistance to specific pathogen races and tend to be stable across environmental conditions (Michelmore et al., 2013). But the disease resistance can also be polygenic, when many genes are involved and provide small but additive effects, such as receptor-like kinases (RLKs) (Kou & Wang, 2010) and primary metabolism genes that play a role in providing energy for the resistance response (Bolton, 2009).

The identification of R genes has become one way to detect disease resistance. Liu (2012), used 454-derived transcriptome sequencing to detect the common bean (*P. vulgaris* = Pv) resistance-gene-like sequences (PvRGLs). A total of 364 PvRGLs were successfully identified, 65% of which were integrated into the common bean genetic map. Miklas et al (2006) discussed the resistance genes and quantitative trait loci (QTL) related to several major diseases and insects. In general, 15 clusters of monogenic disease resistance genes are located on seven chromosomes (Pv01, Pv02, Pv04, Pv06, Pv07, Pv08, and Pv11); eight defense-related genes are located on five chromosomes (B1, B2, B3, B5, and B7); and one lectin and alpha-amylase inhibitor gene is located on chromosome Pv04 (Miklas et al., 2006). In the bean reference genome study, large clusters of NB-LRR genes were found at the ends of chromosomes Pv04, Pv10, and Pv11.

Among a total of 376 identified NB-LRR genes, 106 are TNLs-encoding genes and 108 are CNL-encoding genes (Schmutz et al., 2014).

Genotypic Sources of FRR Resistance

Among root rot control strategies, development of cultivars with root rot resistance has been generally considered the best long-term management option (Tu 1992; Park and Rupert 2000; Abawi et al. 2006; Conner, 2014). The genetic inheritance of FRR resistance in dry bean and breeding for resistant cultivars have been studied extensively. Boomstra & Bliss (1977) suggested that the recurrent breeding method is more suitable than pedigree selection or backcross breeding to develop resistant cultivars, because of the quantitative inheritance of FRR resistance. Boomstra et al. (1977) tested a collection of Plant Introduction (PI) lines from Mexico for FRR resistance with a nutrient culture method, and found 17 lines that had varied levels of resistance, among which the black bean line PI203958 (N203) was the most resistant. However, Beebe et al. (1981) reported that N203 only displayed an intermediate resistance in field tests in Colombia, while the bean lines from Latin America (Mesoamerican genotypes) showed more common FRR resistance, which could be due to natural selection by exposure to soil-borne pathogens for long period of time.

Studies have shown that the susceptibility to FRR is more common in large-seeded Andean genotypes than in Mesoamerican genotypes of beans (Schneider et al. 2001; Román-Avilés & Kelly, 2005), and seed size is positively related to root rot severity (Schneider & Kelly, 2000). The snap bean cultivar FR266, which was developed from a cross between N203 and *Phaseolus coccineus,* has been used in different genetic studies on FRR resistance and to transfer root rot

resistance from the Mesoamerican genepool to the Andean genepool (Schneider et al., 2001; Román-Avilés & Kelly, 2005; Cichy et al., 2007). Five bean genotypes of Mesoamerican origin, MLB-49-89A, RWR719, G2333, G685 and MLB-48-89A, were recommended as good sources of resistance to FRR in a study of improving FRR resistance in bean cultivars in Uganda (Mukankusi, 2008; Obala et al., 2012). Two small red bean lines, RWR719 and Vuninkingi, were also found to have resistance to FRR, and it was estimated that the root rot resistance trait was controlled by two to nine genes and multiple QTL (Mukankusi et al., 2011). Conner et al. (2014) identified potential cultivar sources of resistance to root rot in western Canada, among which cranberry bean cultivars 'Etna' and 'Cran09' were found to have highest root rot resistance, and the navy bean cultivar 'Navigator' and two black bean cultivars 'Black Violet' and 'CDC Jet' were detected to have partial root rot resistance.

QTL Analysis for FRR Resistance

In previous studies, QTL analysis was conducted on different bean populations to identify the genetic basis of resistance to FRR. Different molecular markers and analysis methods were developed and utilized.

Schneider et al. (2001) used two RIL populations from crosses of the resistant Andean large-seeded snap bean line FR266 with dark red kidney susceptible lines 'Montcalm' and 'Isles' for detecting QTL related to FRR resistance using random amplified polymorphic DNA (RAPD) markers. As a result, 16 QTL were identified and two of the RAPD markers that showed significant association with resistance were detected on linkage group Pv02 of the genetic map, and the pathogenesis-related (PR) protein *Pv*PR2 was found close to the two

makers (Schneider et al., 2001). Chowdbury et al. (2002) used a RIL population derived from a cross between the susceptible navy bean cultivar 'AC Compass' and resistant line NY2114-12 for mapping with RAPD markers. Two QTL were detected by Interval Mapping (IM) and the phenotypic variations explained by these QTL were 30% and 20%, respectively (Chowdhury et al., 2002). Another QTL analysis with RAPD markers was conducted in two inbred backcross-derived populations from a cross of 'Red Hawk' x 'Negro San Luis' (NSL) and a cross of C97407 x NSL. A total of 12 QTL associated with FRR resistance were detected and three of them were assigned to Pv02 and Pv05, and 7.3 to 53% of the phenotypic variations were explained (Román-Avilés & Kelly, 2005). Kamfwa et al. (2013) investigated QTL for FRR resistance in the RILs of MLB49-89A (resistant) x K132 (susceptible) with simple sequence repeat (SSR) markers. A QTL significantly associated with FRR resistance was found between the two SSR makers PVBR87 and PVBR109 on linkage group Pv03 (Kamfwa et al., 2013).

In recent years, single nucleotide polymorphism (SNP) markers have been replacing other molecular markers in QTL analysis. SNPs are bi-allelic and co-dominant, and reflect the single DNA base differences between homologous DNA fragments (Souza, 2012). The 6000 SNP *Phaseolus vulgaris* Illumina Infinium BeadChip (BARCBean6K_3) was developed by the Bean Coordinated Agriculture Project (BeanCAP) at USDA-ARS Soybean Genomic and Improvement Laboratory (Beltsville, MD). The resulting genetic map from SNP analysis has higher density of marker coverage than maps with other markers and the physical position in the genome of each SNP marker can be located. Hagerty et al. (2015) identified QTL associated with FRR resistance and *Aphanomyces euteiches* root rot resistance in a RIL population of RR6950 (resistant) x OSU5446 (susceptible) using SNP markers. RR6950 is a small brown-seeded bean with type III growth habit and OSU5446 is a bush green bean breeding line with type I growth habit. A total

of 1,689 SNP markers were used in genetic map construction. Two QTL for FRR resistance were found on Pv03 and Pv07, explaining 9 and 22% of phenotypic variation. Some other agronomic traits related to root rot resistance were measured, such as taproot diameter (TD) and shallow basal root angle (SBRA). One QTL was found for TD on Pv02, explaining 10% of phenotypic variation and one QTL was found for SBRA on Pv05, explaining 19% of phenotypic variation (Hagerty et al. 2015). A RIL population of Puebla 152 (resistant) x Zorro (susceptible) was used to detect QTL related to FRR resistance and root architecture traits in a study of Nakedde (2015). The two parents were both black bean but with different architecture traits. Four QTL were identified for root architecture traits on Pv01, Pv05, and Pv09, and one QTL associated with FRR resistance on Pv05 was found. The QTL for total root weight and shallow root weight colocalized on Pv09, and the QTL for deep root dry weight colocalized with the QTL for total biomass on Pv05. The phenotypic variation explained by these QTL varied from 8.3% to 12.7%.

Transcriptome Analysis

Transcriptome analysis is the study of the changes in total transcripts in a cell at a specific stage of development or physiological condition. It has been an asset for better understanding gene expression, genetic variation, and gene structure annotation associated with plant traits (Wang et al., 2009). With the improvements in high-throughput sequencing technology over recent years, gene expression information can be obtained through whole transcriptome profiling, which is generated by sequencing messenger ribonucleic acid (mRNA) (Davidson, 2011). To conduct the RNA sequencing (RNA-seq) procedure, extracted RNA is converted to complementary DNA (cDNA), which is then processed by massive parallel sequencing that produces numerous 36- to 150-bp short reads (Wang et al., 2009). Those reads

are then aligned to a reference genome by using algorithm programs such as TopHat (Trapnell, 2009) or SOAP (Li, 2008). The profiles generated from these programs can then be used for further gene expression analysis.

Investigation of the gene expression profiles associated with economically and biologically important traits in common bean will facilitate functional genomic studies and the utilization of specific genes. Transcriptome analysis related to disease resistance genes will contribute to the understanding of gene expression patterns and the identification of candidate disease resistance genes. However, pertinent studies on dry beans are limited compared to those on model plants and other crops. Some examples of fungal resistance genes are the *RPW8* genes in *Arabidopsis thaliana* for mildew resistance (Xiao et al., 2001); the *Cf* and *Ve* genes in tomato (*Lycopersicum esculentum*) that confer resistance to a broad spectrum of diseases (Kawchuk et al., 2001); and the *mlo* and *Rpg* genes in barley (*Hordeum vulgare*) (Büschges et al., 1997; Chełkowski et al., 2003).

Gene expression responses under disease stress can cover a broad range in plant metabolism pathways. Iqbal et al. (2005) studied soybean root responses to *Fusarium solani* f. sp. *glycines*. The variation in transcript abundance in inoculated or non-inoculated roots was detected using expressed sequence tags (ESTs). ESTs are fragments of mRNA sequences derived from single sequencing reactions performed on clones randomly selected from cDNA libraries (Parkinson & Blaxter, 2009). The result showed a significantly increased transcript abundance in root tissue 10 days after inoculation and the expressed genes were associated with resistance, signal transduction, plant defense, cell wall synthesis, and transport of metabolites (Iqbal et al., 2005). Xue et al. (2014) studied the differentially expressed genes in common bean responding to *Fusarium oxysporum* f. sp. *phaseoli,* using the cDNA amplified fragment length polymorphism

(cDNA-AFLP) technique. From 8,730 generated transcript-derived fragments, 423 (4.9%) displayed altered expression patterns. The expressed genes with known putative function were found to be related to metabolism, signal transduction, protein synthesis and processing, cytoskeletal development and organization, transport of proteins, gene expression and RNA metabolism, redox reactions, defense and stress responses, energy metabolism, and hormone responses (Xue et al., 2014).

CHAPTER 1: SCREENING *FUSARIUM* ISOLATES FOR VIRULENCE ON COMMON BEANS (*PHASEOLUS VULGARIS*)

INTRODUCTION

Fusarium root rot (FRR) is one of the commonly recognized root rot diseases that can severely reduce bean yield and constrain bean productivity (Abawi & Corrales, 1990). In East African countries, farmers have experienced significant loss in bean production because of FRR and the problem is becoming worse over time (Ongom, et al., 2012). In most production regions of dry bean and snap bean in the United States, FRR has also been considered a major soil-borne disease that causes severe yield loss (Chatterjee, 1958; Keenan et al., 1974; Saettler, 1982; Cichy, 2007). The major species of *Fusarium* that causes bean root rot is *F. solani* , which is now known as the *F. solani* species complex (FSSC) (O'Donnell 2000). *F. phaseoli* and *F. cuneirostrum* are two FRR causal pathogen and they are paraphyleticly related with soybean sudden death syndrome (SDS) causal pathogens (*F. virguliforme, F. tucumaniae, F. brasilien*se, and undescribed *Fusarium sp*.), and these six species are all belong to Clade 2 of the FSSC (O'Donnell et al., 2010). Some other species that were not commonly known as FRR causal pathogens have also been found to cause bean root rot. *Fusarium oxysporum* known to cause wilt or yellowing was identified as the major root rot pathogen in northeast Brazil (Abawi, 1989). *Fusarium oxysporum* was also reported in North America as FRR causal pathogens (Estevez De Jensen et al., 1999; Bilgi et al., 2011).

The first symptoms of *Fusarium* infection usually appear about 7 – 10 d after germination, with narrow, longitudinal, red to brown streaks on hypocotyls and taproots. Then cortex tissues develop lesions and necrosis (Hall, 1991). The infection may become more severe as the plant develops and complete rotting of root systems can eventually occur. Infected plants are usually

stunted and may develop adventitious roots from hypocotyl for survival (Román-Avilés et al., 2003; Román-Avilés & Kelly, 2005). The severity of FRR is related to environmental conditions such as drought, flooding, soil compaction, and low soil fertility (Hall, 1994). FRR disease incidence increases under drought conditions when the pathogens have the ability to survive at low water potentials while the growth of the plant host is inhibited (Cook, 1973; Schoeneweiss, 1975). Under excess water and flooding conditions, root growth is restricted due to oxygen deficiency, which also causes toxic metabolites to accumulate in roots, and thus defense reactions of the host plant are suppressed (Stolzy et al., 1965). In compacted soil, which has decreased porosity and degraded soil structure, root growth is restricted and uptake of water and nutrients is constrained, thus the disease susceptibility of plants increases (Allmaras et al., 1988).

The susceptibility of beans to FRR varies among genotypes and the susceptibility is more common in Andean bean genotypes than Middle American genotypes (Mukankusi et al., 2008). MLB-49-89A, a medium-seeded black Middle American bean variety with an indeterminate type-III growth habit, was reported to have resistance to FRR (Mukankusi et al., 2011). CAL96, a large-seeded, red mottled Andean bean genotype in East Africa with type-I upright determinate bush growth habit, was reported to be susceptible to FRR (Kamfwa et al., 2013). 'Chinook', a light red kidney bean cultivar with type-I upright determinate growth habit, was developed and released by the Michigan Agricultural Experiment Station and the USDA-ARS in 1998 (Kelly et al., 1999). 'Chinook' was reported to be susceptible to FRR (Schneider & Kelly, 2000). 'Zorro', a black bean variety with type-II upright short-vine with indeterminate growth habit, was developed and released by the Michigan Agricultural Experiment Station in 2008 and exhibited resistance to some root rot pathogens in field tests (Kelly et al., 2009).

Different methods were utilized in previous studies for screening common beans for FRR resistance and two typical greenhouse screening methods were compared to test their repeatability (Chaudhary et al., 2006). One of these methods was the liquid inoculum method (Schneider & Kelly, 2000), where a *Fusarium* spore suspension is applied around the hypocotyl; the other was the inoculum layer method (Schmitthenner & Bhat, 1994), where the plants are inoculated by inserting a layer of inoculum in the growth media at planting. According to Chaudhary et al. (2006), the liquid inoculum method showed low repeatability compared to the inoculum layer method. However, a layer of inoculum in the growth media does not represent real field conditions, where the pathogen tends to be more evenly distributed. In this study, both greenhouse and laboratory tests were used for screening virulence of *Fusarium* species isolated from infected common bean plants in East Africa and the USA, and the screening methods are different from previous studies.

The objectives of this study were to (a) determine the virulence variations among *Fusarium* isolates; (b) select the most effective *Fusarium* isolate to use in screening for FRR resistance in the RIL population of MLB-49-89A x CAL96; (c) devise an effective and efficient screening strategy to use in screening the RIL population; (d) discover the genotypic differences in susceptibility of a representative Middle American and Andean bean cultivar.

EXPERIMENT 1 Greenhouse Screening

Materials and methods

Fusarium isolates

A collection of *Fusarium* species were obtained from Dr. Martin Chilvers' plant pathology laboratory at Michigan State University (MSU) (Table 1.1). The first three isolates in the list were obtained from USDA-ARS Northern Regional Research Laboratory (NRRL). The MIMTC- strains were collected from infected soybean roots from MSU Montcalm Research Farm in Michigan.

Table 1.1 *Fusarium* isolates used in greenhouse screening for virulence (Experiment 1).

Code	Species	Plant Source	Origin
31156	*F. phaseoli*	Dry Bean	USDA-ARS, NRRL
31157	*F. cuneirostrum*	Dry bean	USDA-ARS, NRRL
Mont-1	*F. virguliforme*	Soybean	USDA-ARS, NRRL
MIMTC-A3	*F. brasiliense*	Soybean	Michigan, USA
MIMTC-A9	*F. brasiliense*	Soybean	Michigan, USA
MIMTC-B11	*F. brasiliense*	Soybean	Michigan, USA

Plant material

Common bean cultivars, CAL96 and MLB-49-89A, were obtained from the Center for International Tropical Agriculture (CIAT), Kampala, Uganda and planted in a greenhouse (average temperature of 28℃ during the day and 22℃ at night, 12 h of light) at MSU to produce seeds.

Inoculum preparation

Pure *Fusarium* isolates were cultured on slants of Potato Dextrose Agar (PDA) at 5℃ for long-term storage. To prepare *Fusarium* inoculum, isolates were sub-cultured from slants to Nash-Snyder (NS) plates (Nash & Snyder, 1962) and kept at room temperature for 7 to 10 d. Moistened sorghum kernels were put in bags with about 1.7 kg/bag and autoclaved at 120℃ for 8 h and cooled at room temperature overnight. For each *Fusarium* isolate, five NS *Fusarium* inoculated plates and five NS non-inoculated plates were blended with 500 mL sterile distilled water in a sterile blender to make an inoculum slurry. The slurry was mixed with the autoclaved sorghum kernels at a rate of 100ml/bag, and the bags were sealed and incubated at room temperature. The bags were shaken once every day to allow even mycelium growth. After 20 to 25 d when the mycelium had almost covered all sorghum kernels, the kernels were air-dried in a sterilized tray.

Screening

Perlite and vermiculite medium were mixed in a ratio of 2:1. In 1 L greenhouse plastic pots, 35 ml of *Fusarium* sorghum inoculum was mixed in 700 ml of perlite-vermiculite medium. Bean seeds were placed on the top and covered with another 200 ml of perlite-vermiculite medium. Medium without *Fusarium* sorghum inoculum was used as control. Bean seeds were planted with three replicate pots and three seeds per pot. After emergence, plants were watered once daily and fertilized with half-strength Hoagland's solution (Hoagland and Arnon, 1950) twice weekly. Greenhouse temperature was set at 26℃ during the day and 22℃ at night. The

experiment was set up as a completely randomized design (CRD) with two experimental factors (isolates and genotypes).

Fourteen days after emergence, plants were I removed from the growth media and cleaned and rinsed the roots and hypocotyls in tap water. The roots were evaluated for FRR symptoms using the CIAT 1-9 scale (Table 1.2), which is a nine points scale where 1 indicates no disease and 9 indicates advanced rotting of the root. Plants with score of 1-3 were considered as resistant, with score of 4-6 were considered as moderately resistant and 7-9 were considered as susceptible. After visual evaluation, plants were oven-dried at 60℃ for 3 d and shoots and roots were weighed separately. The percentage reduction in root and shoot dry weight of inoculated plants were calculated compared to non-inoculated controls.

Table 1.2 CIAT 1-9 *Fusarium* root rot rating scale used in experiment 1[#]

Score	Symptom Description
1	No visible disease symptoms
2	Approximately 5% of the hypocotyls and root tissues covered with lesions
3	Light discoloration either without necrotic lesions or with approximately 10% of the hypocotyls and root tissues covered with lesions
4	Approximately 17.5% of the hypocotyls and root tissues covered with lesions
5	Approximately 25% of the hypocotyls and root tissues covered with lesions, but tissues remain firm with deterioration of the root system and heavy discoloration symptoms may be evident
6	Approximately 37.5% of the hypocotyls and root tissues covered with lesions
7	Approximately 50% of the hypocotyls and root tissues covered with lesions, combined with considerable softening, rotting, and reduction of the root system
8	Approximately 62.5% of the hypocotyls and root tissues covered with lesions
9	Approximately 75% or more of the hypocotyls and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system

[#] Adapted from Corrales and van Schoonhoven, 1987. CIAT: International Center for Tropical Agriculture.

The experiment was repeated for a second run under the same condition with same bean

genotypes, and experimental data were collected for analysis.

Statistical analysis

The mean of three plants in each replicate for each response variable was calculated and used for data analysis in SAS 9.4 (SAS Institute, Cary NC) with PROC MIXED procedure. The statistical model was established with genotype, treatment (isolates), and experiment repeat as fixed effects with replicate as random effect. The normal distribution of data was checked with normal quantile plots and residual plots. The effects and interactions of genotype, treatment, and experiment repeat were checked through Least Squares Means (LSM) with the least significant differences (LSD) value at 0.05. The PROC CORR command was used to analyze Pearson correlations among variables.

Results

The bean genotypes, the different *Fusarium* isolates, and their interaction had significant effects on all response variables with P-value less than 0.05 (Table 1.3). The experiment repeat had no significant effect on all response variables except shoot dry weight reduction, but the interaction of genotypes and run had no significant effect. Run was treated as a random effect in the statistic model in all statistical analysis of this experiment.

All six tested *Fusarium* isolates had caused root rot symptoms with mild to severe virulence on both bean genotypes (MLB-49-89A and CAL96). The non-inoculated controls of both genotypes were completely healthy with no visible disease symptoms. All inoculated plants had root rot symptoms and were significantly different from the non-inoculated controls (Table 1.4).

Table 1.3 Analysis of variance (ANOVA) of the mean response for *Fusarium* root rot scores (Score), average root dry weight (Root_DW), average shoot dry weight (Shoot_DW), root dry weight reduction (Root_Loss), and shoot dry weight reduction (Shoot_Loss) of CAL96 and MLB-49-89A inoculated with six different *Fusarium* isolates in two runs of experiment.

		P-value				
Source	Df	Score	Root_DW	Shoot_DW	Root_Loss	Shoot_Loss
Genotype[€]	1	0.0213	0.0004	<.0001	<.0001	<.0001
Trt	5	<.0001	<.0001	<.0001	<.0001	<.0001
Exp repeat	1	0.3794	0.3781	0.6736	0.3868	0.006
Geno*Trt	5	0.004	<.0001	<.0001	<.0001	<.0001
Geno*Exp Repeat	1	0.4703	0.4811	0.5841	0.5354	0.1666
Geno*Trt*Exp Repeat	11	0.0016	0.9995	0.9996	0.9999	0.9572

[€] Geno: Bean genotypes (CAL96 and MLB49-89A); Trt: Different *Fusarium* isolates; Exp Repeat: Two runs of experiment; Geno*Trt: Interaction between Geno and Trt; Geno*Exp Repeat: Interaction between Geno and Exp Repeat; Geno*Trt*Exp Repeat: Interactions among Geno, Trt, and Exp Repeat.

Table 1.4 The comparison of average disease symptom scores (two runs of experiment) of two bean genotypes MLB-49-89A (MLB) and CAL96 (CAL) without inoculation (Non-inoc) and inoculated with six different *Fusarium* isolates.

