IDENTIFICATION AND MAPPING OF QTL ASSOCIATED WITH FUSARIUM ROOT ROT RESISTANCE AND ROOT ARCHITECTURE TRAITS IN BLACK BEANS (*Phaseolus vulgaris* L.)

By

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ABSTRACT

IDENTIFICATION AND MAPPING OF QTL ASSOCIATED WITH FUSARIUM ROOT ROT RESISTANCE AND ROOT ARCHITECTURE TRAITS IN BLACK BEANS (*Phaseolus vulgaris* L.**)**

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This study identified and mapped QTL associated with physiological resistance to Fusarium Root Rot (FRR) disease, along with root architecture traits in a black bean Recombinant Inbred Line (RIL) population. The parents of this population were the resistant landrace 'Puebla 152' and the cultivar 'Zorro' that had no previously known resistance to FRR. One QTL associated with FRR resistance and four QTL associated with root architecture traits were detected. Total root weight and shallow root weight were associated with the same QTL on Pv09. A QTL associated with root length was detected on Pv01, and was found to be independent of the *fin* determinacy locus. The QTL associated with FRR resistance, along with another associated with deep root weight and total plant biomass were detected 260 kbp apart on Pv05. None of the detected QTL accounted for more than 13% of phenotypic variation, indicative of the fact that FRR resistance and root traits are governed by several genes of minor influence. Puebla 152 was the source of all the beneficial alleles governing the QTL detected. Additionally, two field surveys were conducted in the common bean-growing areas of Uganda to update the available information on the extent and severity of bean diseases. Root rots, caused by Sclerotium and Fusarium species, along with insect damage by the bean beetle (*Callosobruchus maculatus*) were found to be the most significant biotic constraints in the areas surveyed.

To my parents, Mr. and Mrs. Charles Nakedde, and to my siblings.

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KEY TO ABBREVIATIONS

BLAST- Basic Local Alignment Search Tool

CIAT- International Center for Tropical Agriculture.

IPM-*PIPE*- Integrated Pest Management –Pest Identification Platform for Extension and Education.

NIFA- National Institute of Food and Agriculture.

P C- Personal Communication.

QTL- Quantitative Trait Locus/Loci.

Chapter One

IDENTIFICATION AND MAPPING OF QTL ASSOCIATED WITH FUSARIUM ROOT ROT RESISTANCE AND ROOT ARCHITECTURE TRAITS IN BLACK BEANS (*Phaseolus vulgaris* L.)

General Introduction

Common bean (*Phaseolus vulgaris)* is the most important legume in human diets (Beebe et al. 2012). It provides an inexpensive source of high quality protein and minerals, especially in sub-Saharan Africa, where common bean is usually called "the meat of the poor". Common bean is also a source of income for many families in Latin America and Africa (Wortmann et al. 1998). Common bean production intensity in Africa (Ratio of gross cropped area to area cropped by common bean) is highest in areas of high population density where few sources of adequate protein exist (Turner et al. 1978; Wortmann et al. 1998).

Annual common bean yields in the developing world average 0.5 million metric tons/Ha, compared to about 2.0 metric tons/ Ha in the developed world (FAOSTAT, 2015). Lower yields in the developing world are due to both biotic and abiotic stresses. The abiotic stresses may include drought, salinity, and low soil fertility (Beebe et al. 2012; Beaver et al. 2003). One of the most important biotic stresses is disease, such as root rots. Root rots are caused by a complex of *Fusarium solani Rhizoctonia solani,* and *Pythium* species, which result in stunting, chlorosis, poor stands, rotting of roots, and yield reduction up to

85% (Abawi and Pastor-Corrales, 1990; Burke and Miller, 1983; O'Brien et al. 1991). The most widespread of root rots is caused by *Fusarium solani* f sp. *phaseoli* (FSP) (Abawi and Pastor-Corrales, 1990; Burke and Miller, 1983). In Uganda, bean root rots have been reported to cause 100% loss (Tusiime, 2003). Root rots have also gained importance in other East African countries like Kenya, The Democratic Republic of Congo, Malawi, and Rwanda (Mukankusi et al. 2011; Otsyula et al. 2003; Rusuku et al. 1997).

Fusarium root rot (FRR), characterized by reddish lesions on the root and hypocotyl, is particularly severe on large-seeded Andean genotypes due to their lack of genetic resistance (Abawi and Pastor-Corrales, 1990; Dickson, 1973). Control of Fusarium root rot is difficult for two main reasons. First, it is a soil-borne fungus and so fungicide use is expensive, highly regulated and not always effective, (Snapp et al. 2003). Second, in the developing world, resource-poor farmers who are not able to incorporate crop rotation or fallow into their cultivation practices produce beans.

Due to these constraints, the most effective way to control this disease is to exploit host plant resistance. FRR is quantitative in nature and appears to be a combination of physiological mechanisms and root system avoidance due to architecture traits such as root dry weight, root length and root mass (Cichy et al. 2009; Kamfwa et al. 2013; Mukankusi et al. 2011; Román-Avilés et al. 2004; Snapp et al. 2003). This resistance has been reported in small-seeded Middle-American genotypes but is absent in the large-seeded Andean genotypes, which are generally preferred in Africa (Román-Avilés et al. 2011; Schneider et al. 2001; Wortmann et al. 1998). Finding Quantitative Trait Loci (QTL) that

explain a large amount of the variation is critical to enable eventual introgression of resistance from the resistant Middle-American genotypes into the susceptible but preferred large-seeded Andean genotypes via marker-assisted backcrossing.

Literature review

Origin, domestication and gene pools of common bean

Common bean belongs to family Fabaceae (Leguminosae), the third largest family of flowering plants, after the Orchidaceae and Asteraceae (Smýkal et al. 2014). The crop belongs to the genus Phaseolus which has 76 species divided into two clades A and B (Delgado-Salinas et al. 2006). The five most important cultivated crop species of the genus, *P. vulgaris* L. (common bean), *P. coccineus* (scarlet runner bean), *P. polyanthus* Greenm. (Year bean), *P. acutifolius* (tepary bean), and *P. lunatus* L. (lima bean) all belong in clade A. (Delgado-Salinas et al. 2006). Common bean, grown in all continents of the world except Antarctica, is a diploid (2n=2x=22), short-day predominantly self-pollinating species. The main products of common bean are shell beans (harvested at physiological maturity), dry beans (harvested at complete maturity) and snap beans (pods harvested before seed development) (Gepts, 1998; Koenig and Gepts, 1989).

The cultivated common bean originated from wild common bean, that can be found in Northern Mexico, all the way to Northwestern Argentina (Singh et al. 1991). The wild ancestors of common bean are self-pollinating and easily hybridize with cultivated common bean producing viable offspring (Gepts and Debouck, 1991). Wild common bean is organized in two geographically isolated and genetically differentiated gene pools (Middle-American and Andean) that diverged from a common ancestral wild population more than 100,000 years ago, before domestication (Bitocchi et al. 2013; Mamidi et al. 2013) Wild common beans are divided into two sub-groups, *P. vulgaris* var. *arborigineus* and *P. vulgaris* var. *mexicanus*, the wild progenitors of the Andean and Middle-American gene pools respectively. These two sub-groups are distinguishable at both molecular and

morphological levels (Gepts and Debouck, 1991).

Wild common bean has undergone major changes during domestication to cultivated common bean, some of which include appearance of indeterminate and determinate upright bush growth habits, gigantism of leaf, pod, and seed characteristics; suppression of explosive pod dehiscence, loss of seed dormancy, appearance of a vast variety of seed sizes, shapes, and colors, and selection for insensitivity to photoperiod (Gepts, 1998; Singh et al. 1991).

Cultivated common bean is made up of two genetically and morphologically distinguishable gene pools, the Andean and Middle-American (Beebe et al. 2001; Gepts and Debouck, 1991). These two gene pools appear to have diverged before domestication, each with its own geographical distribution. The Middle-American gene pool is native to Central America, Mexico and Colombia, while the Andean is from southern Peru, Bolivia and northern Argentina (Gepts, 1998; Koenig and Gepts, 1989). The members of the Middle-American gene pool typically have smaller seed size (< 25-40g/100 seed) while the Andean gene pool members possess larger seeds (>40 g/100 seeds) with kidney or cylindrical shapes that vary greatly in color (Singh et al. 1991). These gene pools are further classified into different races based on morphological and agronomic adaptation. The Middle-American gene pool contains races Durango, Jalisco and Mesoamerica, while the Andean gene pool is constituted by races Chile, Nueva Granada, and Peru (Singh et al. 1991; Gepts et al. 1991)**.**

The gene pools show incomplete reproductive isolation (Gepts, 1998). One of the reasons for this isolation could result from independent divergence before domestication (Kwak and Gepts, 2009). The other could be due to favorable epistatic combinations for each one

of the gene pools, which would be undone in the recombinant progeny. Moreto et al. (2011) reported significant additive by additive, dominant by dominant and dominant by additive epistasis for yield components in a Mesoamerican x Andean gene pool cross. The presence of these distinct gene pools also suggests that common bean may be in the process of further speciation of each of the gene pools (Kwak and Gepts, 2009; Papa and Gepts, 2003).

Agronomic characteristics of common bean

Dry bean is an annual short-season common bean seed type that matures in 85 to 100 days (Katungi et al. 2009; Kelly et al. 2012). It is largely a self-pollinated plant although cross-pollination is possible if the stigma contacts with pollen coated insects such as bees (Rusuku et al. 1997). Dry bean exhibits four types of growth habits namely, Type I, the determinate bush habit, Type II, the upright indeterminate short vine habit and Type III, the prostrate indeterminate vine type habit and type IV, the climbing bean (Singh, 1991). The non-climbing types are the most common in North America. In the Andes and Africa, most landraces are of either type I or type IV, while in North America, research has resulted in the release of cultivars with type I and type II growth habits, that are more amenable to mechanization (Beebe, 2012; Katungi et al. 2009; Kelly and Cichy, 2013). The crop does best in soils that are reasonably fertile, well drained and does not have conditions that interfere with germination and emergence (Wortmann et al. 1998). Common bean is cold intolerant. It can tolerate high temperatures if adequate soil moisture is available (Avila and Blair, 2012; Katungi et al. 2009; Kelly and Cichy, 2013). In tropical and sub-tropical regions, the crop grows best at altitudes greater than 1000 meters above sea level (masl), however areas lower than 1000 masl have recently been used for dry bean

production due to increasing population pressure and development of more heat tolerant cultivars (Beebe, 2012; Buruchara 2007; Katungi et al. 2009).

Global Distribution of common bean

The main dry bean production regions are the Americas, Caribbean, Eastern and Southern Africa and Asia (Beebe, 2012; Kelly and Cichy, 2013). In the United States, dry beans are largely produced in northern states or at higher elevations in the intermountain and western states (Kelly and Cichy, 2013). In Africa, dry beans are mainly produced in Eastern and Southern Africa (Katungi et al. 2009), in the Caribbean and Latin America, Mexico and Brazil are the main producers. China is also major producer in Asia. India and Myanmar are listed as high producers, although these figures might include tonnage from other species such as lima and butter beans (*P. lunatus* and *P. coccineus*) and as a result may not accurately represent the area planted to common bean (Akibode and Meridia, 2011; Beebe, 2012; Kelly and Cichy, 2013; FAOSTAT, 2015).

In the United States, dry bean is produced under rain-fed conditions in the East, Midwest and Upper Midwest, while mainly all western production in semiarid states is grown under irrigation, with the highest yield in the western irrigated regions (Kelly and Cichy, 2013). The entire production process is mechanized, from planting, to harvest. In Central America, Brazil, and Eastern and Southern Africa beans are often intercropped in traditional mixed systems, often with maize, or occasionally with cassava, sorghum, or pigeon peas (Hyman et al. 2008). In these production systems, fertilizers and other inputs like pesticides may or may not be used. In much of Africa, beans are produced for home consumption, although this is slowly changing, to include production for the local and foreign markets. In areas where market-oriented production is practiced, for example in

Brazil, dry bean production is practiced in monocultures and often mechanized (Katungi et al. 2009; Kelly and Cichy, 2013; Maryrose et al. 2014)

Production trends

In the United States, dry bean yields had been stagnant at around 1.5 MT/ha in the 20 years between 1960-1979 (Kelly and Cichy, 2013). This prompted increased interest and effort into both breeding and agronomic practices among the dry bean breeding and farming community to increase yield (Kelly and Cichy, 2013). According to FAOSTAT (2014), the yield has steadily risen from 1.5 MT/ha in 1993 to 2.0MT/ha in 2013. This translates into a 1.5% annual yield increase (Fig1.1) Total production has generally ranged from 1.0 to 1.5 million tons between 1993-2013, except for 2001, 2004 and 2011, when 900,000, 800,000 and 900,000 MT were reported respectively due to variable weather conditions. Vandermark et al. (2014) present an optimistic picture as it pertains to yield. They assert that dry bean cultivars have yet to reach a yield plateau for most market classes, and that continued introgression of germplasm from other races of common bean should provide new sources of genetic diversity to enhance yield in the future.

In Eastern Africa, Tanzania is the leading producer of common bean followed by Uganda and then Kenya (FAOSTAT, 2015). The production in East Africa has generally been on an upward trend (table 1.2) except for Ethiopia in 2013 due to recent intermittent droughts (Viste et al. 2013). This upward trend could be attributed to the breeding efforts from The International Center for Tropical Agriculture (CIAT) along with the National Agricultural Research Stations (NARS). This collaborative effort has resulted in the release of a number of improved cultivars that have been largely adopted by the bean growers (Beebe, 2012).

Common bean production constraints

Two major types of constraints affect common bean production; abiotic and biotic constraints. The main abiotic constraints include drought, high temperatures, excessive and erratic rainfall, nutritional disorders such as nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg), zinc (Zn), and calcium (Ca) deficiencies; and manganese (Mn), aluminum (Al) and salt (NaCl) toxicities (Allen et al. 1996; Schwartz and Pastor-Corrales, 1989; Wortmann et al. 1998). These constraints are more severe in Africa and the tropics where irrigation and fertilizer use is minimal and soil types are usually low in pH (Wortmann et al. 1998). Low pH worsens an already serious situation because it directly leads to the toxicities and deficiencies described above (Thung, 1990).

Diseases and pests are the major biotic constraints. The important diseases include foliar diseases like angular leaf spot (*Pseudocercospora griseola),* halo blight (*Pseudomonas syringae* pv. *phaseolicola*), common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) anthracnose (*Colletotrichum lindemuthianum*). Viral diseases, that is; Bean Common Mosaic Virus (BCMV) and Bean Common Necrosis Virus (BCNV), along with nematodes have also gained importance in recent years. (Rusuku et al. 1997; Schwartz, 2005; Sikora et al. 2005; Mukankusi et al. 2011; Wortmann et al.1998).

The insect pests include foliage pests such as the bean stem maggot (beanfly) (*Ophiomyia phaseoli*), cutworms (larvae of various moths mostly in the genera *Agrotis* and *Spodoptera*), striped bean weevil (*Alcidodes leucogrammus*), foliage beetles (*Ootheca mutabilis* and *O. bennigseni*), black bean aphid (*Aphis fabae*, and *A. craccivora*), common whitefly (*Bemicia tabaci*), leaf hoppers (*Empoasca dolichi* and *E. lybica),* and storage

pests like the bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) (Karel and Antrique, 1989; Allen et al. 1996; Shwartz and Steadman, 1989).

Root rots are a major concern in bean producing areas worldwide and have been frequently reported as major threats to bean crops in northeast Brazil, Mexico, Nicaragua, coastal Peru, and the United States (Abawi and Pastor-Corrales, 1990; Kamfwa et al. 2014; Obala et al. 2012; Mukankusi et al. 2011).

Root rots of common bean

A complex of soil-dwelling pathogens cause a range of root rot diseases. These fungi include *Fusarium solani* spp. complex that causes Fusarium root rot (FRR); *Rhizoctonia solani* that causes Rhizoctonia root rot; *Sclerotium rolfsii* that causes Sclerotium root rot; *Macrophomina phaseolina* that causes Charcoal rot; and *Pythium ultimum* that causes Pythium root rot. (Abawi and Pastor-Corrales, 1990; Burke and Miller, 1983; Mukankusi et al. 2011, Nzungize et al. 2011). The range of symptoms of the bean root rot complex include elongated water-soaked lesions on the roots and hypocotyls that may cause wilt and kill young plants, dark brown or reddish roots, sunken lesions in lower hypocotyls, rotting of lateral roots, and vascular discoloration of the upper taproot or lower stem (Navarro et al. 2008). Fusarium induced root rots are the most widespread and devastating, causing yield losses of up to 84% reported (Abawi and Pastor-Corrales, 1990; Burke and Miller, 1983; Kamfwa et al. 2014; Obala et al. 2012; Mukankusi et al. 2011).

Another *Fusarium* species, *F. oxysporum,* is also soil dwelling and infects roots, however this species complex proceeds to colonize the vascular tissue, causing fusarium wilt. Symptoms include yellowing, wilting, and necrosis of leaf and stem tissue, which often

results in hastened maturity, characterize the disease, along with decreased seed size, and yield loss (Pastor-Corrales and Abawi, 1987; Schwartz et al. 1996).

Fusarium root rot (FRR)

This is the type of root rot caused by Fusarium spp. excluding *F. oxysporum*. *Fusarium solani* f.sp. *phaseoli* (FSP) is the most common of these root rot causing species (Abeysinghe, 2012; Bilgi et al. 2008; Singh et al. 2012). The pathogen infects seedlings, about a week after emergence. Initial symptoms are narrow, long, red to brown streaks on the hypocotyls and taproot, then a black discoloration of the tap and lateral roots. The taproot may die, depending on the virulence of the strain. An 80-100% reduction of root mass has been reported in later stages of the disease (Bilgi et al. 2008). Above ground symptoms include stunting, yellowed leaves and a reduction in number of pods. The severity of the disease is increased when the plants are subjected to environmental stresses like drought, excess moisture, low temperatures, soil compaction, low soil fertility, low pH and other pathogens like Pythium or root-knot nematodes (Abawi and Pastor-Corrales, 1990; Abeysinghe, 2012; Agrios, 2005; Mukankusi, 2008).

Taxonomy of Fusarium solani FSP phaseoli (FSP)

The fungus FSP belongs to the genus Fusarium. Most of the members of this genus are pathogenic to either plants or animals (Leslie et al. 2008). This genus is divided into 11 subdivisions, called sections, depending on conidium and colony morphology (O'Donnell, 2000).

FSP belongs to section *Martiella* and contains species that are either homothallic (self-fertile) or heterothallic (outcrossing) (Leslie et al. 2008).

