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QTL ANALYSIS OF RESISTANCE TO *FUSARIUM* ROOT ROT
IN ANDEAN BEAN POPULATIONS AND THE INFLUENCE OF
ROOT ARCHITECTURE ON DISEASE DEVELOPMENT

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By

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ABSTRACT

QTL ANALYSIS OF RESISTANCE TO *FUSARIUM* ROOT ROT IN ANDEAN BEAN POPULATIONS AND THE INFLUENCE OF ROOT ARCHITECTURE ON DISEASE DEVELOPMENT

By

Belinda Román Avilés

Root rot, caused by the soil-borne fungus *Fusarium solani* f. sp. *phaseoli*, is a serious root disease of common bean (*Phaseolus vulgaris* L.). In the absence of effective control methods, the use of resistant bean cultivars is the preferred strategy for the management of root rot. The objectives of this study were: i) to characterize genetic variation of root architecture in contrasting bean classes and identify root system characteristics that may be related to root rot resistance; ii) to transfer *Fusarium* root rot resistance from small seeded black bean into highly susceptible large seeded Andean kidney and cranberry bean; and iii) to identify QTL-marker associated with *Fusarium* root rot resistance in common bean.

Genetic variation in root architecture among common bean classes was highly significant under field conditions compared to the greenhouse environment where variation in root traits was minimal. Bean breeders interested in enhancing root rot resistance and over all root health should focus on selecting for more lateral and adventitious roots since these traits are relatively easy to quantify under field conditions and for greater root dry weight in the greenhouse.

Two inbred backcross line (IBL) populations from crosses between small seeded resistant and large seeded susceptible genotypes were developed. Continuous variation in

root rot frequency distribution was observed for both kidney and cranberry IBL populations but a broader range of root rot scores were observed for the cranberry population. Data suggest that root rot resistance in bean is under polygenic control and is highly influenced by the environment. Heritability estimates ranged from 0.10 to 0.51 for the kidney and from 0.30 to 0.82 for the cranberry IBL populations. Nine QTL, significantly associated with *Fusarium* root rot resistance in the field and greenhouse, explained from 5 to 53% of the total phenotypic variability. QTL associated with root rot resistance were located on linkage group B2 and B5 of the integrated bean map close to previously identified QTL for resistance. Using a linear regression model a combination of five markers accounted for 73% of the phenotypic variation for root rot resistance. Data from the current study should provide breeders the opportunity to combine, through marker assisted backcrossing, large effect QTL (R^2>50) identified on different linkage groups to enhance root rot resistance in common bean.

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Felipe and Heriberto for their love, support, patience, kindness, and understanding

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LIST OF ABBREVIATIONS

AFLPs	Amplified fragment length polymorphism
ANOVA	Analysis of variance
AVG	Average
B	Black bean
BC	Backcross
BJ	BAT93 x JaloEEP558 RIL population
bp	base pair
BSA	Bulk segregant analysis
C	Cranberry bean
C:N	Carbon to nitrogen ratio
CA	California
CBB	Common bacterial blight
CFU	Colony Forming Units
ChS	Chalcone Synthase
cM	Centimorgan
CV	Coeficient of variation
DAP	Days after planting
DH	Double haploid
DL	Dwarf lethal
DNA	Deoxyribosenucleic acid
DRK	Dark red kidney
DTF	Days to flower
DTM	Days to maturity
f.sp.	Forma speciales
GH1	Greenhouse evaluation 1
GH2	Greenhouse evaluation 2
h^2	Heritability estimate
HW	Hybrid weakness
IBLs	Inbred backcross lines
K	Kidney bean
LG	Linkage group
LOD	Likelihood of the odds ratio
LRK	Light red kidney
LSD	Least significant difference
MAS	Marker assisted selection
MI	Michigan
MN	Minnesota
MRF	Montcalm Research Farm
MSU	Michigan State University
N	Nitrogen
ng	nanograms

NIL's	Near isogenic lines
NSL	Negro San Luis
NYSAES	New York State Agricultural Experiment Station
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PR	Pathogenesis-related protein
PvPR1	Phaseolus vulgaris pathogenesis-related protein 1
PvPR2	Phaseolus vulgaris pathogenesis-related protein 2
QTL	Quantitative trait loci
r	Correlation coefficient
R^2, r^2	Coefficient of determination
RAPD	Randomly amplified polymorphic DNA
RFLP's	Restriction fragment length polymorphisms
RH	Red Hawk
RILs	Recombinant inbred lines
S	Snap bean
SCAR	Sequence characterized amplified region
SRAP	Sequence-related amplified polymorphism
SSR	Simple sequence repeat
SW	100 seed weight
TRAP	Target region amplification polymorphism

Chapter I

ROOT ROT CAUSED BY *FUSARIUM SOLANI* F. SP. *PHASEOLI* IN BEANS (*PHASEOLUS VULGARIS* L.): LITERATURE REVIEW

Introduction

Common bean is the most important grain legume used for direct human consumption in Africa, the Caribbean, and Latin America where it provides a cheap source of protein, calories, vitamins, fiber, and minerals for low-income populations (Maiti, 1997; Jacobsen, 1999; Broughton et al., 2003). The demand for beans as a staple protein diet has been growing, while the yield of this crop has plateaued in recent years due to several biotic and abiotic stresses. Bean diseases constitute the most important constraint to bean production around the world (Beebe and Pastor-Corrales, 1991; Tan and Tu, 1994). Root rot has been reported to reduce bean yield in North America to up to 80% (Burke and Miller, 1983; Burke and Silbernagel, 1965; Burke and Nelson, 1967; Steadman et al., 1975; Keenan et al., 1974; Sippell and Hall, 1982; Dryden and Van Alfen, 1984; Burke and Hall, 1991; Tu and Park, 1993; Schneider et al., 1998; Estevez de Jensen et al., 1998 and 1999; Schneider and Kelly, 2000; Park and Rupert, 2000). The disease has also been reported in Japan and several European countries (Kovachevsky, 1931; Ruokola and Vestberg, 1978; Pastor-Corrales and Schwartz, 1994; Furuya et al., 1999). Root rot caused by *Fusarium* spp. has been identified in Brazil, Colombia, Perú, Venezuela, Costa Rica, México, and other Latin America countries (Crispín-Medina and Campos-Avila, 1976; Beebe, 1981; Pastor-Corrales and Schwartz, 1994).

Large seeded dry bean cultivars widely grown in Michigan are highly susceptible to root rot caused by the soil-borne fungus *Fusarium solani* (Mart.) Sacc. f sp. *phaseoli* (Burkholder) W.C. Snyder and H. N. Hans. *Fusarium* root rot is a major problem for bean growers and is present in all production areas of Michigan causing more yield loss than other diseases (Robertson and Frazier, 1982), especially in the central north east (Presque Isle County), north east (Isabella county) and west (Montcalm county) where sandy or sandy loam soils predominate. Kidney beans planted commercially in similar soil types in central Wisconsin and north central Minnesota are forcing farmers to limit their planting as problems with root rot reduce productivity. The market potential of Michigan dark red kidney bean cultivars in all three states has been limited due to root rot. A root rot resistant kidney cultivar would give bean growers the opportunity to expand and maintain production in Michigan and elsewhere.

Genetic resistance to *Fusarium* root rot has been difficult to incorporate into market-acceptable cultivars of snap bean and large seeded Andean dry beans (Estevez de Jensen et al., 1998). An emphasis on quality traits appears to have significantly reduced the genetic variability in these two bean types, which may contribute to their extreme susceptibility to root rot (Gepts, 1998; Schneider et al., 2001). As plant breeding programs become more sophisticated and modern agricultural techniques require higher degrees of crop uniformity, the genetic diversity within crop varieties is reduced and such varieties may become highly vulnerable to many natural hazards such as diseases (Welch, 1981).

Disease biology

Root rot in common bean is caused by a complex of pathogens that include: *Fusarium solani* f. sp. *phaseoli*, *Fusarium oxysporum* Schlecht f. sp. *phaseoli*, *Rhizoctonia solani* Kuhn, *Pythium* spp., *Thielaviopsis basicola* (Berk. & Br.) Ferr., and *Aphanomyces eufeches* f. sp. *phaseoli* Drechs. The widespread nature and importance of *F. solani* as a predominant root rot pathogen in bean emphasizes the need for effective control against this disease. Control can be achieved through a combination of appropriate cultural management practices and through the development of resistant cultivars.

Root rot caused by the fungus *F. solani* is concentrated on the roots of the plants; however, symptoms often extend up the main root and hypocotyl to the soil surface. If soil moisture levels are excessive or too low, the above ground parts may be stunted and turn yellow, wilt, and die before the plants mature. Unlike other root rotting diseases *Fusarium* root rot does not cause seed rot or damping-off of seedlings or wilt. Symptoms do not appear until two or three weeks after planting. The first symptoms are narrow, long, red to brown lesions in the stems where fissures often develop lengthwise which in many cases can destroy the taproot. Small lateral roots, which normally develop from the taproot, can be killed. Such lateral and taproot destruction is then followed by proliferation of fibrous adventitious roots near the soil surface (Pastor–Corrales and Schwartz, 1994; Singh, 1999; Mulligan, 1983). These fibrous roots enhance survival of the plant if they are not disturbed. Under ideal soil and growing conditions, few above ground symptoms will be visible. The root rot complex affecting beans is more important in its influence upon the lateral root system than upon the hypocotyls and

taproot (Burke and Barker, 1966). Plants may be stunted, have an unhealthy appearance, and grow more slowly than healthy plants. Poor root function deprives plants of nutrients and water, which can result in uneven plant, stands and reduced productivity.