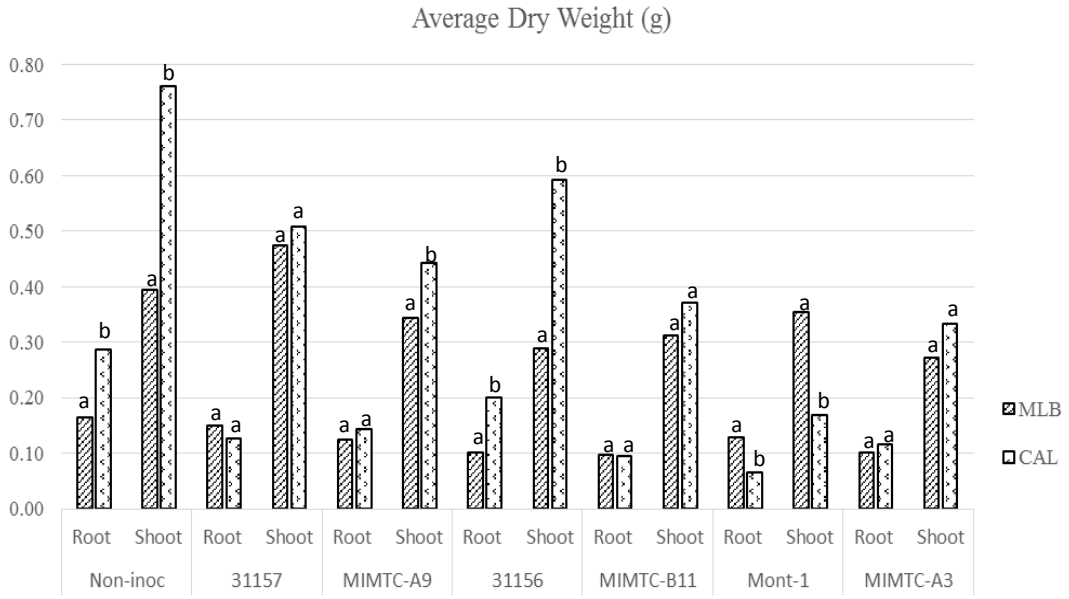
		Average Scores	
Treatment	Species	MLB	CAL96
Non-inoc		1.00 a[&]	1.00 a
31157	*F. cuneirostrum*	**3.41**[#] b	**4.89** b
MIMTC-A9	*F. brasiliense*	5.08 c	5.03 b
31156	*F. phaseoli*	5.36 c	5.47 b
MIMTC-B11	*F. brasiliense*	**5.56** c	**6.83** c
Mont-1	*F. virguliforme*	7.30 d	6.67 c
MIMTC-A3	*F. brasiliense*	7.56 d	7.86 d

[&]the same letters within a column means the disease symptom scores were not significantly different for that bean genotype (LSD, P<0.05).

[#]the bold numbers indicate significant statistic differences between two disease symptom scores for MLB49-89A and CAL96 (LSD, P<0.05).

The disease symptom scores for MLB-49-89A varied from 3.4 to 7.6 and for CAL96 varied from 4.9 to 7.9. Plants inoculated with *F. cuneirostrum* isolate 31157 (*F. cuneirostrum*) had lowest root rot disease symptom scores and plants inoculated with *F. brasiliense* isolate MIMTC-A3 had highest scores for both MLB-49-89A and CAL96. The root rot disease symptoms caused by isolate 31157 (*F. cuneirostrum*) and MIMTC-B11 (*F. brasiliense)* were significantly different between two genotypes (Table 1.4).

Figure 1.1 Average root and shoot dry weight of MLB49-89A (MLB) and CAL96 (CAL) inoculated with *Fusarium* isolates 31157, MIMTC-A9, 31156, MIMTC-B11, Mont-1, and MIMTC-A3[#].



[#]Different Letters indicate statistically significant difference between two genotypes under the treatment of each isolate.)

The non-inoculated controls had higher average root and shoot dry weight than the inoculated plants in both genotypes (Figure 1.1). The average root and shoot dry weight of non-inoculated CAL96 and MLB-49-89A were significantly different, and CAL96 were higher in both root and shoot biomass. The isolate 31156 (*F. phaseoli*) and Mont-1 (*F. virguliforme*)

caused significant difference between two genotypes in root and shoot dry weight. Root dry weight of two genotypes had no difference with inoculation of MIMTC-A9 (*F. brasiliense*), but shoot dry weight were different. The root and shoot dry weight of CAL96 and MLB-49-89A inoculated with the other three *Fusarium* isolates had no significant difference (Figure 1.1).

Table 1.5 Average root and shoot reduction in percentage of MLB49-89A (MLB) and CAL96 (CAL) inoculated with different *Fusarium* isolates compared to non-inoculated controls.

	Average Root Loss (%)		Average Shoot Loss (%)	
	MLB	CAL	MLB	CAL
31157	**9.0** d*	**55.9** bc	**-20.2** c	**33.4** d
MIMTC-A9	**24.3** bc	**50.4** c	**12.6** b	**42.0** cd
31156	38.2 ab	29.9 d	26.4 a	22.3 d
MIMTC-B11	41.4 a	66.8 ab	**21.0** ab	**51.3** bc
Mont-1	**22.4** cd	**76.9** a	**10.0** b	**77.7** a
MIMTC-A3	**38.0** ab	**59.7** bc	30.5 a	56.4 b

*the same letters within a column indicate the average root loss were not significantly different from each other for that bean genotype (LSD, $P<0.05$).

#the bold numbers indicate significant statistic differences between two genotypes for average root loss or average shoot loss (LSD, $P<0.05$).

The root and shoot reduction for both genotypes (MLB-49-89A and CAL96) varied with different *Fusarium* isolates (Table 1.5). The average root reduction ranges from 9% to 41.4% for MLB-49-89A and 29.9% to 76.9% for CAL96. The average shoot reduction ranges from -20.2% to 30.5% for MLB-49-89A and 22.3% to 77.7% for CAL96. MLB-49-89A had less root and shoot reduction than CAL96 with all isolates except 31156 (*F. phaseoli*), but the root and shoot dry weight reduction showed no significant difference between the two genotypes infected by 31156. MIMTC-B11 (*F. brasiliense*) inoculated MLB-49-89A plants had the highest root mass reduction and MIMTC-A3 (*F. brasiliense*) inoculated MLB-49-89A plants had the highest shoot

biomass reduction. Mont-1 inoculated CAL96 plants had the highest root and shoot mass reduction.

The isolates 31157, MIMTC-A9, and Mont-1 caused significant difference in average root loss and shoot loss between two genotypes. Isolate MIMTC-B11 only caused difference between two genotypes on average shoot loss, and isolate MIMTC-A3 only caused difference between two genotypes on average root loss. MLB49-89A inoculated with isolate 31157 had more shoot biomass than the control.

Table 1.6 Pearson correlation coefficients (r) for *Fusarium* root rot scores (Score), root dry weight (Root_DW), shoot dry weight (Shoot_DW), root dry weight reduction (Root_Loss), and shoot dry weight reduction (Shoot_Loss) of MLB-49-89A and CAL96 inoculated with different *Fusarium* isolates.

	Pearson Correlation Coefficients				
	Score	Ave_Root	Ave_Shoot	Root_Loss	Shoot_Loss
Score	-	-0.63**	-0.56**	0.36**	0.50**
Ave_Root		-	0.88**	-0.70**	-0.57**
Ave_Shoot			-	-0.46**	-0.56**
Root_Loss				-	0.89**

[#]– indicates negative correlation and + indicates positive correlation. *P-value=0.1, **P-value=0.05, *** P-value=0.01

Pearson's correlation coefficient (r) indicates proportion of the variance in the dependent variable that is predictable from the independent variable. P-value shows the probability of observing this correlation coefficient under the null hypothesis that the correlation is zero. According to Pearson Correlation Coefficients results, *Fusarium* root rot score was related with average root dry weight, average shoot dry weight, root loss, and shoot loss with varied r (Table 1.6). Average root dry and shoot dry weight were negatively correlated with score, while root and shoot reduction were positively correlated with score. Average root dry weight and shoot dry

weight were highly positively correlated with r = 0.88 ($P<0.05$). Root loss and shoot loss were also highly positively correlated with r= 0.89 ($P<0.05$) (Table 1.6).

EXPERIMENT 2 Laboratory Screening

Materials and methods

Plant materials

According to previous laboratory screening experiments for bean seedling response to *Rhizoctonia* (Dr. Linda Hanson's USDA-ARS Plant Pathology Laboratory), common bacterial blight caused by *Xanthomonas* occurs on non-western grown bean plants in the Magenta boxes that are used to screen bean seedlings. Western-grown seeds (developed on irrigated desert land in Washington, Oregon, California, and Idaho) were used because they are free of bacterial blight diseases (Webster et al. 1983). Plant materials used in this experiment for *Fusarium* isolates screening were two western-grown common bean cultivars 'Chinook' and 'Zorro', obtained from the MSU Dry Bean Breeding and Genetics Program. 'Chinook' is an Andean kidney cultivar, similar to CAL96, whereas 'Zorro' is a Middle American black bean cultivar, similar to MLB-49-89A.

Fusarium isolates

Dr. Linda Hanson (USDA-ARS, East Lansing, MI) provided a collection of Fusarium isolates obtained from infected dry bean roots in East Africa (Rwanda and Uganda). Dr. Linda Hanson's laboratory also characterized morphologically (Table 1.7), except F14-24 which was identified by Dr. Martin Chilvers' Plant Pathology laboratory at MSU. Hanson's laboratory also confirmed identification to species by molecular methods for most of the isolates on the list.

Table 1.7 Fusarium isolates obtained from infected dry bean roots in East Africa (Rwanda and Uganda) and used in laboratory screening for virulence in this experiment.

Code	African Code	Origin	Species
F14-8	G7-142(2) sps	Rwanda	*F. incarnatum*
F14-10	G1A-25 sps1	Rwanda	*F. oxysporum*
F14-11	Mbl 12	Uganda	*F. oxysporum*
F14-12	BKD 21	Uganda	*F. oxysporum*
F14-13	Srtx,1101	Uganda	*F. oxysporum*
F14-14	Lwr 73	Uganda	*F. oxysporum*
F14-15	Mbl 57	Uganda	*F. oxysporum*
F14-21	G2-88-23(isolate 1)	Rwanda	*F. oxysporum*[&]
F14-23	G2-88-23(isolate 2)	Rwanda	*F. oxysporum*[&]
F14-24	Fsp1	Uganda	*F. cuneirostrum*[#]
F14-25	G1-25-5(2)sps1	Rwanda	*F. oxysporum*
F14-26	G3-35-5	Rwanda	*F. oxysporum*
F14-27	G2-313-68B	Rwanda	*F. solani*
F14-28	G1-23-4	Rwanda	*F. oxysporum*
F14-29	G2-284-62	Rwanda	*F. oxysporum*
F14-31	G1-18	Rwanda	*F. oxysporum*
F14-33	G2-129-21A	Rwanda	*F. equiseti*[&]
F14-37	G1-25-5(3)sps2	Rwanda	*F. oxysporum*
F14-38	G2-114-28(isolate2)	Rwanda	*F. oxysporum*
F14-39	G2-42-9(1)	Rwanda	*F. oxysporum*

[&]identified with morphological method only by Dr. Linda Hanson's laboratory.
[#]identified by Dr. Martin Chilvers' laboratory.

Inoculum Preparation

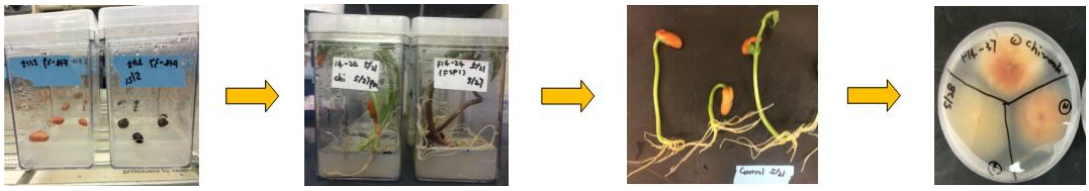
Pure *Fusarium* isolates were sub-cultured on half-strength V8 agar plates and kept at room temperature for 7 to 10 d. Magenta boxes (77 x 77 x 97 mm) with 60 ml of 1% water agar were autoclaved and cooled to about 40 to 50℃. Antibiotic suspension (300 µl) with a concentration of 50 µg/mL of both penicillin and streptomycin was added to each Magenta box. A 5-mm plug of mycelium was taken from each *Fusarium* plate and placed in the center of the water agar. A plug of non-inoculated V8 agar of the same size was placed in Magenta box in the same way to

use as control. The *Fusarium* isolates were grown at room temperature until the mycelium covered the whole agar surface.

Screening

Bean seeds were soaked in a solution of 10% bleach and 0.1% Tween 20 for 15 min and rinsed in sterile water. Then the seeds were placed in sterile germination box on a layer of moist autoclaved paper towel. Germinated seeds were transferred to Magenta boxes inoculated with *Fusarium* isolates or sterile water agar media as control (Figure 1.2). For each *Fusarium* isolate, both 'Chinook' and 'Zorro' were used for the virulence test. For each bean cultivar, three replicates of Magenta boxes, each containing three bean plants, were established.

Figure 1.2 Diagram of Magenta box lab test showing growth medium, plants at harvest stage, harvested plants for rating and re-isolated *Fusarium* strains.



Plants were removed after 7 d from Magenta boxes and evaluated for FRR symptoms using a 0-to-5 scale (Table 9), where zero indicates no visible disease symptom and five indicates severe disease damage. After visual evaluation, three pieces of 3-5 mm root tissue was cut from symptomatic roots of each replicate and soaked in the solution of 10% bleach and 0.1% Tween 20 for 1 min for surface disinfection. After surface disinfection, the root tissue was placed on PDA plates to re-isolate *Fusarium* strains for infection confirmation (Figure 1.2).

Table 1.8 Fusarium root rot disease severity rating scale for Magenta box screening[#].

0	No visible lesions or discoloration
1	<20% lesions or discoloration
2	20-40% lesions and some discoloration
3	40-60% lesions and heavy discoloration
4	60-80% lesions and severe discoloration
5	>80% lesions and severe damage

[#] Adapted from a previous experiment for screening *Rhizoctonia* for bean seedling response in Dr. Linda Hanson's USDA-ARS Plant Pathology Laboratory.

Plants inoculated with *Fusarium* isolates F14-8, F14-11, F14-13, F14-21, F14-25, F14-29, F14-37, and F14-38 were oven-dried at 60℃ for 3 days after visual evaluation, and shoots and roots were weighed separately. The mean root and shoot dry weight of non-inoculated controls were calculated and used to calculate root and shoot loss for inoculated plants. Only the root rot disease severity scores of inoculated plants were recorded for other *Fusarium* isolates. The screening of F14-14, F14-25, F14-37, and F14-39 were repeated for a second run.

Statistical analysis

The mean of three plants in each replicate was calculated for response variables, and used for data analysis in SAS 9.4 (SAS Institute, Cary NC) with PROC MIXED procedure. The statistical model was established with genotype and treatment (isolates) as fixed effects while replicate and experiment repeat (two runs of experiment, when applicable) as random effects. The normal distribution of data was checked in normal quantile plot and residual plot. The effects and interactions of genotype and treatment were checked through LSM with the LSD

value at 0.05. The PROC CORR command was used to analyze Pearson correlations among variables.

Results

Average root and shoot dry weight and root and shoot dry weight reduction were measured for Zorro and Chinook inoculated with isolates F14-8, F14-11, F14-13, F14-21, F14-25, F14-29, F14-37, and F14-38. The average root dry weight was not significantly correlated with disease severity scores, while average shoot dry weight was positively correlated with scores with slight significance (P-value=0.015). Root reduction showed slightly significant positive correlation with score, while shoot reduction showed significant negative correlation with score (Table 1.9).

Table 1.9 Pearson correlation coefficients (r) for comparing root rot disease severity scores (Score) with average root dry weight (Root_DW), shoot dry weight (Shoot_DW), root dry weight reduction (Root_Loss), and shoot dry weight reduction (Shoot_Loss) of Zorro and Chinook inoculated with *Fusarium* isolates F14-8, F14-11, F14-13, F14-21, F14-25, F14-29, F14-37, and F14-38.

	Score	Root_DW	Shoot_DW	Root_loss	Shoot_loss
Score	-	-0.09[#]	0.35**	0.29**	-0.48***
Ave_Root		-	0.24	-0.54***	0.18
Ave_Shoot			-	0.57***	-0.75***
Root_loss				-	-0.59***

[#]– indicates negative correlation and + indicates positive correlation. *P-value=0.1, **P-value=0.05, *** P-value=0.01

All but two tested *Fusarium* isolates caused root rot symptoms. Virulence ranged from mild to severe on both bean genotypes (Zorro and Chinook) as shown in root rot score results (Figure 1.3). The root rot scores for Zorro varied from 0.0 to 2.4, with isolates F14-14 (*F. oxysporum*) and F14-33 (*F. equiseti*) did not cause any disease symptom. Root rot scores varied from 0.2 to

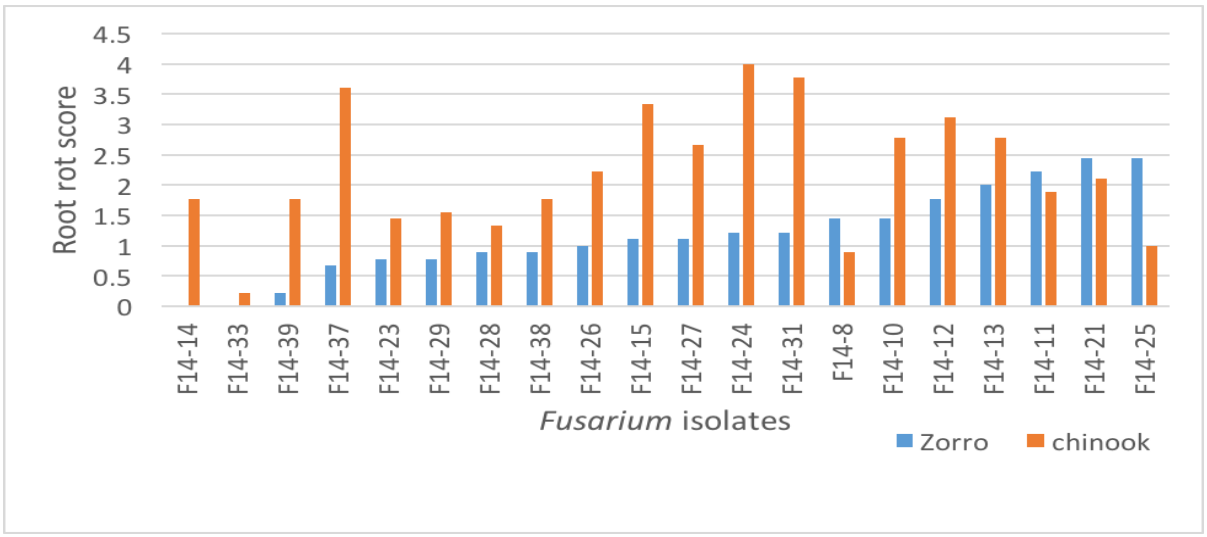
4.0 for Chinook with isolate F14-33 as the lowest score and F14-24 (*F. cuneirostrum, Fsp*1) as the highest. In total, 11 out of the 20 tested isolates caused significantly different root rot symptoms between the two bean genotypes (Table 1.10). The Andean variety Chinook had more severe symptoms than the Middle American variety Zorro for all but one isolate (F14-25).

Table 1.10 Comparison of average disease severity score (with standard deviation in parenthesis) between two bean genotypes (Zorro, Chinook) inoculated with 20 different *Fusarium* isolates. Plants were evaluated for FRR symptoms using a 0-to-5 scale, where zero indicates no disease and five indicates severe disease damage.

Isolates	**Score (Sd.)**	
	Zorro	**Chinook**
F14-8	1.4 (0.19)	0.9 (0.51)
F14-10	**1.4 (1.17)**	**2.8 (0.38)**
F14-11	2.2 (0.51)	1.9 (0.69)
F14-12	**1.8 (0.19)**	**3.1 (0.38)**
F14-13	2.0 (0)	2.8 (0.51)
F14-14	**0.0 (0)**	**1.8 (0.38)**
F14-15	**1.1 (0.19)**	**3.3 (0.58)**
F14-21	2.4 (0.84)	2.1 (0.19)
F14-23	0.8 (0.38)	1.4 (0.19)
F14-24	**1.2 (0.19)**	**4.0 (0)**
F14-25	**2.4 (0.19)**	**1.0 (0.33)**
F14-26	**1.0 (0.33)**	**2.2 (0.69)**
F14-27	1.1 (0.19)	2.7 (0.33)
F14-28	0.9 (0.77)	1.3 (0.33)
F14-29	0.8 (0.38)	1.6 (1.26)
F14-31	**1.2 (0.19)**	**3.8 (0.19)**
F14-33	0.0 (0)	0.2 (0.38)
F14-37	**0.7 (0.33)**	**3.6 (0.54)**
F14-38	**0.9 (0.51)**	**1.8 (0.69)**
F14-39	**0.2 (0.19)**	**1.8 (0.19)**
Mean	1.2 (0.72)	2.2 (1.03)
CV%	67.7	49.8
LSD (0.05)	0.57	0.66

[#]Bold numbers indicate significant differences between two bean genotypes (LSD, α=0.05).

Figure 1.3 Average root rot disease severity scores of two bean genotypes (Zorro, Chinook) under disease stress of 20 different *Fusarium* isolates, sorted as root rot scores from low to high (left to right) for Zorro.



The isolates F14-14, F14-25, F14-37 and F14-39 were selected for a second screening because statistically significant difference was observed in root rot scores between the two bean genotypes. According to the analysis of variance, the experiment repeat or the interaction with run did not have significant effect on root rot score results (Table 1.11). Run was treated as a random effect in the statistical model for following analysis.

Table 1.11 ANOVA of the average root rot disease severity scores of Zorro and Chinook inoculated with *Fusarium* isolates F14-14, F14-25, F14-37 and F14-39 in two runs of experiment.

Source	D*f*	P-value
Geno*	1	<.0001
Trt	3	<.0001
Run	1	0.3738
Geno*Trt	3	<.0001
Geno*Exp Repeat	1	0.5767
Geno*Trt*Exp Repeat	6	0.3796

*Geno: Bean genotypes (Zorro and Chinook); Trt: Different *Fusarium* isolates; Exp Repeat: Two runs of experiment; Geno*Trt: Interaction between Geno and Trt; Geno*Exp Repeat: Interaction between Geno and Exp Repeat; Geno*Trt*Exp Repeat: Interactions among Geno, Trt, and Exp Repeat.

The root rot scores of the two bean genotypes inoculated with the four isolates in the second screening ranged from 0.2 to 3.3. The different disease symptoms between the two bean genotypes were observed in the second screening as in the first screening with variations among the isolates.

Table 1.12 Average root rot scores of two bean genotypes (Zorro, Chinook) inoculated with *Fusarium* isolates F14-14, F14-25, F14-37 and F14-39 in two runs of the experiment.

	Zorro	Chinook
F14-14	**0.2 a**[#]	**1.9 b**
F14-25	**2.4 c**	**1.2 a**
F14-37	**0.8 b**	**3.3 c**
F14-39	**0.3 ab**	**1.8 b**

[#]the same letters within a column indicate the average root loss were not significantly different from each other for that bean genotype (LSD, α=0.05) and bold numbers indicate significant differences between two bean genotypes (LSD, α=0.05).