FSP is very persistent in soil as chlamydospores, and capable of surviving in infested soils for long periods of time (Abawi and Pastor-Corrales, 1990; Abeysinghe, 2012; Hasanzade et al. 2008). The fungus can also survive as mycelium or spores in infected or dead tissues (Abawi and Pastor-Corrales, 1990; Leslie et al. 2008). Nutrient exudates from germinating seeds and root tips trigger FSP spore germination. The fungus then invades the plants by secreting enzymes that degrade the cell wall (cutinases) and other plant defense chemicals like phytoalexins, and growing through cortical tissues (Belete et al. 2013; Burke et al. 1980; Leslie et al. 2008; Mukankusi, 2008). FSP is more virulent on large-seeded dry beans than the small-seeded (Abawi and Pastor-Corrales 1990; Buruchara et al. 1999). Hyakumachi et al. (1995), reported larger sized FSP conidia and chlamydospores induced from kidney bean root exudates, and speculated that these exudates contain more carbohydrates and protein than exudates from other cultivars, which probably explains the higher virulence of FSP on kidney beans.

Management of fusarium root rot (FRR)

There is no single management strategy that is completely effective against FRR. Through a combination of strategies, that is cultural, biological and chemical, the disease can be managed. Cultural practices like crop rotation with non-hosts, reducing compaction, improving soil fertility, and using disease free seed. However, seed treatment with fungicides is only partially effective (Abawi and Pastor-Corrales, 1990; Kraft et al. 1981; Miller and Burke, 1985).

Biological control has been reported to be effective. This has been accomplished by the use of organisms that are antagonistic to FSP. Some of these include arbuscular mycorrhizae, rhizospheric bacteria and fungi. Filion et al. (2003) reported a reduction of disease

incidence and FSP propagule numbers in soil inoculated with arbuscular mycorrhizae. Mudawi et al. (2014) and Abeysinghe (2012) reported significant reduction of fusarium infection when soil was inoculated with rhizobacteria *Trichoderma harzianum* and *Bacillus* spp. These are still largely experimental findings and are yet to be effectively commercialized.

Genetic control of FRR resistance

The most effective way to control FRR is through host plant resistance (Kamfwa et al. 2014; Mukankusi et al. 2011; Obala et al. 2012; Román-Avilès et al. 2011). Fusarium root rot resistance has been associated with small seed size, late maturity and indeterminate growth habit. All these are traits commonly found in genotypes of the Middle-American gene pool (Burke et al. 1980; Mukankusi et al. 2011; Schneider et al. 2001; Román-Avilès and Kelly, 2005).

A number of sources of FRR resistance have been reported, mainly from the Middle-American gene pool, like cultivars MLB-49-89A, Puebla-152, Negro San Luis or in *P. coccineus* derived materials, like cultivars FRR 266, and NY2114-12. (Beebe et al. 1981; Kamfwa et al. 2013; Miklas et al. 2006; Navarro et al. 2008; Román-Avilés and Kelly, 2005 Ronquillo-López et al. 2010).

The genetic control of FRR resistance has been a subject of extensive research efforts since the 1920's. This is probably because root rot had long been recognized as an important disease. McRostie et al. (1921) hypothesized that two duplicate recessive genes control FRR resistance. Yerkes et al. (1956) concluded that major dominant genes condition resistance, after all F_1 progeny from a cross between *P. coccineus* and *P. vulgaris* were

resistant. Bravo et al. (1969) reported that 3 dominant genes from *P. coccineus* conditioned the resistance. However Boomstra and Bliss (1977) reported that FRR resistance was quantitatively inherited and was recessive to susceptibility. Mukankusi et al. (2011) reported that genes with additive and non-additive effects governed FRR inheritance, implying a combination of recessive and dominant genes, and that maternal effects, along with epistasis also contributed to the phenotype. Obala et al. (2010) developed three double cross recombinant inbred line (RIL) populations with six cultivars, four of which were FRR resistant. The authors reported two, three and four genes independently conditioned resistance in each of the RIL populations, respectively. This disparity and disagreement in findings only proves just how complex the genetic control to FRR resistance is, and that it is affected by source of genetic resistance, screening method, environment and epistatic interactions. Hence the need to implement marker-assisted selection when breeding for FRR resistance so as to increase gain from selection is essential.

The advent of molecular markers has enabled mapping of the quantitative loci (QTL) associated with FRR resistance. Several studies have reported QTL associated with FRR; Schneider et al. (2001) used 156 RAPD markers to identify seven QTL in two RIL populations from FR 266, an Andean, FRR resistant line derived from the resistance source PI 203958, a weedy bean landrace from Mexico, and 'Montcalm', a susceptible cultivar. The QTL were located on linkage groups B2 and B3, close to Pathogenesis-Related (PR) protein genes. These are low molecular weight compounds produced by the plant in response to pathogen attack (Van Loon, 1985). Individual QTL detected in this study didn't explain more than 15% of phenotypic variation.

Román-Avilés and Kelly (2005), used 350 RAPD markers to identify nine QTL on linkage

groups B2 and B5, that accounted for 5-53% of phenotypic variation in two inbred backcross line populations derived from the susceptible cultivars 'Red Hawk' and 'C97407' each crossed with the resistant black bean line 'Negro San Luis'. This study confirmed the efficacy of Marker Assisted Backcrossing (MABC) in the introgression of beneficial alleles across gene pools.

Navarro et al. (2008) also identified six QTL using RAPD markers in a RIL population from 'Eagle' and 'Puebla 152', a FRR resistant, black-seeded dry bean from Mexico. Ronquillo-Lopez et al. (2010) using the same Eagle and Puebla 152 RIL population reported that QTL associated with *F. oxysporum* f. sp. *phaseoli*, FSP and *A .euteiches* are independent. Puebla 152 was first reported as FRR resistant by Beebe et al. (1981).

Navarro et al. (2009) identified a major FRR resistance QTL explaining 25-49% of phenotypic variation that is associated with RAPD markers AD9.950 and S18.1500 in repulsion phase. Using MABC, the authors were able to introgress this QTL into four BC_1F_3 derived from crossing Puebla 152 with susceptible cultivars 'Hercules' and 'Nicelo'. Kamfwa et al. (2013) identified a major QTL explaining 34% of the variation on linkage group B3, using Simple Sequence Repeat (SSR) markers. In this study, two RIL populations were used from a cross between 'K132' and 'K20', both from the Andean gene pool with large red mottled-seed types with 'MLB-49-89A', a medium-seeded black bean from the Middle-American gene pool as the recurrent FRR resistant parent.

The use of single nucleotide polymorphism (SNP) markers promises to further elucidate the genomic location of resistant loci. SNPs are more numerous and cover more of the genome than any other marker hence can provide a better resolution of the QTL positions.

Hagerty et al. (2015) located QTL associated with FRR resistance, Aphanomyces root rot resistance along with root traits like tap root diameter in a snap bean RIL population, using 1,689 SNP markers. The RIL population was a cross between the highly root rot resistant line RR6950, a small seeded black indeterminate type III accession of unknown origin and OSU5446, a highly root rot susceptible determinate type I bush snap bean cultivar. The QTL associated with FRR resistance was located on Pv03 and explained 9% of the variation. Both QTL for Aphanomyces root rot resistance and taproot diameter mapped closely on Pv02. Additional QTL for Aphanomyces root rot resistance were located on Pv04 and Pv06.

Mechanisms of resistance to FRR

No commercial cultivars have shown complete resistance (immunity) to FRR (Bailey et al. 2003; Foroud et al. 2014; Mukankusi et al. 2011 Obala et al. 2012). The mechanisms of resistance to FRR are subject of much speculation. Pierre et al. (1970) reported a hypersensitive reaction in FRR resistant species of *P. coccineus* and *P. vulgaris.* The highly resistant varieties rapidly accumulated brown deposits (a periderm) in the cortical region. The moderately resistant species also formed a periderm, however, it was much deeper in the endodermis, where the fungus never reaches. It was concluded that in the highly resistant species, the periderm probably contributes to the resistance, but in the moderately resistant species, a chemical pathway, not the periderm, may be responsible for the resistance.

It is speculated that FRR resistance in the small and black-seeded genotypes is due to the fact that they accumulate more of the fungi-toxic phenolic compounds like phytoalexins,

than the large-seeded Andean genotypes (Mukankusi, 2008; Statler, 1970). This theory is further supported by the findings of Schneider et al. (2001), who reported that most of the QTL associated with root rot resistance have been identified in bean genomic regions close to resistance and defense genes like pathogenesis-related proteins (*PVPR-2*), polygalacturonase-inhibiting protein (*Pgip*) and chalcone synthase (*ChS*) are located.

A large and robust root system has also been associated with root rot resistance, mainly due to disease escape. These plants can easily recover from root rot pathogen attack by compensating the destroyed tissue with more healthy tissue, as well as grow away from regions with high pathogen concentration. (Cichy et al. 2007; Kraft and Boge, 2001; Huisman, 1982; Román-Avilés et al. 2004; Snapp et al. 2003).

Snapp et al. (2003) investigated the contribution of vigorous adventitious root towards enhancing root rot resistance in snap bean. The authors found that lateral root number at root:shoot interface and root diameter were correlated with root rot tolerance.

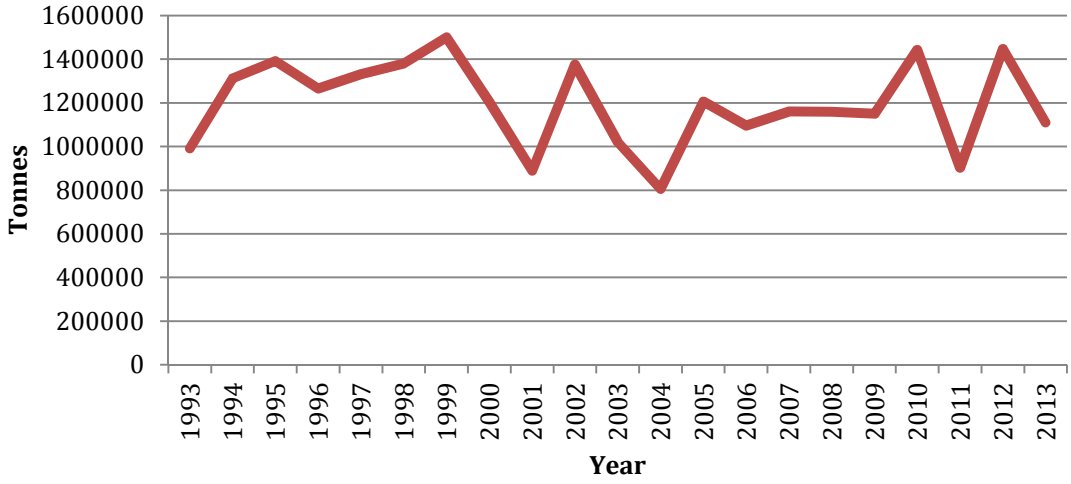
All these findings are further proof that FRR resistance is a quantitatively inherited trait, with different sources of resistance having different combinations of the beneficial genes that contribute to the observed partial resistance. This along with the great influence of environmental factors to the trait present a challenge to bean breeders working to enhance root rot resistance (Hadwiger, 2015; Ma et al. 2013; Michelmore et al. 2013).

The overall goal of this thesis was to identify QTL associated with FRR resistance and root traits in common bean (*P. vulgaris L.*) using SNP markers. The reported large effect QTL,

and their associated markers would aid in the understanding of the genetic control of FRR resistance and assist future marker-assisted breeding efforts for FRR resistance.

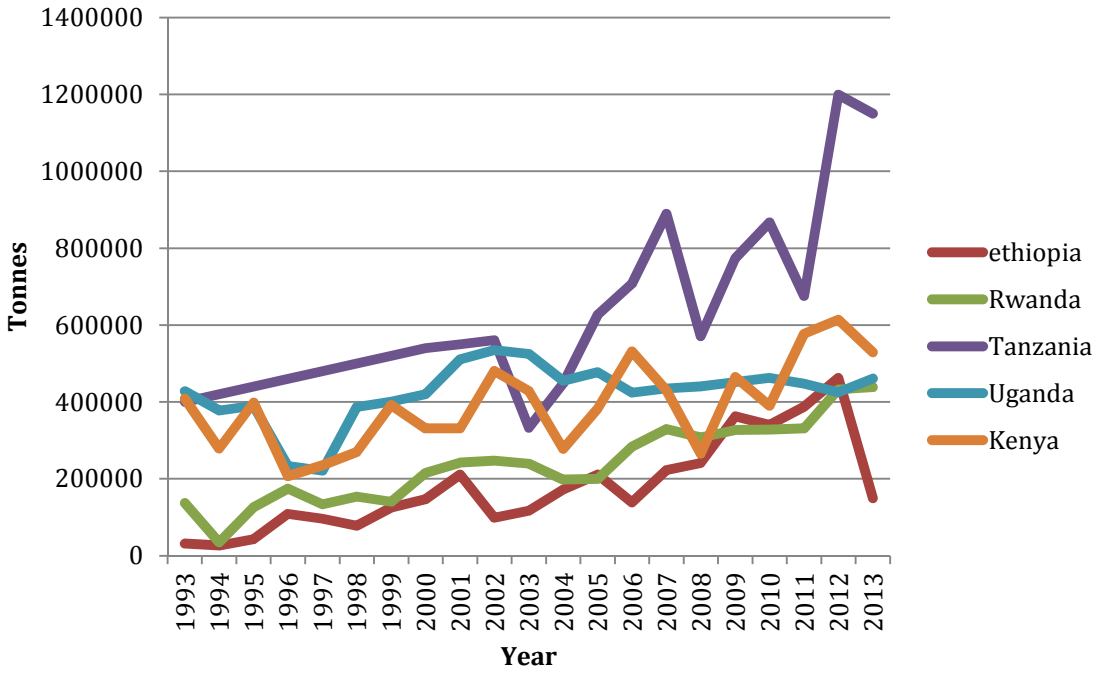
APPENDIX

Figure 1.1: Dry bean production in the USA (Tonnes) from 1993-2013



Source, FAOSTAT, 2015

Figure 1.2: Dry Bean production trends in Eastern Africa (1993-2013)



Source, FAOSTAT, 2015

REFERENCES

REFERENCES

Abawi, G. S., and Pastor-Corrales, M. A. (1990). Root rots of beans in Latin America and Africa, Diagnosis, research methodologies, and management strategies. CIAT publication No. 35, ISBN, 958-9183-14-X.

Abeysinghe, S. (2012). Biological control of *Fusarium solani f. sp. phaseoli* the causal agent of root rot of bean using *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01. Ruhuna Journal of Science, 2(1).

Agrios, G. A. Plant Pathology (Fifth ed.) Elsevier Academic Press (2005), p.922.

Akibode, S., and Maredia, M. (2011). Global and regional trends in production, trade and consumption of food legume crops. Department of Agricultural, Food and Resource Economics, Michigan State University, 87.

Allen, D. J., Ampofo, J. K. O., and Wortmann, C. S. (1996). Pests, diseases, and nutritional disorders of the common bean in Africa, A field guide. CIAT publication No. 260, ISSBN, 958-9439-55-1 CIAT, Cali, Colombia.

Avila, T., Blair, M. W., Reyes, X., and Bertin, P. (2012). Genetic diversity of bean (*Phaseolus*) landraces and wild relatives from the primary centre of origin of the Southern Andes. Plant Genetic Resources, 10 (01), 83-92.

Bailey, K. L., Gossen, B. D., Gugel, R. K., Morrall, R. A. A. (2003). Diseases of field crops in Canada (3rd edn). University Extension Press, University of Saskatchewan, Saskatoon.

Beebe, S. E., Bliss, F. A., and Schwartz, H. F. (1981). Root rot resistance in common bean germplasm of Latin American origin. Plant Disease, 65, 485-489.

Beebe, S., Rengifo, J., Gaitan, E., Duque, M. C., and Tohme, J. (2001). Diversity and origin of Andean landraces of common bean. Crop Science, 41(3), 854-862.

Beebe, S. (2012). Common bean breeding in the tropics. Plant Breeding Reviews, 36, 357–426.

Beaver, J. S., Rosas, J. C., Myers, J., Acosta, J., Kelly, J. D., Nchimbi-Msolla, S., Misangu, R., Bokosi, J., Temple, S., Arnaud-Santana, E., and Coyne, D. P. (2003). Contributions of the Bean/Cowpea CRSP to cultivar and germplasm development in common bean. Field Crops Research, 82(2), 87-102.

Berta, G., Sampo, S., Gamalero, E., Massa, N., and Lemanceau, P. (2005). Suppression of Rhizoctonia root-rot of tomato by *Glomus mossae* BEG12 and *Pseudomonas fluorescen*s A6RI is associated with their effect on the pathogen growth and on the root morphogenesis. European Journal of Plant Pathology, 111(3), 279-288.

Bilgi, V. N., Bradley, C. A., Khot, S. D., Grafton, K. F., and Rasmussen, J. B. (2008). Response of dry bean genotypes to Fusarium root rot caused by *Fusarium* solani f. sp. *phaseoli,* under field and controlled conditions. Plant Disease, 92(8), 1197-1200.

Bitocchi, E., Bellucci, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E., Santilocchi, R., Zeuli, P.S., Gioia, T., Logozzo, G., Attene, G., Nanni, L., Papa, R. (2013). Molecular analysis of the parallel domestication of the common bean (*Phaseolus vulgaris*) in Mesoamerica and the Andes. New Phytologist, 197(1), 300-313.

Bravo, A., Wallace, D. H., and Wilkinson, R. E. (1969). lnheritance of resistance to Fusarium root rot of beans. Phytopathology, 59, 1930-1933.

Burke, D. W., Miller, D. E., and Barker, A. W. (1980). Effects of soil temperature on growth of beans in relation to soil compaction and Fusarium root rot. Phytopathology, 70(11), 1047-1049.

Burke, D.W., and Miller, D., E. (1983). Control of Fusarium root rot with resistant beans and cultural management. Plant Disease, 67, 312-1317.

Cichy, K., Snapp, S., Kirk, W. (2007). Fusarium root rot incidence and root system architecture in grafted common bean lines. Plant and Soil, 300, 233–244.

Cichy, K., A., Blair, M. W., Galeano Mendoza, C. H., Snapp, S, S., and Kelly, J. D. (2009). QTL analysis of root architecture traits and low phosphorus tolerance in an Andean bean population. Crop Science, 49(1), 59-68.

Delgado-Salinas, A., Bibler, R., and Lavin, M. (2006). Phylogeny of the genus *Phaseolus* (Leguminosae): a recent diversification in an ancient landscape. Systematic Botany, 31(4), 779-791.

FAOSTAT, F.A.O. (2015). Statistical Databases. Food and Agriculture Organization of the United Nations.

Filion, M., St-Arnaud, M., Jabaji-Hare, S. H. (2003) Quantification of *Fusarium solani* f. sp. *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using real time polymerase chain reaction and direct isolations on selective media. Phytopathology, 93, 229-235.

Foroud, N. A., Chatterton, S., Reid, L. M., Turkington, T. K., Tittlemier, S. A., and Gräfenhan, T. (2014). Fusarium diseases of Canadian grain crops, impact and disease management strategies. In: Future challenges in crop protection against fungal pathogens. (pp. 267-316). Springer New York.

Gepts, P. (1998). Origin and evolution of common bean, past events and recent trends. HortScience, 33, 1124-1130.

Gepts, P., and Debouck, D. G. (1991). Origin, domestication, and evolution of the common bean (Phaseolus vulgaris L.). Common beans, Research for Crop Improvement, 7-53.