Other root rot pathogens

Fusarium oxysporum Schlecht f. sp. *phaseoli* Kendrick & Snyder: *Fusariu*m yellows or wilt which usually appears on older plants and begin as a yellowing and wilting of the lower leaves (Beebe, 1981; Abawi, 1994). The wilting and yellowing progress up the plant until the entire plant turns yellow. This is readily observed in the field. Plants also may be stunted, particularly if infected at an early stage. In this disease roots and stem show few external symptoms in contrast to *F. solani*. Discoloration of the vascular system is a diagnostic symptom of *Fusarium* yellows, and damage can be readily seen by cutting into the lower stem and looking for a red-brown streaking in the vascular tissues. The discoloration is usually present in plants showing foliar symptoms and is particularly evident in the lower stem and at stem and petiole nodes. Severely infected plants become yellowed, wilted and die prematurely, which may cause yield reduction or total crop loss (Schwartz et al., 2001).

Rhizoctonia solani Kuhn: The pathogen exists primarily as mycelium that is colorless when young and becomes tan to light brown with age (Abawi, 1994). The perfect basidial state is less common, forming blackish, barrel-shaped spores on a thin layer of mycelium at or near the soil level under conditions of high humidity. *Rhizoctonia* root rot can cause seedling death, root and stem rot, stem cankers, and pod rot. The first symptoms appear on the stems or roots as linear or circular reddish, sunken lesions with a

brown to reddish-brown border (Beebe, 1981). The cankers enlarge with age, and retard normal plant growth. Pods may also develop lesions and rot if in contact with moist soil. This pathogen can survive in infected plant residue and continuous cropping with susceptible crops such as beans can increase pathogen population levels in soils. Irrigation water and soil movement spreads spores of *Rhizoctonia*, and moderate to high soil moisture and low soil temperatures favors seedling disease development.

Pythium spp.: *Pythium* species may infect planted seeds prior to germination, germinating seedlings, young plants, or older plants during blossoming and pod formation (Abawi, 1994). This pathogen can cause seed decay and seedling death. Damage is most common in wet soils and initial root rot symptoms appear as elongated water soaked areas on the hypocotyl and roots, within one to three weeks after planting. The pathogen will extensively prune roots, reduce overall plant growth, and destroy much of the hypocotyl and main root system. Roots infected by *Pythium* are typically light brown in color and soft and watery to the touch. This infected region will eventually dry out, become somewhat sunken, and tan to brown in color. Disease development late in the season results when pods in contact with the soil become infected. *Pythium* root rot and wilt is favored by high moisture and moderate to high temperature (Beebe, 1981). Pathogen survival and inoculum buildup are favored by soils with high organic matter and poor drainage. The pseudo-fungus can be transported within and between fields by contaminated irrigation water and is also favored by limited crop rotation and by root damage during cultivation, or by other soil related problems.

Thielaviopsis basicola (Berk. and Br.) Ferr. (Syn. *Chalara elegans* Nag Raj and Kendrick): *Thielaviopsis* black root rot, caused by *Thielaviopsis basicola* is characterized

by a dark brown to black necrotic tissue that develops on the below ground stems and root (Abawi, 1994). Fissures often develop in necrotic cortical tissue. Low temperatures (15-20°C) as well as high humidity seem to favor fungal development. *Thielaviopsis* can be identified in diseased tissue by the production of black chlamydospores, which survive for over a year in soil. This disease occurs on blackeye cowpea (*Vigna unguiculata)* as well as on other bean types.

Aphanomyces euteiches f. sp. *phaseoli* Drechs: Symptoms caused by this pathogen can easily be confused with those of *Pythium* spp. Initial root rot symptoms appear as elongated water soaked areas on the hypocotyl and root, and under favorable conditions, these lesions will rapidly extend leading to a soft rot of the tissue, which looks brown in color. Infected plants exhibit dwarfing, chlorosis, and premature defoliation (Abawi, 1994). The disease is favored by temperatures of 24-28°C and high humidity. This pathogen can cause severe damage to beans when combined with *Pythium*. *A. euteiches* f. sp. *phaseoli*, does not infect peas (*Pisum sativum* L.), but is pathogenic to alfalfa (*Medicago sativa* L.; Pfender and Hagedorn, 1982).

Persistence and Transmission of Fusarium root rot

The pathogen *F. solani* usually survives as thick-walled chlamydospores in the soil (Nash et al., 1961; Hall and Phillips, 1992 and 2004b; Cardona et al., 1995; Mondal et al., 1996) but is not disseminated by seed (Nash and Snyder, 1964). The chlamydospores germinate when stimulated by nutrients exuded by germinating seeds and root tips, which proceeds the fungus mycelium penetration of the plant tissue. The fungus also produces chlamydospores within the cortical tissue of infected bean roots

(Burkholder, 1919). Chlamydospores of *F. solani* can germinate and reproduce near seed and roots of many non-host plants and also in organic matter. As a consequence the pathogen can survive in the field for over a year (Schroth and Cook, 1964; Pastor-Corrales and Schwartz, 1994). The mycelial form of the pathogen predominates in the soil and is more tolerant of residues, antibiotics, and possibly crop rotations (Maloy, 1960); although both chlamydospores and mycelium forms grew well on root exudates from a number of non-host plants (Maloy and Burkholder 1959).

When host tissue is not available, *F. solani* has been reported to have a limited saprophytic growth and can perpetuate itself in a soil environment (Nash et al.1961; Schroth and Hendix, 1962). The observation was suggested by Schroth and Snyder (1961) and Cook and Schroth (1965a) who reported that *F. solani* chlamydospores germinated when various amino acids and sugars were added to the soil. Common amino acids and sugars were added to stimulate non-host root exudates, which may be capable of providing limited nutrients for saprophytic growth. Chlamydospores germinated when placed near root tips or germinating seeds; however, root exudates from mature bean roots did not stimulate germination. Around 60% of the chlamydospores germinated when adjacent to seeds of *P. vulgaris* (Cook and Snyder, 1965b). The germ tubes were lysed quickly by other microorganisms unless susceptible host tissue was available, which suggests that limited saprophytic growth is not a major factor in inoculum production.

Many reports are available on the mode of penetration and host-parasite relations of *F. solani* (Burkeholder, 1919; Christou and Snyder, 1962; Chatterjee, 1958; Bywater, 1959). Most researchers agree that initial infections occur through stomata in the

hypocotyl. The hyphae may also penetrate the roots and infect either directly through the epidermis or through mechanical or natural wounds. Wounds formed by the emergence of the lateral roots are a common site for penetration. Infrequently, hyphae can enter through the bases of damaged bean root hairs.

The pathogen typically forms a thallus on the host surface before penetrating host tissue (Christou and Snyder, 1962). Glucose was essential for hypocotyl penetration and nitrogen stimulated pathogenesis and penetration (Toussoun et al., 1960). Organic nitrogen was more effective then inorganic nitrogen in stimulating both the development of the thallus on the hypocotyls and the expression of symptoms (Maurer and Barker, 1965). Further, the ability of the nitrogen form to influence root rot depended on the soil type and pH (Papavizas et al., 1968).

F. solani is moved around the field by wind and rain, irrigation water, farm implements, and any other agent or process capable of moving soil. With each successive crop of beans, pathogen population increase and the disease becomes more severe (Kraft et al., 1981).

Plant damage is usually increased under environmental conditions that stress plants on maximized plant densities. These conditions include deep planting and plant spacing, soil compaction, hardpan layers, cool temperature, high or low pH, low fertility, pesticide or fertilizer injury, and flooding or extended drought (Burke et al., 1972b and 1980; Burke, 1965a, b, and c; Burke and Nelson, 1965; Burke and Barker, 1966; Burke, 1968 and 1969; Cook and Papendick, 1972; Cook and Schroth, 1965a; Huber, 1963).

Soil compaction, occurring as a result of seasonal tillage, seems to be an important factor in the *Fusarium* root rot problem. Plants have limited root growth as a

result of compaction and experience water stress in years when rainfall is deficient (Schumacher and Smucker, 1981). In addition, excessive water, or flooding, often causes compacted soils to become oxygen deficient (Miller and Burke, 1977). Low oxygen supply causes the development of anaerobic respiration and is associated with the activity of certain plant diseases such as root rot (Henry, 1962; Miller and Burke, 1977). As moisture is removed by the transpiring surfaces of the plants, a potential gradient is established from the leaf to the root and into the soil. Since water moves in the soil by bulk or mass flow, and depends on pore size, changes in the soil moisture content have marked changes on the hydraulic conductivity of water. Water which remains in the smaller pores and as thin films around the soil particles is largely unavailable to plants. Excessive tillage decreases the large pores in the soil and results in an increase of tiny pores which cause water to drain faster possibly favoring the pathogen. Matrix potentials influence the amount of bean seed exudation and hence the activity of *F. solani* and *Pythium ultimum* (Stanghellini and Hancock, 1970; Reid, 1979).

Control

When putting root rot control measures into practice, growers must remember that the pathogen is not seed borne, but is strictly a soil borne organism. *Fusarium* root rot of bean can be controlled in several ways. The best control measure is to plant into a warm temperature (minimum temperature >15°C), well-prepared, well-drained, and well-fertilized seed bed capable of supporting rapid bean growth. Conditions that promote plant health and growth will reduce losses associated with *Fusarium* root rot of common

bean (Pastor-Corrales and Schwartz, 1994; Singh, 1999; Tan and Tu, 1994; Snapp et al., 2003; Román-Avilés, 2004;).

Irrigation should be managed to provide enough water to the developing plant without causing moisture stress or excess. By manipulating water supply, growers can achieve maximum yield with minimal root rot (Tu et al., 1992). Plants may be predisposed to infection after being stressed by moisture or temperature extreme. Rotation of beans with non-host crops such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), or alfalfa will usually reduce root rot severity (Pastor-Corrales and Schwartz, 1994). Soil solarization can be effective in sterilizing the soil when environmental conditions are favorable. In production systems where polyethylene film is used as mulch, pre-plant application of chemical fumigants is the principal method employed for control of soil borne pathogens, but is too expensive for most bean production systems (Chellemi and Olson, 1994).