EXPERIMENT 3 Greenhouse Screening with Varied Inoculum Amount

Materials and methods

Plant materials and *Fusarium* isolates

Plant materials used in this experiment were the same common bean cultivars as described in experiment one, CAL96 and MLB49-89A.

Dr. Martin Chilvers' Plant Pathology Laboratory (MSU) provided five *Fusarium* isolates. They collected these *Fusarium* species from infected dry bean or soybean roots in different research fields in Michigan and characterized them morphologically (Table 1.13).

Table 1.13 Fusarium isolates used for greenhouse screening with varied inoculum levels, and their identifications, original farms and host plants.

Code	Species	Origin	Host Plant
F_14-7	*F. solani*	PLP Farm*	Dry bean
F_14-26	*F. oxysporum*	Montcalm Research Farm[#]	Soybean
F_14-38	*F. oxysporum*	Montcalm Research Farm	Dry bean
F_14-40	*F. cuneirostrum*	Montcalm Research Farm	Dry bean
F_14-42	*F. brasilense*	Montcalm Research Farm	Dry bean

*PLP Farm: Plant Pathology Farm of MSU in East Lansing, Michigan.
[#]Montcalm Research Farm: MSU research farm in Montcalm, Michigan.

Inoculum preparation

Dr. Martin Chilvers' Plant Pathology Laboratory (MSU) prepared sorghum inoculum for each of the *Fusarium* isolates using the same method as described in experiment one. Autoclaved sorghum was prepared to use in non-inoculated experiment controls. Sorghum inoculum and autoclaved sorghum were ground into powder (1mm particle) before use.

Screening

The containers used to plant bean seeds were 354 ml coffee cups with three holes on the bottom for drainage. Different levels of inoculum amount for each *Fusarium* isolate were tested, according to a preliminary experiment conducted by Chilvers' laboratory. Different levels of autoclaved sorghum were also used to observe the effect of sorghum in the screening. Ground sorghum inoculum or autoclaved sorghum with desired amount (Table 1.14) was mixed thoroughly with 200 ml vermiculite (medium) in each cup with another 70 ml of vermiculite added to the top. Bean seeds were placed on the top and covered with another 70 ml vermiculite. Experiment controls were set up in the same method with autoclaved sorghum in four levels (Table 1.14). Five replicates and five seeds per replicate were established for each *Fusarium* isolate and control. The experiment was set up as a completely randomized design (CRD) with two experimental factors (genotype and treatment). Greenhouse temperature was set at 26℃ during the day and 22℃ at night. Plants were watered once daily in the morning.

Table 1.14 Amount of sorghum inoculum used for each *Fusarium* isolate and autoclaved sorghum used for controls in the greenhouse screening with varied inoculum levels.

Isolate	**Species**	**Levels (g/cup)**
F_14-7	*F. solani*	1, 2 and 3
F_14-26	*F. oxysporum*	1.5, 3 and 5
F_14-38	*F. oxysporum*	1.5, 3 and 5
F_14-40	*F. cuneirostrum*	0.5, 1, and 2
F_14-42	*F. brasilense*	0.5, 1, and 2
Control	Autoclaved Sorghum	0, 2, 3 and 5

Fourteen days after experiment set-up, whole root system were pulled out and cleaned by

washing the hypocotyls and roots in tap water. The number of plants germinated and number of plants with disease symptom in each replicate were recorded. The roots were evaluated for FRR disease severity using a five scores scale (Table 1.15), where 1 indicates no disease and 9 indicates dead plant, and disease severity scores (DS) were given to each plant. The disease severity scores of controls were also converted to disease index (DX) using the formula DX = (DI×DS)/9, in which DI = Disease Incidence, the percentage of plants with disease symptom. DX was not calculated for inoculated plants with *Fusarium* isolates because the DI was 100% for all replicates. After visual evaluation, plants were oven-dried at 60℃ for 3 d and shoots and roots were weighed separately. The percentage reduction in root and shoot dry weight of inoculated plants compared to controls were calculated.

Table 1.15 Fusarium root rot rating scale used in greenhouse screening with varied inoculum levels.

Score	Symptom description*
1	Healthy - no lesion on the roots or hypocotyls
3	Discrete, light or dark-brown, superficial necrotic lesion
5	Necrosis and decay of the adventitious roots or taproot but with good root biomass
7	Extensive root rot with obvious root loss
9	Plant dead

*Adapted from the Rhizoctonia root rot severity rating scale in Dr. Martin Chilvers' Plant Pathology Laboratory, MSU.

The experiment was conducted for a second run with *Fusarium* isolate F_14-38 and F_14-40 at the same three levels, and with F14-42 at levels of 0.25g, 0.5g and 1g. For F_14-38 and F_14-40, CAL96, MLB49-89A and two progenies of RIL population of CAL96 x MLB49-89A were used as plant materials. For F_14-42, CAL96, MLB49-89A and six progenies of RIL population of CAL96 x MLB49-89A were used as plant materials.

Statistical analysis

The experimental data of control and each *Fusarium* isolate treatment were analyzed separately in SAS 9.4 (SAS Institute, Cary NC). The statistical model was established with genotypes and levels of treatment as fixed effects, and replications as random effect. The normal distribution of data was checked in normal quantile plot and residual plot. The effects of genotypes, levels of treatment, and interactions were determined by the analysis of variance in PROC MIXED procedure. The differences of LSM was checked for comparison of differences among mean responses with the LSD at α=0.05. The PROC CORR command was used to determine Pearson correlation coefficients among variables.

Results

Bean genotype had significant effect on all four measurements for the controls with four levels of autoclaved sorghum. The amount levels of autoclaved sorghum had significant effect on disease index and disease score, but only slight effect on root dry weight and no effect on shoot dry weight (Table 1.16).

The non-inoculated controls planted in vermiculite mixed with varying amount of autoclaved sorghum showed variance in DX and DS, while the plants showed no difference in average root dry weight or shoot dry weight for both bean genotypes (Table 1.17). CAL96 was found to have slight discoloration on roots with all three levels of autoclaved sorghum added in plant growing media. However, the DX and DS were not significantly different between 0g and 2g levels for CAL96. MLB-49-89A had no disease symptoms when grown with 0g autoclaved sorghum, but plants showed some disease symptoms from slight discoloration to moderate necrosis on roots when sorghum was added and the DS varied among different sorghum levels.

The DS and DX were higher in MLB49-89A than CAL96 at all sorghum amount levels except the 0g level (Table 1.17).

Table 1.16 ANOVA of average root rot disease index (DX), disease severity score (DS), root dry weight (Root_DW), and shoot dry weight (Shoot_DW) of experimental controls of two bean genotypes (CAL96 and MLB-49-89A) planted with four levels of autoclaved sorghum.

		P-value			
Source	Df	DX	DS	Root_DW	Shoot_DW
Geno[&]	1	<.0001	<.0001	0.0005	<.0001
Level	3	<.0001	<.0001	0.0169	0.1272
Geno x Level	3	0.0002	<.0001	0.2496	0.0236

[&]Geno: Bean genotypes (CAL96 and MLB49-89A); Level: four different amount levels of autoclaved sorghum. Geno x Level: Interaction between Geno and Level.

Table 1.17 Average root rot disease index (DX), disease severity score (DS), root dry weight (Root_DW), and shoot dry weight (Shoot_DW) of two bean genotypes (CAL96 and MLB49-89A) planted with four levels of autoclaved sorghum as experiment control.

	DX		DS		Root_DW (g)		Shoot_DW (g)	
	CAL	MLB	CAL	MLB	CAL	MLB	CAL	MLB
0g	0.1 a[&]	0.0 a	1.6 a	1.0 a	0.10 a	0.08 a	0.39 a	0.19 a
2g	0.1 a	0.3 b	2.0 ab	3.3 b	0.07 a	0.06 a	0.46 a	0.20 a
3g	0.3 b	0.5 c	3.2 c	4.5 c	0.09 a	0.04 a	0.38 a	0.29 a
5g	0.2 ab	0.6 c	2.4 b	5.2 c	0.08 a	0.05 a	0.36 a	0.21 a

[&]Values that followed with same letter within columns are not significantly different from each other (LSD, α=0.01)

No significant difference was found in DS, percentage of root reduction or shoot reduction at all three levels for CAL96 inoculated with three different levels of isolate F_14-7 (*F. solani*) (Table 1.18, F_14-7). Variations were found in root and shoot reduction for MLB-49-89A inoculated with F_14-7, and shoot reduction was negative which indicated the increase of shoot biomass of inoculated plants. No significant difference was found between CAL96 and

MLB49-89A inoculated with F_14-7 for all measurements except shoot reduction at 3g level (Table 1.18, F_14-7). No significant difference was found between CAL96 and MLB-49-89A inoculated with isolate F_14-26 (*F. oxysporum*) for all measurements at all three levels of inoculum, except DS at 1.5g level, which was lower than the other two levels. Variation was found in DS for both genotypes inoculated with F_14-26(Table 1.18, F_14-26).

Difference in root and shoot reductions at the 1.5g level between the two genotypes inoculated with isolate F_14-38 (*F. oxysporum*) was found, such that MLB49-89A had reduced root biomass while CAL96 had slightly increased root biomass. CAL96 had reduced shoot biomass while MLB49-89A had increased shoot biomass (Table 1.18, F_14-38). The data for MLB49-89A at the 3g level was missing due to mistake. Differences between two genotypes inoculated with F_14-40 (*F. cuneirostrum*) were found in DS at 2g level, in root reduction at 0.5g and 2g levels, and in shoot reduction at 1g level. Variations were also found among inoculum levels for both genotypes in DS. CAL96 had lower DS and less root reduction than MLB-49-89A, but MLB-49-89A had shoot biomass increased or slightly decreased while CAL96 had shoot biomass decreased (Table 1.18, F_14-40). Differences of DS and shoot reduction were found between two genotypes inoculated with F_14-42 (*F. brasilense*) at 0.5g. MLB-49-89A had less root and shoot reduction than CAL96 at all three inoculum levels of F_14-42(Table 1.18, F_14-42).

Table 1.18. Average disease severity score (DS), percentage root reduction (Root_loss) and shoot reduction (Shoot_loss) of two bean genotypes (CAL96 and MLB49-89A) inoculated with three different levels of *Fusarium* isolates F_14-7, F_14-26, F_14-38, F_14-40, and F_14-42.

F_14-7	DS		Root_loss (%)		Shoot_loss (%)	
	CAL	MLB	CAL	MLB	CAL	MLB
1g	3.4	2.7	29.1	23.7 a	10.2	-6.8 b
2g	3.3	2.6	49.7	55.3 b	28.7	-2.0 b
3g	3.8	3.4	36.6	33.7 a	**26.8**	**-36.0 a**

F_14-26	DS		Root_loss (%)		Shoot_loss (%)	
	CAL	MLB	CAL	MLB	CAL	MLB
1.5g	2.9 a	3.2 a	39.2	39.2	13.5	-10.6
3g	4.4 b	4.2 b	34.1	45.2	-21.8	-15.7
5g	4.3 b	4.1 b	29.9	53.4	-3.6	-14.8

F_14-38	DS		Root_loss (%)		Shoot_loss (%)	
	CAL	MLB	CAL	MLB	CAL	MLB
1.5g	3.2 a	3.2 a	**-6.3 a**	**36.4**	**15.7**	**-41.0**
3g	3.8 ab	.	30.8 b	.	19.0	.
5g	4.5 b	4.7 b	38.9 b	45.8	5.7	-19.1

F_14-40	DS		Root_loss (%)		Shoot_loss (%)	
	CAL	MLB	CAL	MLB	CAL	MLB
0.5g	5.1 b	5.0 ab	**3.0**	**34.6**	7.1	-18.5
1g	4.6 b	4.7 a	11.9	22.3	**6.8**	**-18.7**
2g	**3.8 a**	**5.6 b**	**25.2**	**52.3**	19.7	4.8

F_14-42	DS		Root_loss (%)		Shoot_loss (%)	
	CAL	MLB	CAL	MLB	CAL	MLB
0.5g	**4.6 a**	**5.7**	32.7	29.6	**35.0**	**5.1**
1g	5.7 b	5.6	30.7	13.6	19.5	9.9
2g	6.0 b	6.4	13.8	24.4	29.3	12.0

a. Values that followed with different letters within columns are significantly different from each other (LSD, α=0.05)

b. Bold numbers indicate significant differences between two bean genotypes (LSD, α=0.05).

Table 1.19 Average disease severity score (Ave_DS), root reduction (Root_loss), and shoot reduction (Shoot_loss) for two parents (MLB49, CAL96), and two progenies (15, 267) inoculated with *Fusarium* isolate F_14-38 and F_14-40 at three different levels.

F_14-38	Ave_DS			Root_loss (%)			Shoot_loss (%)		
	1.5g	3g	5g	1.5g	3g	5g	1.5g	3g	5g
MLB49	2.0	3.5	4.9	14.9	17.3	32.9	9.9	-14.6	0.0
CAL96	2.9	4.2	5.1	0.4	35.5	57.5	-6.8	16.1	-1.4
CxM_15	2.9	4.2	4.7	21.8	42.3	57.5	-0.3	1.2	-2.8
CxM_267	2.4	3.4	4.3	0.6	31.1	48.8	-6.1	-7.6	-10.3
Mean	2.6	3.8	4.8	9.4	31.5	49.2	-0.8	-1.2	-3.6
CV%	32.7	24.1	18.5	21.0	48.0	31.7	-17.4	12.3	-5.1
LSD(0.05)	1.07	1.21	1.19	0.25	0.17	0.17	0.18	0.13	0.26

F14-40	Ave_DS			Root_loss (%)			Shoot_loss (%)		
	0.5g	1g	2g	0.5g	1g	2g	0.5g	1g	2g
MLB49	2.3	3.6	3.4	7.7	0.5	12.8	-7.8	2.7	11.3
CAL96	3.0	3.5	3.9	-3.2	-5.8	8.2	-1.1	-6.9	5.8
CxM_15	1.5	2.9	3.2	5.1	23.8	8.4	-7.6	4.6	6.4
CxM_267	2.7	3.1	3.7	18.2	13.4	15.7	8.9	1.2	14.2
Mean	2.4	3.3	3.5	7.0	7.9	11.3	-1.6	0.4	9.4
CV%	32.7	17.8	18.1	2.3	2.7	1.2	-9.2	47.7	1.2
LSD(0.05)	0.82	0.74	0.84	0.21	0.26	0.19	0.20	0.26	0.15

In the second run of testing isolate F_14-38 and F_14-40 on CAL96, MLB-49-89A, and two progenies of their RIL population, results of average DS, root reduction, and shoot reduction of four genotypes were shown in Table 1.19. The DS ranged from 2.0 to 5.1, which indicated that the plants were resistant or moderately resistant to this isolate. The root reduction ranged from 0.4% to 57.5% and shoot reduction ranged from -10.3% to 16.1%. For both average DS and root reduction, the values increased as the amount of inoculum increased (Table 1.19). With inoculation of F_14-40, the DS ranged from 1.5 to 3.9, which indicated the plants inoculated with this isolate showed no root rot symptom or slight discoloration. The root reduction ranged from -5.8% to 23.8% and shoot reduction ranged from -7.8% to 14.2%. Generally, the root and shoot loss of the inoculated plants were reduced as the amount of the inoculum increased.

In the second run of testing isolate F_14-42 on CAL96 x MLB49-89A and six progenies of their RIL population, five measurements related to root rot disease resistance: DS, root reduction, shoot reduction, root dry weight, and shoot dry weight were analyzed for variance within eight bean genotypes and three different levels of inoculum (Table 1.20). Variations were found among genotypes in all five measurements with P-value less than 0.0001, but level had no significant effect in all measurements except in DS (Table 1.20).

Table 1.20 ANOVA of average disease severity score (DS), root reduction (Root_loss), shoot reduction (Shoot_loss), root dry weight (Root_DW), and shoot dry weight (Shoot_DW) of CAL96 and MLB49-89A, and six progenies in RIL population of CAL96 x MLB49-89A inoculated with *Fusarium* isolates F14-42 at three different levels.

		P-value				
Source	D*f*	DS	Root_loss	Shoot_loss	Root_DW	Shoot_DW
Geno	7	<.0001	<.0001	<.0001	<.0001	<.0001
Level	2	<.0001	0.2828	0.1951	0.6316	0.4195
Geno x Level	14	0.0227	0.3298	0.3575	0.0553	0.0812

Geno: Bean genotypes; Level: three different amount levels of inoculum; Geno x Level: Interaction between Geno and Level.

The DS among eight genotypes ranged from 3.4 to 4.9 at 0.25g level, from 4.7 to 5.9 at 0.5g level, and from 5.6 to 7.2 at 1g level. MLB-49-89A had lowest DS at 0.5g level, and the lowest DS was in the progenies at the other two tested levels. MLB-49-89A was lower than CAL96 at 0.5g and 1g levels for root and shoot reduction, but higher at 0.25g level. The DS increased as the amount of inoculum increased for both parent lines, and MLB-49-89A always had less root and shoot reduction than CAL96 at all three inoculum levels (Table 1.21).

Table 1.21 Summary of average disease severity score (Ave_DS), root reduction (Root_loss), and shoot reduction (Shoot_loss) for two parents (MLB49, CAL96), and range and mean of six progenies inoculated with *Fusarium* isolate F14-42 at three different levels.

	Ave_DS			Root_loss (%)			Shoot_loss (%)		
	0.25g	0.5g	1g	0.25g	0.5g	1g	0.25g	0.5g	1g
MLB49	3.8	4.7	6.7	30.0	29.6	21.5	17.9	17.5	8.8
CAL96	4.5	6.5	6.6	28.3	35.5	46.7	12.7	23.1	26.8
Lowest	3.4	5.1	5.6	-12.1	-26.0	-45.5	-5.8	-15.0	-28.6
Highest	4.9	5.9	7.2	35.0	42.6	31.4	27.3	20.3	18.4
Mean	4.2	5.2	6.5	18.9	18.2	9.8	8.4	9.8	2.7
CV%	17.8	17.1	10.9	1.5	1.7	3.9	2.6	1.8	8.3
LSD (0.05)	0.81	0.58	0.68	0.35	0.36	0.39	0.26	0.21	0.23

DISCUSSION

All of the tested *Fusarium* species, whether having been reported to be associated with FRR of common bean or not, had caused root rot symptom on tested common bean plants in this study, except two isolates tested in Experiment II which did not cause root rot symptom on Zorro. Virulence varied among tested *Fusarium* species. In all three experiments in this study, significant differences were found on the response of plants inoculated with different *Fusarium* species. Variations of virulence were also found within different isolates of the species. The three *F. brasiliense* isolates tested in Experiment I showed variation in virulence from mild to severe on both MLB49-89A and CAL96 genotypes. In Experiment II, the 16 tested *F. oxysporum* isolates showed varied virulence from mild to severe on both Zorro and Chinook. In virulence screening of *Fusarium* species with other crops, variations were also found. *F. brasiliense, F. cuneirostrum, F. phaseoli,* and *F. virguliforme* were known to cause soybean sudden death syndrome (SDS) with intraspecific variation in pathogenicity (Aoki et al., 2005). Different

isolates of *F. oxysporum* and *F. solani* were found to be varying in virulence on pea according to Ondrej et al. (2008).

In the laboratory screening, *F. oxysporum* occupied 80% of all the tested isolates. *F. oxysporum* is a *Fusarium* species that is different from the species belonging to FSSC and is known to be the causal pathogen of Fusarium wilt of common bean (Kendrick & Snyder 1942). A possible reason that *F. oxysporum* accounted for a large number of the African isolates is that *F. oxysporum* reproduces faster which resulted in sampling bias when researchers isolated and collected the pathogen from infected plants.

The genetic variation among the bean genotypes result in the difference of resistance or susceptibility of the host-plants, which results in different reaction to the same *Fusarium* isolate. In Experiment I, with inoculation of isolate 31157 and MIMTC-B11, MLB-49-89A and CAL96 have showed statistical significant differences in root rot scores. In Experiment II, more than half of the tested isolates caused significantly different disease symptoms on Zorro as compared to Chinook in terms of root rot score. In Experiment III, genotype has also had effect on results of one or more of the measurements. Studies have shown that Middle American genotypes more commonly show FRR resistance and susceptibility to FRR is more common in large-seeded Andean genotypes (Beebe et al. 1981; Schneider et al., 2001; Román-Avilés & Kelly, 2005). In Experiment I, Mesoamerican genotype MLB49-89A had better resistance than Andean genotype CAL96 inoculated with isolate 31157 and MIMTC-B11. In Experiment II, the Mesoamerican variety Zorro had better resistance than Andean variety Chinook inoculated with all but one of the isolates that caused different disease symptoms on the two genotypes. MLB-48-89A was recommended as a good source of resistance to FRR among bean cultivars in Uganda (Mukankusi, 2008). However, in Experiment I, MLB49-89A showed resistance or moderate

resistance to four of the isolates and susceptibility to two other isolates. The results indicate that the resistant genotypes only have resistance to a limited range of species or isolates of the pathogen, and this information should be available when to defining the resistance of a bean cultivar to a certain disease. It was showed in this study that the results of resistance or susceptibility of two genotypes can be opposite when tested with different *Fusarium* species or isolates. In Experiment II, with inoculation of isolate F14-25, Zorro had more severe disease symptom than Chinook. In Experiment III, MLB-48-89A had more severe disease symptom than CAL96 with inoculation of isolate F_14-40.

Plants infected with *Fusarium* are usually stunted and grow more slowly than healthy plants (Román-Avilés et al., 2003). Thus, the plant biomass and biomass reduction, especially the roots are suitable measure of the resistance or susceptibility of different bean genotypes to root rot diseases. In both greenhouse screening (Experiment I and III), the results have reflected the reduction of root biomass of the plants inoculated with all the tested *Fusarium* isolates. However, the shoot dry weight of the inoculated plants was sometimes higher than the non-inoculated controls. The possible reason could be that the isolate only had mild virulence and only causes superficial symptom on the roots and was not able to cause severe biomass reduction; or the inoculated plants grew slower than the control plants as the cotyledon was still attached to the stem when measuring the dry weight, which made the shoot biomass higher.

Due to the difference in dry weight of the non-inoculated control plants of the two parent bean genotypes, the percentage of dry weight reduction compared to controls becomes a better measurement to show the virulence of the tested *Fusarium* isolates, and the reaction of the bean genotypes. Additionally, the root and shoot dry weight and their reduction can be complementary measurements to the root rot scores, since the evaluation of root rot symptom by root rot scores

is more subjective. In Experiment I, the root and shoot dry weight and root and shoot loss of the two bean genotypes inoculated with isolate Mont-1 were significantly different while the root rot scores did not show the difference. Cultivars that produce more adventitious roots and larger basal roots tend to be more tolerant to root rot (Snapp et al., 2003; Cichy et al., 2007), and the weak roots of the infected plants were unable to absorb and transport water and nutrient effectively (Román-Avilés et al., 2003). Thus, the root and shoot biomass is expected to be negatively related to root rot score and the root and shoot biomass reduction is expected to be positively related to root rot score. The results in Experiment I confirmed this hypothesis (Table 1.6). However, in experiment II, the correlation does not agree with this hypothesis. The possible reason could be the limited space in the Magenta boxes that constrained the growth of the plants, which resulted in that the plant biomass that did not correspond to the root rot scores, so the root and shoot dry weights were not measured for the other isolates in Experiment II.