Hadwiger, L. A. (2015). Anatomy of a non-host disease resistance response of pea to *Fusarium solani*, PR gene elicitation via DNase, chitosan and chromatin alterations, Frontiers in Plant Science, 6, 373.

Hagerty, C. H., Cuesta-Marcos, A., Cregan, P. B., Song, Q., McClean, P., Noffsinger, S., and Myers, J. R. (2015). Mapping *Fusarium solani* and *Aphanomyces euteiches* and root rot resistance and root architecture quantitative trait loci in common bean. Crop Science, 55(5), 1969-1977.

Huisman, O. (1982). Interrelations of root growth dynamics to epidemiology of root invading fungi. Annual Review of Phytopathology, 20:303-327

Mudawi, H. I., and Idris, M. O. (2014) The Efficacy of *Trichoderma* spp. and *Bacillus* Isolates in the Control of Chickpea Wilt. Science Publishing group, 3(5), 346-351.

Hasanzade, F., Rastegar, M.F., Jafarpou, B., Kermani, M. (2008). Identification of *Fusarium solani* f .sp. pisi the cause of root rot in chickpea and assessment of its genetic diversity using AFLP in northeast Iran. Research Journal of Biological Sciences, 3, 737-741.

Hyman, G., Fujisaka, S., Jones, P., Wood, S., De Vicente, M. C., and Dixon, J. (2008). Strategic approaches to targeting technology generation. Assessing the coincidence of poverty and drought-prone crop production. Agricultural Systems, 98(1), 50-61.

Iqbal, M. J., Yaegashi, S., Ahsan, R., Shopinski, K. L., and Lightfoot, D. A. (2005). Root response to *Fusarium solani* f. sp. *glycines*, temporal accumulation of transcripts in partially resistant and susceptible soybean. Theoretical and Applied Genetics, 110(8), 1429-1438.

Karel, A.K., and Antrique, A. (1989). Insects and other pests in Africa, p. 455-504. In, Shwartz, H.F., and Pastor-Corrales, M.A. eds. Bean production problems in the tropics. CIAT, Cali, Colombia

Kamfwa, K., Mwala, M., Okori, P., Gibson, P., and Mukankusi, C. (2013). Identification of QTL for Fusarium root rot resistance in common bean. Journal of Crop Improvement, 27(4), 406-418.

Katungi, E., Farrow, A., and Chianu, J. (2009). Common bean in Eastern and Southern Africa, a situation and outlook analysis. International Centre for Tropical Agriculture (CIAT), 61.

Kelly, J.D. and Cichy, K.A (2013). Dry Bean Breeding and Production Technologies, pp. 23-54. In: Dry Beans and Pulses, Production, Processing, and Nutrition. Editors, Siddiq, M., Uebersax, M.A., Wiley-Blackwell Publishing Co. Oxford U.K.

Kendra, D. F., Christian, D., and Hadwiger, L. A. (1989). Chitosan oligomers from *Fusarium solani*/pea interactions, chitinase/β-glucanase digestion of sporelings and from fungal wall chitin actively inhibit fungal growth and enhance disease resistance. Physiological and Molecular Plant Pathology, 35(3), 215-230.

Koenig, R., and Gepts, P. (1989). Allozyme diversity in wild *Phaseolus vulgaris*, further evidence for two major centers of genetic diversity. Theoretical and Applied Genetics, 78(6), 809-817.

Kraft, J. M., and Boge, W. (2001) Root characteristics in pea in relation to compaction and Fusarium root rot. Plant Disease, 85, 936–940.

Kraft, J.M., Burke, D.W., and Haglund, W.A. (1981). Fusarium diseases of beans, peas and lentils. p. 142-156. In P.E. Nelson, T.A. Toussoun and R.J. Cook (eds.) Fusarium: Diseases, biology and taxonomy. Penn State University (PSU) Press, USA.

Kwak, M., and Gepts, P. (2009). Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae). Theoretical and Applied Genetics, 118 (5), 979-992.

Leslie, J. F., and Summerell, B. A. (2006). The Fusarium laboratory manual. Blackwell publishing.

Ma, L. J., Geiser, D. M., Proctor, R. H., Rooney, A. P., O'Donnell, K., Trail, F., and Kazan, K. (2013). Fusarium pathogenomics. Annual Review of Microbiology, 67, 399-416.

Mamidi, S., Rossi, M., Moghaddam, S. M., Annam, D., Lee, R., Papa, R., and McClean, P. E. (2013). Demographic factors shaped diversity in the two gene pools of wild common bean *Phaseolus vulgaris L*. Heredity, 110 (3), 267-276.

Maryrose, N., Palapala, V., Steele, K., and Otsyula, R. (2014). Analysis of AFLP markers for screening resistance to common bean roots rot (*Pythium* spp.). American Journal of Experimental Agriculture, 5(4), 374–391.

McRostie, G. P (1921). Inheritance of disease resistance in the common bean. Journal of American Society of Agronomy, 13, 15-32.

Michelmore, R. W., Christopoulou, M., Caldwell, K. S. (2013). Impacts of resistance gene genetics, function, and evolution on a durable future. Annual Review of Phytopathology, 51, 291-319.

Miklas, P. N., Kelly, J. D., Beebe, S. E., and Blair, M. W. (2006). Common bean breeding for resistance against biotic and abiotic stresses, from classical to MAS breeding. Euphytica, 147(1-2), 105-131.

Miller, D.E., and Burke, D.W. (1985). Effect of soil physical factors on resistance in beans to Fusarium root rot. Plant Disease 69:324-327.

Mohr, U., Lange, J., Boller, T., Wiemken, A., and Vögeli□lange, R. (1998). Plant defence genes are induced in the pathogenic interaction between bean roots and *Fusarium solani*, but not in the symbiotic interaction with the arbuscular mycorrhizal fungus *Glomus mosseae.* New Phytologist, 138(4), 589-598.

Moreto, A. L., Ramalho, M. A. P., and Bruzi, A. T. (2011). Epistasis in an Andean × Mesoamerican cross of common bean. Euphytica, 186(3), 755–760.

Mukankusi, C., Derera, J., Melis, R., Gibson, P. T., and Buruchara, R. (2011). Genetic analysis of resistance to Fusarium root rot in common bean. Euphytica, 182 (1), 11-23.

Navarro, F. M., Sass, M. E., and Nienhuis, J. (2009). Marker-Facilitated Selection for a Major QTL Associated with Root Rot Resistance in Snap Bean (*Phaseolus vulgaris* L.). Crop Science, 49(3), 850-856.

Navarro, F., Sass, M. E., and Nienhuis, J. (2008). Identification and confirmation of quantitative trait loci for root rot resistance in snap bean. Crop Science, 48(3), 962-972.

Nzungize, J., Gepts, P., Buruchara, R., Buah, S., Ragama, P., Busogoro, J. P., and Baudoin, J. P. (2011). Pathogenic and molecular characterization of *Pythium* species inducing root rot symptoms of common bean in Rwanda. African Journal of Microbiology Research, 5(10), 1169-1181.

O'Brien, R. G., O'Hare, P. J., and Glass, R. J. (1991). Cultural practices in the control of bean root rot. Animal Production Science, 31(4), 551-555.

O'Donnell, K. (2000). Molecular phylogeny of the Nectria haematococca-*Fusarium solani* species complex. Mycologia(2000), 919-938.

Obala, J., Mukankusi, C., Rubaihayo, P. R., Gibson, P., and Edema, R. (2012). Improvement of resistance to Fusarium root rot through gene pyramiding in common bean. African Crop Science Journal, 20 (1).

Otsyula, R., Rubaihayo, P., and Buruchara, R. (2003). Inheritance of resistance to *Pythium* root rots in beans (Phaseolus vulgaris) genotypes. African Crop science Conference Proceedings (Vol. 6, pp. 295-298).

Pastor-Corrales, M.A., and Abawi, G. S. (1987). Reactions of selected bean germplasms to infection by *Fusarium oxysporum* f. sp. *phaseoli*. Plant Disease, 71, 990-993.

Papa, R., and Gepts, P. (2003). Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris L.)* from Mesoamerica. Theoretical and Applied Genetics, 106(2), 239-250.

Román-Avilés , B., Lewis, J. M., and Kelly, J. D. (2011). Fusarium genetic control, A long term strategy. In, Control of fusarium diseases, Alves-Santos F. M., Diez J., eds. Research Signpost, Kerala, India, 65-108.

Román-Avilés, B., Snapp, S. S., and Kelly, J. D. (2004). Assessing root traits associated with root rot resistance in common bean. Field Crops Research, 86(2), 147-156.

Ronquillo-López, M. G., Grau, C. R., and Nienhuis, J. (2010). Variation in reaction to spp. identified in a common bean (*Phaseolus vulgaris*) population developed for field-based resistance to root rot and wilt. Crop Science, 50 (6), 2303-2309.

Rusuku, G., Buruchara, R. A., Gatabazi, M., and Pastor-Corrales, M. A. (1997). Occurrence and distribution of soil-borne fungi pathogenic to common bean in Rwanda. Plant Disease, 81, 445-449.

Schneider, K. A., Grafton, K. F., and Kelly, J. D. (2001). QTL analysis of resistance to Fusarium root rot in bean. Crop Science, 41(2), 535-542.

Schwartz, H. F., Brick, M. A., Nuland, D. S., and France, G. D. (ed.1996). Dry bean production and pest management. Reg. Bull. No. 562A. Colorado State Univ., Fort Collins, CO.

Schwartz, H.F., and J.R. Steadman. (1989). White mold, p. 211-230, In, H.F. Shwartz and M.A. Pastor-Corrales, eds. Bean production problems in the tropics. CIAT, Cali, Colombia.

Sikora, R. A., Greco, N., Silva, J. V., Luc, M., and Bridge, J. (2005). Nematode parasites of food legumes. In Plant parasitic nematodes in subtropical and tropical agriculture, (Ed. 2), 259-318.

Singh, S. P., and Schwartz, H. F. (2010). Breeding common bean for resistance to diseases, a review. Crop Science, 50(6), 2199-2223.

Singh, S. P., Gepts, P., and Debouck, D. G. (1991). Races of common bean (*Phaseolus vulgaris*, Fabaceae). Economic Botany, 45(3), 379-396.

Singh, S. P., Nodari, R., and Gepts, P. (1991). Genetic diversity in cultivated common bean, I. Allozymes. Crop Science, 31(1), 19-23.

Smýkal, P., Coyne, C. J., Ambrose, M. J., Maxted, N., Schaefer, H., Blair, M. W., Berger, J., Greene, S. L., Nelson, M. N., Besharat, N., Vymyslický, T., Toker, C., Saxena, R. K., Roorkiwal, M., Pandey, M. K., Hu, J., Li, Y. H., Wang, X. L., Guo, Y., Qiu, L. J., Redden, R. J., and Varshney, R. K. (2014). Legume crops phylogeny and genetic diversity for science and breeding. Critical Reviews in Plant Sciences, 34(1-3), 43–104.

Snapp, S.S., Kirk, W., Román-Avilés, B., and Kelly, J.D. (2003). Root traits play a role in integrated management of FRR in snap beans. HortScience, 38,187-191.

Statler, G. D. (1970). Resistance of bean plants to *Fusarium solani* f. sp. *phaseoli*. Plant Disease Reporter 54, 698-99.

Terán, H., Jara, C., Mahuku, G., Beebe, S., and Singh, S. P. (2012). Simultaneous selection for resistance to five bacterial, fungal, and viral diseases in three Andean × Middle-American inter-gene pool common bean populations. Euphytica, 189 (2), 283–292

Thung, M. (1990). Phosphorus, A limiting nutrient in bean (*Phaseolus vulgaris L.*) production in Latin America and field screening for efficiency and response. In Genetic Aspects of Plant Mineral Nutrition (pp. 501-521). Springer Netherlands

Tusiime, G. (2003). Variation and detection of *Fusarium solani* f. sp. *phaseoli* and quantification of soil inoculum in common bean fields. PhD thesis. Makerere University, Kampala, Uganda

Vandermark, G. J., Brick, M., Kelly, J. D., Osorno, J., and Urrea, C. (2014). Yield gains in edible grain legumes. In Edible grain legumes. Yield Gains in Major US Field Crops. CSSA special publications, 33. 87-124. ISBN, 978-0-89118-620-5

Van Loon, L. C. (1985). Pathogenesis-related proteins. Plant Molecular Biology, 4 (2), 111-116.

Viste, E., Korecha, D., and Sorteberg, A. (2013). Recent drought and precipitation tendencies in Ethiopia. Theoretical and Applied Climatology, 112 (3-4), 535-551.

Windeis, C. E. (1991). Current status of Fusarium taxonomy. Phytopathology, 81, 1048-1051.

Wortmann, C. S,. Kirkby, R. A., Eledu, C. A., and.Allen, D, J. (1998). Atlas of common bean, *Phaseolus vulgaris*. CIAT Publication No. 297.

Xue, R. F., Wu, J., Wang, L. F., Blair, M. W., Wang, X. M., De Ge, W., Zhu, D. Z., and Wang, S. M. (2014). Salicylic Acid Enhances Resistance to *Fusarium oxysporum* f. sp. *phaseoli* in Common Beans (*Phaseolus vulgaris L.*). Journal of Plant Growth Regulation, 33 (2), 470-476.

Yerkes, W. D., and Freytag, G. F. (1956). *Phaseolus coccineus* as a source of root-rot resistance for the common bean. Phytopathology, 46 (1), 32-32

Chapter Two

IDENTIFICATION AND MAPPING OF QTL ASSOCIATED WITH FUSARIUM ROOT ROT RESISTANCE AND ROOT ARCHITECTURE TRAITS IN BLACK BEANS (*Phaseolus vulgaris L)*

Introduction

Common bean (*Phaseolus vulgaris L.)* is the most important legume in human diets (Beebe et al. 2012). It provides an inexpensive source of high quality protein and minerals, especially in sub-Saharan Africa (Beebe et al. 2012).

Annual common bean yields in the developing world average 0.5 metric tons/Ha, compared to about 2.5 metric tons/Ha in the developed world (FAOSTAT, 2015). Lower yields in the developing world are due to both biotic and abiotic stresses. The abiotic stresses may include drought, salinity, and low soil fertility (Abawi and Pastor-Corrales, 1990). One of the most important biotic stresses is disease, particularly root rots. Root rots are caused by a complex of pathogens, which include *Fusarium solani* f. sp. *phaseoli, Rhizoctonia solani, Sclerotium, Aphanomyces* and *Pythium* species that result in stunting, chlorosis, poor stands, rotting of roots, and yield reduction up to 85 % (Abawi and Pastor-Corrales, 1990; Obala et al. 2012). In Uganda, bean root rots have been reported to cause 100% loss (Tusiime, 2003).

The most widespread of root rots is caused by *Fusarium solani* f sp. *phaseoli* (FSP.) (Schneider et al. 2001). Root rots continue to gain importance in many Eastern African

countries like Kenya, The Democratic Republic of Congo, Malawi, and Rwanda (Mukankusi et al. 2011; Otsyula et al. 2003; Rusuku et al. 1997) where beans are widely grown by subsistence farmers.

Fusarium root rot (FRR), characterized by reddish lesions on the root and hypocotyl, is particularly severe on large-seeded Andean genotypes due to their lack of genetic resistance (Abawi and Pastor-Corrales, 1990; Dickson, 1973; Román-Avilés et al. 2011). Control of FRR is difficult for two main reasons. First, it is a soil-borne fungus and so fungicide use is expensive, highly regulated and not always effective, (Snapp et al. 2003). Second, in the developing world, resource-poor farmers are not able to incorporate adequate crop rotation or fallow into their bean cultivation practices due to small land holdings. Consequently, the most effective way to control this disease is to exploit host plant resistance. Resistance to FRR has been reported in small-seeded Middle-American genotypes. This resistance is lacking in the large-seeded Andean genotypes, which are generally preferred in Africa (Mukankusi et al, 2011; Roman-Avilés et al. 2011; Schneider et al. 2001; Wortmann et al. 1998). The resistance is quantitative in nature and appears to be a combination of physiological mechanisms and root system avoidance due to architecture traits such as root dry weight, root length and root mass (Cichy et al. 2009; Kamfwa et al. 2013; Mukankusi et al. 2011; Román-Avilés et al. 2004; Snapp et al. 2003).

Finding Quantitative Trait Loci (QTL) that account for a large amount of the variation is critical to enable eventual introgression of FRR resistance from the resistant Middle-American genotypes into the susceptible but preferred large-seeded Andean genotypes.

The deployment of molecular markers has enabled mapping of QTL associated with FRR resistance. The use of Single Nucleotide Polymorphism (SNP) DNA markers promises to further elucidate this long-standing research question. SNP markers have a physical position in the genome, are more numerous and provide better genome coverage than prior markers, thereby increasing the likelihood of detecting QTL

A large and robust root system has also been associated with root rot resistance (Snapp et al. 2003). This may suggest a disease escape or avoidance mechanism, where roots find regions in the soil profile with less disease pressure It is also thought that when the primary root dies due to infection, roots that arise from the shoot–root transition zone, could replace the function of the taproot. These roots are typically called basal or adventitious roots and they are oriented horizontally rather than vertically. However, they rarely meet the plant's requirement for nutrient and water uptake (Cichy et al. 2007; Jackson, 1955; Kraft and Boge, 2001; Román-Avilés et al. 2004; Snapp et al. 2003; Estevez de Jensen et al. 2004). Environmental conditions such as excessive soil compaction, which cause plant stress and constrain optimal root development potentially aggravate Fusarium root rot development (Burke, 1968; Estevez de Jensen et al. 2004). Snapp et al. (2003) investigated the contribution of vigorous adventitious root towards enhancing root rot resistance in snap bean. The authors reported that lateral root number at root:shoot interface and root diameter were correlated with root rot tolerance.

Genetic variation has been observed among common bean germplasm for root architecture traits (Beebe et al. 2006; Cichy et al. 2009; Román-Avilés et al. 2004). Hagerty et al.

(2015) reported an association of tap root diameter with root rot resistance along with QTL associated with basal root angle and tap root diameter. Several additional root architecture traits have been reported to be positively associated with root rot resistance. Some of these include root length, root length density, root surface area, number of adventitious roots and total root dry weight (Cichy et al. 2007, 2009; Román-Avilés et al. 2004). Cichy et al. (2007) reported that genes expressed in the root, when the plant is grown in non-compacted soils might control root rot resistance. These findings lend support to the hypothesis of the contribution of root architecture traits toward root rot avoidance.

However, phenotyping these root architecture traits is difficult and complicated. Entire roots are difficult to extract from the soil, root architecture traits are phenotypically plastic and many sampling procedures are both destructive and inadequate (Beebe et al. 2006). Screening for root traits makes breeding for improved root architecture traits particularly challenging. Marker-assisted selection would greatly enhance breeding for these traits because it would enable indirect selection without the need for the expensive and laborious root phenotyping. Finding QTL that explain a large amount of the phenotypic variation is critical to enable marker-assisted eventual introgression of resistance from the resistant Middle-American genotypes into the susceptible but preferred large-seeded Andean bean genotypes.