Plants should be widely spaced within the row to reduce competition for water (Burke, 1965a). Widely spaced plants have been associated with less root rot than those growing closer together. The beneficial effects of wide spacing are thought to be due to high plant growth rate and limited plant competition; however, this decrease in disease severity is nullified if soil temperatures are low (Burke, 1965a). Less than optimal soil temperatures will reduce the ability of bean roots to penetrate compacted soils.

Fungicides are generally not effective in controlling *Fusarium* root rot. Chemicals such as Thiram and Benlate used as seed treatments are recommended to reduce the severity of root rot in several countries (Pastor-Corrales and Schwartz, 1994), but chemical control may be too expensive for some farmers. Captan 400 + Streptomycin +

Lorsban 50 is the standard seed treatment used in the United States but it has failed to effectively control the disease where resistant cultivars are unavailable (Kirk and Snapp, unpublished data). The application of *Bacillus subtilis* strain GBO3 (Kodiak) with *Rhizobium* as a seed treatment is a promising new approach to enhance root rot control in beans (Estevez et al., 2002).

In some countries of Latin America bean straw is fed to animals which can result in serious disease problems if that straw is infected by *F. solani*. Bean straw, carrying the pathogen, should never be fed to animals, as the manure will carry the organism and serve as a source of infection in the future (Pastor-Corrales and Schwartz, 1994). One recommendation is that the bean residues should be removed from the field, to areas where beans probably will not be grown. Any diseased bean residue left on the field should be turned under deeply by fall plowing (Pastor-Corrales and Schwartz, 1994; Snyder et al., 1959).

Root traits as an approach for root rot resistance

Factors such as the ability of a bean seed to germinate in the cold, ability of the plant to develop a large, vigorous root system, and the presence of inhibitory substances in the seed coat and hypocotyls, would increase the level of resistance to root rot in common bean (Statler, 1970). Quantitative information on root system traits associated with root rot tolerance would improve selection criteria for root vigor.

Root rot resistance is expected to be related in part to root system traits. Limited knowledge on the relative importance and function of various root classes complicates the prospects of breeding for specific root traits in common bean (Lynch and van Beem,

1993). Field studies suggest that root system traits play a role in resistance to root rot (Burke and Barker, 1966). The effect of root rot on yield was not ameliorated by complete protection of hypocotyls and taproot, where beans were grown in raised beds of pathogen-free soil compared to infested raised beds, from which most of the root system extended into *Fusarium* infested soil (Burke and Miller, 1983). Vigorous, unrestricted roots outgrew and overcame local hypocotyl and taproot infections in this study. These results indicate that this fungus seems to have limited effect on plants with vigorously growing roots. A field study comparing raised and flat beds showed that root vigor of commercial snap bean cultivars was relatively high ($>0.2cm^{-3}$) and root rot caused by *F. solani* was less in the raised beds compared to flat beds (Snapp et al., 2003). These results suggested that integrated management practices can improve root vigor and as a result reduce root rot severity.

In a study conducted with peas large-rooted lines regenerated more roots when one-third or two-thirds of the root system was removed or when one cotyledon was removed from 5-day-old plants. These same large rooted lines produced more roots and had more root surface area when exposed to a compacted layer (1.6 g cm^{-3} bulk density) infected with *Fusarium* root rot (*Fusarium solani* f. sp. *pisi*) pathogen (Kraft and Boege, 2001). In conclusion, large-rooted pea lines should have an advantage over other line under adverse growing conditions of compaction and the presence of *Fusarium* root rot (Kraft and Boege, 1996 and 2001).

The physiological, architecture, and morphological features of individual root systems affect many processes such as the composition of soil organisms, turnover of soil organic matter, plant anchorage, acquisition of water, and nutrients by plants. These

features will also affect the establishment, growth, reproduction and mortality of plant populations (Bledsoe et al., 1999). Root systems are difficult to study, primarily because they are spatially and temporally complex and because available methods for their study are labor-intensive and destructive. The evaluation of roots for *Fusarium* root rot has been difficult due to the large number of intermediates in levels of susceptibility for traits strongly influenced by environmental factors (Schneider et al., 2001). A complex genotype by environment interaction has made it difficult to make progress in controlling this disease through resistance breeding.

Root damage is a more accurate indicator of yield reductions caused by *Fusarium* root rot, than the conventional rating of hypocotyl lesions (Beebe et al., 1981; Schneider et al., 2001). Root architecture traits, such as secondary and tertiary root branches, adventitious roots, basal roots (arising from the basal region of the hypocotyl), angles, and radii, should also be considered in evaluating root systems. Adventitious roots appear to play an important role in water and nutrient transport and can ameliorate multiple interacting stresses of the field environment (Stoffella et al., 1979; Leskovar and Stoffella, 1995).

Detecting differences in root architecture and growth patterns among bean genotypes may provide unique selection criteria for genetic resistance to *Fusarium* root rot. Genetic variation existed in root architecture among common bean classes and was highly significant under field conditions (Román- Avilés et al., 2004), indicating the value of screening in the field environment. It appears that the introgression of root traits or root vigor from small seeded to large seeded bean genotypes could be effective. Large seeded bean genotypes, such as Chinook 2000 and FR266 that possess a vigorous root

system suggests that breeders have been effective in introgressing desirable rooting traits from small seeded genotypes (Román- Avilés et al., 2004).

Root and shoot weights have been used to determine root rot resistance in edible beans (Hall and Phillips, 2004a). Since shoot weight is easier and quicker to measure than root weight, resistance to *Fusarium* root rot can be expressed as percentage reduction in fresh shoot weight of plants inoculated after emergence compared to uninoculated plants. This relationship of shoot to root growth has been studied in climbing bean genotype HAB 229 possessing a more taproot dominant root system, when compared to the prostrate genotype Carioca with a highly branched, non-tap root dominant system with large number of root meristems (Lynch and van Beem, 1993).

Moreover, knowledge of the genetic determinants of root traits and how they influence yield would allow for a more targeted breeding approach utilizing technologies such as QTL analysis. In general developing a vigorous root system could help improve root rot resistance in large seeded bean genotypes.

Breeding for *Fusarium* root rot resistance (past and present)

Long recognized as a problem in the U. S., *Fusarium* root rot is the most serious root rot disease of common bean. Breeding for resistance to root rot caused by *F. solani* in common bean has been challenging (Wallace and Wilkinson, 1965; Nelson, 1973). Unlike some other fungi, there is no-clear indication from present distribution of the origin for *Fusarium* based on the diversity, distribution of species, and telemorphs (Backhouse et al., 2001). The diversity of *Fusarium* species and their telemorphs suggest that the species is an ancient group, probably appearing fairly early in the evolution of the

Ascomycetes. However there is no fossil record to substantiate this fact, and no calculations exist on the rate of molecular evolution.

Fusarium root rot of bean was first recorded in 1916 by Burkholder, in the state of New York, (Burkholder, 1916 and 1919) and is one of the most prevalent diseases in the world (Boomstra et al. 1977; Wallace and Wilkinson, 1965; Wallace and Wilkinson, 1973; Pastor-Corrales and Schwartz, 1994). McRostie (1921) reported that susceptibility to *Fusarium* root rot was dominant over resistance and that two factors were involved in the inheritance in common bean. Conflicting reports exist in the literature on the number of genes involved in resistancce and levels of dominance, depending on the parental genotypes used in the different genetic studies. Resistance is usually associated with late maturity, small seeds, and large, indeterminate vine growth habit (Kraft et al., 1981). Tolerance was recessive in crosses between *P. vulgaris* and *P. coccineus* and probably controlled by three major genes or two genes with modifying factors. Results from this study also indicated no relation between root vigor and resistance (Azzam, 1958).

Earlier studies indicated that resistance to root rots existed in *P. coccineus* (Yerkes and Freytag, 1956) and in a black seeded *P. vulgaris* accession, N203 (PI 203958), introduced from Mexico by Oliver Norwell of the Carnegie Institute of Washington and later assigned the accession number PI 203958 (Azzam, 1958; Wallace and Wilkinson, 1965; Singh, 1999; Wallace and Wilkinson, 1973). Genetic studies with the dry bean lines PI 165435 and PI 203958 showed that resistance to *Fusarium* root rot in *P. vulgaris* was controlled by one dominant gene and one recessive gene independently inherited (F_1 all susceptible and F_2 showed a ratio of 13 susceptible: 3 resistant; Smith and Houston, 1960). Resistance derived from *P. coccineus* crosses showed nearly complete

dominance, and only a few genes were responsible for its expression (Wallace and Wilkinson, 1965). Data collected from the *P. coccineus* (Scarlet Runner) crosses showed a high degree of resistance, but higher resistance was observed in PI 203958, and other breeding lines; and extreme susceptibility in existed yellow-eye and red kidney Andean genotypes. Scarlet Runner is highly resistant, but not as resistant as PI 203958. This resistance whether derived from PI 203958 or Scarlet Runner, is incompletely dominant (Bravo et al., 1969). Additive gene effects are larger than dominant gene effects. In 1949 Dr. T.L. York at Cornell University, Ithaca, N.Y. crossed Scarlet Runner with common red kidney beans (after subsequently backcrossing to common bean) and he obtained a common bean line (NY2114-12) with slightly improved resistance over Scarlet Runner (Wallace and Wilkinson, 1973). Estimates of the number of genes controlling resistance ranged from three to seven, and the effect of individual genes could not be distinguished (Nelson, 1973). Using NY2114-12 as a source of resistance, quantitative inheritance of resistance to *Fusarium* was observed, which was inherited independently from genes conferring resistance to other root rots (Dickson and Boettger, 1977).