From a cultural management perspective, the use of bean cultivars with resistance is one of the most important and effective methods to control FRR (Abawi, 1990). To discover resistant sources and to study on disease related genes or quantitative trait loci (QTL), screening bean genotypes or populations with suitable virulent *Fusarium* isolate is critical, and the selection of the isolate affects the effectivity and reliability of the phenotyping process. From the screening results, the *Fusarium* isolates that could distinguish between two parent genotypes for resistance to FRR can be used to detect the resistance in the recombinant inbred line (RIL) population derived from the crossing of two contrasting parents. According to results from experiment III, significant differences were found between CAL96 and MLB-49-89A inoculated with F_14-42 in DS and shoot reduction, and F_14-42 was the only one among the five tested isolates that caused consistent root and shoot reduction at all three inoculum levels for the two bean

genotypes. In the second run of testing isolate F_14-42 on CAL96 and MLB-49-89A and six progenies of their RIL population, we observed variability for resistance. The root and shoot reduction of CAL96 x MLB-49-89A agreed with the results from the first run that MLB-49-89A always had lower root and shoot reduction than CAL96 at all three inoculum levels. The variation of reaction of the eight genotypes to F_14-42 in root rot score, root reduction and shoot reduction showed that the *F. brasilense* isolate F_14-42 was suitable to be used in phenotyping the RIL population of CAL96 x MLB49-89A for FRR resistance.

Three different screening methods were used in the three experiments in this study and a specific root rot disease symptom evaluation scale was used for each experiment. In the two greenhouse experiments, both evaluation scales were 1-9 scale, but the difference was the scale in experiment I has nine points and the scale in experiment III has only five points. The first 1-9 scale was developed and published by CIAT (Corrales and van Schoonhoven, 1987), and the five points 1-9 scale was designed to make the evaluation easier to be able to quickly classify the root rot symptom into five categories and to distinguish the tested bean individuals for resistance or susceptibility. The 0-5 scale was developed specifically for the Magenta box test, which was adapted from a previous experiment for screening *Rhizoctonia* for bean seedling response in Dr. Linda Hanson's USDA-ARS Plant Pathology Laboratory. The Magenta box test was suitable for laboratory screening of isolates collected outside of USA, which were limited to be used in laboratory only due to regulation. The greenhouse screening method can be used for screening local isolates of *Fusarium* species. In this study, both the greenhouse and laboratory methods of screening *Fusarium* isolates had consistent results over two runs. The greenhouse strategy is more suitable for phenotyping the RIL population with more than one hundred lines and more measurements can be taken, such as root and shoot dry weight and even the structure of the root

system. The first symptoms of *Fusarium* infection on roots usually appear about 7 – 10 days after germination (Hall, 1991), the greenhouse screening is more suitable for keeping the plants for a longer period than the seven days used in Magenta box method.

Different levels of autoclaved sorghum were used in non-inoculated controls in Experiment III, to investigate the effects of sorghum to the bean plants. The results showed that autoclaved sorghum caused root rot disease symptom on the plants, and the more autoclaved sorghum was added, the more severe the root rot symptom appeared. The possible reason could be that the vermiculite used as plants growing media was not autoclaved and the greenhouse was not an isolated environment, which means there could be spores of fungi existing in the growing media. The autoclaved sorghum mixed in vermiculite provided a nutrition resource for the fungi to germinate and grow. The results showed that the less autoclaved sorghum added in the media, the less root rot disease symptoms detected, and no disease symptom was found when no autoclaved sorghum was added. The effect of the autoclaved sorghum on DX or DS was not significant when the amount of autoclaved sorghum was lower than 2g. Additionally, the biomass of plants in both root and shoot was not affected by adding autoclaved sorghum in the growing media. In future screening of bean plants for *Fusarium* root rot resistance, autoclaved sorghum should not be used in the non-inoculated controls.

For future work, to better understand whether the screening method effect the virulence test results, we can test the same isolate with all three different methods. Additionally, the effect of the combination of different isolates can be investigated. In a study of screening *Fusarium* isolates on pea for their virulence, researchers found that the combination of two virulent isolates do not result in increase in the virulence, and the possible reason could be antagonistic reaction

between the two isolates or lower production of the phytotoxins in the mixture (Ondrej et al. 2008).

CHAPTER 2: IDENTIFICATION OF QTL FOR FUSARIUM ROOT ROT RESISTANCE IN COMMON BEAN USING SNP MARKERS

INTRODUCTION

Common bean (*Phaseolus vulgaris*) is an important grain legume for human consumption because of its high protein content, vegetable fiber, and micronutrients (Siddiq and Uebersax, 2012). Common beans are very diverse in cultivation methods, usage, and the range of environments to which they have adapted. In Africa, common bean is an important food legume as well as a cash crop for small-scale farmers. However, the productivity has been severely constrained by bean diseases and farmers have experienced significant crop losses. The soil-borne disease, Fusarium root rot (FRR), is a major bean production constraint (Ongom et al., 2012). In the regions of dry bean production in the United States, FRR also caused severe yield losses (Chatterjee, 1958; Keenan et al., 1974; Saettler, 1982; Cichy, 2007).

The symptoms of FRR begin with red to brown streaks on taproots and hypocotyls, and lesions and necrosis form gradually. Disease severity increases and eventually complete rotting of root systems occurs as the plant develops (Hall, 1991). Susceptible bean genotypes tend to have weak root systems with limited branching and reduced dry weight when infected by *Fusarium* (Román-Avilés and Kelly, 2004). For breeding purposes, the presence of adventitious roots, as well as other root traits such as total root dry weight, root branching, and number of lateral roots can be used as selection criteria for development of cultivars resistant to root rot.

FRR is affected by environmental conditions that restrict root growth, such as drought, flooding, low soil fertility, and soil compaction (Hall, 1994). In the perspective of cultural management, the use of resistant cultivars, minimization of soil compaction, good drainage, high soil fertility and rotation with other resistant crops can contribute to effective disease control of

FRR (Abawi, 1990; Román-Avilés et al., 2003; Schwartz, 2011). Among root rot control strategies, development of cultivars with root rot resistance has been generally considered the best long-term management option (Tu 1992; Park and Rupert 2000; Abawi et al. 2006; Conner, 2014). Breeding for FRR is challenging because screening must occur in the presence of the pathogen under properly environmental conditions for a disease reaction, and the inheritance of FRR resistance is quantitative (Boomstra & Bliss, 1977). The assessment of root rot symptoms requires destructive sampling, which makes it labor- and time- intensive. Marker-assisted selection (MAS) for lines with root rot resistance would be beneficial as selections can be made in early stages of the breeding process. The identification of quantitative trait loci (QTL) associated with FRR resistance will help identify the genetic basis of resistance and facilitate the MAS process.

Previous studies on QTL related to FRR resistance have been conducted; the earlier studies used random amplified polymorphic DNA (RAPD) markers for linkage map development. Schneider et al. (2001) identified 16 QTL using two recombinant inbred line (RIL) populations of FR266 (resistant) x 'Montcalm'(susceptible) and FR266 x 'Isles'(susceptible). Two RAPD markers showed significant association with resistance and were detected on chromosome B2 (Pv02) of the genetic map near pathogenesis-related (PR) protein PvPR2 (Schneider et al., 2001). Chowdbury et al. (2002) used a RIL population derived from a cross between the susceptible navy bean cultivar 'AC Compass' and resistant line NY2114-12 for mapping with RAPD markers. Interval Mapping (IM) detected two QTL and the phenotypic variations explained by these QTL were 30% and 20% respectively (Chowdhury et al., 2002). Another QTL analysis with RAPD markers was conducted on two inbred backcross-derived populations from a cross of 'Red Hawk' x 'Negro San Luis' (NSL) and a cross of C97407 x NSL. A total of 12 QTL

associated with FRR resistance were detected and three of them were assigned to chromosome B2 and B5 (Pv02 and Pv05), and 7.3 to 53% of the phenotypic variations were explained (Román-Avilés and Kelly, 2005). In summary, previous researchers have discovered over 30 QTL related to FRR resistance using RAPD markers in different bean populations, but not all the QTL were assigned to specific chromosomes. To our knowledge, none of these QTL have been used for MAS. The reason for this could be the identified QTL related to FRR varied from one study to another, and the researchers were not able to compare the positions of those QTL from different studies.

Kamfwa et al. (2013) investigated QTL for FRR resistance with 62 $F_{4:5}$ RIL lines of MLB-49-89A (resistant) x K132 (susceptible) using 12 simple sequence repeat (SSR) markers with polymorphism. The medium-seeded black Middle American bean variety MLB49-89A with Type-III indeterminate growth habit was reported to have moderate resistance to root rot diseases (Mukankusi et al., 2010). The large-seeded, red mottled Andean bean variety K132 (also known as CAL96) with Type-I upright determinate bush growth habit, is commonly consumed in East Africa and is also highly susceptible to root rot. Variable FRR resistance was found in the RILs grown in Uganda, Africa and a significant QTL associated with FRR resistance was found on chromosome Pv03 (Kamfwa et al., 2013). Because the RIL population of MLB-49-89A x CAL96 RIL was rich in variability for root traits and FRR resistance, the population was expanded to 121 lines to evaluate root traits and FRR resistance in Michigan, USA, where FRR has also been a major soil-borne disease that affects bean production.

In recent years, single nucleotide polymorphism (SNP) markers have been replacing other molecular markers. The resulting genetic maps with SNP markers have higher density of marker coverage than maps based on previous marker types (Song et al. 2015). Hagerty et al. (2015)

identified QTL associated with FRR resistance and *Aphanomyces euteiches* root rot (ARR) resistance in a RIL population of RR6950 (resistant) x OSU5446 (susceptible). RR6950 is a small brown-seeded bean with Type III growth habit and OSU5446 is bush green bean breeding line with Type I growth habit. Two QTL for FRR resistance were found on Pv03 and Pv07, one QTL on Pv02 was found for taproot diameter (TD), and one QTL was found for shallow basal root angle (SBRA) on Pv05. The phenotypic variation explained by these QTL varied from 9% to 22%. The QTL for ARR resistance on chromosome Pv02 overlapped with the QTL for TD (Hagerty et al., 2015). Nakedde (2015)identified four QTL associated with root architecture traits on Pv01, Pv05, and Pv09, and one QTL associated with FRR resistance on Pv05 in the RIL population of Puebla 152 (resistant) x Zorro(susceptible). The two parents were both black bean but with different architecture traits. The QTL for total root weight and shallow root weight colocalized on Pv09, and the QTL for deep root dry weight colocalized with the QTL for total biomass on Pv05. The phenotypic variation explained by these QTL varied from 8.3% to 12.7%.

The objectives of this study were to (a) characterize FRR resistance to *Fusarium* species from Michigan in the RIL population derived from a cross of MLB49-89A x CAL96, and (b) detect QTL related to FRR resistance in the RIL population using SNP markers.

MATERIALS AND METHODS

Mapping Population

A cross of MLB-49-89A and CAL96 was made at Center for International Tropical Agriculture (CIAT) Kampala, Uganda. The RIL population of MLB49-89A x CAL96 (F_3 generation seeds) was sent to USDA-ARS common bean breeding and genetics laboratory at Michigan State University (MSU) and were advanced to F_4 generation through single seed descent. A single plant of each F_4 line was harvested and seeds from each plant were bulked to get $F_{4:5}$ RILs. The individuals that had germination problems, seedling death, or maturity problems were discarded. $F_{4:5}$ RILs was planted in field to increase seeds. A total of 121 lines were maintained in $F_{4:6}$ RILs and the phenotypic evaluation were conducted on this population. DNA of the RIL population was extracted from the F_4 generation for SNP sequencing.

Field Data Collection

In 2014, the RIL population (F_4 generation) was planted at Michigan State University Saginaw Valley Research Farm (Frankenmuth, MI) with one plot for each line. The agronomic data including days to flower (DF), days to maturity (DM), lodging (LG) and growth habit (GH) of the population were recorded through the growing season in the field. DF was recorded as the number of days from planting to when approximately 50% plants in a plot have at least one opened flower. DM was recorded as number of days from planting to when approximately 50% of plants in a plot have at least one dry pod. LG was recorded at harvest with scores of 1 to 5, where 1=100% plants standing erect and 5=100% plants flat on the ground. GH was recorded during flowering and verified when crop is senescing as type I=determinate erect or upright, II=indeterminate erect, and III=indeterminate prostrate. One plant from each plot was uprooted

and evaluated for root rot symptom with the CIAT 1-9 scale (Corrales and van Schoonhoven, 1987) at harvest, where one indicates no disease and nine indicates the root is completely rotted.

In 2015, the RIL population ($F_{4:5}$) was planted at Michigan State University Montcalm Research Farm (Entrican, MI), where was previous identified as infected by FRR. Forty-five days after planting, 3 plants of each line were uprooted with a shovel to avoid any damage to the root system. The roots were washed with tap water to clean off soil and evaluated for root rot symptom with the CIAT 1-9 scale. Some physical traits of the population were also measured in laboratory, including root length, root diameter, root dry weight, and shoot dry weight. Root length was measured as the length from tap root tip to middle point of hypocotyl. Root diameter was measured at the middle point of hypocotyl with a digital caliper. Root and shoot dry weights were measured after drying plants in oven at 60℃ for 3 d. Some agronomic traits were recorded through the growing season in the field, including days to flower (DF), days to maturity (DM) and lodging (LG).

Greenhouse Experiment

Inoculum

The *Fusarium brasilense* isolate F_14-42 (as described in Chapter One) was selected to use as inoculum for phenotyping the RIL population for root rot resistance. The isolate, stored on slants of Potato Dextrose Agar (PDA), was provided by Dr. Martin Chilvers' Plant Pathology Laboratory at MSU and was sub-cultured to Nash-Snyder (NS) plates. The sub-culture was kept at room temperature for 7 to 10 d and then used to make sorghum inoculum with the method described in Experiment One of Chapter One.

Screening

The 354 ml coffee cups with three holes on the bottom for drainage were used as containers for plants to grow. One gram of ground sorghum inoculum was mixed thoroughly with 200 ml vermiculite (medium) per cup with 70 ml of vermiculite added to the top. Bean seeds were then placed on the top and covered with additional 70 ml vermiculite. Pure medium vermiculite without inoculum was used as an experimental control. Five replicates and five seeds per replicate were established for each individual RIL for both inoculated screening and control. Greenhouse temperature was set at 22℃ during the day and 22℃ at night. Plants were watered once daily in the morning. The entire experiment was repeated for a second run with the same conditions.

Fourteen days after experimental set-up, whole root system were pulled out and cleaned by washing the hypocotyls and roots in tap water. The number of plants germinated and number of plants with disease symptom in each replicate were recorded. The roots were evaluated for FRR disease severity using the 1-9 scale as used in experiment three in Chapter One (Table 2.1), where 1 indicates no disease and 9 indicates plant died and only five points were used in the evaluation. After visual evaluation, plants were oven-dried at 60℃ for 24 h and shoots and roots were weighed separately. The percentage reduction in root and shoot dry weight of inoculated plants compared to controls were calculated.

Table 2.1 Root rot disease severity rating scale for screening RIL population of MLB49-89A x CAL96 for FRR resistance in greenhouse.

Score	Symptom description
1	Healthy - no lesion on the roots or hypocotyls
3	Discrete, light or dark-brown, superficial necrotic lesion
5	Necrosis and decay of the adventitious roots or taproot but with good root biomass
7	Extensive root rot with obvious root loose
9	Plant dead due to disease

***** Adapted from the Rhizoctonia root rot disease severity rating scale in Dr. Martin Chilvers' Plant Pathology Laboratory.

Statistical Analysis of Phenotypic Data

The mean value of response variables of five plants in each replicate of each RIL line was calculated and analyzed in SAS 9.4 (SAS Institute, Cary NC). The statistical model was established with genotype as fixed effect and replicate, date of planting and run as random effects. The normal distribution of data was checked in normal-quantile plot and residual plot, and the effects of genotype on response variables were determined with PROC MIXED procedure. The PROC CORR command was used to analyze Pearson correlations among variables.

DNA Extraction and SNP Genotyping

Fresh first trifoliate young leaves were collected from the F_4 RIL population in the field for DNA extraction. DNA was extracted by following the Bregitzer Laboratory Miniprep DNA isolation method and was genotyped through the Illumina 6000-SNP BARCbean6K_3 SNP chip (USDA-ARS Soybean Genomic and Improvement Laboratory, Beltsville, MD). The SNP alleles were called using the GenomeStudio Genotyping Module v1.8.4 (Illumina, Inc.) and the SNPs with polymorphism were selected.

Genetic Map Construction and QTL Analysis

A total of 1150 polymorphic SNPs between CAL96 and MLB49-89A were used to construct the linkage map in MapDisto (Lorieux, 2012) using Kosambi's mapping function with logarithm of odds (LOD) score of 4.0. The linkage map with 11 chromosomes was then compared with physical positions of the markers to check the order on each chromosome.

Windows QTL Cartographer 2.5 (Wang et al., 2007) was used to detect QTL related to each phenotypic trait. The composite interval mapping (CIM) procedure was performed with the parameters set to be 10 cM window size, 1 cM walkspeed, 5 significant background markers, forward and backward multiple linear regression method, and LOD threshold of 2.5. After finding QTL, 1000 permutation was performed to determine the LOD threshold and QTL with LOD value lower than the LOD threshold generated from permutation were discarded. Linkage map and detected QTL were displayed using MapChart v.2.3 (Voorrips, R.E., 2002)

Broad-sense heritability (h_B^2) for each trait was calculated according to heritability equation in terms of variance component:

$$h_B^2 = \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_\varepsilon^2 / r)},$$

where σ_G^2 is the genetic variance, σ_ε^2 is variance due to the environment, and r is the number of replications. The calculation was conducted with the following formula (Wang, 2014) using mean square values from ANOVA table:

$$h_B^2 = \frac{M1 - M2}{M1},$$

where M1 is the mean square value among genotypes and M2 is the mean square value of error (within each genotype).

RESULTS

Phenotypic Variation in Field Data

The RIL population showed continuous normal distribution in all measurements of root rot scores, DF, DM, GH and LG based on the phenotypic data in 2014 (Figure 2.1). Root rot scores ranged from 1 to 9, which covered all points in the evaluation scale. DF ranged from 33 to 57 d, and most of the population flowered in 40 to 50 d. DM ranged from 88 to 104 d, and most of the population reached maturity in 90 to 100 d. LG of the population ranged from 1 to 5, and most of the population had a lodging score of 2 or 3. GH of the population included all three growth habit types with most of the population had Type II (indeterminate erect) growth habit while the two parents had Type I and III growth habit.

DF was correlated with GH ($r = 0.26$, $P<0.02$) and LG ($r = 0.20$, $P<0.01$) in the 2014 field data (Table 2.2). DM was correlated with LG ($r = 0.28$, $P<0.05$). GH was significantly correlated with LG ($r = 0.57$, $P<0.001$). However, disease severity score did not show correlation with any of those measurements (Table 2.2).

Figure 2.1 Frequency and distribution of disease severity score, days to flower, days to maturity, growth habit and lodging of the RIL population (F4 generation) of MLB49-89A x CAL96 according to the field data in 2014.

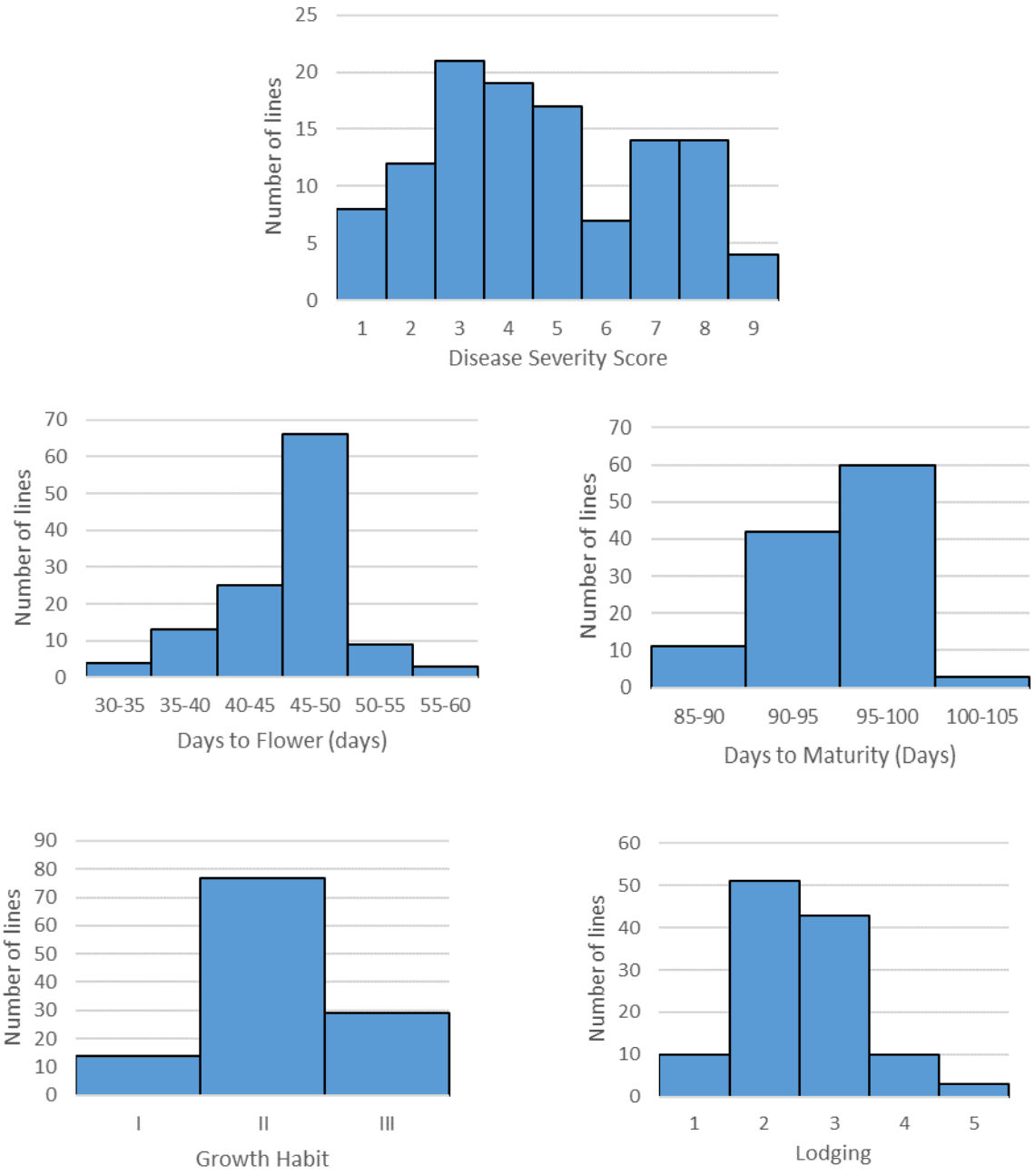


Table 2.2 Pearson correlation coefficients of disease severity score (Score), days to flower (DF), days to maturity (DM), growth habit (GH) and lodging of RIL population of MLB49-89A x CAL96 in the field in 2014.