The objectives of this study were twofold,

1) To identify QTL associated with FRR resistance in a Puebla 152 x Zorro RIL population using SNP markers.
2) To identify QTL associated with root architecture traits in a Puebla 152 x Zorro RIL population using SNP markers.

Materials and Methods

Mapping population

Three phenotyping experiments (I, II and III) were carried out using a Recombinant Inbred line (RIL) population derived from a cross of two black bean cultivars. The population consisted of 121 F_4-derived F_7 ($F_{4,7}$) RILs developed by single seed descent from a cross between Puebla 152, a black-seeded indeterminate type III Mexican bean landrace cultivar identified as a source of root rot resistance (Beebe et al. 1981; Navarro et al. 2008), and 'Zorro', a widely grown black bean type II cultivar with no known resistance to FRR (Kelly et al. 2009). Additionally, the two parents displayed striking differences in root architecture. Puebla 152 had significantly larger root mass than Zorro. The initial cross was made in fall 2007 and F_1 were grown out in a greenhouse in spring 2008. The F_2 were planted in a field at the Saginaw Valley Research and Extension Center (SVREC), Richville MI and F_3 seed (1 pod from each plant in the field) were harvested and planted in the greenhouse in fall 2009. Two seeds (F_4), harvested from each F_3 plant, were planted in the greenhouse and thinned to one seedling per pot. F_5 seed from each F_4 plant was harvested separately and increased as progeny rows of each line. Progeny rows were harvested separately and have been maintained through selfing as individual RILs.

Inoculum preparation

A suspected FSP strain, FSP-3, which was later confirmed by to be *Fusarium cuneirostrum* (Chilvers Plant Pathology lab, MSU) was used in experiment I of this study. The strain was isolated from the South-Western highlands of Uganda (Mukankusi, 2008)

Fusarium cuneirostrum Strain NRRL 31157 was used in experiment II of this study. The strain was isolated from bean fields in Presque Isle, Michigan and deposited in the USDA ARS culture collection (NRRL) (Aoki et al. 2005)

The strains were increased on Potato Dextrose Agar (PDA) media plates at room temperature for 10 d, and then used for inoculum preparation. The strains were allowed to colonize autoclaved sorghum seeds for 4wk and the sorghum was then used as inoculum. The NRRL 31157 strain was maintained by inoculating the susceptible cultivar 'Red Hawk', while FSP-3 was inoculated on susceptible cultivar K132. Each strain was then re-isolated from the infected plants, grown on PDA, and examined morphologically to confirm its integrity.

Experiment I

The study was carried out in Uganda in a screen house at the International Center for Tropical Agriculture (CIAT) Kampala based on the protocol described by Kamfwa et al. (2013). The plants were grown in wooden trays measuring 74 x 42 x 12 cm filled with sterilized soil. 500 g of sorghum inoculum was added to each tray and thoroughly mixed to ensure even distribution. The susceptible variety K132 was grown in each of the inoculated trays for 14 days. An even infection in all the trays further confirmed the presence, even distribution and virulence of the strain. The experiment consisted of a single row (42-cm) with 20 plants each and each RIL was replicated twice. Each tray contained five RILs along with one row each of a susceptible check (CAL 96) and a resistant check (MLB-49-89A).

The RILs were planted on July 02, 2014 in a completely randomized design with two replicates. After 28 d, 18 plants per row were uprooted with a trowel and disease severity was rated according to the CIAT scale (Abawi and Pastor-Corrales, 1990), where, 1= no visible symptoms; 3=light discoloration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissue covered with lesions; 5= approximately 25% of the hypocotyl and root tissue covered with lesions, although tissues remain firm, with some deterioration of the root system; 7= approximately 50% of the hypocotyl and root tissues covered with lesions along with considerable softening, rotting and root system reduction, and 9= approximately 75% or more of the hypocotyls and root tissues affected in advanced stages of rotting along with severe reduction in root mass. Ratings of 1-3 indicate resistance, 4-5 moderate resistance, and 6-9 susceptible.

Experiment II

The RIL population, along with the parents was planted on November 28th 2014 at Michigan State University greenhouse facilities. Greenhouse was set to 25°C day and 20°C night time temperatures. Individual lines were planted (at a rate of 3 seeds per line) in 1L plastic pots filled with a potting mixture composed of peat and soil, both pasteurized and evenly mixed at a ratio 3:1. 10ml of sorghum seeds colonized with the pathogen FSP strain NRRL 31157 was added into each pot, and evenly mixed with the potting mixture prior to planting. The strain was kindly provided by Dr. M. Chilvers Plant pathology lab at Michigan State University. The experiment was set up as a completely randomized design with two replicates and a control (non-inoculated) and was watered three times a week. On December 27th 2014, 30d later, whole plants were harvested, roots washed and the plants individually bagged. The bags were placed in an oven at 60°C for 3d. Total root dry weight

and total biomass of each line was then taken. The percentage reduction in biomass and root dry weight between inoculated and non-inoculated lines, along with the percent root weight were determined.

Experiment III

The RIL population was evaluated under greenhouse conditions for root traits by collaborators at the University of California-Riverside. A split plot design with four replications was used to evaluate the individual RILs and parents. Three root and shoot sampling times were assigned to the main plot and the lines to the subplots. Seeds were germinated in Petri dishes on May 9, 2013. Seven days later, seedlings of similar growth were transplanted into 8.5 kg of dry silica sand in polyethylene tube bags sleeved into polyvinyl chloride (PVC) tubes, 80 cm long and 10 cm in diameter. Each bag was brought to water-holding capacity for three consecutive days for sand settlement using half-strength Hoagland's solution provided in the glasshouse. Plants were harvested on three different sampling days after planting; namely, 35 (pre flowering), 47 (flowering) and 62 days (pod setting) after planting. Roots were gently washed by hand with tap water. Number of basal lateral roots including the maximum root length were counted and measured. The entire root system was cut into two parts, the shallow roots developed between 0-30 cm and the deep roots below 30 cm depth. Shallow root biomass, deep root biomass, total root biomass and shoot biomass were determined after the samples were dried in a forced-air drier for 24 h at 80ºC. Data collected at pod setting (62d) was analyzed and used in the QTL analysis in this study.

Statistical Analysis of Phenotypic Data

The mean score for each RIL was calculated and analysis of variance (ANOVA) was calculated using PROC MIXED (SAS 2013) for each trait. For experiment II, a categorical variable (inoculation) with 2 levels yes or no, was added to the data to enable comparison between the lines and the controls. The percent root dry weight trait from experiment II

and the mean disease severity ratings obtained from experiment I were log transformed to achieve normal distribution. The least square (LS) means were compared by a LS means T-test or LS means F-Test where applicable. The PROC CORR (SAS 2013) command was used to analyze Pearson correlations among variables. Broad-sense heritability (H^2) of each trait was calculated based on the mean of the line (Bernardo, 2002) as,

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_e/r)}$$

Where σ_G^2 = genetic variance, σ_e = variance due to the environment, and r = the number of replications

These genetic variance components for each trait were calculated from the SAS 2013 ANOVA generated Expected Mean Squares as follows,

σ_G^2= Mean Square of residual (along with mean square of interactions where applicable) subtracted from Mean Square of genotypes and the result divided by number of replications

σ_e= Since there was only one environment in each experiment, the Mean Square of the error was considered as σ_e.

DNA extraction and Single Nucleotide Polymorphism genotyping

This was done by James Heilig in 2010. A leaf tissue sample from three seedlings of each line and parents of the RIL population was bulked and used for DNA extraction following the miniprep method of Afanador et al. (1993). DNA was quantified using a NanoDrop 8000 V2.3.1 spectrophotometer (Thermo Fisher Scientific Inc., DE), diluted to a final concentration 100 ng/µl, and the DNA quality was checked by loading 5 µl on a 1%

agarose gel.

The RIL population was genotyped with 5398 single nucleotide polymorphism (SNP) markers, on the BARCBean6K_3 BeadChip. The genotyping was conducted through the BeanCAP (www.beancap.org) project at the Soybean Genomics and Improvement USDA Laboratory (USDA–ARS, Beltsville Agricultural Research Center) in Maryland (Hyten et al. 2010). The SNP genotyping was conducted on the Illumina platform by following the Infinium HD Assay Ultra Protocol (Illumina Inc.). The Infinium II assay protocol includes the procedures to make, incubate, and fragment amplified DNA, prepare the bead assay, hybridize samples to the BARCBean6K_3 BeadChip, extend and stain samples, and image the bead assay. The SNP alleles were called using the GenomeStudio Genotyping Module v1.8.4 (Illumina, Inc.). The data were manually adjusted for allele calls.

Genetic mapping and QTL analysis.

The JOINMAP 4.0 program (Van Ooijen, 2006) was used to create a genetic map for the population. Polymorphic SNPs (1095, 20.3%) were loaded into the program. Prior to mapping, SNPs were further analyzed for co-segregation and segregation distortion. SNPs that co-segregated at the same locus, and those that showed severe segregation distortion ($P<0.01$) were discarded. A total of 378 (7.0%) SNPs were used to generate the genetic map. In order to divide the SNPs into the 11 known linkage groups of *P. vulgaris*, the sequence of each SNP was searched from dbSNP database (Sherry et al. 2001) and BLAST searched in the bean genome at the PHYTOZOME (v10.2.1) database, (Goldstein et al. 2012) to determine its physical location. The SNPs that are located on the same chromosome were grouped together and mapped separately to produce 11 linkage groups.

The mapping was done using regression mapping method while recombination frequencies were calculated by Kosambi function. The QTL analysis was conducted using Win Cartographer V2.5–011 (Wang et al. 2012) by composite interval mapping (CIM) method. The Model 6 with 10 cM window size, 2 cM walk speed, five significant background markers, and analysis by forward and reverse multiple linear regressions for each chromosomal position were used. Probability thresholds of 0.05 were used for the CIM method. Both individual replications and combined (average value of each RIL for both replications) were analyzed. The threshold LOD for each trait was determined after running 1000 permutations (Churchill and Doerge, 1994). A QTL was only reported as significant if it appeared in the analysis of each replication along with the mean. The MapChart program (Voorrips, 2002) was used to display maps and QTL positions.

Results

Experiment I

The ANOVA revealed significant differences between the RILs for disease severity (Table 2.7). Mean disease severity among the RILs displayed a continuous variation that was skewed towards resistance (Fig.2.8). The severity rating ranged from 2.0 to 7.22, with a mean of 3.05. Both parents displayed a resistant reaction to the FSP strain used (FSP-3), despite Zorro having no known resistance to FRR (Table 2.8, Supplemental 1).

Zorro had a mean rating of 3.36 while Puebla 152 had 2.75. Many of the RILs displayed a resistant reaction, however mild transgressive segregation towards susceptibility was displayed, whereby 42% of the RILs were rated as moderately resistant (rating 4-6).The trait had heritability of 0.98 and coefficient of variation (CV) 32.7% (Table 2.8). A consistent rating of each of the 18 plants sampled from each RIL was not obtained (Appendix 1). The plants appeared to segregate for susceptibility and resistance within each RIL. However, the resistant check (MLB-49-89A) and susceptible check (CAL 96) each displayed a consistently resistant (mean 2.0) and susceptible (mean 8.8) rating respectively (Supplemental 1).

Experiment II

Results from the analysis of variance (ANOVA) (Table 2.1) revealed highly significant differences between the 109 RILs for total biomass and root dry weight and percent root dry weight (Table 2.2). Root dry weight, total biomass and percent root dry weight each were not significantly different between the parents but varied greatly between the RILs (Table 2.2). Puebla 152 had a mean root dry weight of 0.4g, while the mean root dry weight of Zorro was 0.30g. The trait showed a normal distribution (Fig. 2.8). Root dry

weight ranged from 0.23 to 0.65g among the RILs. 56% of the RILs had a higher root dry weight than the mean of the parents, indicative of positive transgressive segregation (Table 2.2). Total biomass also displayed a normal distribution (Fig.2.8). Puebla 152 had a mean total biomass of 2.07g, while the mean total biomass for Zorro was 1.30g. The progeny total biomass ranged from 0.80 to 3.45g (Table 2.2). The trait displayed negative transgressive segregation, whereby 60% of the progeny had a mean total biomass less than the parental mean. Percent root mass had the narrowest range of variation within the RILs (18.8-32.4%). Puebla 152 had percent root mass of 25.3% while Zorro had 27.7% (Table 2.2). Heritability estimates were calculated for root dry weight (0.34), total biomass (0.37) and percent root dry weight (0.27) and showed low to moderate values (Table 2.2). The coefficient of variation varied from 19.8% to 38.6% across traits.

Mean comparisons of the parents and controls for all three traits are shown in Table 2.3. In all combinations tested inoculated or non-inoculated, root dry weight was not significantly different between the parents, however the difference between the inoculated parents (73.3%) approached the significance level ($P = 0.067$). Puebla 152 had consistently higher root dry weight than Zorro in both inoculated and control treatments.

For total biomass, Zorro showed the only significant difference (84.6%, $P<0.0407$) between inoculated and non-inoculated treatments, suggesting that it lacked resistance to this strain of FSP. In all comparisons of inoculated versus control lines, the inoculated line had the higher percent root dry weight. Root dry weight and total biomass traits were strongly and positively correlated ($r=0.498***$).

Experiment III

The ANOVA revealed highly significant differences between the 121 RILs for all the root traits measured (Table 2.4), although the parents Puebla 152 and Zorro were not significantly different for many of the traits (Table 2.5). All of the traits displayed a continuous variation, along with major transgressive segregation (Fig. 2.1). The number of lateral roots ranged from 4 to 11 among the RILs while Puebla 152 had 7.5 and Zorro 7.0. Shallow root weight ranged from 0.98g to 6.28g among the RILs, while Puebla 152 weighed 5.7g and Zorro 5.61g. Deep root weight ranged from 0.03g to 21.21g among the RILs, Puebla 152 weighed 7.3g and Zorro 5.29g. Total root weight ranged from 1.01g to 30.76g among the RILs, while Puebla 152 weighed 13.03g and Zorro 10.90g. Total plant biomass showed the highest variation among RILs. Biomass ranged from 4.24 to 124.8g, while Puebla 152 weighed 103.03g and Zorro 72.38g. Root length also displayed substantial variation within the RILs. RIL means ranged from 38.8g to 102.0g, while root length in Puebla 152 was 84.75cm and Zorro 93.59cm. Heritability estimates for number of lateral roots (0.4), shallow root weight (0.55), deep root weight (0.49), total root weight (0.56), total plant biomass (0.69) and root length (0.35) were moderate to high suggesting that gain from selection should be possible (Table 2.5). The coefficient of variation (CV) ranged from 30 to 64% across the root traits measured underscoring the difficulty of making repeatable measurements of root traits even under controlled greenhouse conditions. This can be improved by the use of computer software to measure the roots, rather than the manual method (Kraft and Boge, 2001).

Pearson's correlation coefficients of all the traits assayed are shown in Table 2.6. The most strongly correlated traits were between total root weight and deep root weight (r=0.87***), total root weight and shallow root weight (r=0.84***), total root weight and total plant biomass (r=0.75***). Shallow root weight and total plant biomass were also strongly correlated (r=0.65***), along with deep root weight and total plant biomass (r=0.65***). Shallow root weight and deep root weight were strongly correlated (r=0.488***) whereas deep root weight and root length were also strongly correlated (r=0.39***).

Genetic mapping and QTL analysis

A genetic map of the Puebla 152 x Zorro RIL population was generated (Appendix 2). The map spanned 687.7 cM in length and was fairly saturated, with an average SNP coverage of 1 SNP per 1.82 cM. The map covered approximately 57% of the 1200 cM bean genome. However, marker coverage was not even across chromosomes. Chromosomes Pv02, Pv04, Pv05, Pv06, Pv07, and Pv09 had the most dense marker coverage, while the remaining chromosomes had some areas over 10cM in length that were not covered by the markers. Chromosomes Pv01 and Pv08 had the least marker coverage, with only 19 and 36 SNP markers, respectively.

Five QTL were revealed by Composite Interval Mapping (CIM) analysis of non-inoculated root traits in experiment III. The QTL were associated with total root weight, root length, deep root weight, shallow root weight, and total plant biomass (Table 2.9, Figs. 2.2 to 2.8). The QTL associated with root length was located on Pv01 in a 1.82 cM interval between SNP ss715649523 located at 20.28 cM and SNP ss715647677 located at 22.19cM (Table

2.9, Figs. 2.2 and 2.6). This QTL accounted for 13.7% of phenotypic variation and was at a LOD peak of 3.45.The LOD peak was at SNP ss715649523 located at 20.28 cM.

The QTL associated with total root weight was located in Pv09 in a 0.5 cM interval between SNP ss715650429 located at 0.01cM and SNP ss715649127 located at 0.51 cM (Table 2.9, Figs. 2.3 and 2.4). This QTL accounted for 8.3% of phenotypic variation and had a LOD peak of 3.2. The LOD peak was at SNP ss715650429 located at 0.01cM.

The QTL associated with shallow root weight was located in the same genomic region on Pv09 as the QTL associated with total root weight. That is, in a 0.5 cM interval between SNP ss715650429 at 0.01 cM and SNP ss715649127 located at 0.51 cM (Table 2.9, Figs. 2.2 and 2.7). This QTL had a LOD peak of 3.2 and accounted for 8.3% of phenotypic variation. The LOD peak was at SNP ss715650429 located at 0.01cM.

The QTL associated with deep root weight was located on Pv05 in a 5.93 cM interval between SNP ss715645322 at 51.73cM and SNP ss715645341 at 57.66 cM (Table 2.9, Figs. 2.2 and 2.8). This QTL had a LOD peak of 3.05 and accounted for 9.2% of phenotypic variation. The peak LOD was at SNP ss715645340 located at 55.0cM.

The QTL associated with total plant biomass was also located on Pv05. The QTL was located in the same genomic region on the genetic map as the QTL associated with deep root weight. (Table 2.9, Figs. 2.2 and 2.5). This QTL accounted for 12.7% of phenotypic variation, with a peak LOD of 5.02..

CIM analysis also revealed a QTL associated with FRR resistance on Pv05 from data generated in the inoculated experiment I conducted in Uganda (Table 2.9, Figs. 2.2 and 2.3). This QTL was located in a 4.27 cM interval between SNP ss715645343 located at 56.82 cM and SNP ss715645377 at 61.09 cM. The QTL had a LOD peak of 3.2 for SNP

ss715645368 located at 60.05 cM, and accounted for 10.1% of the variation in FRR scores among the RILs. The beneficial allele in all of the QTL detected was contributed by Puebla 152. No QTL or genomic regions significantly associated with root dry weight, biomass or percent root dry weight were detected in experiment II using CIM analysis (Supplemental 3 and 4)

Discussion

Phenotypic data

The distribution of the disease severity ratings in experiment I (Fig. 2.8) further confirmed previous reports that FRR resistance is quantitatively controlled. Puebla 152 displayed resistance to the FSP-3 strain as was expected. However the resistance reaction (3.36 rating) of Zorro was unexpected, because Zorro had not been known to have FRR resistance. An explanation could be that Zorro, being a black bean, probably has the genes that confer resistance to FRR. This finding is in agreement with some previous reports that have identified black bean Middle-American genotypes as root rot resistance sources (Beebe et al., 1981; Bravo et al. 1969; Mukankusi et al. 2011; Obala et al. 2012).