Resistance to *Fusarium* root rot has been identified in tropically adapted bean lines (Boomstra and Bliss, 1977, Hassan et al., 1971). The widely used source of *Fusarium* root rot resistance, PI 203958 (N203), bean from México is a wild, black-seeded, and semi vine with short, flat stringy pods (Dickson, 1973). N203 has the highest level of resistance among all known accessions of the common bean, *Phaseolus vulgaris*. Resistance in both the Scarlet Runner and PI 203958 sources was inherited quantitatively (Boomstra and Bliss, 1977; Hassan et al., 1971). Support for this theory is indicated by

numerous distinct degrees of resistance in the breeding lines and varieties studied as well as by the large environmental variation (Wallace and Wilkinson, 1965; Schneider et al., 2001).

Resistance genes from PI 203958 have been incorporated into pink, pinto, and small red cultivars such as: USWA 19, Rosa, Viva, Gloria, Rufus, NW 59, NW 63, Cahone, NW 410, NW 590, Pindak, and Holberg (Burke, 1975; Burke and Miller, 1983; Burke, 1982; Schneiter et al., 1982 and 1983; Silbernagel and Hang, 1997; Wood et al., 1983). A bush snap bean (*P. vulgaris*) breeding line (FR266- Fusarium Resistant 266), was developed with resistant to *Fusarium* root rot caused by *F. solani* (Silbernagel, 1987). FR266 possesses a large root system, white seed, and the source of *Fusarium* root rot resistance was PI 203958.

Middle American bean germplasm has higher levels of root rot resistance compared to the highly susceptible large seeded beans of Andean origin (Beebe, 1981). Field experiments conducted by Schneider et al. (1998) demonstrated that dark red kidney seed types were the most susceptible and that FR266 and PI 203958 were among the most resistant genotypes. Moderate to high heritability (0.48 to 0.66) estimates for lines derived from PI 203958 and low to high heritability estimates (0.13 to 0.85) for lines derived from crosses between Montcalm x FR266 indicated that *Fusarium* root rot resistance can be controlled through genetic resistance (Schneider and Kelly, 1999). Previous work shows that adequate levels of root rot resistance in beans has been limited (especially in large seeded kidney beans and snap beans). The evaluation for root rot requires more appropriate experimental designs for the analysis of quantitative traits with

replicated trials and different locations (Smith and Houston, 1960; Wallace and Wilkinson, 1965; Schneider et al., 2001).

Bean breeders have attempted to transfer the resistance from the Middle American gene pool to current commercial kidney bean cultivars from the Andean gene pool, but gene transfer across gene pools takes time and success is not guaranteed. Previous studies in resistance breeding exhibit partial resistance to *Fusarium* root rot, large differences in levels of susceptibility and large genotype by environment interaction. Both of these facts support the proposed quantitative inheritance of *Fusarium* resistance (Tan and Tu, 1994; Schneider et al., 2001). Quantitatively inherited traits result from a genetic component that under normal conditions of measurement cannot be controlled by individually recognizable quantitative trait loci. The inability to recognize individual loci depend on the reliability of scoring phenotypes which can be challenging. Since the traits of interest are, by nature, genetically complex, environmental factors and genetic background potentially have a major impact on their expression.

Due to the complex inheritance of resistance to *F. solani* in common bean breeders must use only crosses with highly resistant selections. One breeding method for improving resistance to *Fusarium* root rot is by backcrossing to commercial types which is essential for the development of acceptable root rot resistant varieties. Advanced backcross quantitative trait loci (QTL) analysis has been proposed as a method of introgressing QTL from wild species into a cultivated genetic background (Tanksley and Nelson, 1996). The advantage of such a method for the development of populations is that the genetic background is largely identical and the influence of other negative genes on the analysis is limited.

The polygenic inheritance along with strong environmental effects has limited the improvement of root rot resistance under field conditions (Wallace and Wilkinson, 1965; Bravo et al., 1969; Hassan et al., 1971; Boomstra et al., 1977; Beebe et al., 1981; Sibernagel, 1990; Tu and Park, 1993; Schneider and Kelly, 1999). Narrow sense heritability of 26% to 44% with crosses using PI 203958 and Scarlet Runner are high enough to suggest that selection can be effective but low enough to imply that effective selection requires progeny testing, and evaluation of root rot as a quantitative character (Hassan et al., 1971). Intermediate to high levels of root rot resistance were reported in Middle American germplasm in small-seeded genotypes (Beebe et al., 1981). Resistance may have resulted from natural selection during repeated exposure to soil-borne pathogens.

Problems associated with intergene-pool crossing

Transferring *Fusarium* root rot resistance from Middle American gene pool into Andean gene pool results in a major challenge. Evidence of divergence between Middle American and Andean cultivated gene pools is provided by observed hybrid weakness or dwarf lethality in the F_1 generation of certain crosses between these two cultivated genotypes (Gepts and Bliss, 1985). Crosses showing F_1 dwarf lethals always involved a small-seeded genotype showing an 'S' phaseolin type (of Middle American origin) and a medium to large seeded genotype with a 'T' (Tendergreen) or 'C' (Contender) phaseolin type (of Andean origin). Genetics of dwarf lethals based on results of a backcrossing experiment were described by Shii et al. (1980). Their results indicate that the phenotype is controlled by complementary gene loci, one expressed in the root (*DL*1 or Dosage

dependent Lethal 1) and the other in the shoot (*DL*2). Also the incompatibility was due to nuclear and not to cytoplasmic effects, and the lethality increases with increasing dosage of dominant alleles of *DL*2 in the scion and of *DL*1 in the root stock. Small seeded bean genotypes from Middle American gene pool carried *DL*1 and the medium to large seeded genotypes from Andean gene pool carried *DL*2. In addition, high temperatures (30°C/25°C, day/ night) regime are responsible for hybrid weakness, which restricted root growth and could be overcome by the addition of cytokinin (Shii et al., 1980 and 1981). This suggested that the *DL*1 and *DL*2 might be related to the regulation of hormonal function or metabolism. Symptoms included decrease leaf and root growth, leaf chlorosis, the developmental of adventitious roots on the stems, eventual death. The recessive gene *Icr* causes trifoliate leaf crippling in the presence of either *DL*1 or *DL*2 gene (Singh and Molina, 1996). When both *DL*1 and *DL*2 are present in F_1 from inter-gene pool crosses, a dwarf-lethal or semi-lethal phenotype is produced, irrespective of the alleles at the *icr* locus. For example the genotypes of the recombinant inbreed lines (RILs) used to study this *Icr* gene are, for WA7807-305: *DL1DL2dl2dl2IcrIcr*, and for TY5578-220: *dl1dl1DL2DL2icr icr*.

Dwarfism of F_1 hybrids was observed in over 100 diallel crosses derived by crossing between entries within germplasm groups of various geographical origins of dry beans at the International Center of Tropical Agriculture (CIAT), Cali, Colombia (Singh and Gutiérrez, 1984). Upon close examination of the characteristics of the parents of all dwarf F_1 hybrids, it was found that, in each case, one of the parents had small seed (100 seed weight 25g or less) and the other medium (26 to 40g) or large seeded (>40g) seeds. All F_1 hybrids within small seeded and between medium and large seeded types were

normal. Dwarfism only occurred when the small seeded was crossed with a medium or large seeded type. The incompatibility barrier would have served as a partial or complete isolation mechanism and has limited genetic incompatibility between these two distinct bean gene pools.

To determine if the *DL*1 and *DL*2 were present in the wild *P. vulgaris* gene pool and whether they exhibit the same geographical distribution as in their cultivated counterparts, wild accessions chosen for their geographical range distribution and phaseolin and allozyme diversity were crossed to two testers known to have genes controlling dwarf lethality (Koinange and Gepts, 1992). Results from test crosses showed that the *DL* genes were present in the wild beans and that they followed the same geographical distribution pattern as in the cultivated, whereas previously they had only been reported in cultivated *P. vulgaris*. Possible origin of the *DL* genes may have been by introduction into the cultivated gene pool from the wild ancestor of *P. vulgaris*, presumably through domestication, although outcrossing subsequent to domestication cannot be excluded (Koinange and Gepts, 1992).

Reiber and Neuman (1999a) found that abnormal growth and development of F_1 bean hybrids might involve interruption of the regulation of cytokinin allocation, thereby disrupting the root-shoot feedback loop between root-sourced cytokinins and shoot-produced factors. Although zeatin ribose concentrations are reduced in roots and leaves of the F_1 hybrids, stems had very high concentrations of zeatin ribose, suggesting that there is a breakdown in the transport or allocation of this cytokinin instead of a deficiency of cytokinins. To better understand the mechanisms regulating the integration between roots and shoots in dwarf lethality changes in the concentrations of zeatin ribose in two

cultivars, Redkloud and Batt and F_1 crosses carrying the *DL* phenotypes were studied (Reiber and Neuman, 1999b). Applications of benzylaminopurine (BAP) to roots of F_1 hybrids increased the number of root tips and leaves. Estimates of the transport of zeatin ribose type cytokinin from roots of F_1 hybrids indicated that transport out of hybrid roots was reduced compared with those transported out of Middle American or Andean roots. This suggested that zeatin ribose type cytokinins are involved in hormonal integration between roots and shoots of common bean and that one of the barriers to hybridization between Andean and Mesoamerican landraces is related to hormone transport.

Deleterious effects of gene pool crossing (crosses between Middle American and Andean gene pool) were circumvented through careful management of the F_1 (hybrid) root system as described by Beaver (1993), who successfully harvested F_2 seeds from F_1 plants which were expected to be all dwarf lethal, $F_1=DL_1/DL_2$ (Shii et al., 1981 and 1980). With the application of 'hormex' (0.24% 1-napthaleneacetic acid and 0.013% 3-indolebutyric acid) to the soil before planting, F_1 plants began to grow and recover a healthy appearance (Beaver, 1993).