	Score	DF	DM	GH	Lodging
Score	-	0.067	-0.145	-0.022	-0.001
DF		-	0.136	0.261**	0.204*
DM			-	0.130	0.280*
GH				-	0.566***

*significant at 0.05 level; **significant at 0.01 level; ***significant at 0.001 level.

The field phenotypic data of the RIL population ($F_{4:5}$ generation) in 2015 presented continuous normal distribution in root length, root diameter, root dry weight, shoot dry weight, DF, DM, and lodging (Figure 2.2). The distribution of root rot scores was left skewed, that most of the population had root rot score of 7 to 9 and the lowest score in the population was 5, and the two parents were not significantly different in root rot symptom (Figure 2.2). Root length of the population ranged from 4.9 to 23.1 cm and root diameter ranged from 1.9 to 7.5 mm. Root dry weight ranged from 0.06 to 1.9 g and shoot dry weight ranged from 0.8 to 14.5. Root and shoot dry weight of the two parents were not significantly different and the shoot dry weight of MLB-49-89A was slightly higher than CAL96. DF ranged from 40 to 60 d with most of the population flowered in 45 to 55 d, which is about five days longer than that in 2014. DM ranged from 88 to 124 d and most of the population reached maturity in 105 to 115 d, which is about 15 days longer than that in 2014. LG of the population ranged from 1 to 5, and LG score of 3 was the most common score in the population. The seed weight ranged from 24 to 58 g/100 seeds in the RIL population, and CAL96 had highest seed weight of 69.1 g/100 seeds (Figure 2.2).

Figure 2.2 Frequency and distribution of disease severity score, root length, root diameter, root dry weight, shoot dry weight, days to flower, days to maturity, lodging and seed weight of the RIL population ($F_{4:5}$ generation) of MLB-49-89A x CAL96 according to the field data in 2015. Black and white arrows indicated the phenotypic values of parents MLB-49-89A and CAL96, respectively.

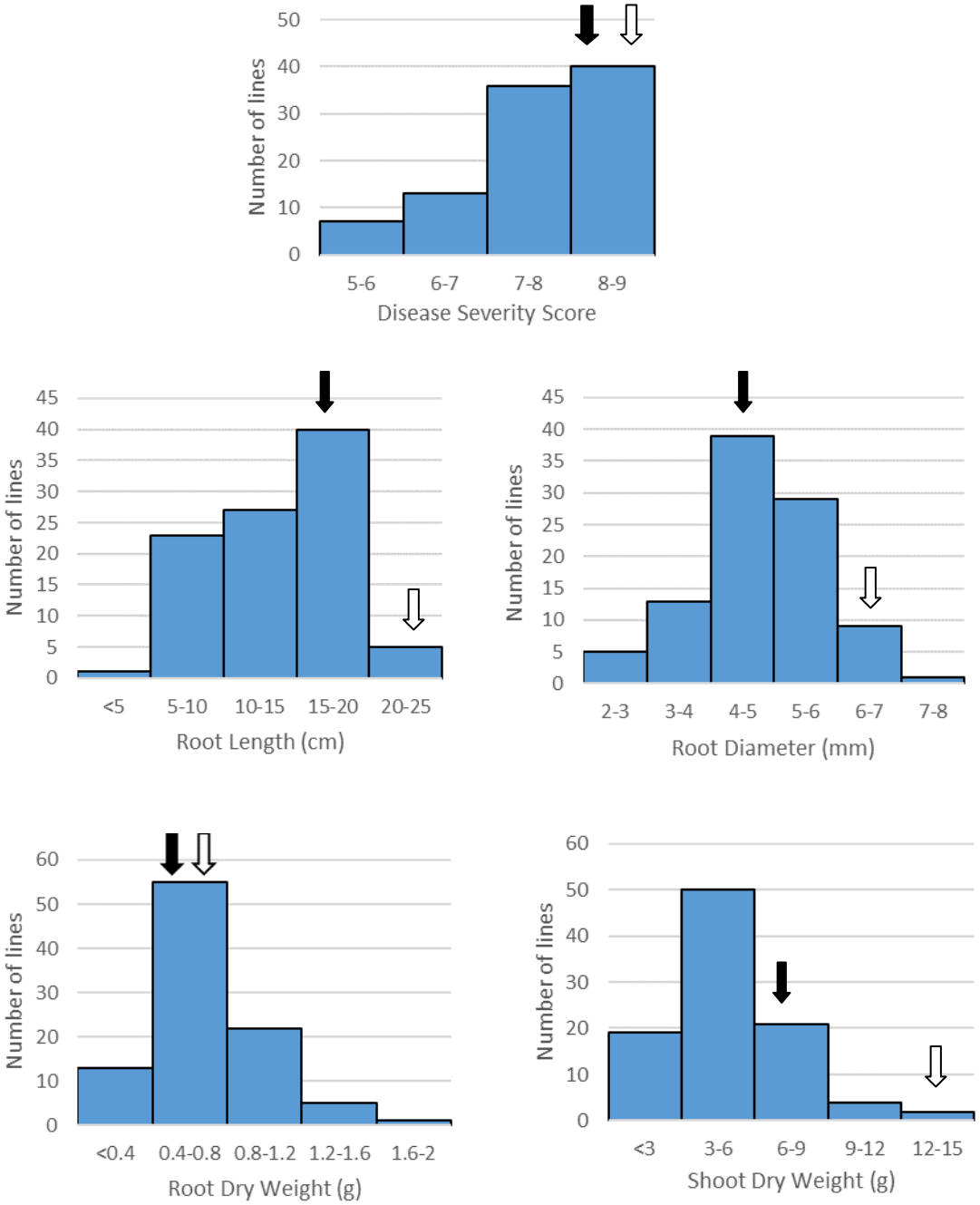
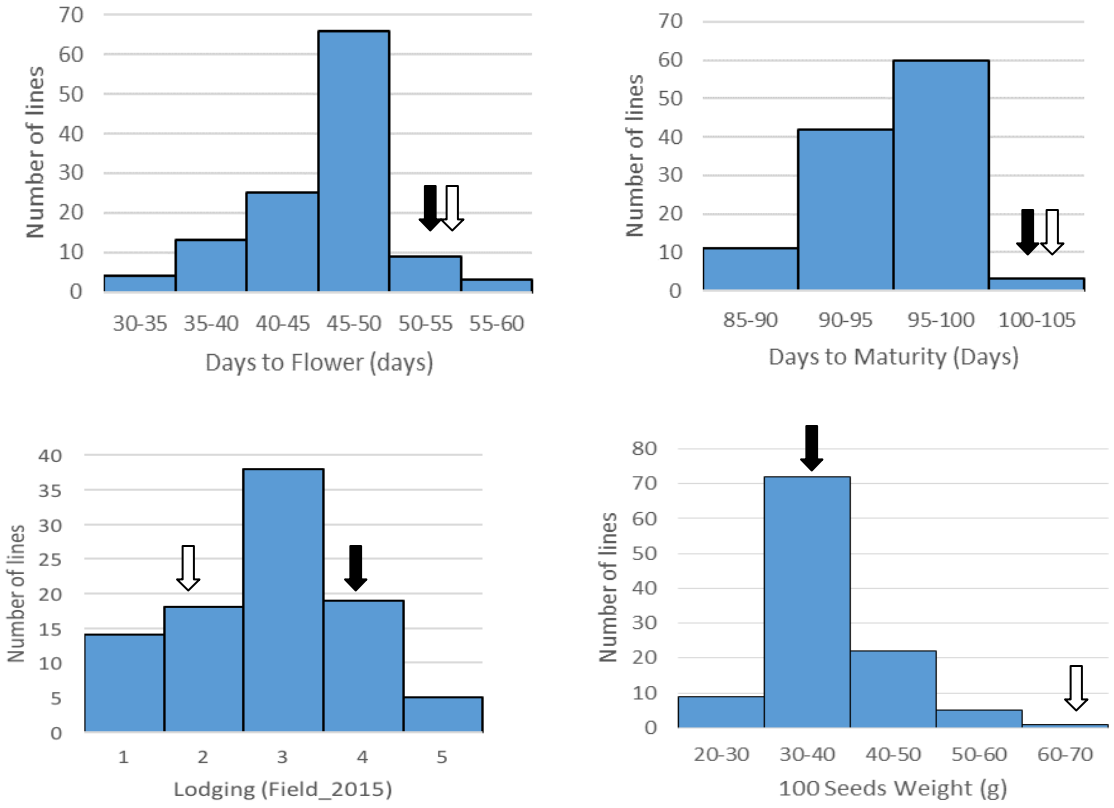


Figure 2.2 (cont'd)



Root diameter was significantly negatively correlated with disease severity score (r =0.35, P<0.001) and positively correlated with root length (r = 0.26, P<0.1) (Table 2.3). Shoot dry weight and root dry weight were significantly positively correlated (r = 0.64, P<0.01). DM was negatively correlated with shoot dry weight, and seed weight was positively correlated with root dry weight. Correlation was not found between the LG scores and DF or DM as found in 2014 (Table 2.3). LG scores from the two years (2014, 2015) were significantly correlated (r = 0.36, P<0.001), but the correlation of DF and DM from two years were not found (Table 2.4).

Table 2.3 Pearson correlation coefficients of disease severity score (Score), root length (Root_Lgth), root diameter (Root_Dia), root dry weight (Root_DW), shoot dry weight (Shoot_DW), days to flower (DF), days to maturity (DM), lodging and seed weight (Seed_Wght) of RIL population of MLB49-89A x CAL96 in Montcalm Research Farm (Entrican, MI) in 2015.

	Root_Lgth	Root_Dia	Root_DW	Shoot_DW	DF	DM	Lodging	Seed_Wght
Score	-0.074	-0.354***	-0.015	0.105	-0.075	-0.036	0.046	0.063
Root_Lgth	-	0.260*	0.132	0.085	-0.044	0.053	-0.049	-0.048
Root_Dia		-	0.097	-0.046	-0.116	0.001	0.018	-0.146
Root_Wght			-	0.635***	-0.119	-0.062	0.009	0.212*
Shoot_Wght				-	-0.095	-0.213*	-0.082	0.180
DF					-	0.187	0.174	-0.073
DM						-	0.162	-0.063
Lodging							-	0.078

*significant at 0.05 level; **significant at 0.01 level; ***significant at 0.001 level.

Table 2.4 Pearson correlation coefficients of days to flower (DF), days to maturity (DM) and lodging of the RIL population of MLB49-89A x CAL96 in field data from 2014 and 2015.

	DF_2014	DM_2014	Lodging_2014
DF_2015	0.056		
DM_2015		0.087	
Lodging_2015			0.361***

***significant at 0.001 level.

Phenotypic Variation in Greenhouse Experiment

Genotype had a significant effect on all measurements while experiment repeat had no significant effect at P = 0.001 level in greenhouse phenotyping. The average values of from two experiment repeats were used for the QTL analysis with all these traits.

Table 2.5 ANOVA of the mean response for disease severity scores (DS), inoculated root dry weight (RootDW_Inoc), non-inoculated control root dry weight (RootDW_Ctrl), root dry weight reduction (Root_Loss), inoculated shoot dry weight (ShootDW_Inoc), non-inoculated control shoot dry weight (ShootDW_Ctrl), shoot dry weight reduction (Shoot_Loss) of 121 RILs of MLB49-89A x CAL96 and two parents.

		P-value						
Source	D*f*	DS	RootDW_Inoc	RootDW_ Ctrl	Root_loss	ShootDW_Inoc	ShootDW_Ctrl	Shoot_loss
Geno	122	<.0001	<.0001	<.001	<.0001	<.0001	<.0001	<.0001
Exp_repeat	1	0.302	0.063	0.046	0.044	0.030	0.024	0.429

Geno: genotypes (121 RILs and two parents); Exp_repeat: two runs of experiment.

In greenhouse experiment, the RIL population displayed continuous distribution in all measurements including disease severity scores, inoculated root dry weight, inoculated shoot dry weight, control root dry weight, control shoot dry weight, root loss, and shoot loss (Figure 2.3). The average root rot scores ranged from 2.4 to 7.6 (Table 2.6) in the population evaluated with the 1-9 scale. The inoculated root dry weight ranged from 0.02 to 0.1 g, with an average of 0.05 g in the population. The inoculated shoot dry weight ranged from 0.04 to 0.2 g with an average of 0.09 g. The control root dry weight ranged from 0.04 to 0.14 g with an average of 0.08 g. The control shoot dry weight ranged from 0.06 to 0.22 with a mean of 0.13 g. The root loss ranged from -13.6 to 70.7% and shoot loss ranged from -4.3 to 58.4%, where the negative values indicated the inoculated plants had higher root or shoot dry weight than the control plants (Table 2.6).

Figure 2.3 Frequency and distribution of disease severity scores, inoculated root dry weight, inoculated shoot dry weight, control root dry weight, control shoot dry weight, root loss, and shoot loss of RIL population of MLB49-89A x CAL96 in greenhouse screening. Black and white arrows indicated the phenotypic values of parents MLB49-89A and CAL96, respectively.

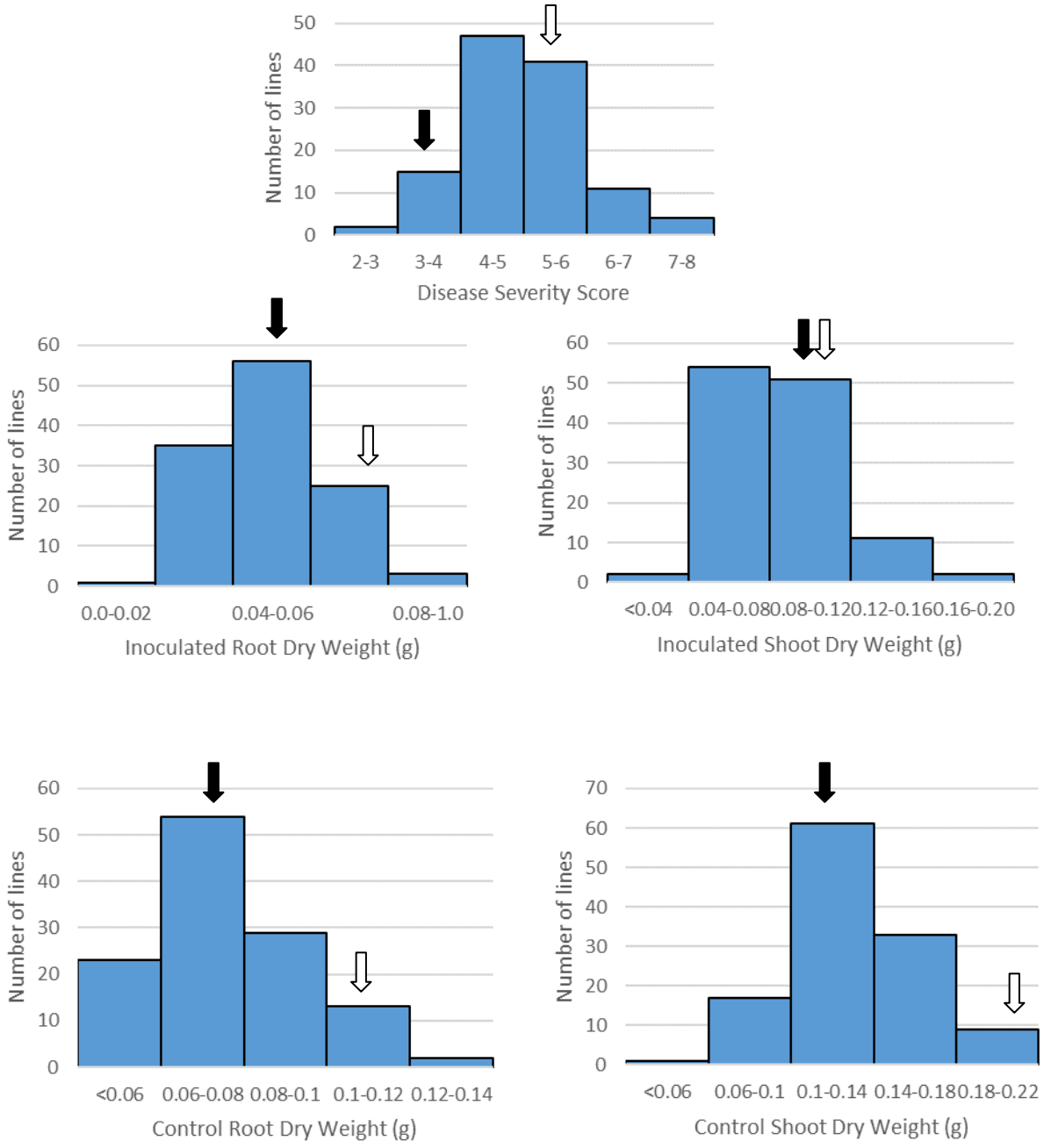
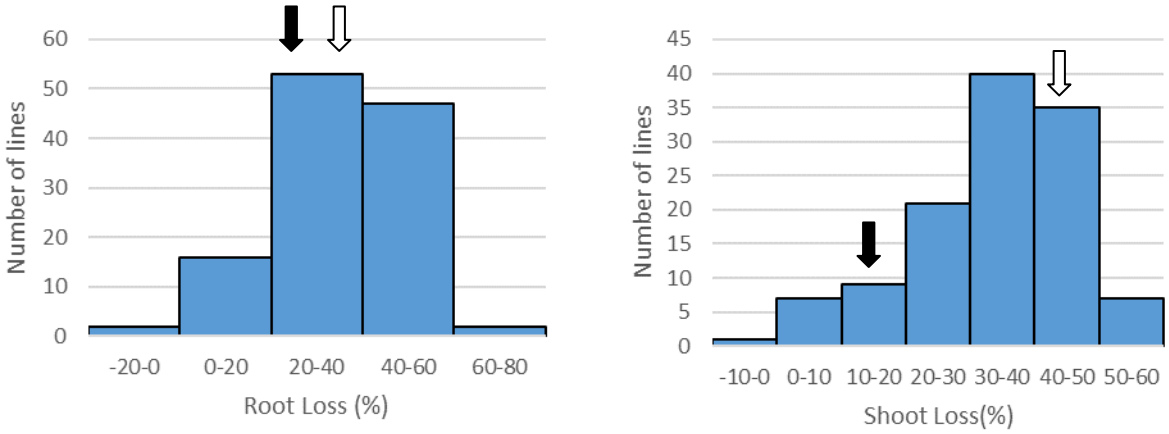


Figure 2.3 (cont'd)



Both MLB-49-89A and CAL96 showed moderate resistance to the *F. brasilense* isolate F_14-42 with disease severity scores of 3.5 and 4.8 respectively, while the RIL population varied from resistant to susceptible with disease severity scores varied from 2.4 to 7.6 (Table 2.6). MLB49-89A had less root and shoot biomass than CAL96 either inoculated or non-inoculated. Both MLB49-89A and CAL96 inoculated with F_14-42 had reduction in root and shoot biomass and MLB89-89A had less reduction than CAL96 in both root and shoot. The coefficient of variation (CV) varied from 19 to 43% and the CV for root and shoot reduction were the highest. The estimated broad-sense heritability for all traits tended to be high and ranged from 0.63 to 0.89, in which disease severity scores showed highest estimated heritability (Table 2.6).

Table 2.6 Summary of average disease severity scores (DS), inoculated root dry weight (RootDW_inoc), inoculated shoot dry weight (ShootDW_inoc), control root dry weight (RootDW_ctrl), control shoot dry weight (ShootDW_ctrl), root loss (Root_Loss), and shoot loss (Shoot_Loss) for two parents (MLB49-89A, CAL96), range and mean for the RIL population of MLB49-89A x CAL96, and their heritability estimates (***h^2*).**

	DS	RootDW _inoc (g)	ShootDW _inoc (g)	RootDW _ctrl (g)	ShootDW _ctrl (g)	Root_loss (%)	Shoot_loss (%)
MLB49	3.5	0.059	0.101	0.084	0.118	30.0	14.5
CAL96	4.8	0.068	0.112	0.108	0.218	37.3	48.7
Lowest	2.4	0.018	0.035	0.043	0.055	-13.6	-4.3
Highest	7.6	0.090	0.196	0.138	0.218	70.7	58.4
Mean	5.0	0.050	0.087	0.077	0.131	34.9	33.7
CV (%)	18.9	31.5	31.9	24.3	23.4	42.8	38.4
LSD(0.05)	1.10	0.02	0.05	0.03	0.05	0.29	0.30
h^2	0.89	0.83	0.77	0.81	0.82	0.71	0.63

CV: coefficient of variation; LSD (0.05): least significant difference at $\alpha = 0.05$ level; h^2: heritability estimates.

The disease severity score in the greenhouse experiment was significantly correlated with all other measured traits, with r score ranged from 23% to 39%. Significant correlation was also found between inoculated root and shoot dry weight, control root and shoot dry weight, and root and shoot dry weight reduction (r ranged from 0.65 to 0.79) (Table 2.7).

Table 2.7 Pearson correlation coefficients of disease severity score (DS), inoculated root dry weight (RootDW_Inoc), inoculated shoot dry weight (ShootDW_Inoc), root loss (Root_Loss), shoot loss (Shoot_Loss), control root dry weight (RootDW_Ctrl), and control shoot dry weight (ShootDW_Ctrl) of the RIL population derived from a cross of MLB49-89A x CAL96.

	DS	RootDW_ Inoc	ShootDW _Inoc	Root_Loss	Shoot_Loss	RootDW_ Ctrl	ShootDW_ Ctrl
DS	-	-0.392***	-0.359***	0.280***	0.258***	-0.244***	-0.232***
Root_inoc		-	0.787***	-0.714***	-0.540***	0.439***	0.435***
Shoot_inoc			-	-0.476***	-0.709***	0.450***	0.566***
Root_Loss				-	0.647***	0.063*	0.001
Shoot_Loss					-	-0.078*	-0.057
Root_Ctrl						-	0.739***

*significant at 0.05 level; ***significant at 0.0001 level.

Linkage Map and QTL Analysis

The genetic map of 11 chromosomes with total length of 925.5cM was constructed with SNP markers. Chromosome Pv01 was the longest in map distance and chromosome Pv06 was the shortest. The total number of markers of the genetic map was 528 after deleting extra markers within map distance of one centimorgan. Chromosome Pv09 had the highest number of markers, and chromosome six had the lowest number of markers and was the shortest in distance (Table 2.8).

Table 2.8 Number of markers and map distance by chromosome of the genetic linkage map developed from recombinant inbred lines (RILs) of the F_4 population of MLB49-89A x CAL96 with single nucleotide polymorphism (SNP) markers.