The mild transgressive segregation toward susceptibility observed (42% of the RILs were rated as moderately resistant, rating 4-6, Fig.2.8) might be due to the fact that Puebla 152, being a landrace, may have actually been mixture of pure lines. This heterogeneity may have provided a higher opportunity for any recessive susceptibility genes within these lines to segregate among the progeny, hence the observed transgressive segregation. This same explanation may also be the cause of the observed segregation within each RIL (Supplemental 1). The fact that the checks displayed consistent reactions in each tray (Supplemental 1) rules out the possibility of non-even inoculum distribution as the cause of the inconsistent ratings of the RILs.

The high heritability reported in this study (Table 2.8) may be due to the fact that the experiment was conducted in a highly controlled environment, and as a result, the effect of

confounding factors greatly reduced. Kamfwa et al. (2013) reported similarly high broad sense heritability values in a similar greenhouse study to detect for FRR resistance-associated QTL. These results suggest that a greenhouse screen may be better for selecting early generation breeding materials, as more gain from selection is realized. However field screening of later generation materials is still strongly recommended.

In experiment II, the normal distribution of root weight and total biomass confirmed that the two traits are quantitatively controlled (Fig. 2.8). The fact that that the traits have low heritability estimates (Table 2.2) is indicative of the very high influence of environmental factors on the expression of these traits. Puebla 152 and Zorro did not exhibit significant differences in biomass, percent root dry weight or root dry weight, despite prior studies that reported a large root and total biomass in Puebla 152 (Navarro et al. 2008). The lack of statistically significant differences may have resulted from the short duration of the experiment (30 days). The 30-day period might have been insufficient to detect differences in these traits. In addition, the small 1l pots in which the plants were grown might have restricted root growth, hence contributing to the lack of significant differences between the cultivars. Due to space limitations in the greenhouse, only two replicates (6 plants) were evaluated, consequently the power of the statistical tests to detect the differences was limited. In viewing the raw data, there were substantial percentage differences between the two cultivars for these traits, despite the fact that they were not statistically significant. Puebla 152 consistently had higher root dry weight and total biomass than Zorro (Table 2.3). Puebla 152 had 73% larger root dry weight than Zorro following inoculation treatment, and 59.2% higher total biomass. These findings indicate that there is indeed a

difference between the parents for these traits, but the experiment may have required greater replication and duration to capture these differences. Comparisons between inoculated and non-inoculated lines of the same cultivar revealed that the non-inoculated lines had higher biomass and root dry weight (Table 2.3). This finding is in agreement with previous studies that reported that root rots negatively affect root growth and total biomass (Abawi and Pastor-Corrales, 1990). The finding that inoculated lines consistently had higher , percent root weight than their non-inoculated counterparts (Table 2.3) may point towards a compensation reaction in both parents, whereby the plants challenged by the pathogen allocated a greater percentage of total dry matter for root production. This compensation reaction was reported by Jackson (1955) in tomato (*Solanum lycopersicum* L.), and by Kraft and Boge, (2001) in pea (*Pisum sativum* L.*)*.

Finally, the strong positive correlation of root traits weight and total biomass (Table 2.6) may indicate that pleiotropic genes control the two traits. Hence indirect selection for root dry weight may be accomplished by selecting for total biomass, which is a much easier trait to evaluate. In addition to the lack of significant differences between the parents for the traits measured in experiment II (Table 2.2), it is suspected that the particularly low heritability values, implying a low genetic contribution towards the expression of the traits, may also have contributed to the fact that no QTL were detected in this study.

The frequency distributions of the root traits measured in experiment III confirm the quantitative nature of the traits (Fig 2.1). There were highly significant differences and variation between the RILs for all traits, unlike the parents that did not differ significantly

in any trait (Tables 2.4 and 2.5). If the parents had different alleles for each of these traits then the segregation of these alleles within the RILs could have accounted for the observed variation. Despite the fact that Puebla 152 and Zorro are both black beans and therefore genetically similar in many traits, differences have been previously observed between them for biomass and root architecture (Ibarra-Perez et al. unpublished). It is suspected that some of this genetic variation contributed to the particularly high coefficient of variation (CV) values for these traits (Table 2.5). Total root dry weight and total plant biomass, measured in both experiment II and III, had higher heritability estimates in experiment III than in experiment II (Tables 2.2, 2.5). This difference may have resulted from the increased variability from the reaction of the lines to the pathogen in experiment II, which reduced the heritability estimates. This is further evidence of the influence of environment on heritability of traits, and that heritability studies must be made in environments where the crop is grown.

Genetic mapping and QTL analysis

The fact that both the parents of this population belong to the same race in the Middle-American gene pool, and may possess identical alleles at many loci could explain why so many (79.7%) of the genotyped SNPs were monomorphic. This loss of marker coverage may also be the reason why only 57% of the genome was mapped in this population. This population however typifies a breeding population where crosses are made between similar elite parents, but despite the similarities between parents, QTL were detected for many traits in this population (Table 2.9, Fig.2.2).

Most of the QTL detected in these studies are located in genomic regions that have been previously associated with other traits of agronomic importance. The QTL associated with root length on Pv01 (Table 2.9, Fig.2.2) was initially suspected to be located near the phenotypic locus *fin,* which has been reported to confer determinacy in *Phaseolus vulgaris* (Koinange et al. 1996; Repinski et al. 2012). However a BLAST search in PHYTOZOME database (Goodstein et al. 2012) of the sequence of a primer of the PvTFL1y gene, confirmed to be the *fin* gene (Repinski et al. 2012), revealed that the PvTFL1y gene is located at 45.5 Mbp, while the indicative marker of the detected QTL for root length on Pv01 is located at 3.86 Mbp (Table 2.9). The two genes are over 40Mb apart and therefore do not reside at the same locus. In addition the *fin* gene was not segregating in this population, as both parents are indeterminate possessing the dominant *Fin* gene. Among the annotated genes in the vicinity of the root length associated QTL on Pv01 is a cytokinin oxidoreductase gene. This gene regulates the levels of cytokinin, a plant hormone associated with root and shoot growth, among other functions (Goldstein et al. 2012; Wang et al. 2014). This finding is in agreement with Cichy et al. (2007), who reported that resistance to root rot was conditioned by genes expressed in the root. A deeper root may contribute to disease escape by enabling the plant roots to explore a larger total soil volume (Berta et al. 2005), thereby increasing chances of growing away from areas with high pathogen concentrations in soil surface layers, since inoculum concentrations are not evenly distributed throughout the field.

The same QTL detected on Pv05 was associated with both deep root weight and total plant biomass in the non-inoculated experiment III (Table 2.9, Fig. 2.2). Heilig (2015), using the

same Puebla 152 x Zorro RIL population, also detected a large effect QTL (R^2 = 13.1%) associated with root: shoot ratio in an interval on Pv05 that overlapped with the interval in which the QTL detected in this study resides. This study was done in the green house. The QTL detected by Heilig, (2015) had a peak on SNP ss71565320, at 39.0 Mbp, which is 172.6 Kbp from the QTL detected in this study. These QTL may suggest that pleiotropic genes govern traits for root and shoot biomass, as was evidenced by the strong correlation (r= 0.65***) between these traits (Table 2.6). The fact that the QTL detected in this study has a different contribution to the total variation in both traits (R^2=12.7% for total biomass and R^2=9.2% for deep root mass, Table 1.9) may suggest that both traits share some but not all genes that govern their expression.

Annotated genes in the vicinity of this QTL are associated with cell division and differentiation (Goldstein et al. 2012). Liao et al. (2004) and Hagerty et al. (2015) also reported QTL associated with root length and shallow basal root growth angle respectively on Pv05, the latter under infected field conditions. A large basal root angle is associated with shallow roots whereas a small basal root angle implies deep roots (Liao et al. 2004). A direct comparison of this QTL at SNP ss715645390, located at 39.21 Mbp (Table 2.9) with the QTL reported by Hagerty et al. (2015) at SNP ss715645443, located at 38.42 Mbp, revealed that the two SNPs are only 725 kb apart, suggesting the same genomic region may be controlling root depth.

Another QTL associated with resistance to root rot pathogen FSP-3 was detected on Pv05 in experiment I in Uganda (Table 2.9). The QTL appears to be located in a region previously reported to harbor QTL associated with FRR resistance and root architecture

traits. Román-Avilés and Kelly (2005) reported several large effect QTL (R^2= 7.0- 53.3%) on Pv05 associated with FRR resistance using RAPD markers. The black bean variety Negro San Luis contributed the beneficial alleles in the QTL reported on Pv05. Two markers associated with the largest effect QTL (53% and 30%) were previously reported by Schneider et al. (2001) as linked to QTL associated with FRR resistance in FR266 that originated in black bean landrace PI203958. Liao et al. (2004), studying root traits in a non-inoculated *P. vulgaris* RIL population from DOR364, a deep-rooted genotype and G19833, a shallow-rooted genotype, reported a QTL associated with root length on Pv05.

A direct comparison of this QTL associated with FRR resistance (SNP ss715645368, at 39.46 Mbp, Table 2.9) with the QTL associated with deep root weight and total biomass (SNP ss715645390, at 39.20 Mbp, Table 1.9), showed the two QTL to be in the same interval, but with peaks 260 Kb apart.. The QTL associated with FRR resistance was more distant (985 Kb) from the SNP ss715645443 (38.47 Mbp) reported by Hagerty et al. (2015) as associated with FRR resistance. This is confirmation of earlier reports that genes governing related traits are usually found in clusters (Overbeek et al. 1999). A direct comparison with the QTL detected by Liao et al. (2004) and Román-Avilés and Kelly (2005) was not possible due to a difference in marker systems used. It is possible that the large effect QTL reported by Román-Avilés and Kelly (2005) may be a combination of these QTL (Flint and Mott, 2001). The co-localization of QTL associated with FRR resistance and root architecture traits may point to the fact that a combination of physiological mechanisms and root architecture traits is responsible for the partial root rot resistance observed.

An identical QTL, on Pv09 was revealed as associated with total root dry weight and shallow root dry weight at 0.29 Mbp (Table 2.9, Fig.2.2). This is another genomic area that has genes related to cell division and elongation (Goodstein et al. 2012). This QTL provides confirmation of previous reports that shallow basal roots are the largest contributors to root weight (Lynch and Van Beem, 1993). However, this QTL appears to be of minor effect (R^2=8.3%). This QTL may also imply that shallow root traits are governed by minor genes whose expression is greatly influenced by the environment, as was reported by Stam, (1998). This is supported by the different heritability values of the traits between experiments II and III (Table 2.2 and 2.5).

In conclusion, five QTL associated with FRR resistance and root traits were detected in this study. Total root weight and shallow root weight were associated with the same QTL on Pv09. A QTL associated with root length was detected on Pv01, and found to be independent of the *fin* locus. Two QTL, one associated with FRR resistance, another with deep root weight and total plant biomass were detected on Pv05, 260 kbp apart. These findings suggest that QTL associated with related traits are usually clustered together. The co-localization of QTL associated with root weight and total plant biomass may imply that pleiotropic genes control some root and shoot traits, and hence indirect selection for the hard to measure root traits may be accomplished by selecting for shoot traits, such as biomass. The finding that none of the detected QTL accounted for more than 13% of phenotypic variation is indicative of the fact that FRR resistance and root traits are governed by several genes of minor influence. Finally, the fact that all the beneficial alleles governing the QTL detected were contributed by Puebla 152 (Table 2.9) is confirmation

that the cultivar is a good source of root architecture traits that may be valuable in breeding for root rot avoidance in common bean.

APPENDIX

Table 2.1: Results of ANOVA for biomass, root dry weight and percent root dry weight of the Puebla 152 x Zorro RIL population, inoculated with FSP. in the greenhouse, East Lansing MI (Expt. II).

		P-Value		
Source	DF[§]	Root dry weight	Total biomass	% Root dry weight
RILs.	108	<0.0001***	0.0105***	<0.0001***
Inoculation	1	0.0003***	0.0002***	0.069

DF[§], Degrees of freedom

*** P- value significant (α= 0.001)

Table 2.2: Range and mean of root dry weight, total biomass and percent root dry weight of parents and RIL population (inoculated) in Expt. II.

Genotype	Root dry weight (g)	Total biomass (g)	% Root dry weight
		Parents	
Puebla 152	0.4a	2.07a	25.3a
Zorro	0.3a	1.3a	27.7a
		RILs	
Highest value	0.65	3.45	32.4
Lowest value	0.23	0.80	18.8
Mean	0.43	1.42	29.7
CV (%)	38.6	34.6	19.8
Heritability	0.34	0.37	0.27

[1]Means in the same column followed by the same letter are not significantly different (Least Square means F- test, $\alpha = 0.05$).

Table 2.3: Results of analysis of pairwise differences between Zorro and Puebla 152 Parents for root dry weight, total plant biomass and percent root dry weight inoculated or non-inoculated with FSP. strain NRRL 31157 (Expt. II).

Trait	Cultivar	Inoc.†	Mean/g	Cultivar	Inoc.†	Mean/g	% diff.§	P Value
Root dry weight	Puebla	y	0.4	Puebla	n	0.5	30.0	0.243
	Puebla	y	0.5	Zorro	y	0.3	73.3	0.067
	Zorro	y	0.3	Zorro	n	0.3	0	1.00
Total biomass	Puebla	y	2.1	Puebla	n	2.3	11.0	0.672
	Puebla	y	2.1	Zorro	y	1.3	59.2	0.541
	Zorro	y	1.3	Zorro	n	2.4	84.6	0.040*
% Root dry weight	Puebla	y	25.1	Puebla	n	17.3	45.1	0.575
	Puebla	y	25.1	Zorro	y	12.5	100.8	0.641
	Zorro	y	12.5	Zorro	n	23.5	88.1	0.993

1 P value reported from Least Square means F-test. * P value significant (α= 0.05).

Inoc.†- Inoculation y- Inoculated n- not inoculated (control).

% diff.§- Percentage difference.

Table 2.4: Results of ANOVA for No. lateral roots, shallow root weight, deep root weight, total root weight, total plant biomass and root length of the Puebla 152 x Zorro RIL population (Expt. III).

		P value					
Source	DF[§]	No. Lateral Roots	Shallow root weight	Deep root weight	Total root weight	Total plant biomass	Root length
RIL	119	0.0005***	<0.0001***	0.0001***	<0.0001***	<0.0001***	0.0078***
Residual	120	-	-	-	-	-	-

*** Significant at α= 0.05

DF[§], Degrees of freedom

Table 2.5: Range, heritability, coefficient of variation (CV) and mean of No. lateral roots, shallow root weight, deep root weight, total root weight, total plant biomass and root length of parents and RIL population of Puebla 152 x Zorro (Expt. III)

Genotype	No. lateral roots	Shallow root wt (g)	Deep Root wt (g)	Total root wt (g)	Total plant biomass (g)	Root length (cm)
			Parents			
Puebla 152	7.5a	5.70a	7.33a	13.03a	103.03a	84.75a
Zorro	7.0a	5.61a	5.29a	10.90a	72.38a	93.50a
			RIL Progeny			
Highest value	11.00b	16.28b	21.21b	30.76b	124.75b	102.00b
Lowest value	4.00a	0.98a	0.03a	1.01a	4.24a	38.88a
Mean	6.83	6.39	4.68	11.09	73.80	81.49
CV (%)	30.2	50.7	64.0	55.4	40.4	47.7
H^2	0.45	0.55	0.49	0.56	0.69	0.35

[1] Values in the same column followed by the same letters are not significantly different (Least Square Means T-test α= 0.05).

Table 2.6: Pearson Correlation coefficient (r) matrix of No. lateral roots, shallow root weight, deep root weight, total root weight, total plant biomass and root length of RIL population of Puebla 152 (Expt. III).

	No. lateral roots.	Shallow root wt.	Deep root wt.	Total root wt.	Total plant biomass.	Root length.
No. Lateral roots.	1.000	0.357***	0.198	0.312***	0.358***	0.127
Shallow root wt.	0.357***	1.000	0.483***	0.844***	0.655***	0.164
Deep root wt.	0.198	0.483***	1.00	0.876***	0.649***	0.391***
Total root wt.	0.312***	0.844***	0.876***	1.000	0.757***	0.325***
Total plant biomass.	0.358***	0.655***	0.649***	0.757***	1.000	0.351***
Root length.	0.127	0.164	0.391***	0.325***	0.351***	1.000

[1]*** significant at α=0.001.

Table 2.7: ANOVA results of disease severity in the 'Puebla 152 x 'Zorro' RIL population (Expt. I)

Source	DF†	Mean-Square	Expected-Mean-Square	Error Term	Error DF	F Value	P value
ID no.	109	2.309	$\sigma_e + r(\sigma_G^2)$	MS(Residual)§	110	1251190	<.0001***
Residual	110	1.845e -5	σ_e				

***, P value significant at $\alpha = 0.001$

DF†, Degrees of freedom

R, number of replications.

σ_G^2, Genetic variance of RILs.

σ_e, Error variance.

MS (Residual) §, Mean Square of residuals (error)

Table 2.8: Fusarium root rot scores of parents, checks and progeny means, range, and heritability and coefficient of variation (CV) estimates of resistance of the'Puebla 152 X 'Zorro' RIL population (Expt. I).

Genotypes	Mean rating†
Parents	
Puebla 152	2.75a
Zorro	3.36b
Mid-parent value	3.05
Checks	
Resistant	2.00
Susceptible	8.68
Progeny	
Highest value	7.22
Lowest value	2.00
Mean	3.25
CV (%)	32.7
Heritability	0.99

[1]Values followed by the same letters are not significantly different (Least Square means T-test $\alpha = 0.05$). Mean rating†, Mean disease severity rating of 40 plants (2 replications). Disease severity was rated according to the CIAT scale (Abawi and Pastor-Corrales, 1990). Ratings of 1-3 indicate resistance, 4-5 moderate resistance, and 6-9 susceptible.

Table 2.9: QTL detected for root traits and FRR resistance in the Puebla 152 x Zorro RIL population (Expts. I and III).

Trait	Chrom.	Position (cM)	Peak LOD†	Add††	R^2 (%)§	Indicative marker	Physical Position (bp)
Total root wt.	Pv09	0.01	3.2	0.37	8.3	ss715650429	298,696
Root length	Pv01	20.28	3.45	0.29	13.7	ss715649523	3,862,571
Deep root dry wt.	Pv05	55.0	3.05	0.28	9.2	ss715645340	39,200,027
Shallow root wt.	Pv09	0.01	3.2	0.37	8.3	ss715650429	298,696
Total biomass	Pv05	55.0	5.02	0.42	12.7	ss715645340	39,200,027
FRR Resistance	Pv05	60.05	3.2	0.276	10.06	ss715645368	39,460,326

†LOD, Log of odds.

R^2(%)§, Proportion of the phenotypic variance explained by the QTL estimated as, $1 - 10^{\frac{-2*LOD}{n}}$.