The transfer of quantitative traits between gene pools has had limited success as a result of dwarf lethality (Kelly, 1988). Not all bean types carry the dwarf lethal genes and an inventory of bean lines known to carry the null (*dl*) genes was proposed by Kelly (1989). The purpose of the inventory was to facilitate researchers in their selection of parents to use as a bridge between gene pools.

Types of genetic markers

Genetic markers can be classified into morphological, biochemical, and DNA-based markers. Morphological traits controlled by a single locus can be used as genetic markers provided their expression is reproducible over a range of environments. Morphological trait markers have had limited application in plant breeding when used for indirect selection of a trait, because they may result in an altered phenotype that conflicts with growers needs, unfavorable pleiotrophic interactions, late expression, rare polymorphisms, and the potential negative effect on fitness or desirability of an individual, dominant inheritance, and they can be influenced by environmental and genetic factors (Staub et al., 1996; Weber and Wricke, 1994; Hospital et al., 1992; Singh et al., 1991b). The limitations to the application of morphological markers can be overcome however by the application of more abundant biochemical (enzyme and protein markers) and DNA markers (RFLP's, AFLP's, RAPD's, and SSR).

The use of genetic markers for indirect selection was first realized with the discovery of simple inherited, structural variation present in isozymes and the phaseolin seed storage proteins in common bean. Isozymes are differently charged protein molecules that can be separated using electrophoretic procedures (Staub et al., 1996; Singh et al., 1991a; Weber and Wricke, 1994). Allozymes are conditioned by genes that affect subtle secondary characteristics of the enzyme with little or no effect on enzymatic properties and they do not impair the viability of the mutant organism. Isozymes, as opposed to molecular markers, are restricted by a limited number of markers available and by the number of diverse biochemical assays needed to detect enzyme activity, which limits the number of genes able to be assessed (Stuber, 1991).

The construction of linkage maps based on DNA markers has been completed for a number of crop species including common bean (Vallejos et al., 1992). Restriction fragment length polymorphisms (RFLP's) methodology is based upon the availability of cloned sequences, which can be used to probe homologous regions of specific genomes for the presence of variation at the DNA level. This variation is monitored as polymorphisms in the length of the defined DNA fragments, caused by deletion and insertions that are produced by digestion of the DNA with restriction endonucleases (Weber and Wricke, 1994; Staub et al., 1996). However, this methodology is expensive, laborious, and requires the use of radioactivity, and thus is not readily adapted as a selection tool by most breeders working with large populations (Young, 1995; Kelly and Miklas, 1998; Nienhuis et al., 1994). RFLP markers, however, are critical in anchoring the linkage groups in the integrated bean map and combining linkage maps.

Amplified fragment length polymorphism (AFLPs) methodology is based on PCR and selective amplification of restriction enzymes digested DNA fragments (Savelkoul et al., 1999). Specific adapter molecules are ligated to the restriction fragments (Staub et al., 1996). AFLPs have been shown to detect low levels of polymorphism in many different organisms including bean (Tohme et al., 1996). Selection can be facilitated using molecular marker methodologies to identify unique parental germplasm within the wild gene pools.

More recently, sequence-related amplified polymorphism (SRAP) markers have been developed for the amplification of genomic regions associated with defined open reading frames (Li and Quiros, 2001). These markers target coding sequences in the genome and results in moderate numbers of co-dominant markers. The SRAP technique

is a PCR based marker system with two-primers, a forward (17 bases) and a reverse primer (18 bases), run on acrylamide gels. SRAP as well as AFLPs and RAPDs techniques make use of no specific sequence information, and the markers generated are randomly distributed across the genome. Another marker technique known as target region amplification polymorphism (TRAP) was derived from the SRAP technique. Contrary to the SRAP technique, TRAP markers are developed using bioinformatics tools and expressed sequence tag database information to generate polymorphic markers around targeted candidate gene sequences (Hu and Vick, 2003). TRAP techniques are useful for the screening of germplasm collections and for tagging genes controlling desirable traits such as disease resistance.

Microsatellites or simple sequence repeats (SSR) are PCR based markers that have been developed for a wide range of plant species. SSR use known DNA sequence information to produce highly polymorphic and allele specific markers. These markers detect length polymorphisms at genetic loci that exhibit SSRs that are highly variable (Morgante and Olivieri, 1993). A genetic map for common bean based on microsatellites was developed at CIAT to facilitate marker-assited selection (MAS) and genetic studies (Blair et al., 2003). Tandem repetitive DNA sequences on microsatellites occur as di-, tri-, and tetra-nucleotide microsatellites (Ma et al., 1996). The repeat is called a dinucleotide microsatellite because the sequence that is repeated multiple times is only two bases long (e.g. AGGTCACACACACAGCG), a trinucleotide is only three bases long (e.g. GTTACTACTACTACTACAGT), and the tetranucleotide microsatellite is four bases long (e.g. GGTGGCAGGCAGGCATT) and is repeated multiple times. The number of times the microsatellite sequence is repeated is unique to individual genotypes.

When studying the frequency of di-, tri-, and tetra-nucleotides in wheat AC-SSR occurred every 292 kb whereas AG-SSR occurred every 212 kb (Ma et al., 1996). Trinucleotide repeats were about ten times less common than the dinucleotide tandem repeats, and tetranucleotide repeats were rare in wheat and most crops (Ma et al., 1996). In addition dinucleotide repeats tend to be fairly long. On average, dinucleotides SSRs have a higher number of repeats than trinucleotides (Gaitán-Solís et al., 2002).

Random amplified polymorphic DNA (RAPDs), is a PCR-based assay amplification of random genomic DNA segments with single short oligodeoxynucleotide of arbitrary sequence that detects nucleotide sequence polymorphisms. The reaction products are separated on agarose gels and then visualized by ethidium bromide staining. RAPDs have been used widely for map construction and linkage analysis in many crops (Staub et al., 1996). They are usually dominant markers with polymorphisms between individuals defined as presence or absence of a particular RAPD band (Staub et al., 1996). RAPDs are quick and simple to perform, have no requirement of DNA sequence information but have problems with reproducibility between different laboratories, use fluorescence instead of radioactivity, and small quantities of DNA are required.

Application of RAPD technology is highly suitable for detecting polymorphism between related germplasm and between bean genotypes of the same market class (Haley et al., 1994). Although their potential usefulness is limited because of their dominant nature, RAPDs are suitable for analysis involving RIL populations in which progeny are near homozygous. Marker assisted selection, used to select indirectly for resistant genotypes, may facilitate improvement of disease resistance when field selection is laborious and destructive.

RAPD markers associated with QTL for field resistance to ashy stem blight (*Macrophomina phaseolina*) in common bean were identified by Miklas et al. (1998). These QTL-linked RAPD's and the *Mp-1* and *Mp-2* resistance genes and linked RAPD markers from breeding line BAT 477 identified by Olaya et al. (1996) for ashy stem blight, would provide initial tools for testing MAS for control of ashy stem blight in common bean. Achenbach and Patrick (1996) used RAPD markers as a diagnostic tool for the identification of *F. solani* isolates that cause sudden death in soybean (*Glycine max* (L.) Merr.). Schneider et al. (2001) identified RAPD markers associated with QTL controlling *Fusarium* root rot in common bean that can be used to select progeny possessing complementary QTL for intermatting.

The advantage provided by molecular markers over classical genetic markers is the ability to identify and utilize information on naturally occurring polymorphisms within populations. The identification of individuals, which have accumulated a number of desirable agronomic characteristics during the breeding process, constitutes the most important activity of a plant breeder. Low heritability, epistasis, undesirable linkages, pleiotropic effects, and inadequate screening procedures are a few of several factors that may interfere with the successful incorporation of important traits into new cultivars (Nienhuis et al., 1994; Staub et al., 1996).

The identification of molecular markers closely linked to and preferentially flanking the gene(s) of interest would allow selection to be carried out based on the molecular marker genotypes (Simpson, 1999). Genetic markers allow the detection of polymorphisms due to small changes in DNA sequence, and have proven extremely efficient in the discrimination of individuals. In addition, developmental, tissue specific

and environmental factors do not influence the detection of these polymorphisms, making them excellent markers (Simpson, 1999; Staub et al., 1996). Since root rot disease ratings require destructive evaluation and are often confounded by climatic factors, indirect selection using linked markers would improve the efficiency of breeding for resistance.

In general development and advancement in genetic marker technology have been central in the search for QTL. Until the discovery of DNA-based genetic markers, allozymes used initially as biochemical markers had insufficient protein variation for high-resolution mapping. The discovery of DNA-based markers has largely replaced allozymes in mapping studies as DNA-based markers are unlimited in terms of their genetic location or number.

Application of molecular markers to the study of quantitative traits

Quantitative traits loci (QTL) for disease resistance

Traits controlled by more than one gene are complexly inherited and highly affected by the environment. A quantitative trait loci (QTL) is an area of the genome that is associated with the control of quantitative trait (e.g. root rot resistance). QTL analysis is a valuable tool for genome exploration and the investigation of complexly inherited traits (Young, 1996). QTL analysis has enhanced the breeders understanding of quantitative resistance by revealing the location and size of loci controlling complex disease resistance. An example of how QTL analysis the breeders understanding would be by confirming the interaction between resistance traits that control physiological and those influencing plant morphological and phenology traits that influence disease

avoidance and escape mechanism for white mold (*Sclerotinia sclerotiorum*) of bean in a field environment (Kolkman and Kelly, 2002 and 2003). Markers associated with a QTL could assist in the understanding of quantitative disease resistance by identifying loci that influence resistance to more than one disease (Ariyarathne et al., 1999). The possible function of a QTL can be assumed due to co-localization of the QTL with defense response genes (Lozovaya et al., 2004; Kolkman and Kelly, 2003; Schneider et al., 2001; Geffroy et al., 2000; Mohr et al., 1998). The possible co-localization of QTL with major genes for resistance suggested that these QTL might be allelic versions of qualitative resistance genes (Geffroy et al., 2000).