Chromosome	No. of Markers	Distance (cM)
Pv01	51	106.5
Pv02	53	87.7
Pv03	49	106.3
Pv04	38	78.3
Pv05	48	83.2
Pv06	36	62.7
Pv07	43	79.3
Pv08	62	94.2
Pv09	65	95.2
Pv10	42	58.3
Pv11	41	73.7
Total	528	925.5

All phenotypic data from field and greenhouse studies were used for QTL analysis. With field data in 2014, QTL were found for DF and LG. With field data in 2015, QTL were found for disease severity score, root and shoot dry weight, DF, LG and seed weight (Table 2.9), among which disease severity score, root and shoot dry weight were considered as traits that related with FRR resistance. With greenhouse experiment phenotypic data, QTL were found for disease

severity score, inoculated root and shoot dry weight, control root dry weight, and root and shoot loss (Table 2.9).

A QTL associated with LG in 2014 was found on Pv01, explaining 14% phenotypic variation. Four QTL for DF were found on Pv03, Pv05, Pv07 and Pv09 with explained phenotypic variation ranged from 7 to 19% (Table 2.9). A QTL associated with disease severity score in 2015 was found on Pv02, explaining 11% of phenotypic variation. A QTL associated with root dry weight in 2015 was found on Pv07, explaining 11% of phenotypic variation. Two QTL associated with shoot dry weight were found on Pv05 and Pv10, explaining 17 and 11% of phenotypic variation. One R gene was found close to the QTL for disease severity score on PV02. For QTL of shoot dry weight, one R gene was found in the region of QTL on Pv05 and five R genes were found in the region of QTL on Pv10 with the one closest to the closest SNP showed in the table. A QTL for days to flower was also found on Pv11 and a QTL associated with seed weight was found on Pv05, explaining 17% of phenotypic variation (Table 2.9).

In the greenhouse experiment, a QTL associated with disease severity score was found on chromosome Pv11, explaining 9% of phenotypic variation. QTL associated with inoculated root dry weight was also found on Pv11, explaining 11% of phenotypic variation. Three QTL associated with inoculated shoot dry weight were found on Pv01, Pv07 and Pv09, explaining 9, 12 and 7% of phenotypic variation. Two QTL associated with control root dry weight were found on Pv02 and Pv09, explaining 11% of phenotypic variation for both QTL. Two QTL associated with root and shoot loss were found on the same location of Pv03 and Pv07, respectively, explaining phenotypic variation ranged from 8 to 12%. A cluster (in total of 39 genes) of nucleotide-binding and leucine-rich repeat (NB-LRR) domain encoding genes (NL-encoding genes) were found in the region between two flanking markers of the QTL of disease

severity score with the one closest to the closest SNP showed in the table (Table 2.9). Other disease resistance related genes (R genes) were also found in the regions of QTL for inoculated shoot dry weight on Pv09, control root dry weight on Pv02, and QTL for root and shoot loss on Pv03 (Table 2.9).

On Pv02, QTL for control root dry weight in greenhouse overlapped with QTL for disease severity score in 2015. On chromosome Pv07, QTL for inoculated shoot dry weight, root loss, and shoot loss in greenhouse, root dry weight in 2015 field, and DF in 2014 field were overlapping with each other. QTL for disease severity score, inoculated root dry weight, and DF in 2015 were overlapping with each other on Pv11 (Figure 2.4).

For the QTL of DS_Inoc, the beneficial allele contributed to lower disease severity score was from CAL96 (Additive = -0.31). For the QTL of inoculated root dry weight, the beneficial allele contributed to higher inoculated root dry weight was from CAL96 (Additive = 0.006). For the QTL of ShootDW_Inoc, the QTL on Pv01 and Pv09 had beneficial allele from MLB-49-89A to contribute to higher inoculated shoot dry weight, and the QTL on Pv07 had beneficial allele from CAL96 to contribute to higher inoculated shoot dry weight. For QTL of control root dry weight, the QTL on Pv02 had beneficial allele from CAL96 to contribute to higher control root dry weight and the QTL on Pv09 had beneficial allele from MLB-49-89A. The QTL for root and shoot loss on Pv03 had beneficial allele from MLB-49-89A that contributed to less root loss and QTL for root and shoot loss on Pv07 had beneficial allele from CAL96. The QTL of disease severity score in 2015 had beneficial allele from MLB-49-89A that contributed to lower disease severity score. The QTL for root dry weight in 2015 had beneficial allele from CAL96 to contribute to higher root dry weight. The QTL of shoot dry weight on Pv05 had beneficial allele from MLB-49-89A that contributed to higher shoot weight, and the QTL of shoot dry weight on

Pv10 had beneficial allele from CAL96. The QTL of LG in two years had beneficial allele from CAL96 that contributed to lower lodging score (Table 2.9).

Table 2.9 Quantitative trait loci (QTL) related to Fusarium root rot resistance detected in RIL population derived from a cross of MLB49-89A x CAL96 for disease severity scores (DS_Inoc), inoculated root dry weight (RootDW_Inoc), inoculated shoot dry weight (ShootDW_Inoc), control root dry weight (RootDW_Ctrl), root dry weight reduction (Root_Loss), and shoot dry weight reduction (Shoot_Loss) in greenhouse experiment, and disease severity scores (DS_2015), root dry weight (Root DW_2015), shoot dry weight (ShootDW_2015) and seed weight in 2015 field study, lodging and days to flower (DF) in 2015 and 2014 field, and the disease-related genes within/close to the QTL if available.

Trait	Chrom.	LOD	R^2	Map Position (cM)	Physical Position (Mb)	Closest SNP	Disease related gene in this region	Anotation	Physical Position (bp) of Genes		Additive
									Start	End	
DS_Inoc	11	2.96	0.09	60.51	48.89	ss715647839	Phvul.011G203100	LRR and NB-ARC domains-containing disease resistance protein	48,152,413	48,155,957	-0.314
RootDW_Inoc	11	3.94	0.11	63.41	50.2	ss715640613					0.006
ShootDW_Inoc	1	3.12	0.09	59.91	45.25	ss715647367					-0.009
	7	4.12	0.12	18.41	2.62	ss715646498					0.011
RootDW_Crtl	2	3.49	0.11	21.11	31.31	ss715639514	Phvul.002G166400	Disease resistance protein (CC-NBS-LRR class) family	30,827,644	30,831,066	0.007
	9	3.6	0.11	4.81	7.63	ss715645741					-0.008
Root_Loss	3	2.74	0.09	50.41	33.05	ss715647751	Phvul.003G129700	disease resistance protein (TIR-NBS-LRR class), putative	31,823,207	31,828,985	0.06
	7	3.94	0.12	15.51	2.37	ss715648692					-0.058

Table 2.9 (cont'd)

Shoot_Loss	3	3.23	0.08	50.41	33.05	ss715647751	Phvul.003G129700	Same as Root_Loss			0.038
	7	3.1	0.09	15.51	2.37	ss715648692					-0.042
DS_2015	2	3	0.11	14.11	29.3	ss715647527	Phvul.002G152100	Leucine-rich repeat protein kinase family protein	29,314,263	29,318,463	0.839
RootDW_2015	7	3.08	0.11	12.11	1.81	ss715648636					0.111
ShootDW_2015	5	4.67	0.17	48.41	32.37	ss715649151	Phvul.005G117900	NB-ARC domain-containing disease resistance protein	33,909,268	33,914,355	-1.262
	10	3.21	0.11	19.71	33.34	ss715640868	Phvul.010G091100	NB-ARC domain-containing disease resistance protein	33,625,707	33,642,450	1.082
Lodging_2015	1	12.68	0.42	57.91	45.25	ss715639272					-0.76
Lodging_2014	1	4.82	0.14	57.91	45.25	ss715639272					-0.364
DF_2015	11	3.04	0.11	60.51	48.89	ss715647839	Phvul.011G203100	Same as DS_Inoc			-1.271
DF_2014	3	3.12	0.07	32.11	25.78	ss715641974					1.3
	5	7.1	0.19	76.61	38.54	ss715645449					2.229
	7	2.92	0.07	19.41	2.67	ss715646495					-1.363
	9	3.99	0.12	39.61	21.24	ss715650556					1.677
Seed Weight	5	5.44	0.17	69.51	38.22	ss715645421					-2.67

LOD: logarithm of odds.
R^2: coefficients of determination represent the phenotypic variance explained by the QTL.
Additive: Negative additive value indicates RILs with allele from MLB-49-89A had greater sample mean in that phenotypic measurement, while positive additive value indicates RILs with allele from CAL96 had greater sample means in that phenotypic measurement.

Figure 2.4 Quantitative trait loci (QTL) of traits related to *Fusarium* root rot with an LOD threshold of 2.5, and their locations on the genetic linkage map developed from recombinant inbred lines (RILs) of MLB49-89A x CAL96.
(The traits include disease severity scores (DS_Inoc), inoculated root dry weight (Root_Inoc), inoculated shoot dry weight (Shoot_Inoc), control root dry weight (Root_Ctrl), control shoot dry weight (Shoot_Ctrl), root dry weight reduction (Root_Loss), and shoot dry weight reduction loss(Shoot_Loss) in greenhouse experiment, and disease severity scores (DS_2015), root dry weight (Root_2015), shoot dry weight (Shoot_2015) and seed weight (100seedwght) in 2015 field study, lodging (Lodging_2014, Lodging_2015) and days to flower (DF_2014, DF_2015) in 2014 and 2015 field.)

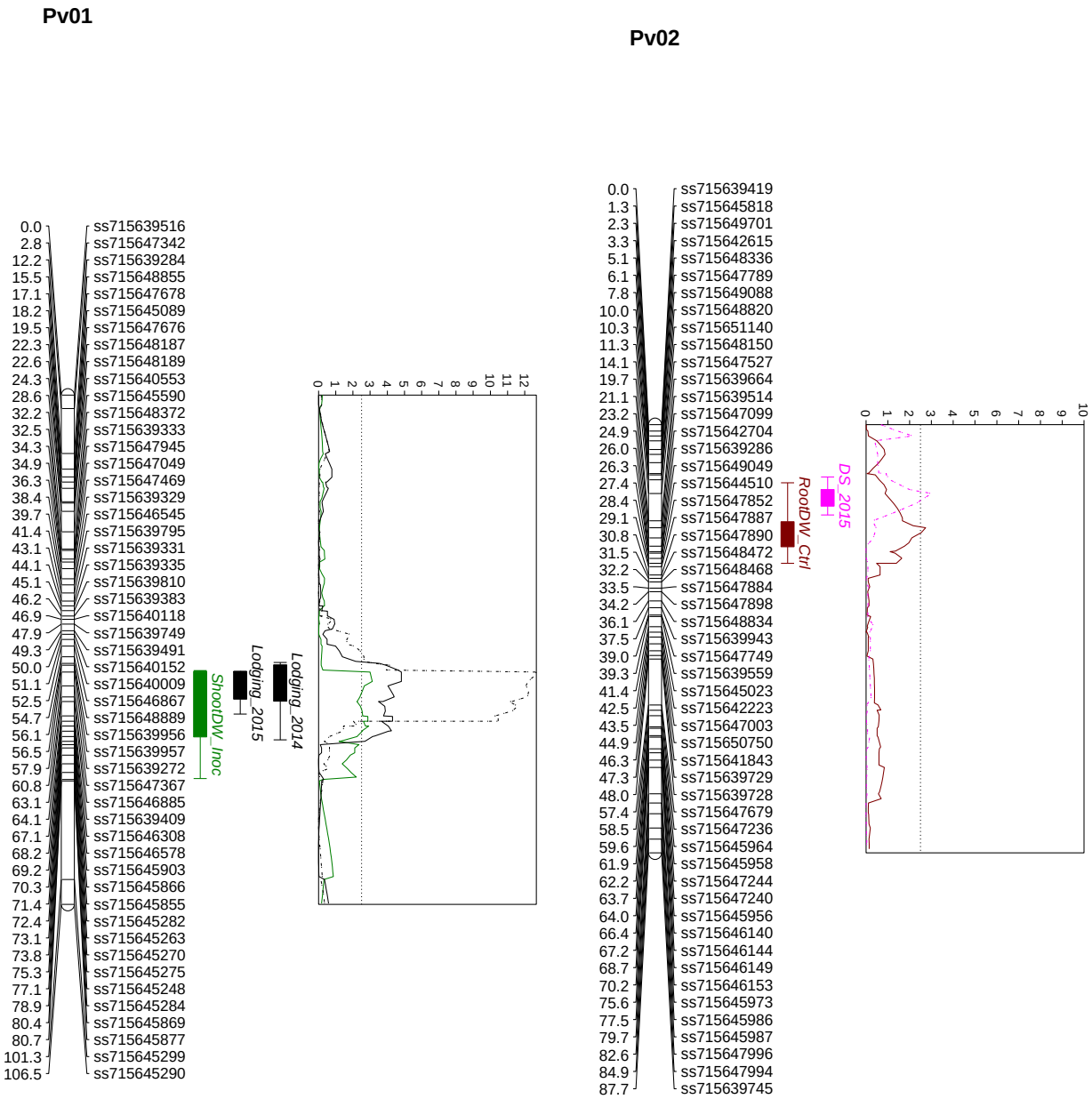


Figure 2.4 (cont'd)

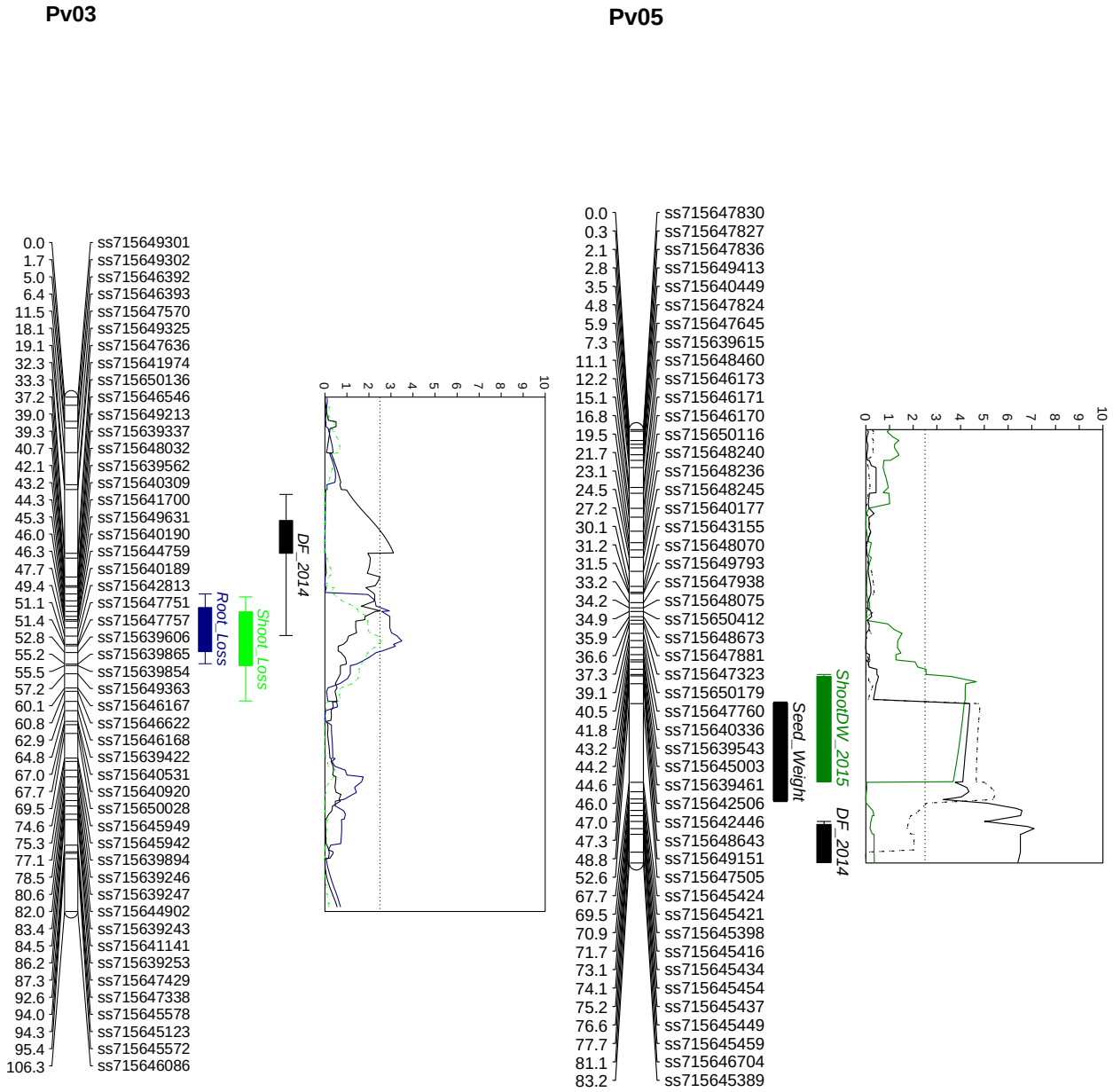


Figure 2.4 (cont'd)

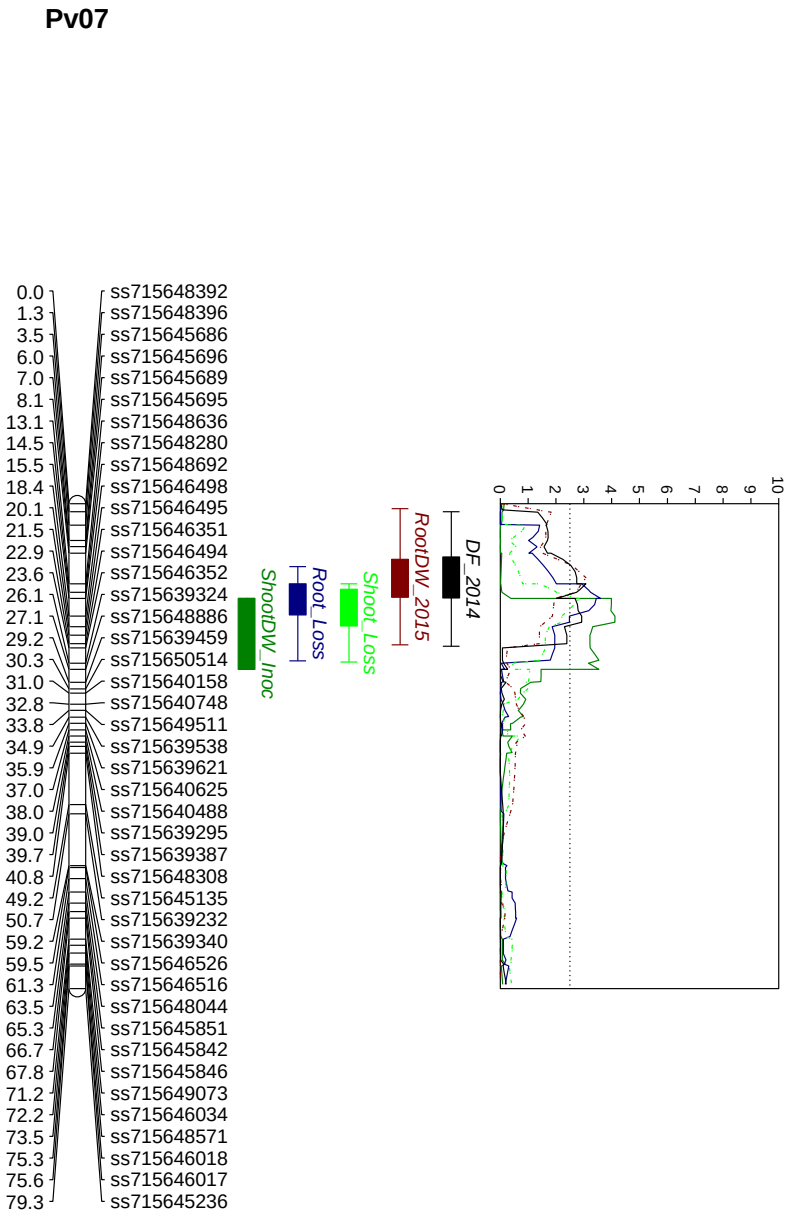


Figure 2.4 (cont'd)