Add††, Effect of substituting a single allele from one parent to another. Positive values indicate alleles from the parent Puebla 152.,

Table 2.10: Root weight comparison of the inoculated and control RILs of the Puebla 152 X Zorro population in experiment II

	Root weight (g)			
ID no.	Control	Inoculated	% Difference	P value
501	0.45	0.2	25.0	0.10
502	0.38	0.4	-2.5	0.87
503	0.43	0.2	22.5	0.13
504	0.40	0.6	-20.0	0.18
507	0.40	0.15	25.0	0.10
508	0.55	0.5	5.0	0.74
509	0.40	0.6	-20.0	0.18
510	0.50	0.4	10.0	0.50
511	0.30	0.3	0.0	1.00
512	0.25	0.3	-5.0	0.74
513	0.40	0.5	-10.0	0.50
514	0.45	0.2	25.0	0.10
515	0.30	0.3	0.0	1.00
516	0.50	0.6	-10.0	0.50
517	0.55	0.4	15.0	0.32
518	0.45	0.3	15.0	0.32
519	0.25	0.3	-5.0	0.74
520	0.35	0.3	5.0	0.74
521	0.45	0.2	25.0	0.10

Table 2.10 (cont'd.)

522	0.40	0.3	10.0	0.50
523	0.35	0.3	5.0	0.74
524	0.53	0.6	-7.5	0.61
525	0.40	0.3	10.0	0.50
527	0.25	0.4	-15.0	0.32
528	0.50	0.4	10.0	0.50
530	0.40	0.15	25.0	0.61
531	0.40	0.2	20.0	0.08
532	0.45	0.4	5.0	0.74
533	0.50	0.15	35.0	0.02*
534	0.30	0.15	15.0	0.32
536	0.43	0.15	27.5	0.07
537	0.40	0.8	-40.0	0.01**
539	0.55	0.6	-5.0	0.74
540	0.40	0.15	25.0	0.10
541	0.30	0.3	0.0	1.00
542	0.35	0.2	15.0	0.32
543	0.25	0.45	-20.0	0.18
544	0.40	0.3	10.0	0.50
545	0.35	0.3	5.0	0.74
546	0.40	0.3	10.0	0.50
547	0.45	0.3	15.0	0.32

Table 2.10 (cont'd.)

548	0.30	0.1	20.0	0.18
549	0.40	0.3	10.0	0.50
550	0.35	0.3	5.0	0.74
551	0.30	0.15	15.0	0.32
552	0.30	0.45	-15.0	0.32
553	0.40	0.1	30.0	0.05*
555	0.50	0.2	30.0	0.05*
556	0.40	0.75	-35.0	0.02*
557	0.35	0.3	5.0	0.74
558	0.35	0.3	5.0	0.74
559	0.50	0.3	20.0	0.18
560	0.50	0.45	5.0	0.74
561	0.50	0.15	35.0	0.02*
562	0.35	0.15	20.0	0.18
563	0.40	0.2	20.0	0.18
564	0.40	0.3	10.0	0.50
565	0.45	0.3	15.0	0.32
566	0.30	0.45	-15.0	0.32
567	0.55	0.6	-5.0	0.74
568	0.40	0.6	-20.0	0.18
569	0.45	0.75	-30.0	0.05*
570	0.50	1.2	-70.0	<.0001***

Table 2.10 (cont'd.)

571	0.45	0.3	15.0	0.32
573	0.45	0.3	15.0	0.32
574	0.35	0.45	-10.0	0.50
575	0.45	0.9	-45.0	0.01**
576	0.40	0.5	-10.0	0.50
577	0.50	0.5	0.0	1.00
578	0.45	0.6	-15.0	0.32
579	0.40	0.4	0.0	1.00
580	0.35	0.2	15.0	0.32
581	0.40	0.4	0.0	1.00
582	0.50	0.4	10.0	0.50
583	0.30	0.4	-10.0	0.50
584	0.45	0.4	5.0	0.74
585	0.30	0.4	-10.0	0.50
586	0.45	0.4	5.0	0.74
588	0.23	0.3	-7.5	0.61
589	0.65	0.5	15.0	0.32
590	0.35	0.3	5.0	0.74
591	0.55	0.1	45.0	0.01**
592	0.55	0.4	15.0	0.32
593	0.50	0.1	40.0	0.01**
595	0.50	0.4	10.0	0.50

Table 2.10 (cont'd.)

596	0.40	0.4	0.0	1.00
598	0.60	0.5	10.0	0.50
599	0.40	0.4	0.0	1.00
600	0.45	0.3	15.0	0.32
601	0.45	0.6	-15.0	0.32
602	0.60	0.2	40.0	0.01**
603	0.45	1.05	-60.0	0.01**
604	0.63	0.1	52.5	0.01**
605	0.45	0.4	5.0	0.74
607	0.60	0.3	30.0	0.05*
609	0.60	0.2	40.0	0.01*
611	0.35	0.6	-25.0	0.10
612	0.55	0.3	25.0	0.10
613	0.55	0.6	-5.0	0.74
614	0.35	0.2	15.0	0.32
615	0.55	0.4	15.0	0.32
616	0.50	0.6	-10.0	0.50
617	0.25	0.1	15.0	0.32
619	0.45	0.4	5.0	0.74
620	0.40	0.15	25.0	0.10
621	0.40	0.45	-5.0	0.74
622	0.55	0.3	25.0	0.10

Table 2.10 (cont'd.)

[1] * P value significant (Least Square means F-test $\alpha = 0.05$)

[2] ** P value significant (Least Square means F-test $\alpha = 0.001$)

[3]*** P value significant (Least Square means F-test $\alpha = 0.0001$)

Figure 2.1: Histograms showing distribution of No. of lateral roots, shallow root weight, deep root weight, total root weight, total plant biomass, root length and FRR ratings in the Puebla 152 x Zorro RIL population (Expt. III).

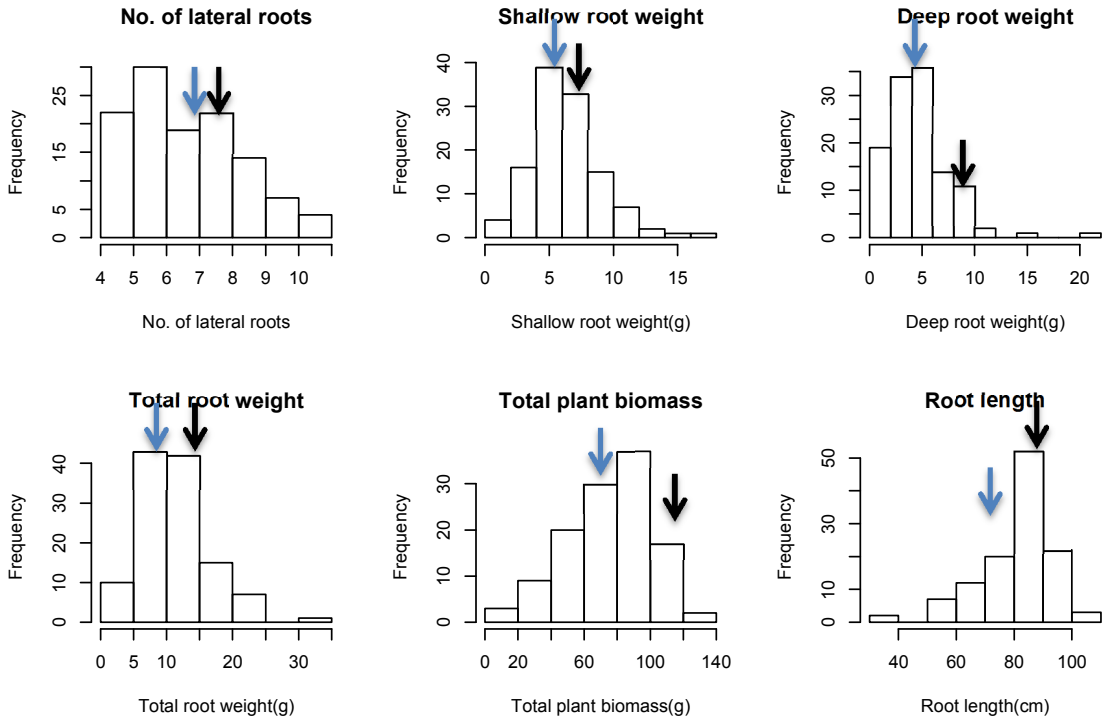
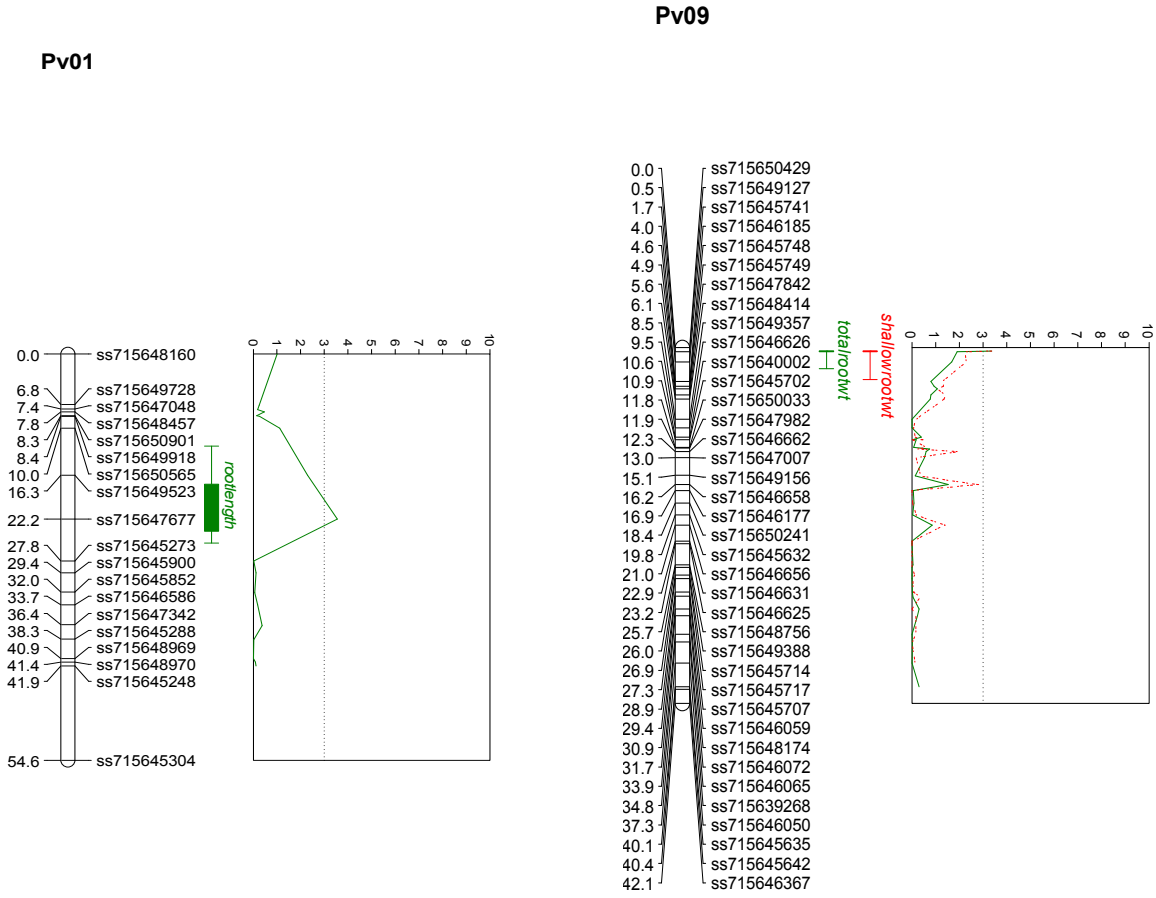


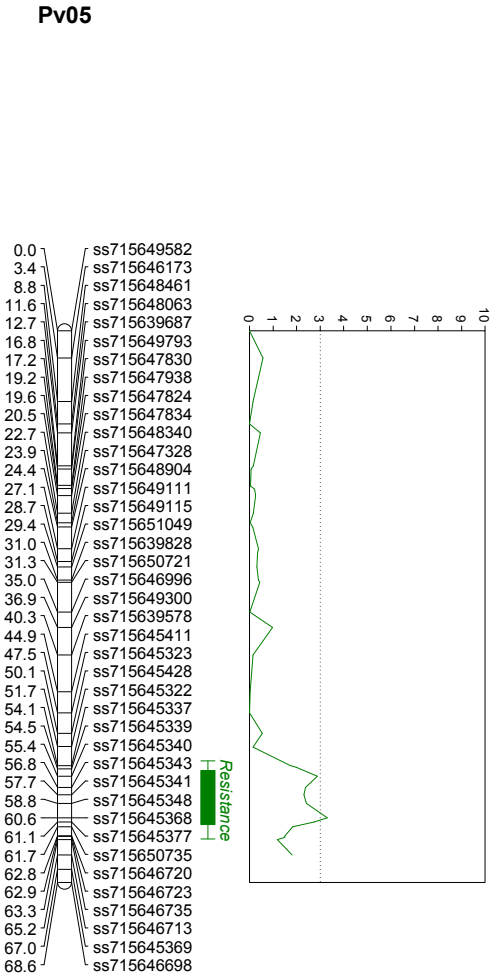
Figure 2.2: QTL positions detected by CIM analysis of Puebla 152 X Zorro RIL population, (a) root length (Pv01) in expt. III (b) shallow root weight and total root weight (Pv09) in expt. III, (c) FRR resistance on (Pv05) in expt.I, (d) deep root weight and total biomass (Pv05) in expt.III



(a) (b)

Figure. 2.2 (cont'd)

(c)



(d)

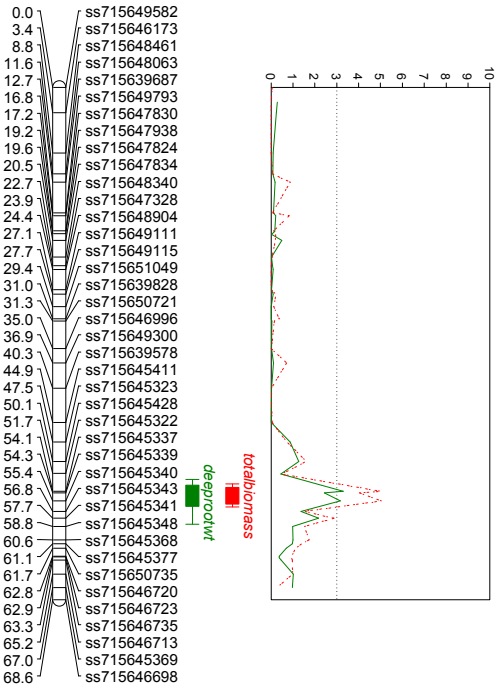


Figure 2.3: LOD graph of FRR disease severity rating from CIM of the Puebla 152 X Zorro RIL population (Expt. I).

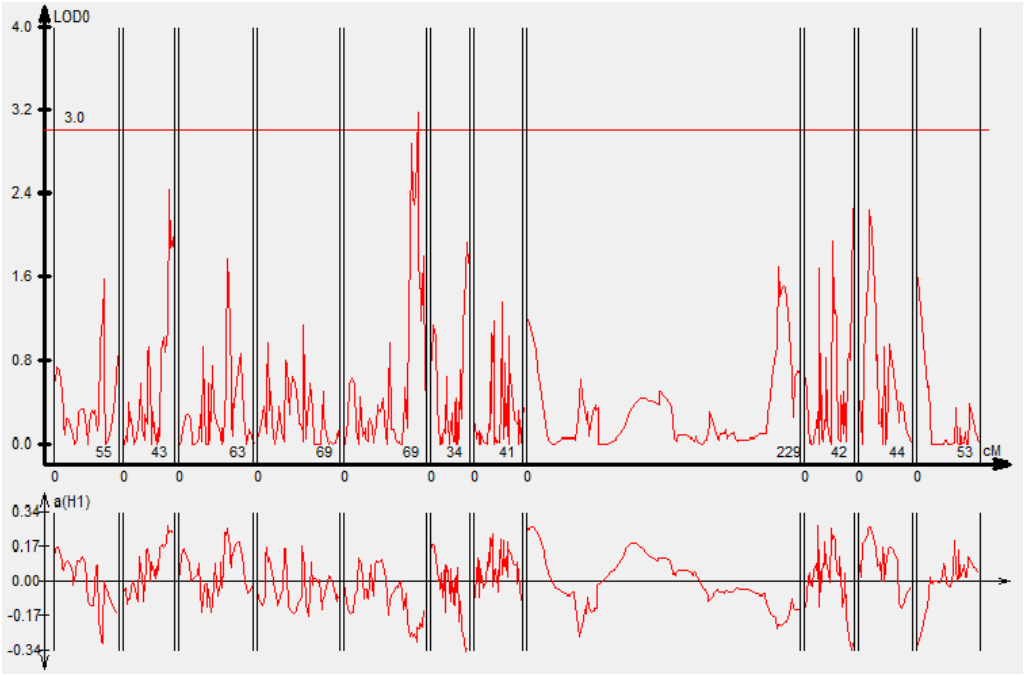


Figure 2.4: LOD graph of total plant biomass (Expt. III) from Composite Interval Mapping (CIM) of the Puebla 152 x Zorro RIL population.

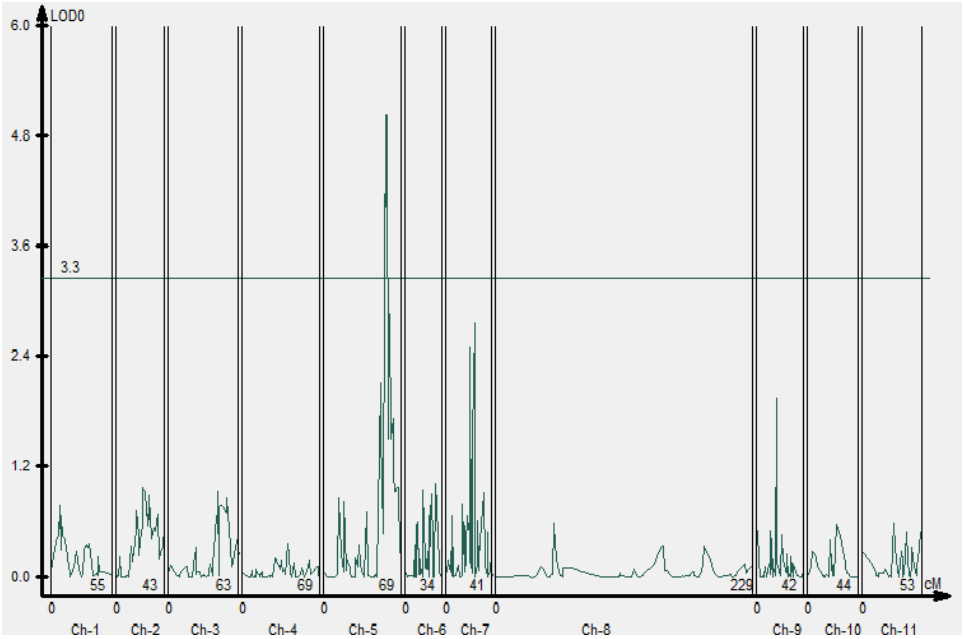


Figure 2.5: LOD graph of root length (Expt. III) from Composite Interval Mapping (CIM) of the Puebla 152 X Zorro RIL population.