One major objective when conducting QTL analysis is the identification of the location of genes which account for high levels of genetic variation (R^2) of agriculturally important phenotypes and the use of QTL in MAS in plant improvement. The use of MAS in breeding for quantitative resistance would have greater impact when breeding for root rot resistance and white mold resistance in common bean as screening for resistance is highly influenced by plant morphological traits that can contribute to disease avoidance or escape (e.g. for white mold) which complicates selection procedure (Tanksley et al., 1989). In the case of *Fusarium* root rot selection can be complicated by the interaction of other soil pathogens. By using MAS breeders can accelerate progress toward breeding objectives since breeders will know what they are selecting for (Asíns, 2002). A second objective of QTL analysis is to answer basic questions about evolutionary processes. A third objective would be to provide breeders with information on the uniqueness of resistance sources not previously known. QTL analysis is accomplished by estimating the probability that a QTL is present at a position on the genome over the chance that a

QTL is absent. QTL mapping experiments involve a common basic procedure in many taxa. The analysis has two essential stages: the association of the trait with the marker and the mapping of the marker (Kearsey and Farquhar, 1998).

QTL mapping attempts to locate areas on the genome that affect the trait in question, given the measurable phenotypes (trait values), the individual genotypes (genetic markers), and population structure. Many QTL mapping protocols are time consuming as one evaluates the association of a QTL with a marker or marker interval while ignoring the effects of other segregating QTL in the mapping population (Mackay, 2001). A very important consideration in the analysis and interpretation of QTL data is the threshold LOD (log of odds) employed, for inferring that a QTL is statistically significant (Paterson, 1995; Lindhout, 2002; Kearsey and Farquhar, 1998). If the QTL peak is above the established threshold than the QTL is consider being statistically significant.

Three approaches can be taken to map QTL: selective genotyping, comparative QTL mapping, and DNA pooling. Selective genotyping is an excellent approach for efficiently mapping QTL that influence a single phenotype only. Specifically selective genotyping involves the identification of a subset of individuals from a genetic mapping population, which represent the most extreme phenotypes in the population. By phenotyping a large population, and then selecting only the most extreme individuals (bulking both top and bottom extremes) for genotyping, one can obtain equal or greater information about QTL. Since the use of selective genotyping for complex traits may be hindered by the limitation of a set of DNA bulks based on disease reaction alone, single and multi-trait bulking strategy have been used for disease such as white mold) of

common bean (Kolkman and Kelly, 2003). Multiple trait bulking was used to identify QTL conditioning resistance in an agronomically acceptable plant type, by avoiding the detection of QTL associated with undesirable avoidance mechanisms such as early flowering or maturity or short dwarf plants that restrain disease development but limit yield potential (Kolkman and Kelly, 2002). Comparative QTL mapping is based on the finding that diverse taxas within common taxonomic families that often share similar gene order over large chromosomes of different taxa based on common reference loci (Paterson, 1995). The comparative maps of rice (*Oriza sativa* L.) and maize provide a basis for interpreting molecular, genetics, and breeding information between these two important species and establish a framework for ultimately connecting the genetics of all grass species (Ahn and Tanksley, 1993).

Bulked segregant analysis (BSA) is similar to selective genotyping, in that it consists of DNA bulks (resistant and susceptible bulks) but with the exception that resistant and susceptible bulks are based on actual extreme genotypes. BSA is the pooling of DNA from individuals sharing a particular characteristic and is used on single gene analysis. BSA was proposed as a strategy to overcome some of the disadvantages of developing near isogenic lines (NILs; Michelmore et al., 1991). This technique uses a segregating population, F_2, F_3, or backcross, where segregants are characterized for the trait of interest and separated into groups or bulks (Michelmore et al., 1991). Within each pool or bulk (resistant and susceptible bulks), the individuals are identical for the trait or gene of interest but are arbitrary for all other genes. Bulk segregant analysis has been used to find RAPD markers linked to disease resistance genes and tagging disease resistant genes in lettuce (*Lactuca sativa* L.; Michelmore et al., 1991), common bean

(Haley et al., 1991; Miklas et al., 1993 and 1996) and other crops (Quarrie et al., 1999; Tabor et al., 2000).

Selective genotyping and BSA are often combined in QTL studies as have been the case in studies of root rot and white mold resistance in dry bean (Schneider et al., 2001; Miklas et al., 2001; Park et al., 2001; Kolkman and Kelly, 2003). The method of selective genotyping is a powerful method to detect association for a quantitative trait (Van Gestel et al., 2000). Selective genotyping of individuals, on the basis of both single-trait and multiple trait DNA pooling strategies, was utilized to create several sets of bulks in their breeding populations. Selective genotyping combined with BSA have been used to study *Fusarium* head blight in wheat, Shen et al., 2002); for agronomic traits in oilseed rape (*Brassica napus* L., Butruille et al., 1999); molecular mapping of chromosome segments introgressed from wild nightshade (*Solanum lycopersicoides*) in cultivated tomato (*Lycopersicon esculentum*, Chetelat and Meglic, 2000); *Alternaria solani* resistance in tomato (Zhang et al., 2003); and for *Cercospora maydis* resistance in corn (Gordon et al., 2004).

Sixteen QTL for *Fusarium* root rot resistance were identified using a $F_{4:5}$ RIL population derived from a cross between the susceptible large red kidney Montcalm and the root rot resistant snap bean breeding line FR266 (Schneider et al., 2001). QTL for resistance to root rot had also been identified in linkage group B2 and B3 of the bean core map (Schneider et al., 2001), close to the *PvPR-1* and *PvPR-2*, pathogenicity related proteins, which are thought to be involved in the defense response mechanism against these pathogens. Interval mapping of QTL revealed two QTL for *Fusarium* root rot resistance using an $F_{2:6}$ RIL populations derived from a cross between A.C. Compass,

highly susceptible to *Fusarium* root rot, and NY2114-12, a germplasm highly resistant to *Fusarium* root rot (Chowdbury et al., 2002). Six QTL for *Fusarium* root rot resistance were identified using a RIL population derived from a cross between the root rot susceptible snap bean 'Eagle' and 'Puebla 152' a root rot resistant, black seeded dry bean (Navarro et al., 2003).

Unbalanced populations an approach for studying quantitatively inherited traits

Experimental designs have been categorized according to the type of population used to generate disequilibrium and the unit of marker analysis. In the classification of experimental designs, some basic fundamental assumptions made. The primary assumption is that the putative QTL is genetically linked to a marker with recombination fraction (r) and based on this primary assumption other minor assumptions are made. Within the minor assumptions one would find: the underlying distribution of the trait is normal, no selection for either marker or QTL; only a single segregating QTL is linked to each marker or marker pair; genetic markers do not have pleiotropic effects on the quantitative traits; and no interaction between the QTL and other loci (epistasis); no interaction between the QTL and other non-genetic factors.

When studying complexly inherited traits a different approach in terms of population structure or experimental design is needed compared to simple inherited traits. For low heritable traits more advanced type populations are needed where the trait of interest is more or less fixed in a homozygous background. In contrast simple inherited traits are of high heritability, not influenced by the environment, and can be evaluated in early generation populations (e.g. F_2, BC_1). QTL studies for complex traits have used

balanced populations in which both parental alleles are in high frequency, for example RILs, F_2 populations, backcross (BC_1), and double haploid populations (*Brassica napus*, Butruille, et al., 1999). Backcross populations, however, are more informative (at low marker saturation) when compared to RIL populations as the distance between linked loci increases in RILs. By using balanced populations the position of the QTL on the chromosome is determined efficiently, estimation of numbers of QTL affecting a trait of interest is possible, and this allows for the estimation of the relative contribution of each QTL to the expression of the trait of interest.

Alternative to the balanced population is the unbalanced population such as the advanced backcross populations or inbred backcross lines (IBLs), also known as backcross RILs, where the alleles of one parent are present at a much lower frequency (Doganlar et al., 2002). IBLs population was first described when studying quantitative characters in wheat, facilitating isolation and identification of the genes involved in the inheritance of quantitative traits (Wehrhahn and Allard, 1965). IBLs are generated by backcrossing multiple times to the recurrent parent and then the backcross F_1 lines are advanced by single seed descent to fix any segregating loci and reach the desired level of homozygosity, in the same manner as is used to develop RILs. No selection pressure is applied during IBL development. IBL are evaluated in replicated trials to identify those lines that deviate significantly from the recurrent parent for the trait of interest.

Unbalanced populations have been used in QTL mapping to determine the number of genes controlling a trait and for the introgression of desirable QTL from unadapted to more adapted germplasm (Fulton et al., 2000; Chetelat and Meglic, 2000; Doganlar et al., 2002; Tanksley and Nelson, 1996). When using unbalanced populations

for mapping and identifying QTL there is a loss of resolution and efficiency (Butruille et al., 1999; Tanksley and Nelson, 1996), but IBL have the advantage of being more genetically and phenotypically similar to the recurrent parent, which generally has market-desirable traits.