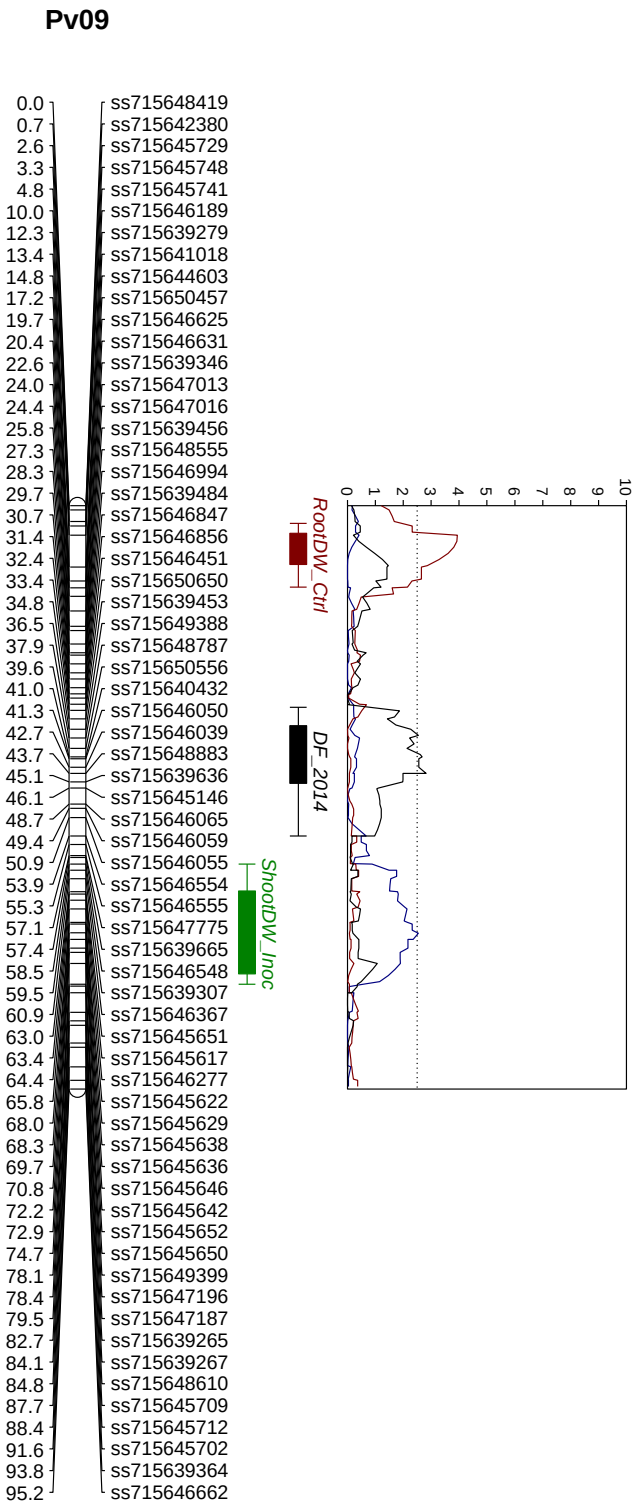
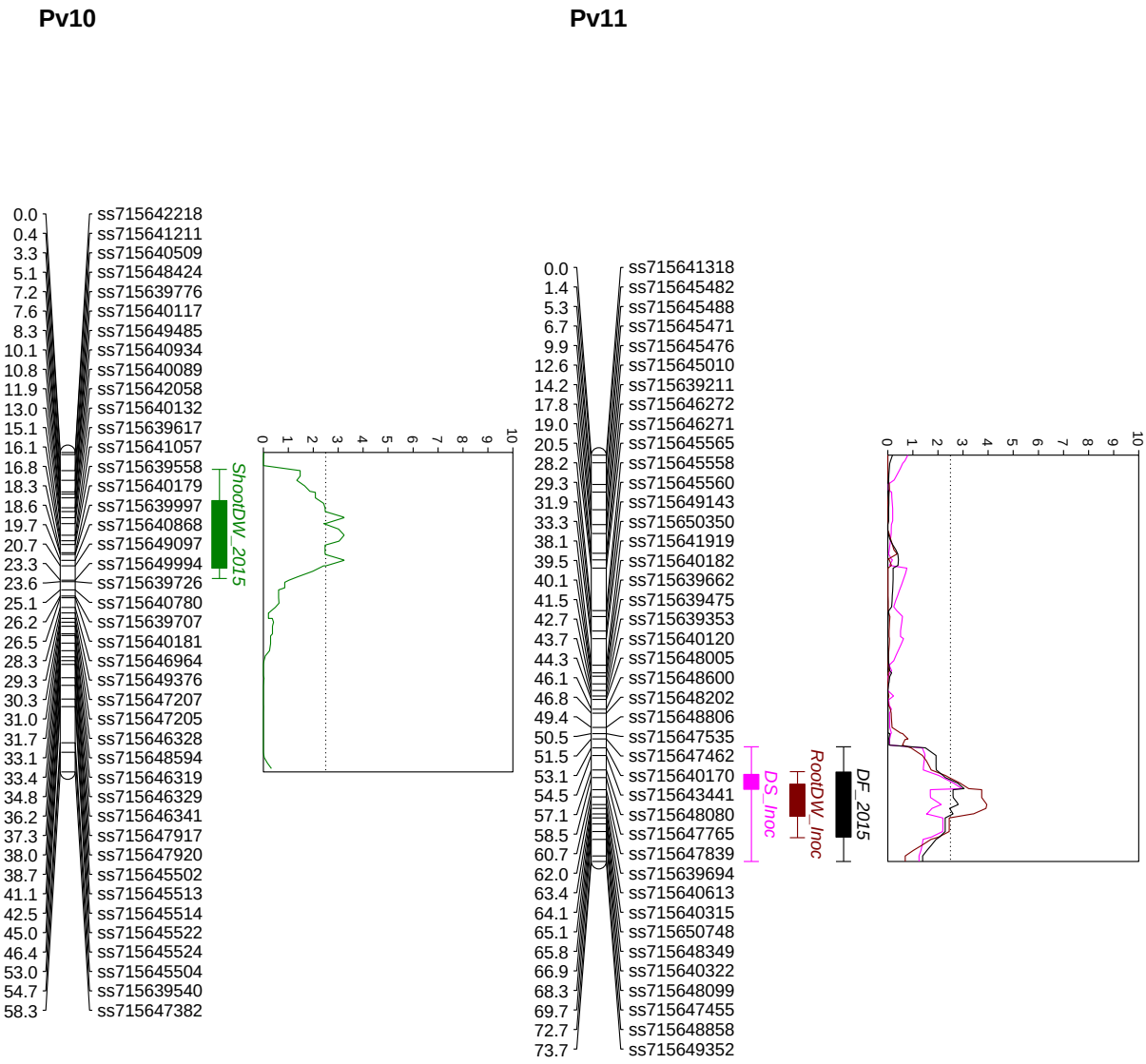


Figure 2.4 (cont'd)



Five most resistant and five most susceptible RILs to FRR were selected from each environment conditions based on their DS. The five most resistant ones were not exactly the same in different environment, but there were three lines (CxM_15, CxM_425 and CxM_433) found as most resistant lines in more than one environment. The five most susceptible RILs were also not exactly the same in three environments, but two lines (CxM_222 and CxM299) were found in more than one environment. The seed type of either resistant or susceptible lines varied from red-mottled to black. For either resistant or susceptible lines, the lodging scores varied from 1 to 4. The growth habit of the resistant lines was TypeII or TypeIII, while the susceptible lines covered all three types of growth habit. It would appear that the FRR reaction of the RILs was independent of the growth habit or lodging. The seed weight also appear not to have direct relationship with resistance or susceptibility, since the seed weight for resistant lines or susceptible lines both varied from low to high (Table 2.10).

The RILs were checked for SNP variation on the location of six important QTL in this study, including the QTL for DS_Inoc on Pv11, RootDW_Inoc on Pv11, Root & Shoot Loss on Pv03 and Pv07, RootDW_2015 on Pv07, and DS_2015 on Pv02. Six RILs (CxM_15, CxM_122, CxM_142, CxM_198, CxM_425, and CxM_517) were found to have alleles for all six QTL. Six RILs were found to have alleles for five of the QTL and 14 RILs were found to have alleles for four of the QTL (Table 2.11).

Table 2.10 The five most resistant and five most susceptible lines to FRR in the RIL population of MLB-49-89A x CAL96 in different environment and their seed type, lodging score, growth habit (GH), and seed weight, selected based on disease severity scores (DS).

		RILs	DS	Seed Type	GH	Lodging	Seed Weight (g/100 seeds)
Greenhouse	Resistant	CxM_248	3.1	Medium, Black	II	2	42
		CxM_267	4.7	Small, Red Mottled	II	2	27.3
		CxM_425	**4.0**	**Medium, Pink**	**III**	**4**	**33.8**
		CxM_433	**4.8**	**Medium, Red Mottled**	**II**	**1**	**39.5**
		CxM_521	2.5	Medium, Black	III	3	54.3
	Susceptible	CxM_121	7.0	Small, Purple Mottled	I	1	31.9
		CxM_122	7.6	Medium, Red Mottled	II	2	41
		CxM_208	6.9	Medium, Black	III	4	44.6
		CxM_222	**8.0**	**Medium, Black**	**I**	**3**	**32**
		CxM_246	7.2	Medium, Caramel	III	4	36.8
Field_2015	Resistant	**CxM_15**	**4.7**	**Medium, Black**	**II**	**2**	**35.1**
		CxM_425	**4.3**	**Medium, Pink**	**III**	**4**	**33.8**
		CxM_433	**5.3**	**Medium, Red Mottled**	**II**	**1**	**39.5**
		CxM_506	4.3	Medium, Black	III	3	38.1
		CxM_520	5.0	Medium, Black	II	3	39.5
	Susceptible	CxM_42	8.7	Small, Purple Mottled	II	2	23.8
		CxM_125	7.7	Small, Black	III	3	37.1
		CxM_205	8.3	Small, Black	II	1	30.8
		CxM_222	**8.0**	**Medium, Black**	**I**	**3**	**32**
		CxM_299	**8.0**	**Medium, Red Mottled**	**II**	**4**	**54.7**
Field_2014	Resistant	**CxM_15**	**1.0**	**Medium, Black**	**II**	**2**	**35.1**
		CxM_21	1.0	Medium, Black	II	2	-
		CxM_187	2.0	Medium, Purple Mottled	II	2	39.9
		CxM_411	1.0	Small, Black	II	3	31.5
		CxM_433	**1.0**	**Medium, Red Mottled**	**II**	**1**	**39.5**
	Susceptible	CxM_204	9.0	Medium, Pink	I	1	45.4
		CxM_285	9.0	Small, Brown	II	3	33.9
		CxM_299	**8.0**	**Medium, Red Mottled**	**II**	**4**	**54.7**
		CxM_204	9.0	Medium, Pink	I	1	45.4
		CxM_421	9.0	Small, Black	II	4	39.7

*RIL in bold means it was found as most resistant or susceptible under more than one environment conditions.

Table 2.11 RILs of MLB-49-89A x CAL96 that have combined alleles for several or all of six QTL related to FRR resistance, including disease severity score (DS_Inoc), inoculated root dry weight (RootDW_Inoc), root and shoot loss (Root&Shoot_loss) in greenhouse, and root dry weight (RootDW_2015) and disease severity score (DS_2015) in 2015 field.

	DS_Inoc	**RootDW_Inoc**	**Root&Shoot_loss**	**Root&Shoot_loss**	**RootDW_2015**	**DS_2015**
RIL	**Pv11**	**Pv11**	**Pv07**	**Pv03**	**Pv07**	**Pv02**
CxM_15[#]	√	√	√	√	√	√
CxM_122[&]	√	√	√	√	√	√
CxM_142	√	√	√	√	√	√
CxM_198	√	√	√	√	√	√
CxM_425	√	√	√	√	√	√
CxM_517	√	√	√	√	√	√
CxM_108	√	√	√		√	√
CxM_123	√	√	√	√	√	
CxM_187	√	√	√	√	√	
CxM_233	√	√	√		√	√
CxM_433	√	√	√		√	√
CxM_521	√	√	√		√	√
CxM_17		√	√	√	√	
CxM_191		√	√	√		√
CxM_199	√	√	√			√
CxM_205[&]	√	√		√		√
CxM_211	√	√		√		√
CxM_267	√	√		√		√
CxM_280	√	√	√		√	
CxM_284	√	√		√		√
CxM_285[&]	√	√		√		√
CxM_290	√	√		√		√
CxM_339	√	√	√		√	
CxM_340	√	√	√		√	
CxM_342	√	√	√		√	
CxM_506		√	√		√	√

√ indicates the RIL has the allele for that QTL.

[#]RILs in bold are resistant to FRR according to table 2.10.

[&] RILs found to be susceptible to FRR according to table 2.10.

DISCUSSION

The phenotypic results in 2014 field data suggested the genetic diversity of the RIL population and the quantitative inheritance of all measurements. The GH and LG score results indicated successful segregation in the RIL population from the two parents with contrasting GH and LG type. The high positive correlation between GH and LG score indicated that it was obvious that the plants with indeterminate growth habit tend to be prostrate and flat on the ground compared to bean genotypes with determinate growth habit. Bean genotypes with indeterminate prostrate growth habit tend to flower later in the season than the determinate erect bean genotypes in this population. The correlation between disease severity score and the other measurements was not observed, the reason could be that Fusarium root rot was not a major problem in the Saginaw Valley Research Farm and the disease severity score did not reflect the resistance or susceptibility of the RIL population to FRR. In previous studies, negative correlations between FRR ratings and days to flower were detected, first by Schneider et al. (2001) in RIL populations of FR266 x 'Montcalm' and FR266 x 'Isles' and second by Román-Avilés and Kelly (2005) in two inbred backcross line (IBL) populations of 'Red Hawk' x 'Negro San Luis' (NSL) and C97407 x NSL. These correlations indicate that by the time plants evaluated for root rot in the field, the more mature ones tended to have more disease symptom. The author suggested that the bean plants were more affected by root rot during reproductive growth stages (flowering and seed development) when plants stopped vegetative growth and less resources were available for host defense response (Schneider et al., 2001; Román-Avilés and Kelly, 2005).

More measurements were taken for the RIL population in 2015 field experiment than in 2014, and the two parents were included in those measurements. For all measurements except

seed weight, transgressive segregation was detected in the RIL population since lines with values lower than the lowest parent or higher than the highest parent were observed. The continuous normal distribution of those measurements indicated the genetic diversity in the population and the complex quantitative inheritance. The distribution of the disease severity score of the population was skewed to susceptibility and the lowest score was five. Most individuals in the RIL population showed moderate to severe susceptibility to FRR in the Montcalm Research Farm in 2015, where the FRR pressure was very high. It suggested that the environment affected the plant reaction to FRR, since the disease severity and the types of soil-borne pathogen varied from field to field. The resistant parent MLB-49-89A in this study also had high disease severity score in the field in 2015. Schneider et al. (2001) suggested that genetic resistance to FRR might be overcome under severe disease pressure, as in her study the resistant parent FR266 showed root rot rating greater than 4.0 in field test in Montcalm Research Farm. Root diameter had significant negative correlation with DS, which suggested that the plants with more severe root rot symptoms tend to have weaker taproots. Both the DF and DM were delayed in 2015 compared to 2014 and a lack of correlation was observed between the years, which suggested that they were subjective to the environments. The population in 2015 suffered from flooding in the seedling stage, which could result in late flowering and maturity. Even though the lack of correlation for DF and DM was observed between the two years, a strong correlation between lodging in 2014 and 2015 was detected. The single *Fin* gene located on the chromosome Pv01 controls the determinacy in growth habit of common bean (Kwak et al., 2008). In this study, QTL detected for lodging scores in both 2014 and 2015 were found at the same location on Pv01 and the closest SNP to the QTL matched with the most significant SNP (ss715639272)

associated with determinacy in the association mapping of the Andean bean diversity panel (Cichy et al., 2015).

In greenhouse experiment, continuous distribution was observed in all measurements as expected and transgressive segregation was also observed. For the two parents, CAL96 had slightly higher diseases severity score than MLB49-89A, and the root and shoot loss of CAL96 were higher than MLB-49-89A, which matched the results from the previous greenhouse screening with *F. brasilense* isolate F_14-42 on the two parents with the same method. In the RIL population, varied reaction to FRR was found and the DS ranged from low to high, which indicated the availability of using this population for QTL analysis for FRR resistance and possibility of selecting resistant lines from this population for breeding purposes. Variations were found for all measurements in this population and the values in root and shoot reduction were more dispersed with higher CV than other measurements, which was because of the values varied from negative to positive. Even though all the inoculated plants showed FRR disease symptom from mild to severe, the resistant lines could have slightly higher biomass in root or shoot than the controls. The reduction of the biomass of the infected resistant lines could be not detected due to the screening method in the greenhouse only kept plants for 14 days after planting and plants were at very early growth stage. Generally, it was observed that the infected plants tended to grow slower than the non-infected plants at the early growth stage and the smaller plants at the early stage were more susceptible to the disease. The significant correlation between disease severity score with plant biomass (root and shoot dry weight) and biomass reduction reflected the possibility of using the root and shoot dry weight and their reduction as indicators to select for root rot resistance in breeding practice. Since the large seeded lines, such as the susceptible parent CAL96 establish root system faster than small seeded lines at early

stage of development, the biomass reduction is a better indicator than biomass. The reduction in plant biomass is also less subjective while the disease severity scores may subject to personal assessment. Additionally, the greenhouse provided a more stable environment and it may increase the gains when selection is made in the greenhouse.

The heritability estimates for all measurements varied from moderate to high, which supported the availability to improve genetic resistance to FRR by introducing resistant genes from resistant Middle American varieties to susceptible Andean varieties. Moderate to high heritability h^2(0.48 – 0.71) were reported in the RIL populations of FR266 x 'Montcalm' and FR266 x 'Isles' by Schneider et al. (2001) over greenhouse and field experiments, and it was expected that the heritability derived from greenhouse to be substantially higher than that from field since the environmental variation was less in the greenhouse. Kamfwa et al. (2013) also observed high heritability (0.86 and 0.99, respectively) in the RIL population of K20 x MLB49-89A and K132 (CAL96) x MLB49-89A, and the authors suggested the major gene effects of the resistance to FRR in these population. Mukankusi et al. (2011) estimated that two genes controlling FRR resistance in the F2 population of K132 x MLB-49-89A.

With QTL analysis for all the measured traits in both field and greenhouse trials, a total of 22 QTL were detected. Among all the QTL, only one was found for greenhouse root rot score and another one for 2015 field root rot score. No QTL was found for root rot score in 2014 field, since only one plant was sampled for each line so the evaluation result was not representative, and the FRR pressure in Saginaw Research Farm was not severe. For most of the traits related to plant biomass, more than one QTL was detected for each trait. The root and shoot dry weight were separated in QTL in either greenhouse or field data, and the root and shoot reduction in greenhouse were found at the same location on the genetic map. Previous studies have indicated

that bean genotypes resistant to FRR tend to accumulate larger root biomass by producing adventitious roots (Román-Avilés et al., 2003) and the vigor of the root system is a valuable trait for selection of FRR resistant genotype (Cichy, 2007). So the QTL for root and shoot biomass and their reduction in this study can be used to study the FRR resistance in the RIL population.

By aligning the genetic linkage map with the *P. vulgaris* genome sequence, candidate genes for FRR resistance within the QTL regions can be identified. In this study, the disease resistance (R) genes identified in QTL are all genes encoding nucleotide-binding-site-leucine-rich-repeat (NB-LRR) structure. Most of those R genes belong to coiled-coil NBS-LRR (CNLs) class and only the gene in the region of QTL for root and shoot reduction on Pv03 belong to TIR-containing NB-LRR proteins (TNLs) class. R genes are usually monogenic or major genes that controls resistance to specific pathogen races (Michelmore et al., 2013), but they can also be polygenic with many genes providing small additive effects, such as primary metabolism genes that play a role in providing energy for the resistance response (Bolton, 2009). Since the phenotypic variation explained by the QTL that with R genes in their region were relatively low, ranging from 7 to 17%, those R genes could be polygenic and function together to response to the disease infection.

The most important QTL in this study resided on chromosomes Pv02, Pv03, Pv07 and Pv11. On Pv02, QTL for non-inoculated control root dry weight in greenhouse overlapped with the QTL for disease severity score in 2015 field, which suggested the relationship between root biomass and FRR resistance. On Pv03, QTL for root loss and shoot loss were located on the same position and a R gene was identified within the QTL region. On Pv07, the region with co-located QTL included QTL for root and shoot dry weight as well as root dry weight in different environment, even though no R genes were found in that region. On Pv11, the region with those

co-localized QTL were overlapping with a cluster of CNL class of R genes as identified in common bean reference genome by Schmutz et al. (2014). The RILs found to have combined alleles for most of these important QTL were also found to have resistance or moderate resistance in field and/or greenhouse study. Among the three RILs that were found to have resistance in more than one environment, two had alleles for all six QTL and one had alleles for five of those QTL. These RILs can be selected for breeding work to introduce FRR resistance to other genotypes.

For the QTL of disease severity score in greenhouse on Pv11, the allele contributed to lower disease severity score was from CAL96, which was known as the susceptible parent with bigger root system. It suggested that CAL96 may carry the resistance gene, but only when this resistance gene is combined with other genes from the resistant parent MLB-49-89A, the plant will show resistance. It is also possible that this QTL is more like an indicator of higher root biomass which contributed to the resistance for the RILs at early growth stage, instead of an indicator of resistance gene to FRR. However, the QTL of root loss on Pv03 and QTL of disease severity score in 2015 field on Pv02 had resource alleles from the resistant parent MLB-49-89A, and R genes were also identified in those regions. Therefore, these QTL could be indicators of resistance genes to FRR that functioned in MLB-49-89A and the RILs inherited from it.

In previous studies for QTL of FRR resistance, significant QTL were identified on chromosomes Pv02, Pv03 and Pv05 (Schneider et al., 2001; Román-Avilés and Kelly, 2005; Kamfwa et al., 2013). Since the markers used in those studies were RAPD or SSR markers and the positions of those QTL were not aligned to a physical map of the genome, it is not possible to directly compare the QTL detected in this study. However, in Schneider et al. (2001)'s study, QTL related to FRR resistance on Pv02 was found to span the region on the genome that encodes

a pathogenesis-related protein (PvPR2). Hagerty (2013) used SNP markers to construct genetic map and identified QTL related to FRR resistance as well as taproot diameter and shallow basal root angle. In that study, the physical position of the closest SNP (ss715641537) of the QTL for FRR resistance on Pv03 is 34.7Mb, which is very close to the QTL on Pv03 found in this study for root and shoot loss, and the other QTL for FRR resistance in that study has the closest SNP (ss715649511) on PV07 with physical position of 7.8Mb, which is distant from the QTL in this study. The closest SNP (ss715646264) at 33.9Mb to the QTL for taproot diameter on Pv02 is very close to the QTL for non-inoculated control root dry weight on Pv02 in this study. The QTL for shallow basal root angle on Pv05 had closest SNP (ss715645443) at 38.5Mb, which is very close to the QTL for seed weight and not far from the QTL for 2015 field shoot dry weight in this study. In general, by comparing the physical position of the QTL identified in Hagerty's (2013) study to this study, the QTL on Pv02, Pv03 and PV05 in this study were located in regions similar to QTL discovered in previous study, while the QTL on Pv07 in this study could be a novel QTL. The QTL detected on Pv11 in this study could also be a novel QTL and disease resistance genes for rust (Ur genes) were also identified near that region on Pv11 (Meziadi et al. 2016). Additionaly, the QTL found on Pv05 for seed weight and DF_2014 with physical position of 38.22Mb were found to be close the location of QTL for deep root weight and total biomass at 39.2Mb, and for FRR resistance at 39.46Mb in Nakedde et al.'s (unpublished) study in FRR resistance in a RIL population of two black bean genotypes.

QTL related to white mold resistance was detected on Pv01 near the *fin* gene (Miklas et al., 2001) where is the location of QTL for inoculated shoot dry weight and QTL for LG score in this study. Miklas et al. (2001) also identified QTL related to white mold resistance on Pv07 near the phaseolin seed protein (*Phs*) locus. Zuiderveen et al. (2016) identified QTL related to

Anthracnose resistance in Andean bean cultivars on Pv02 with SNP position at 48.6Mb, which is close to the QTL for control root dry weight on Pv02 in this study.

In conclusion, the inheritance of root rot resistance and plant biomass in the RIL population of CAL96 x MLB-49-89A was shown to be quantitative and heritability estimates tended to be high. The co-localization of QTL and correlation in root rot score and plant biomass, suggested that the plant biomass especially the root dry weight and root dry weight reduction were indicators of FRR resistance in addition to root rot score. The variation of results from field to field and from field to greenhouse were observed, indicating that the disease incidence was strongly influence by environment conditions. It is possible that environmental factors related to disease development in one field were absent in another field and in greenhouse, so the QTL associated with FRR resistance were different under different conditions. However, those RILs that were found to have resistance in more than one environment and contained alleles for most of the important QTL in this study could be used as good resources of root rot resistance. Additionally, with the availability of the whole bean genome sequence, disease related genes within the QTL can be annotated, which provides resources for further exploration for FRR resistance. For example, with the sequence information of those disease resistance genes, primers can be designed to run real-time quantitative PCR (RT-qPCR) to study the expression of the genes. The QTL detected in this study will facilitate the development of molecular markers for use in breeding for FRR resistance and the RIL population of CAL96 x MLB-49-89A phenotyped for FRR resistance will provide resource for selection of bean genotypes with FRR resistance introgressed from Middle American bean genotypes to Andean genotypes.

CHAPTER 3: CHARACTERIZAITON AND SNP IDENTIFICATION OF DISEASE RESISTANCE GENES VIA TRANSCRIPTOME ANALYSIS

INTRODUCTION

Disease as a biotic stress has been one of the major problem that constrains common bean production, especially for low input small scale farms in developing countries (Miklas et al., 2006). The use of cultivars with resistance has been considered the most economic long-term management option and breeding for the development of genetic resistance has been an important strategy to control disease (Abawi & Corrales, 1990). The identification and characterization of disease resistance genes will help the detection of disease resistance in crops and will facilitate the development of molecular markers for marker-assisted selection.