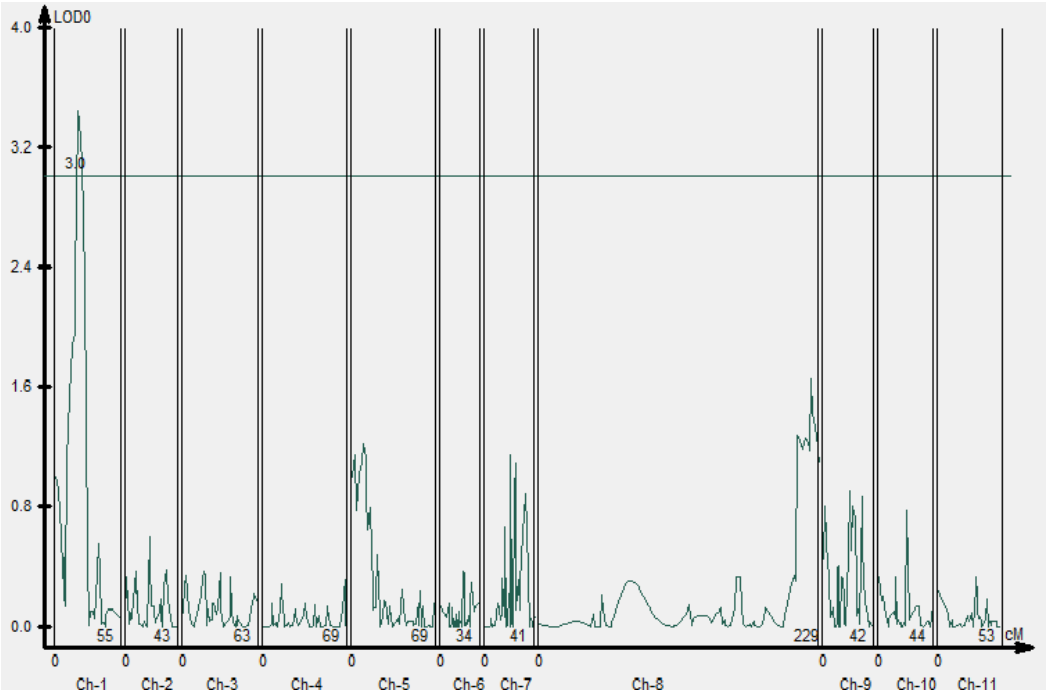


Figure 2.6: LOD graph of shallow root weight (Expt. III) from Composite Interval Mapping (CIM) of the Puebla 152 x Zorro RIL population

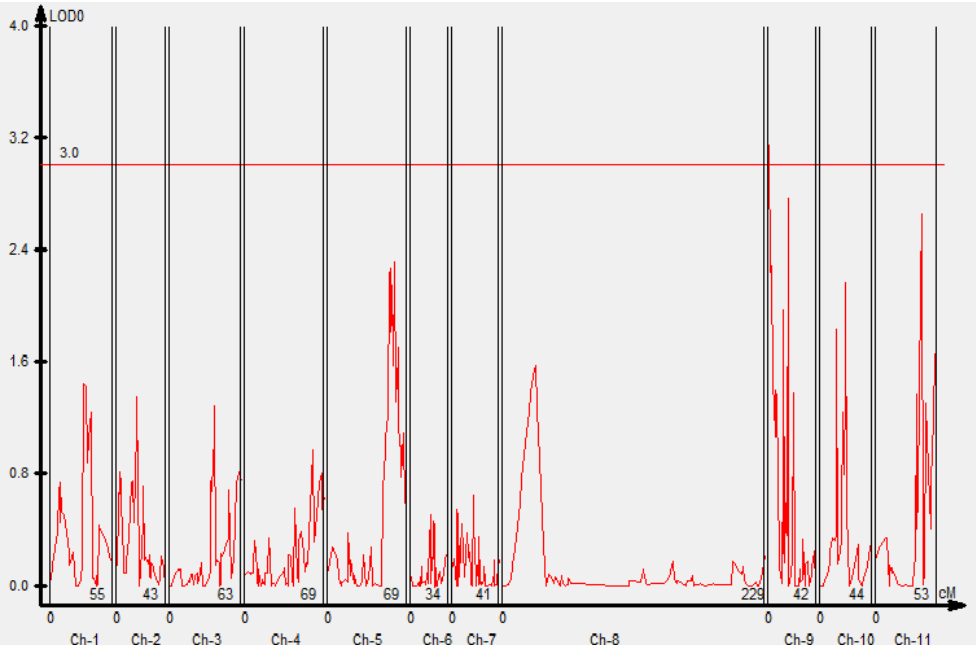


Figure 2.7: LOD graph of deep root weight (Expt. III) from Composite Interval Mapping (CIM) of the Puebla 152 x Zorro RIL population.

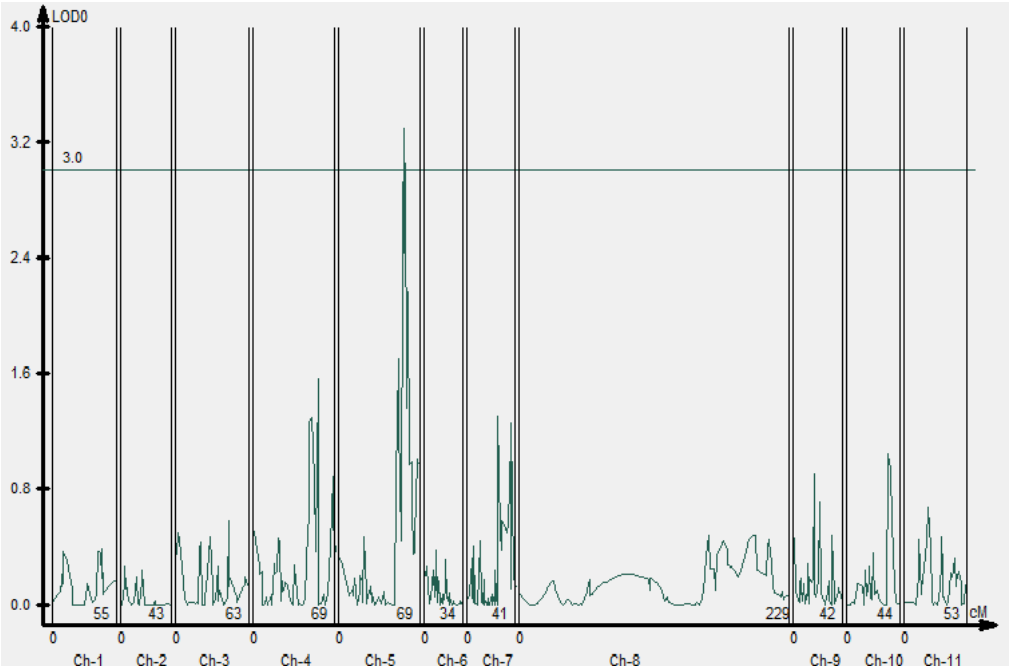
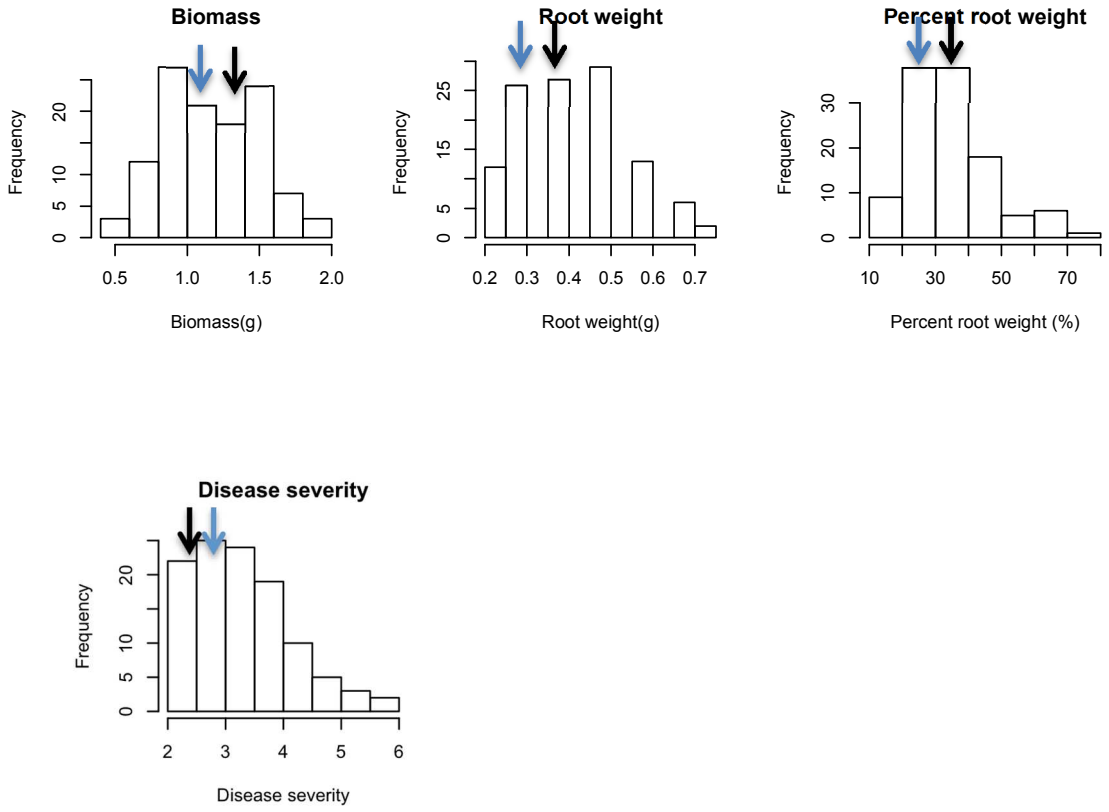


Figure 2.8: Histograms showing distribution of total biomass, root weight, percent root weight and disease severity in the Puebla 152 x Zorro RIL population (Expt. II and I).



REFERENCES

REFERENCES

Abawi, G. S., and Pastor-Corrales, M. A. (1990). Root rots of beans in Latin America and Africa, diagnosis, research methodologies, and management strategies. CIAT publication No. 35, ISBN, 958-9183-14-X.

Afanador, L.K., Haley, S.D., and Kelly, J.D. (1993). Adoption of a "miniprep" DNA extraction method for RAPD marker analysis in common bean (*Phaseolus vulgaris* L.). Annual Report, Bean Improvement Cooperative, 36, 10–11.

Aoki, T., O'Donnell, K., and Scandiani, M. M. (2005). Sudden death syndrome of soybean in South America is caused by four species of Fusarium: *Fusarium brasiliense* sp. nov., *F. cuneirostrum* sp. nov., *F. tucumaniae*, and *F. virguliforme*. Mycoscience, 46(3), 162-183.

Beebe, S. (2012). Common bean breeding in the tropics. Plant Breeding Reviews, 36, 357–426.

Beebe, S.E., Rojas-Pierce, M., Yan X., Blair L., M., W., Pedraza, F., F. Munoz, F., Tohme, J., and Lynch, J.P. (2006). Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Science, 46, 413–423.

Bernardo, R. (2002). Breeding for quantitative traits in plants. Woodbury, MN, Stemma.

Bravo, A., Wallace, D. H., and Wilkinson, R. E. (1969). Inheritance of resistance to Fusarium root rot of beans. Phytopathology 59,1930-1933.

Burke, D. W. (1968). Root growth obstructions and Fusarium root rot of beans. Phytopathology 58, 1575–1576.

Churchill, G. A., and Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. Genetics 138,963–971.

Cichy, K. A., Snapp, S. S., and Kirk, W. W. (2007). Fusarium root rot incidence and root system architecture in grafted common bean lines. Plant and Soil, 300 (1-2), 233-244.

Cichy, K., A., Blair, M. W., Galeano Mendoza, C. H., Snapp, S, S., and Kelly, J. D. (2009). QTL analysis of root architecture traits and low phosphorus tolerance in an Andean bean population. Crop Science, 49(1), 59-68.

Dickson, M. H. (1973). Root rot tolerance in snap beans. NY Food Life Science, 6, 16-17.

Estevez de Jensen, C., Kurle, J.E. and Percich, J.A. (2004). Integrated management of edaphic and biotic factors limiting yield of irrigated soybean and dry bean in Minnesota. Field Crops Research 86, 211–224.

Flint, J., and Mott, R. (2001). Finding the molecular basis of quantitative traits, successes and pitfalls. Nature Reviews Genetics, 2 (6), 437-445.

Freyre, R., Skroch, P. W., Geffroy, V., Adam-Blondon, A. F., Shirmohamadali, A., Johnson, W.C., Llaca, V., Nodari, R. O., Pereira, P. A., Tsai, S. M., Tohme, J., Dron M., Nienhuis J., Vallejos, C. E. and Gepts, P. (1998). Towards an integrated linkage map of common bean (*Phaseolus vulgaris*) 4. Development of a core linkage map and alignment of RFLP maps. Theoretical and Applied Genetics, 97(5-6), 847–856.

Goodstein, D. M., Shu, S., Howson, R., Neupane R. D., Hayes, R. D., Fazo, J., Mitros, T., William, D., Hellsten, U., Putnam, N., and Rokhsar D. S. (2012). Phytozome, a comparative platform for green plant genomics. Nucleic Acids Research. 2012 40 (D1), D1178-D1186.

Hagerty, C. H., Cuesta-Marcos, A., Cregan, P. B., Song, Q., McClean, P., Noffsinger, S., and Myers, J. R. (2015). Mapping *Fusarium solani* and *Aphanomyces euteiches* root rot resistance and root architecture quantitative trait loci in common bean. Crop Science,55 (5), 1969-1977.

Heilig, J. A. (2015). QTL mapping of symbiotic nitrogen fixation in dry bean; Evaluation of dry bean genotypes under organic production systems. Doctoral Dissertation, Michigan State University, East Lansing MI. 150pp.

Hyten, D. L., Song, Q., Fickus, E. W., Quigley, C. V., Lim, J. S., Choi, I. Y., Hwang, E.Y., Pastor-Corrales, M., and Cregan, P. B. (2010). High-throughput SNP discovery and assay development in common bean. BMC genomics, 11(1), 475.

Ibarra-Perez, F.J., Waines, J.G., Ehdaie, B., Heilig, J.A., and Kelly, J.D. (2014) Phenotyping root and shoot traits of Zorro and Puebla 152 common bean (*Phaseolus vulgaris* L.) cultivars. Annual Report Bean Improvement Cooperative 57,107-108.

Jackson, W.T. (1955). The role of adventitious roots in recovery of shoots following flooding of the original root system. American Journal of Botany, 42, 816–819.

Kamfwa, K., Mwala, M., Okori, P., Gibson, P. and Mukankusi, C. (2013). Identification of QTL for Fusarium Root Rot Resistance in Common Bean. Journal of Crop Improvement, 27(4), 406-418.

Kanazin, V., Marek, L. F., and Shoemaker, R. C. (1996). Resistance gene analogs are conserved and clustered in soybean. Proceedings of the National Academy of Sciences, 93(21), 11746-11750.

Kelly, J. D., Varner, G. V., O'Boyle, P., and Long, B. (2009). Registration of 'Zorro' black bean. Journal of Plant Registrations, 3(3), 226-230.

Koinange E.M.K., Singh S.P., Gepts P. (1996) Genetic control to the domestication syndrome in common bean. Crop Science, 36, 1037–1045.

Kraft, J.M., and Boge, W. (2001) Root characteristics in pea in relation to compaction and Fusarium root rot. Plant Disease, 85, 936–940.

Liao, H., Yan, X., Rubio, G., Beebe, S. E., Blair, M. W., and Lynch, J. P. (2004). Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. Functional Plant Biology, 31(10), 959-970.

Lynch, J., and van Beem, J. J. (1993). Growth and architecture of seedling roots of common bean genotypes. Crop Science, 33(6), 1253-1257.

Mukankusi, C., Derera, J., Melis, R., Gibson, P. T., and Buruchara, R. (2011). Genetic analysis of resistance to Fusarium root rot in common bean. Euphytica, 182 (1), 11-23.

Navarro, F., Sass, M. E., and Nienhuis, J. (2008). Identification and confirmation of quantitative trait loci for root rot resistance in snap bean. Crop Science, 48(3), 962-972.

Navarro, F. M., Sass, M. E., and Nienhuis, J. (2009). Marker-Facilitated Selection for a Major QTL Associated with Root Rot Resistance in Snap Bean (L.). Crop Science, 49(3), 850-856.

Obala, J., Mukankusi, C., Rubaihayo, P. R., Gibson, P., and Edema, R. (2012). Improvement of resistance to Fusarium root rot through gene pyramiding in common bean. African Crop Science Journal, 20(1), 1-13

Otsyula, R., Rubaihayo, P., and Buruchara, R. (2003). Inheritance of resistance to Pythium root rots in beans (*Phaseolus vulgaris*) genotypes. African Crop Science Conference Proceedings (Vol. 6, pp. 295-298).

Overbeek, R., Fonstein, M., D'souza, M., Pusch, G. D., and Maltsev, N. (1999). The use of gene clusters to infer functional coupling. Proceedings of the National Academy of Sciences, 96(6), 2896-2901.

Ratnaparkhe, M. B., Tekeoglu, M., and Muehlbauer, F. J. (1998). Inter-simple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theoretical and Applied Genetics, 97(4), 515-519.

Repinski, S. L., Kwak, M., and Gepts, P. (2012). The common bean growth habit gene PvTFL1y is a functional homolog of Arabidopsis TFL1. Theoretical and Applied Genetics, 124(8), 1539-1547.

Román-Avilés, B., and Kelly, J. D. (2005). Identification of quantitative trait loci conditioning resistance to Fusarium root rot in common bean. Crop Science, 45(5), 1881-1890.

Roman-Avilés, B., Lewis, J. M., and Kelly, J. D. (2011). Fusarium genetic control: A long term strategy. In, Control of Fusarium Diseases. Alves-Santos FM, Diez J., eds. Research Signpost, Kerala, India, 65-108.

Román-Avilés, B., Snapp, S. S., and Kelly, J. D. (2004). Assessing root traits associated with root rot resistance in common bean. Field Crops Research, 86(2), 147-156.

Rusuku, G., Buruchara, R. A., Gatabazi, M., and M.A. Pastor-Corrales, M.A. (1997). Occurrence and distribution of soil-borne fungi pathogenic to common bean in Rwanda. Plant Disease 81,445-449.

SAS Institute. (2012). SAS version 9.4. SAS Institute Inc., Cary, NC.

Schneider, K. A., Grafton, K. F., and Kelly, J. D. (2001). QTL analysis of resistance to Fusarium root rot in bean. Crop Science, 41(2), 535-542.

Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., and Sirotkin, K. (2001). dbSNP, the NCBI database of genetic variation. Nucleic Acids Research, 1 29(1), 308-11.

Snapp, S.S., Kirk, W., Román-Avilés, B., and Kelly, J. D. (2003). Root traits play a role in integrated management of Fusarium root rot in snap beans. HortScience 38,187-191.