The advanced-backcross QTL (AB-QTL) analysis was proposed by Tanksley and Nelson (1996) to transfer genetic variability for a quantitative trait from unadapted into adapted pure line or inbred line cultivars using inbred-backcross line method combined with QTL mapping of traits from the unadapted wild parent. This method allows a subset of alleles from the unadapted parent to be examined in the genetic background of elite adapted line. AB-QTL analysis was used successfully to identify alleles for yield, soluble solids, and color from *Lycopersicon hirstum* (wild relative) in a *L. esculentum* (cultivated species) background (Tanksley and McCouch, 1997). Increases in traits ranged from 48% in yield, 22% in soluble solids, and 33% in color. Results also showed that many of the traits were masked in the wild background e.g. *L. hirstum* is green in color. In addition to the above study, using AB-QTL method, two QTL were found in wild species of rice (*Oryza rufipogon*) which increased yield in cultivated rice (*O. sativa*) by 17% (Xiao et al., 1997). A major objective of the AB-QTL population analysis has been to create NILs by genotypic selection that will carry a specific targeted region of the donor parent (Tanksley and Nelson, 1996). With the NILs any phenotypic difference between the recurrent parent and the NIL was attributed to the existence of a specific QTL. On the contrary, an IBL contains several segments from the donor parent. IBLs and AB-QTL differ in the population development but are similar in that they both resemble the recurrent parent of the cross. Results from Tanksley and Nelson (1996)

studies showed that compared with IBLs, the AB-QTL populations may be more efficient in mapping and precisely estimating QTL effects.

A similar approach to the AB-QTL can be use when introgressing resistance from one gene pool into another gene pool in common bean. Unbalanced populations are useful when crossing between common bean gene pools (Middle-American and Andean gene pools) to reduce the frequency of phenotypically inferior genetic material that results from wide crosses. There is a reduction in power for the detection of QTL due to the unequal allele frequency inherited in the inbred backcross populations (Butruille et al., 1999), but the method can provide linkage information to enrich genetic maps (Doganlar et al., 2002). The advantage of using such populations is the recovery of material that resemble the recurrent parent but with the addition of desirable alleles from the donor parent and the influence of other genes on the bioassays is limited (Lindhout, 2002).

When comparing classical F_2 mapping strategy and IBLs to study downy mildew resistance genes in lettuce several advantages for IBL versus F_2 populations were given (Jeuken and Lindhout, 2003). Genes that go unnoticed in the F_2 population may be detected in IBLs, due to effects that can be caused by several mechanisms. First, the homogeneous genetic background found in IBL compared to F_2 populations, will enhance the detection power for single genes. Second, certain genotypes may be underrepresented or over represented in an F_2 population, so that other genes easily mask their effect. Finally, epistatic interaction between unlinked genes may mask the main effect of single genes in an F_2, whereas in an IBL such interactions are absent. One disadvantage of IBL populations could occur when a trait is expressed as a result of genetic interactions from

not closely linked genes the trait is not detectable in IBLs. In general, the IBL strategy seem more efficient for the introgression of quantitative traits and to reveal QTL that are not involved in genetic interactions making the introgression of the trait of interest simpler in commercial cultivars (Jeuken and Lindhout, 2003).

Conclusion

Pathogens such as *F. solani* predominate and limit bean production not only in the U.S. but elsewhere in the world where beans are planted. Chemical seed control is ineffective and expensive, whereas the extreme susceptibility of snap bean and dry bean genotypes to *Fusarium* root rot disease combined with severe yield loss has led to strong interest in developing resistant bean genotypes. Maintaining a vigorous plant by management practices that promote healthy root development can only alleviate initial infection by the pathogen. Stresses that reduce root growth may injure the plant by reducing the volume and extent of soil exploration; water and nutrient acquisition, with a subsequent reduction in shoot growth. In general, stress caused to the root-by-root rot can affect whole plant growth leading to reduced yield in common bean.

Genetic resistance to *Fusarium* root rot has been difficult to incorporate into acceptable cultivars of the large seeded type Andean market classes. Excellent sources of resistance have been identified in small seeded genotypes such as, N203 (PI 203958) and NY2114-12 and several attempts have been made to transfer resistance to other bean classes. Regardless of efforts made to transfer resistance, root rot resistance in large seeded beans has been limited due to the difficulty of crossing between gene pools and the type of populations used. Low to moderate heritabilities indicate that breeding and

selecting for root rot resistance in the field environment is possible but low enough to imply that effective selection requires progeny testing and evaluation of root rot as a quantitative character. Identifying molecular markers linked to QTL associated with resistance to root rot could provide the opportunity to indirectly select for QTL linked to resistance in susceptible genotypes using a marker assisted selection approach. Marker assisted selection, used to select indirectly for resistant genotypes, may facilitate improvement of disease resistance, where field selection is laborious and destructive.

The pathogen species that dominates and limits production differ across bean growing regions (Singh, 1999). *Fusarium* root caused by *F. solani* is one of the most prevalent bean diseases to cause severe yield losses world wide (Pastor-Corrales and Schwartz, 1994) and is a major problem for Michigan growers, causing severe yield loss. Resistance to root rot had been identified in tropically adapted lines, however there is a need to characterize and incorporate that resistance into locally adapted red kidney bean cultivars.

The physiological, architecture, and morphological features of individual root systems affect many processes such as the composition of soil organisms, turnover of soil organic matter, plant anchorage, acquisition of water and nutrients for plant growth. These features will also affect the establishment, growth, reproduction and mortality of plant populations (Bledsoe et al., 1999). Unfortunately, root systems are difficult to study, primarily because they are spatially and temporally complex and because available methods for their study are labor-intensive and destructive. The evaluation of roots for *Fusarium* root rot has been difficult since a score based on an ordinal scale instead of a quantitative scale for a particular genotype is required for traits strongly influenced by

environmental factors (Schneider et al., 2001). A complex genotype by environment interaction has made resistance breeding difficult so progress in controlling this disease has been limited.

Detecting differences in root growth patterns and architecture between bean genotypes may offer unique genetic selection criteria for enhancing tolerance to root diseases and pests, lodging, drought, flooding, stressful root zone temperatures, or edaphic adaptations (Leskovar and Stoffella, 1995). At present there are no models to determine the relative importance of the various root classes to the total root system function in beans, which complicates the prospects for breeding for root traits (Lynch and van Beem, 1993; Kelly, 1998).

The introgression of *Fusarium* root rot resistance into large-seeded kidney germplasm will require the use of resistance sources from other market classes (Schneider et al., 2001). Such introgression should be possible by using the inbred backcross line method to introgress resistance from the Middle American gene pool into the Andean gene pool, in order to reduce the frequency of phenotypically inferior genetic material that results from wide crosses and more rapidly obtain inbred lines that are agronomically acceptable. The study of genetic resistance to root rot in dry beans, especially in kidney beans, will give Michigan bean growers more choices when selecting the most suitable bean types to produce.

General objectives: (1). Characterize genetic variation of root architecture in contrasting bean types exhibited in different bean genotypes. (2). Determine if root architecture is associated with *Fusarium* root rot incidence in kidney bean populations. (3) Study the

effect of temperature on *F. solani* mycelium growth. (4). Use inbred backcross method to transfer *Fusarium* root rot resistance into large-seeded kidney and cranberry bean populations using a small seeded as root rot resistant cultivar from Mexico as the donor parent. (5). Identify and map QTL associated with *Fusarium* root rot resistance in two IBL populations.

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Chapter II

ASSESSING ROOT TRAITS ASSOCIATED WITH FUSARIUM ROOT ROT RESISTANCE IN COMMON BEAN

Introduction

A number of soil-borne, fungal pathogens are widespread throughout common bean (*Phaseolus vulgaris* L.) production areas. One such pathogen is *Fusarium* root rot [caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) W. C. Snyder & H. M. Hans.] which infect and colonizes common bean roots (Burke and Hall, 1991). Pathogen infection acts to reduce root density by killing roots and may attenuate the functional efficiency of the remaining infected roots. Seed yield losses from root rots in susceptible kidney beans can be greater than 50% (Estevez de Jensen et al., 2002). The evaluation of root traits and root rot tolerance mechanisms is particularly challenging, due to high root plasticity in response to environmental factors (Snapp et al., 1995).

Root disease becomes more severe when bean roots are unable to escape the pathogen due to edaphic factors. Low temperatures, drought, flooded or water logged conditions and soil compaction can hamper root growth and predispose bean plants to severe *Fusarium* root rot infection (Thung and Rao, 1999). Seed yield loss is especially severe when the disease occurs during flowering and pod-fill (Schneider et al., 2001). When the primary root dies due to infection, its function could be replaced by roots that arise from the shoot-root transition zone and generally adopt a horizontal rather than a vertical orientation (Jackson, 1955). These basal roots are frequently referred to as adventitious roots, although, by definition, adventitious roots arise only from hypocotyl

tissue (Leskovar and Stoffella, 1995). A root system with many horizontal roots has been termed top-soil foraging architecture with competitive advantage for phosphorus-acquisition in the topsoil (Rubio et al., 2003). Promoting lateral and adventitious roots may also contribute to plant survival in the presence of root rot organisms (Burke and Barker, 1966; Snapp et al., 2003). Integrated management strategies that combine vigorous rooting systems with bio-control seed treatments may be the most profitable and environmentally-appropriate approach to controlling root rot in bean (Estevez de Jensen et al., 2002).

Limited knowledge on the relative importance and function of various root classes complicates the prospects of breeding for root traits in common bean (Lynch and van Beem, 1993; Kelly, 1998). Differences in root elongation and branching rate exist among different plant genera, among species within a genus, and among cultivars within a species (Gabelman et al., 1986; Gallardo et al., 1996; Leskovar and Stoffella, 1995; Lynch and van Beem, 1993; Jackson, 1995; Vercambre et al., 2003; Bingham and Bengough, 2003; Zobel, 1995). Field studies suggest that root system traits play a role in resistance of root rot (Burke and Barker, 1966; Snapp et al., 2003). Detecting genetic differences in root growth patterns and architecture between genotypes may offer a unique selection criteria for tolerance to root diseases enhanced by drought, flooding, and stressful root zone temperatures (Leskovar and Stoffella, 1995).