The mechanisms of the plant innate immune system include two levels: 1) the structural and biochemical barriers to prevent the invasion of pathogens or other pests; 2) the defense response on the molecular level that is triggered by the interaction between pathogen and plant (Michelmore et al., 2013). In gene-for-gene hypothesis, when a resistance gene (*R* gene) in the host plant matches an avirulence gene (*Avr* gene) in the pathogen, incompatibility occurs and the host plant shows resistance (Hammond-Kosack, 1997). The absence of one or both of the genes makes the plant susceptible to the disease.

Most R genes have the nucleotide-binding-site-leucine-rich-repeat (NB-LRR) structure (Michelmore et al., 2013), but there are also genes related to disease resistance that do not contain NB-LRR sequence. In common bean the anthracnose resistance genes *Co-1, Co-12, Co-13, Co-14, Co-15, Co-x,* and *Co-w*, the rust resistance gene *Ur-9* and angular leaf spot resistance gene *Phg-1* on chromosome Pv01, and anthracnose resistance genes *Co-5* and *Bct* gene for

resistance to Beet curly top virus (BCTV) on Pv07 are located in regions without NB-LRR sequences(Meziadi et al., 2016).

Gene expression information can be obtained by sequencing messenger ribonucleic acid (mRNA) and the total transcripts in a cell at a specific stage of development or physiological condition were analyzed through transcriptome analysis (Davidson, 2011). Transcriptome analysis is an asset for better understanding gene expression, genetic variation, and gene structure annotation associated with plant traits (Wang et al., 2009). Transcriptome analysis related to disease resistance genes will contribute to the understanding of gene expression patterns and the identification of candidate disease resistance genes.

Albion and Voyager are two white small seeded navy bean cultivars from Middle American gene pool. Albion was a cultivar released by Asgrow Seed Company in 1987 and Voyager was a cultivar released by Rogers Seed Company in 1995. The two genotypes were selected for the study on inheritance of seed Zn concentration, since Voyager had high seed Zn concentration while Albion had low seed Zn (Cichy et al., 2005). The RNA extraction and sequencing of these two cultivars were conducted previous at Michigan State University (MSU) Dry Bean Breeding and Genetics Program. Even though the total RNA was extracted from pods of Albion and Voyager under high Zn and low Zn treatments, the transcriptome profile contains the sequence information of all genes including disease resistance related genes.

The objectives of this study were to (a) identify and characterize the disease resistance related genes in common bean through the transcriptome profile of two navy bean genotypes Albion and Voyager, and (b) identify single nucleotide polymorphism (SNP) within disease resistance related genes.

MATERIALS AND METHODS

Transcriptome Profiles

Total RNA of Albion and Voyager was extracted from their pods collected 12 days after anthesis by Dr. Karen Cichy at MSU Dry Bean Breeding and Genetics laboratory. RNA sequencing was processed at the MSU Research Technology Support Facility (RTSF) using Illumina Genome Analyzer II (GA II). Carolina Astudillo-Reyes and Andrea Fernandez at MSU Dry Bean Breeding and Genetics laboratory received the transcriptome profiles in FASTQ format with 75-bp paired-end sequencing reads and pre-processing of the raw sequence data was conducted. The pre-processing includes using FASTQ Quality Trimmer and FASTQ Quality Filter to remove the sequences with low quality; using Bowtie v.0.12.7 (Langmead, 2010) and TopHat v 1.4.1 (Trapnell, 2009) for assembly and alignment of the reads to *P. vulgaris* reference genome; using Blast2GO software (http://www.blast2go.com) for gene annotation; using SAMtools (http://samtools.sourceforge.net/mpileup.shtml) for SNP variation detection and filtering. The discovered SNPs had presence in >90% of reads on both forward and reverse strand (Astudillo-Reyes et al. 2015).

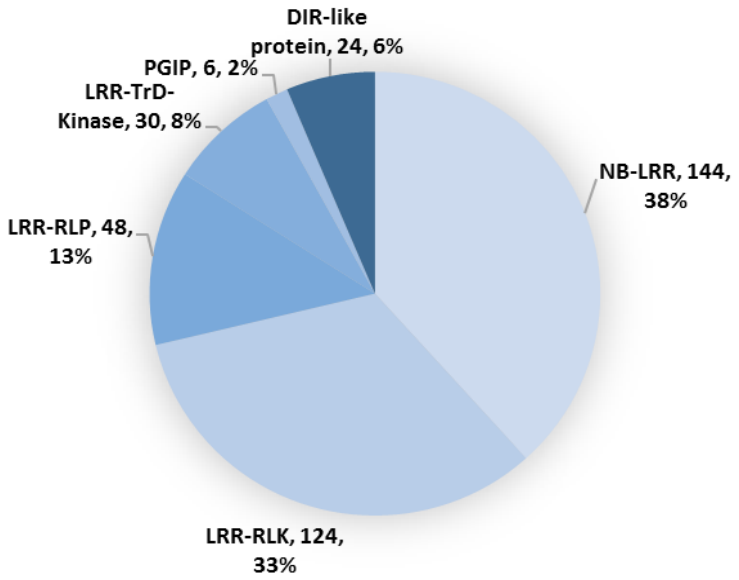
R Genes Identification

Disease resistance related genes were selected from the 19,534 genes with successful annotation by the author, and a map of the 11 common bean pseudomolecules with disease resistance genes and their physical positions was constructed with MapChart (Voorrips, 2002). R genes contained SNPs were selected and searched in Phytozome *P. vulgaris* database (http://www.phytozome.net) to obtain their genomic and coding sequence information.

RESULTS

In total, 376 disease resistance related genes were identified from the Albion and Voyager transcriptome profiles. These genes can be classified into six groups, including 144 genes encoding NB-LRR proteins, 124 genes encoding LRR-containing receptor-like kinases (LRR-RLKs), 48 genes encoding LRR-containing receptor-like protein(LRR-RLP), 30 genes encoding LRR transmembrane protein kinase (LRR-TrD-Kinase), six genes encoding polygalacturonase inhibiting proteins (PGIPs), and 24 disease resistance responsive dirigent-like (DIR-like) protein genes. The first five groups contained the LRR structure and comprised 94% of all these R genes. The groups of NB-LRR protein and LRR-RLK encoding genes were the two largest group that represented 38% and 33% of all those R genes. The DIR-like proteins do not contain LRR structure, and this type of genes occupied only a small portion of all the R genes (Figure 3.1).

Figure 3.1 Classes and numbers of disease resistance related genes (R genes) identified from the Albion and Voyager transcriptome profile. (NB-LRR: nucleotide-binding-site-leucine-rich-repeat; LRR-RLKs: LRR-containing receptor-like kinases; LRR-RLP: LRR-containing receptor-like protein; LRR-TrD-Kinase: LRR transmembrane domain protein kinase; PGIPs: polygalacturonase inhibiting proteins; DIR-like Protein: disease responsive dirigent-like proteins.)



The R genes identified from the Albion and Voyager transcriptome profiles distributed on all 11 chromosomes (Figure 3.2). Chromosomes Pv02, Pv04, Pv08, Pv10, and Pv11 had more R genes than other chromosomes, ranged from 39 to 49 genes per chromosome. The other chromosomes had number of R genes ranged from 19 to 29 genes per chromosome with least amount of R genes on Pv06. Most of these R genes were located at the two ends of the chromosomes, especially on Pv04, Pv08, Pv10 and Pv11. Only one NB-LRR gene was found on Pv09, but other types of R genes including LRR-RLK, LRR-RLP, LRR-TrD-kinase encoding genes were found to be distributed on this chromosome. The anthracnose resistance genes *Co-1*, *Co-12*, *Co-13*, *Co-14*, *Co-15*, *Co-x*, and *Co-w*, the rust resistance gene *Ur-9* and angular leaf spot resistance gene *Phg-1* were found in the region on Pv01where no NB-LRR sequence contained (Meziadi et al., 2016), but genes for LRR-RLK, LRR-TrD-kinase were found in that region. QTL associated with FRR resistance were detected in this study on chromosomes Pv02, Pv03, Pv07 and Pv11. R genes in the type of NB-LRR were found to be in or close to the QTL regions on Pv02, Pv03 and Pv11. No NB-LRR genes were found to be close to FRR resistance related QTL on Pv07, but genes of LRR-TrD-kinase were found in that region (Figure 3.2).

Figure 3.2 Physical map of the 11 common bean pseudomolecules with disease resistance genes identified from transcriptome profile of Albion and Voyager. (Red squares indicate the approximate positions of NB-LRR genes; Blue arrows indicate the locations of important Fusarium root rot resistance related QTL detected in Chapter Two; Red arrows indicate the approximate position of R genes with SNP; Bracket on Pv01 points out the approximate position of the atypical R genes in common bean.)

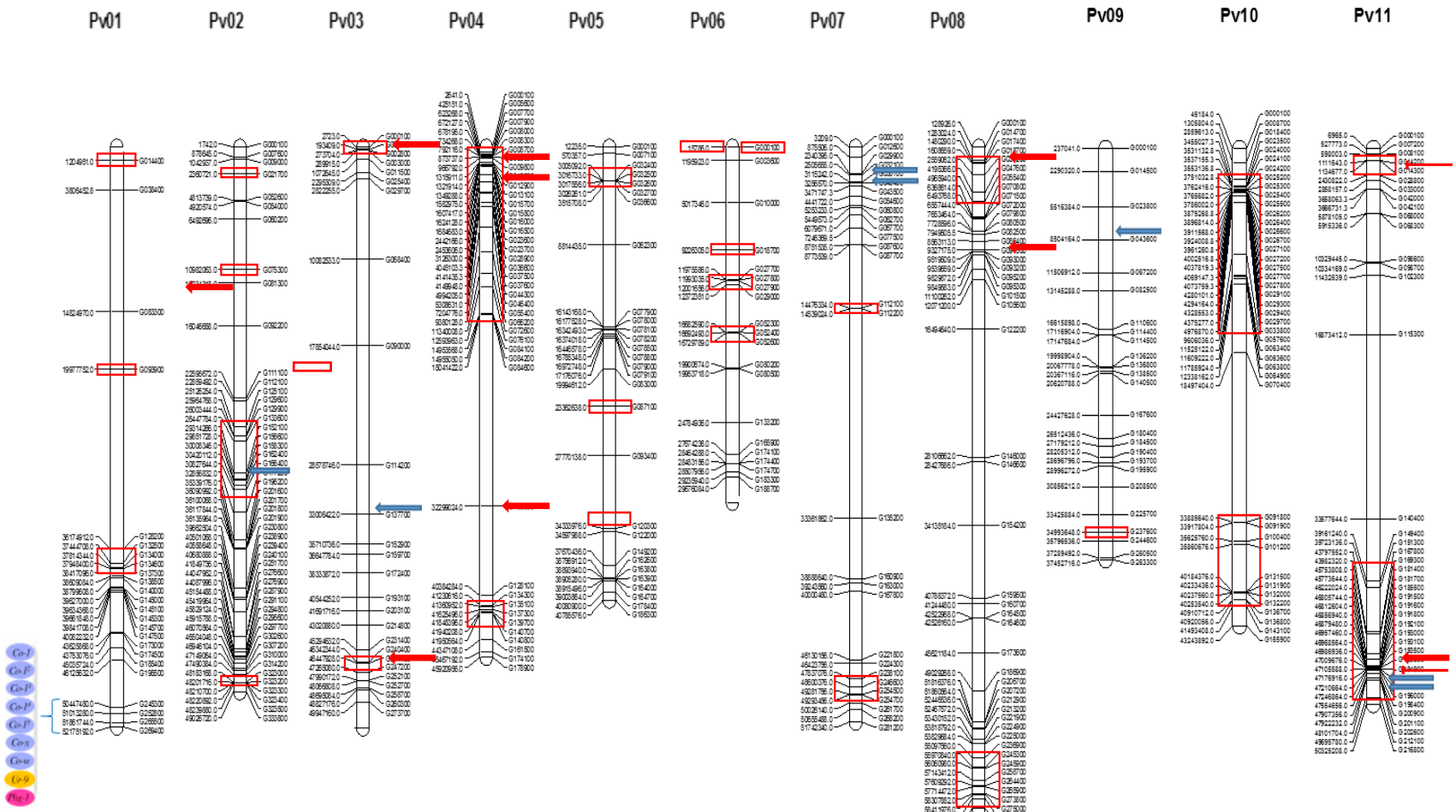


Table 3.1 Disease resistance related genes/gene family with SNPs also identified from the transcriptome profiles of Voyager and Albion, with the coding sequence (CDS) length, genomic length, number of SNPs, and physical position on the chromosomes.

P. vulgaris Genome ID	Family	CDS Length	Genomic length	SNPs in CDS	Chrom.	Physical Position(bp)	
						Start	End
Phvul.002G129700	CC-NB-LRR	3309	3581	1	Pv02	25,976,333	25,979,913
Phvul.002G131000	CC-NB-LRR	3291	3456	1	Pv02	26,192,610	26,196,065
Phvul.002G131200	CC-NB-LRR	3282	5856	1	Pv02	26,231,274	26,237,129
Phvul.003G002300	CC-NB-LRR	7674	15956	36	Pv03	193,409	209,364
Phvul.003G002400	CC-NB-LRR	6051	9483	1	Pv03	210,460	219,942
Phvul.003G002600	CC-NB-LRR	5700	12779	7	Pv03	245,309	258,087
Phvul.003G003000	CC-NB-LRR	5433	16126	8	Pv03	289,915	306,040
Phvul.003G247200	CC-NB-LRR	2511	5759	1	Pv03	47,265,082	47,270,840
Phvul.004G005600	CC-NB-LRR	3354	4533	3	Pv04	425,151	429,683
Phvul.004G008100	CC-NB-LRR	3123	4675	1	Pv04	688,650	693,324
Phvul.004G009300	CC-NB-LRR	3210	3997	2	Pv04	873,737	877,733
Phvul.004G012800	CC-NB-LRR	2907	3107	8	Pv04	1,315,911	1,319,017
Phvul.004G012900	CC-NB-LRR	3348	5386	7	Pv04	1,321,914	1,327,299
Phvul.004G013000	CC-NB-LRR	3366	5523	4	Pv04	1,338,425	1,343,947
Phvul.004G135100	TIR-NB-LRR	3423	7593	1	Pv04	41,360,953	41,368,545
Phvul.008G018700	DIR-like Protein	1191	1707	4	Pv08	1,608,659	1,610,365
Phvul.008G091600	DIR-like Protein	567	1176	1	Pv08	9,327,175	9,328,350
Phvul.011G014200	CC-NB-LRR	2748	3029	1	Pv11	1,111,643	1,114,671
Phvul.011G014300	CC-NB-LRR	2844	2927	4	Pv11	1,134,677	1,137,603
Phvul.011G193100	CC-NB-LRR	3561	7844	10	Pv11	46,968,564	46,976,407
Phvul.011G193500	CC-NB-LRR	3570	5179	2	Pv11	46,986,934	46,992,112
Phvul.011G193600	CC-NB-LRR	3396	3788	1	Pv11	47,009,677	47,013,464
Phvul.011G198400	CC-NB-LRR	3564	4509	2	Pv11	47,554,654	47,559,162
Phvul.011G200300	CC-NB-LRR	3540	3687	1	Pv11	47,805,798	47,809,484

Disease resistance genes with annotated SNPs were also identified from the transcriptome profiles of Voyager and Albion. A total of 24 disease related genes were found to contain SNPs and the number of SNPs in each gene ranged from 1 to 36. These genes are distributed on Pv02, Pv03, Pv04, Pv08 and Pv11. Only one of these genes was TIR-NB-LRR class and two of the genes were DIR-like protein coding genes, and the other 21 genes belonged to CC-NB-LRR class (Table 3.1). The Minor allele frequency (MAF) of these 108 SNPs ranged

from 6 to 100% (not shown in table), where the MAF below 5% is considered as rare variant and higher than 5% is considered as common variants.

DISCUSSION

The R-gene mediated pathogen resistance is an important defense strategy of plants against pathogen invasion. Many plant disease resistance (R) genes have been studied and utilized in plant improvement. In this study, 94% of the R genes contain LRR (Leucine-rich repeats) motif and the LRRs-containing proteins are diverse in structure and function. It is known that LRR-containing proteins are usually involved in protein-ligand and protein-protein interactions that detect the presence of potential pathogens and active the immune response signaling in plants (Kobe and Kajava, 2001). Many studied R genes encode NB-LRR domains and the number of NB-LRR proteins varied depending the plant species (Michelmore, 2013). In this study, 144 NB-LRR genes were highly expressed in the two bean cultivars, which is less than half of the 376 NB-LRR genes identified in the common beans reference genome. The possible reason for the difference resulted from that the RNA extracted from the two bean cultivars was not under any disease pressure. The distribution of the 144 NB-LRR genes on the 11 chromosomes is similar to the NB-LRR genes in the reference genome (Meziadi et al., 2016).

The LRR-RLK and LRR-RLP encoding genes also comprised a large group of R genes in this study and they play an important role in disease defense response in plants. The LRR-RLKs usually contain an extracellular LRR region with an N-terminal signal peptide, a single transmembrane-spanning region, and an intracellular serine-threonine kinase domain. LRR-RLPs has similar structure to LRR-RLKs, but they have a short cytoplasmic tail instead of the kinase

region in LRR-RLKs (Matsushima & Miyashita, 2012). In *Arabidopsis thaliana* genome, more than 600 RLKs have been annotated, but most of which have not been functionally characterized (Michelmore, 2013). PRR (Pattern Recognition Receptors) is a subset of RLKs and function as intercellular communicators in plant immune system. An example of PRR in plants is the *Xa21* in rice that confers broad-spectrum resistance. Examples of LRR-RLPs R genes include *Cf* and *Ve* genes in tomato and *RPP27* gene in Arabidopsis. In some studies, these genes were also considered as a sub-class of PRR that do not contain intracellular domains (Michelmore, 2013). The region of a cluster of R genes on Pv01 where no NB-LRR sequence contained was found to have genes of LRR-RLKs and also on Pv07 where QTL associated with FRR resistance was found to have genes of LRR-RLKs.

Six PGIPs genes were discovered in this study. PGIPs belong to LRR protein family and are plant cell wall proteins that interact with endopolygalacturonases secreted by pathogenic fungi and inhibit their enzymatic activity. PGIPs are also found to favor the accumulation of oligogalacturonides, which activate plant defense responses, and play roles in plant development and recognition of beneficial microbes (Di Matteo et al., 2003). The last group of the R genes detected in this study contains 24 DIR-like protein genes. DIRs are extracellular glycoproteins, which are thought to play important roles in plant secondary metabolism and ligand biosynthesis (Pickel & Schaller, 2013). Examples of DIR-like genes related to disease resistance include *Gbd 1* and *Gbd 2* isolated from cotton infected by *Verticillium dabliae* that encoded a class of cell-surface proteins related to receptor-mediated plant defense (Zhu et al., 2007), and a DIR-like gene (At1G64160) identified as a novel gene in a study of Arabidopsis with *Fusarium oxysporum* infection (Zhu et al., 2013).

The location of these genes with SNPs showed that they tend to locate at the two ends of the chromosomes within clusters of R genes. A gene (Phvul.011G200300) with SNP detected on Pv11 is close to a QTL related to FRR resistance in MLB-49-89A x CAL96 population with physical position of 48.9Mb. Since the transcriptome profile is from Albion and Voyager, it will be interesting to discover SNPs of R genes in the MLB-49-89A x CAL96 population to see if more correlation of the R genes with SNPs and the QTL of disease resistance will be found, and to compare the R genes discovered in different populations.

In conclusion, plant R genes are diverse in structure. Even though the NB-LRR encoding genes are the most prevalent class of R genes, there are also R genes that do not contain NB-LRR structure or do not contain LRR structure. The R genes are varied in function and the most common functions include pathogen recognition, signal transduction and resistance responses. Further structural and functional analysis of disease resistance related genes in common bean will be important for effective utilization of the resistance sources for cultivar improvement.

CONCLUSIONS

Soil borne diseases, such as Fusarium root rot, have been major constraints to common bean production in many production areas in the world. The characterization of the causal pathogen and identification of genetic resistance are needed for disease management and bean cultivar improvement.

In this study, the objectives of Chapter One were to determine the variations of virulence among different *Fusarium* isolates, and to select the suitable isolate and strategy to use in screening the RIL population of MLB-49-89A x CAL96 for FRR resistance. A total of 11 Michigan isolates were tested in the greenhouse and all were virulent from mild to severe on both MLB-49-89A and CAL96. Twenty African isolates were tested in a laboratory screening and 18 of them caused mild to severe disease severity on Zorro and Chinook cultivars. The different genotypes were also found to have variations in response to the inoculum. The FRR resistance of a bean genotype appear to be specific to the *Fusarium* isolate. The Michigan *F. brasilense* isolate F_14-42 which belongs to the *F. solani* species complex (FSSC) was selected for screening the RIL population of MLB-49-89A x CAL96 since significant difference of disease severity was found between the two parents. A greenhouse paper cup screening method was determined to be the most suitable for screening the RIL population with *F. brasilense* isolate F_14-42.

The objectives of Chapter Two were to characterize FRR resistance in the RIL populations to *Fusarium* species from Michigan and to identify related QTL with SNP markers. The FRR resistance in common bean was confirmed as a quantitative trait, and the environment had a significant effect on the FRR resistance of the population. The results also suggested that the plant biomass, especially root biomass and its reduction was a less subjective measurement than disease score to assess the FRR resistance of individual bean plants. QTL on Pv03 for

root/shoot reduction and QTL on Pv02 for root dry weight were found located close to the QTL in previous study for FRR resistance and taproot diameter, respectively, and the QTL on Pv07 for root/shoot reduction found in this study could be a novel QTL. The QTL on Pv02 for disease score and root dry weight, QTL on Pv03 for root/shoot reduction, and QTL on Pv11 for disease score in greenhouse should be further investigated for disease resistance genes verification since R genes were found close or in those regions. Additionally, those RILs that were found to have resistance in more than one environment and contained alleles for most of the important QTL in this study could be used as good resources of root rot resistance in future breeding work.

The objectives of Chapter Three were to characterize the R genes in common bean and identify SNPs in those R genes through the transcriptome profile of two navy bean genotypes. The results suggested that the R genes in common bean are diverse in structure and function. The LRR-containing protein/kinase encoding genes are the majority of the R genes, and the LRR-containing receptor like kinase comprise a significant group in R genes besides the well-known NB-LRR protein encoding genes. The SNPs exist in the R genes could be used for selection of favorable alleles in breeding for disease resistance. Additionally, the distribution of R genes identified in this study towards the two ends of the chromosomes agreed with the type of preference of R gene locations in common bean reference genome.

In general, the results of this study provide information of virulence variations among *Fusarium* isolates, useful genetic resistance resources available in bean cultivars for FRR resistance, and a better understanding of R genes in common bean.

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