Stam, P (1998). Crop physiology, QTL analysis and plant breeding. In: 'Inherent variation in plant growth, physiological mechanisms and ecological consequences'. (Ed.H Lambers) pp.429-440. (Backhuys Publishers, Leiden).

Turner, B. L., and Doolittle, W. E. (1978). The concept and measure of agricultural intensity. The Professional Geographer, 30(3), 297-301.

Tusiime, G. (2003). Variation and detection of *Fusarium solani* f.sp. *phaseoli* and quantification of soil inoculum in common bean fields. Dissertation, Makerere University, Kampala, Uganda.

Van Ooijen. J.W. (2006). JoinMap®4.0 Software for calculation of genetic linkage maps in experimental populations. Kyazma B.V. Wageningen, Netherlands.

Voorrips, R.E. (2002). MapChart, Software for the graphical presentation of linkage maps and QTL. Journal of Heredity, 93,77–78.doi,10.1093/jhered/93.1.77

Wang, S., Basten, C. J., and Zeng, Z. B. (2012). Windows QTL cartographer 2.5. Dep. of Statistics, North Carolina State Univ., Raleigh.

Wang, Y., Liu, H., and Xin, Q. (2014). Genome-wide analysis and identification of cytokinin oxidase/dehydrogenase (CKX) gene family in foxtail millet (*Setaria italica*). The Crop Journal, 2 (4), 244-254.

Wortmann, C. S., Kirkby, R. A., Eledu, C. A., and Allen, D. J (1998). Atlas of common bean (*Phaseolus vulgaris*). CIAT Publication, No.297.

Chapter Three

A report on field surveys conducted in common bean-growing regions of Uganda

Introduction

The common bean (*Phaseolus vulgaris L.*) plays an important role in human nutrition throughout rural and urban areas of Eastern Africa (David et al. 2000). This is because common bean is a major source of dietary protein and calories for many resource-poor communities (Ddamulira et al. 2014) Eastern Africa has the highest common bean production in sub-Saharan Africa at 1,297,000 tons per annum (Wortmann et al. 1998).

In Eastern Africa the highest producing countries in descending order are Tanzania, Kenya, Uganda, Rwanda, Burundi, Ethiopia and The Democratic Republic of Congo. (FAOSTAT, 2015). However, fluctuations in dry bean production and yield per hectare have been reported, despite the expansion in area under production. On-farm yields are much less than the expected yields of 1.5–2 tons per hectare on research farms (CIAT, 2008). Lower productivity has been due mostly due to the incidence of diseases (Buruchara et al. 2011). The most notable of these diseases are root rots, and foliar disease like anthracnose and common bacterial blight (Ddamulira et al. 2014). Up to date information on disease constraints is necessary to enable adequate prioritization of research efforts. The aim of this survey was to update the available information on the extent and severity of bean diseases in the bean growing areas of Uganda.

Methods

Two field surveys were conducted in the rainy seasons of 2013 and 2014**.** Field surveys were conducted in 14 selected bean-growing districts in the five regions of Uganda (Table 3.1, Fig.3.1) that is Northern, Eastern, Central, Western and Southwestern Uganda**.** The 2013 survey was carried out during the second annual rainy season from September to October, while the 2014 survey was done during the first annual rains from April to June.

The survey team comprised two pathologists from CIAT Uganda, Fred Kato and Cathy Amongin, along with a local extension agent, picked from each of the counties visited. The author joined the team for the Northern and Eastern leg of the 2014 survey, during 7th-21st June 2014. The surveys were conducted in all sub counties of each district visited.

Bean disease and pest identification hand books (CIAT-PABRA, 2010) and Legume *ipm* PIPE Diagnostic pocket series were distributed to farmers and extension officers in the districts surveyed.

Farmers were interviewed and their information completed in an on-farm survey questionnaire. The questionnaire included questions about their farm sizes, previous crops grown, varieties grown and major constraints faced.

Diseased or damaged plants identified in the fields were randomly picked, the suspected genus of the pathogen causing the disease identified basing on visual signs and symptoms, and then packed in paper bags and labeled.

Despite the fact that the sample collection was conducted at the end of the season for the 2014 survey, a total of 338 bean root rot infected plant samples were obtained, while 290 samples were obtained in the 2013 survey. The majority of the samples were obtained at primary leaves stage (V1 or V2).

Morphological and genotypic characterization of the isolated root rot pathogens was conducted by CIAT staff and reported in the CIAT Uganda NIFA project progress report (CIAT 2014, unpublished). More than 120 single spore isolates of *Sclerotium rolfsii,* 30 *Pythium,* 100 *Fusarium spp.* and 100 *Rhizoctonia spp.* were obtained from the in 2013 collection. Pure cultures of *S. rolfsii* and *Pythium spp. h*ave been preserved on sterile filter papers and stored at 4 and -20^{o}C. Storage of pure cultures of *Fusarium* and *Rhizoctonia spp*. preservation and storage is still ongoing.

Results

The major biotic constraints identified visually during the survey were root rots and insect damage (Fig 3.8). Four bean root rot pathogens that included *Sclerotium spp., Fusarium spp., Pythium spp.* and *Rhizoctonia spp.* were encountered (Fig. 3.2 to 3.4).

Of the samples collected in the 2014 survey, *Sclerotium spp.* was the most widely spread pathogen (60%) followed by *Fusarium spp.* (25%), and *Rhizoctonia spp.* (10%*).* while *Pythium spp* (4%). was the least common based on visible plant symptoms (Fig. 3.9). Insect damage, mainly caused by the bean beetle (*Callosobruchus maculatus*), was severe at all sites visited in the five regions (Fig. 3.5). This was confirmed by laboratory-based pathogen isolations from the 2014 survey samples that produced over 120 distinct isolates of *Sclerotium spp.,* the highest number of isolates from a single pathogen (CIAT, 2014).

Bush beans are the most prevalent type of common beans grown in Uganda. Most common bean farmers are small-scale subsistence farmers, with the average farm size being 0.5 ha. It was also observed that common beans, mainly the bush type, are intercropped with other crops such as banana, corn, coffee, cassava etc. throughout the districts visited.

The most diseased bean samples were collected from the districts in the central region (36%), then western (22%), then southwestern (21%), then northern (11%), then eastern (10%), Fig.2.7. Common beans are not grown extensively in the Northern region compared to other regions visited in Uganda, as it is not considered a priority crop. This may be because the climate in this region is unfavorable for rain-fed common bean growing.

Farmers' ignorance about the bean root rots was noted from the majority of farmers who think that the disease severity in their fields was as a result of abiotic factors such as soil

pH, soil temperature and soil type. While others thought that wilting and death of the beans in the fields was due to unpredictable weather conditions following late planting.

For some farmers visited, it was reported that the diseases have been detected in previous seasons, however no action has been taken since they think it has less impact on the yield.

In a Northern district, we learnt that farmers are not growing beans extensively especially those that are supplied by the national program due to a myth that they cause cancer.

Recommendations and Conclusion

Root rots continue to present a challenge to common bean farmers in Uganda. However, of concern is the increase in incidence of Sclerotium spp. incited root rots. This may be attributed to an increase in inoculum levels over the years. It is recommended that research attention (both breeding and pathology) be turned to this pathogen, since it is expected to gain importance in the near future.

Entomologists should be consulted so as to devise sustainable and practical solutions to the bean beetle (*Callosobruchus maculatus*).

It is suspected that more biotic constraints affect common bean production in Uganda, however, due to the early stage (V1 or V2) at which the survey was done, it was not possible to capture them, as they affect the crop at later stages.

Farmer extension should be strengthened, to enable sensitization of farmers about the need to adopt better farming methods and improved varieties. This could be done by recruiting and facilitating more extension workers with better transportation and communication tools.

Access to clean seed should be improved. This is especially problematic in the remote rural areas of the country. Strengthening the zonal agricultural research centers, both in human and technical capacity, as to carry out advanced generation testing and a clean seed distribution program, could achieve this. This would greatly reduce seed borne disease incidence, and also reduce the amount of disease inoculum transferred between fields as farmers carry seed and plant material like pods and leaves between fields every season.

Fact-finding surveys should be periodically carried out, and results published in easily accessible outlets to enable the collection and dissemination of up to date information on

constraints and opportunities. This ensures that the research is informed by actual and not perceived problems.

The high degree of variability within the root rot pathogens, as is evidenced by the large numbers of isolates of each pathogen (CIAT, 2014), may greatly hamper resistance-breeding efforts. This is an especially serious challenge to resistance breeding toward those pathogens to which resistance is controlled by major genes, like *Pythium.* Hence screening for resistance genes should continue so as to enable discovery of novel genes.

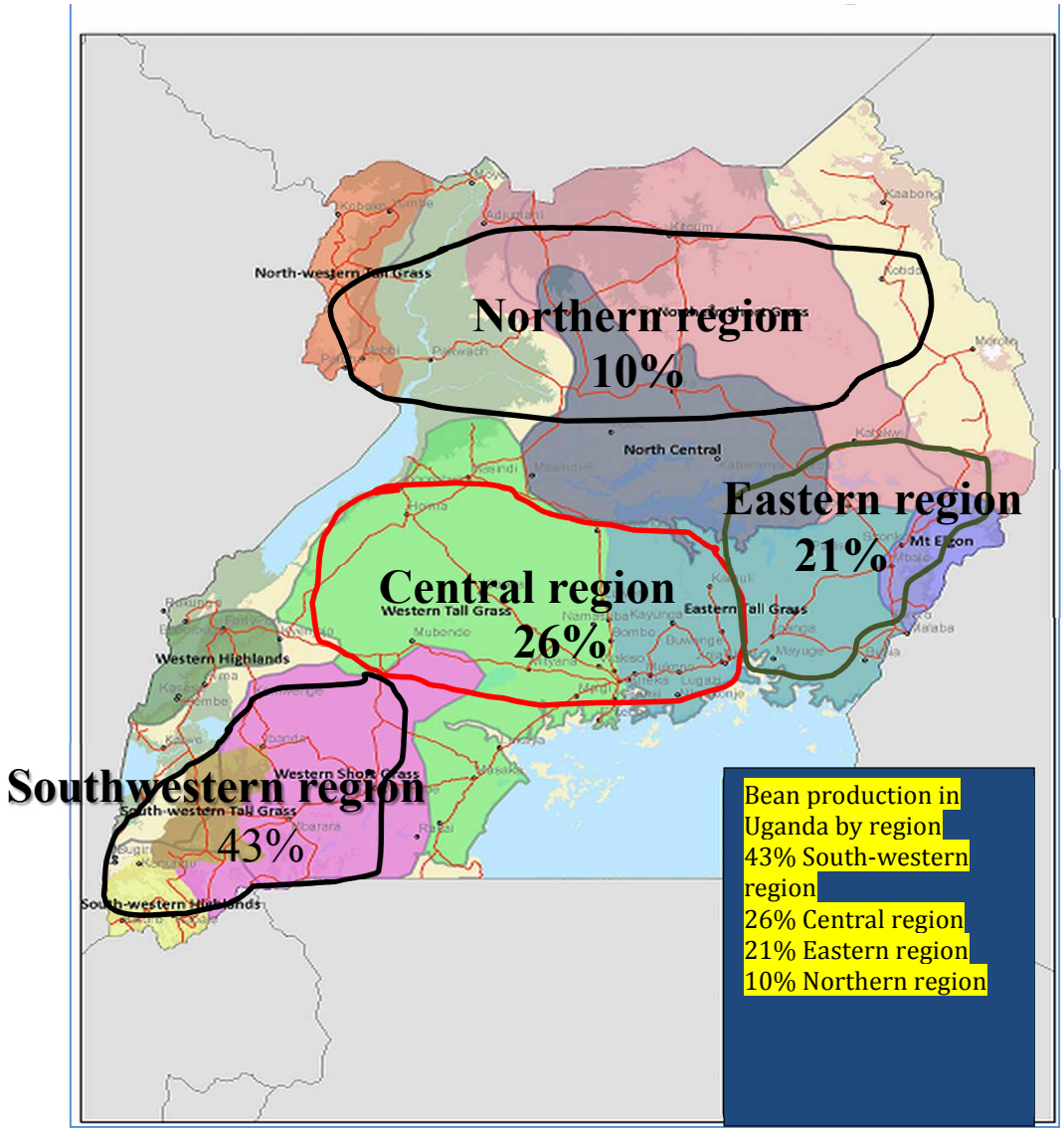
APPENDIX

Table 3.1: Number of farmers' fields visited and samples collected per district in each of the regions during the 2013 and 2014 surveys

2013b				2014a			
Region	District	No. fields	No. samples	Region	District	No. fields	No. samples
Central	Mukono	25	25	Central	Mityana	31	31
Central	Kayunga	15	15	Central	Wakiso	39	39
Central	Luweero	76	76	Central	Mpigi	22	22
Central	Nakaseke	32	32	Eastern	Tororo	6	6
Eastern	Kaberam	14	14	Eastern	Kamuli	21	21
Eastern	Amuria	9	9	Eastern	Jinja	6	6
Eastern	Mbale	31	31	Northern	Pader	10	10
Eastern	Soroti	12	12	Northern	Apac	24	24
Eastern	Sironko	17	17	Northern	Kitgum	22	22
South west	Kisoro	21	21	Northern	Gulu	20	20
South west	Kabale	38	38	South-West	Kabarole	30	30
				South-West	Kyenjojo	20	20
				South-West	Mubende	29	29
				Western	Ibanda	11	11
				Western	Kamwenge	16	16
				Western	Mbarara	31	31
Total	**11**	**290**	**290**		**16**	**338**	**338**

[1] Adopted from CIAT Uganda NIFA project progress report (CIAT 2014, unpublished)

Figure 3.1: A map of Uganda showing the major dry bean production areas



[1] Adapted from Nkalubo (2015, unpublished).

Figure 3.2: A wilted bean plant affected by the bean root rot pathogen (Sclerotium rolfsii). The white mycelia colonized the below ground plant tissues.

Figure 3.3: A Wilted bean plant held showing Pythium root rot symptoms

Figure 3.4: A wilted bean plant affected by suspected Sclerotium root rot (Sclerotium rolfsii) (white fruiting bodies colonizing the soil).

Figure 3.5: Damage by bean beetle (Callosobruchus maculatus)

Figure 3.6: A photo taken from a farmer's bean field during the interview session (Northern Region, 2014).

Figure 3.7: Distribution of samples collected from the five regions surveyed in 2014

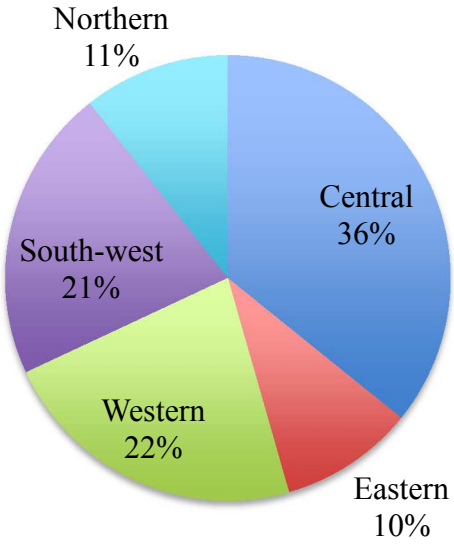


Figure 3.8: Biotic constraints affecting common bean production in the areas surveyed in 2014 (Based on visual identification).

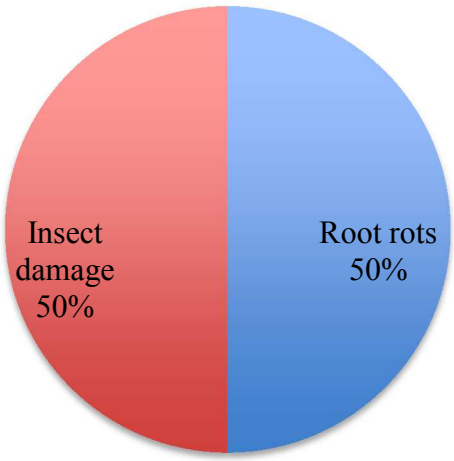
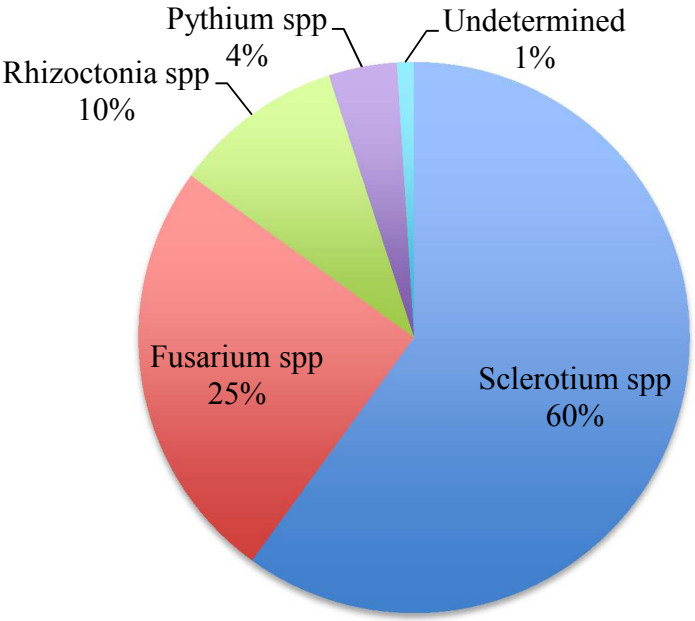


Figure 3.9: Distribution of root rot pathogens in samples collected in 2014 (Based on visual signs and symptoms)



REFERENCES

REFERENCES

Buruchara, R., Chirwa, R., Sperling, L., Mukankusi, C., Rubyogo, J. C., Mutonhi, R., and Abang, M. M. (2011). Development and delivery of bean varieties in Africa, the Pan-Africa Bean Research Alliance (PABRA) model. African Crop Science Journal, 19(4), 227-245.

CIAT (International Center for Tropical Agriculture). (2008). The impact of improved bush bean varieties in Uganda. Highlights - CIAT in Africa. Cali, Columbia. No.43. Available online, http,//webapp.ciat.cgiar.org/africa/ pdf/highlight43.pdf

CIAT (International Center for Tropical Agriculture). (2014, unpublished). Common Bean Productivity Research for Global Food Security NIFA project progress report.

David, S., Kirkby, R., and Kasozi, S. (2000). Assessing the impact of bush bean varieties on poverty reduction in sub-Saharan Africa, Evidence from Uganda (Vol. 31). CIAT, Network on bean research in Africa.

Ddamulira, G., Mukankusi, C., Ochwo-Ssemakula, M., Edema, R., Sseruwagi, P., and Gepts, P. (2014). Identification of new sources of resistance to angular leaf spot among Uganda common bean landraces. Canadian Journal of Plant Breeding. Vol. 2, No.2, 55-65.

FAOSTAT F. A. O. (2015). Statistical Databases. Food and Agriculture Organization of the United Nations.

Nkalubo, S.T. (2015). Power-Point presentation. Unpublished.

Wortmann, C.S, Kirkby, R.A., Eledu, C.A., and Allen D.J. (1998). Atlas of common bean production in Africa, CIAT publication No. 297.