Understanding mechanisms of *Fusarium* root rot resistance in common bean, especially kidney beans, is a major goal of breeding programs. Breeding for resistance has been hampered by the high variability and scorer-bias associated with conventional rating systems, whether using hypocotyl lesion scoring or whole root system scoring

(Beebe et al., 1981; Schneider et al., 2001). Root architecture traits, such as secondary and tertiary root branches, adventitious roots, basal roots (arising from the basal region of the hypocotyl), root angles, and radii, should also be considered in evaluating root systems. Quantitative information on root system traits associated with root rot tolerance would improve selection criteria. Moreover knowledge of the genetic determinants of root traits and how they influence yield would allow for a more targeted breeding approach utilizing technologies such as QTL analysis.

The objectives of this study were to: 1) characterize genetic variation of root architecture in contrasting bean classes under field and greenhouse environments; and 2) identify root system characteristics that may be related to root rot resistance in common bean.

Materials and Methods

Bean genotypes

Ten genotypes representing, four bean seed classes (kidney, cranberry, blacks, and snap beans), were evaluated for reaction to *Fusarium* root rot and root system traits. The characteristics and origin of genotypes chosen in each bean class are described in Table 2.1. Shoot growth habit of the germplasm was characterized according to Singh (1982). The bean genotypes included commercial genotypes grown by Midwestern farmers, recent variety releases (Kelly et al., 1998; Kelly et al., 1999a, b) and black bean genotypes from Mexico, Colombia and Michigan with known resistance to root rot. An exception is FR266 (*Fusarium* Resistant 266), which is a non-commercial snap bean line that is closely related to kidney bean genotypes. FR266 was included as one of the few

Table 2.1. Characteristics of the common bean genotypes used to characterize bean roots during the Summer 2002.

Class	Genotypes	Growth† Habit	Origin¶	Seed‡ Size	Root Rot Reaction
	Andean Gene Pool				
Kidneys	Red Hawk	Type I	MSU	63.8	Susceptible
	Montcalm	Type I	MSU	61.3	Susceptible
	Beluga	Type I	MSU	64.0	Susceptible
	Chinook 2000	Type I	MSU	60.6	Moderately§ Resistant
Cranberries	C97407	Type I	MSU	54.5	Susceptible
	Taylor Hort	Type I	Michigan	56.8	Susceptible
Snap bean	FR266	Type I	ARS/USDA, WA	30.7	Resistant
	Middle American Gene Pool				
Blacks	Negro San Luís	Type III	Mexico	38.5	Resistant
	TLP 19	Type III	CIAT	28.1	Susceptible
	B98311	Type II	MSU	29.8	Moderately Resistant

¶ = MSU= Michigan State University; ARS= Agricultural Research Service; USDA= U.S. Department of Agriculture; CIAT= International Center for Tropical Agriculture;
† = Singh, 1982;
‡ = Seed size is expressed as weight in grams 100 seed-1;
§ = Indicates those genotypes that had intermediate symptoms of *Fusarium* root rot compared to the resistant and susceptible genotypes.

examples of a genotype with reported resistance to *Fusarium* root rot (Silbernagel, 1987). Earlier findings indicate that the FR266 root system is highly branched (Snapp et al., 2003).

Plant growth and management

a. Greenhouse experiments

Plant material was planted on June 30, 2002 and harvested on August 9, 2002 in a greenhouse at Michigan State University in East Lansing, MI. Greenhouse temperature was set to 25°C at day and 20°C at night. Watchdog™ temperature monitoring system (Spectrum Technologies, Inc., 23839 West Andrew Rd. Plainfield, Illinois 60544) was used to monitor soil temperature at 3 cm of depth in the containers. Soil temperatures over the 30 days the experiment was conducted ranged from a low of 18° C to a high of 22° C during the first repetition and from 18 to 25° C during the second repetition.

Genotypes were planted under greenhouse conditions in Treepots™ 40.6 cm in depth and 15.2 cm in width. The potting media consist of a mixture of coconut coir and perlite (1:2). Earlier research indicated the mixture produced representative root systems and root rot symptoms, and simplified root extraction to permit analysis of root growth patterns in the potting media (Snapp et al., 2003). Seeds were germinated 5 days prior to the initiation of the experiment to ensure uniform germination of all genotypes. A modified, low phosphorus half-strength Hoagland's solution was applied at planting and once a week thereafter as needed. Harvesting was conducted once, 30 days after planting (DAP) to minimize concern regarding restriction of root development in the containers.

The greenhouse experiment was arranged in a randomized complete block design. Two repetitions of the greenhouse experiments were conducted, one initiated June 30, 2002 and the other initiated July 8, 2002. There were three replications of each genotype per experiment. Each replication had ten homogeneous experimental units, each consisting of three treepots and a single seedling was transplanted per pot. One way analysis of variance using SAS was performed, evaluating genotype effect (SAS Inst., Inc., Cary, N.C.). Where genotype was significant a means comparison was conducted using a preplanned non-orthogonal comparison by bean class. Pearson correlation coefficients were computed for all data collected.

b. Field experiment

The ten genotypes were evaluated under field conditions at the Montcalm Research Farm located near Entrican, MI (43°20'N; 85°01'W), with an alfisol soil, series name Montcalm/ McBride loamy sand. Plots were planted on June 20, 2002 and harvested on July 27, 2002. The Montcalm location was chosen, as the soil type is representative of bean producing areas in Michigan and the Upper Midwest where *Fusarium* root rot is a major problem, particularly for irrigated bean production sites. *F. solani.* is endemic to the Montcalm site.

The experimental design was a lattice design with three replications. Each experimental unit consisted of 20 plants per row (length of row was 5.0 m, 50 cm between rows and 20 cm between plants within rows). Harvests were conducted at 30 and 60 DAP. At each harvest, nine plants per genotype were randomly chosen from each experimental unit, making sure not to include border plants. Temperature for the field

trial varied from 20° C to 26° C. Precipitation at Montcalm from the time of planting until the time of harvest was 23 mm during the month of June and 28 mm during the month of July. Irrigation provided an additional 41 mm H_20 during the month of July.

Root system quantification

a. Greenhouse

Roots were harvested in the greenhouse as carefully as possible to assure the removal of the whole root system and minimize error due to loss at harvest. Roots were transferred to ice to prevent dehydration and taken to the laboratory where they were processed for analysis.

Roots were washed to remove excess coconut coir and perlite that was attached to the root system. Root architectural traits were analyzed following a slightly modified procedure of Yabba and Foster (2003) and Frahm et al. (2003) using the software WinRHIZO™ (WinRHIZO, Regents Instruments Inc. (2001) Quebec, Canada). Individual root systems were transferred for scanning to a 30 cm x 20 cm plexi-glass plate where they floated in clear water and were carefully dispersed into individual lateral roots and secondary roots with forceps as far as practicable to prevent overlapping (Harris and Campbell, 1989). Care was taken to exclude the sides of the tray from the window area to avoid erroneous counts. Each root was scanned independently twice (front and back) to assure scanning of all roots and the average of two measurements were taken. Although root systems develop a three-dimensional form in the soil, roots were measured in two dimensions in this study. Dry weight (dried at 60° C for 72 hours for dry weight determination) of above ground (vegetative area) and below ground (root system) sections of the plants were taken. The following root morphology parameters

were measured: total root system length (cm), root system surface area (cm^2), root system projected area (cm^2), average root diameter (mm), total root volume (cm^3), crossings, number of meristems (tips), and fractal dimension. Manual counting was conducted to determine the number of adventitious roots. Roots were divided into ten classes, based upon root length and diameter [class A (0-0.5 cm), class B (0.51-1.0 cm), class C (1.01-1.5 cm), class D (1.51-2.0 cm), class E (2.01-2.5 cm), class F (2.51-3.0 cm), class G (3.01-3.5 cm), class H (3.51-4.0 cm), class I (4.01-4.5 cm), and class J (> 4.5 cm)], previously described by Yabba and Foster (2003) and Frahm et al. (2003), with slight modification. The ten root length classes classified as A-J respectively were grouped in three root diameter classes: fine roots or secondary roots (A-C), intermediate roots or laterals (D-G), and taproots (H-J). In this study lateral roots were defined as a major root axis originating at the taproot.

b. Field

Because, field root harvesting is challenging, special care was taken to ensure that roots were dug up carefully and that root systems were as intact as was possible. These roots were harvested to an approximate depth of 0.31 m. After removing roots from the soil they were immediately put in a cooler with water and ice to prevent dehydration. At time of harvest, roots were rated in the field for *Fusarium* root rot symptoms using the root rating scale of 1 to 7, where 1= root system completely free of disease and 7= root system severely affected by disease (Schneider and Kelly, 2000). The roots were taken to the laboratory and cleaned of excess soil. They were processed for image analysis following the procedures described for the greenhouse study.

Results and Discussion

Root system variation: genotypes

There were marked differences in adventitious rooting of genotypes, which varied from 4 to 43 adventitious roots per plant among the bean genotypes screened in the field, and from 0 to 8 adventitious roots per plant under greenhouse conditions (Figure 2.1A). The root rot resistant genotype Negro San Luís (NSL) exhibited a root system that accumulated biomass rapidly and had many adventitious roots, as did the moderately resistant genotypes B98311 and Chinook 2000. Under greenhouse conditions, Chinook 2000 had the highest number of adventitious roots followed by the susceptible TLP19 and the most resistant line, NSL, whereas some of the resistant genotypes had large diameter roots (Figure 2.1A). B98311 had the highest number of adventitious roots under field conditions followed by TLP19, NSL, and Chinook 2000. B98311 is a drought resistant line that has been reported as having a vigorous, deep-penetrating taproot (Frahm et al., 2003). Although shallow adventitious roots are more susceptible to drought, adventitious roots are likely to persist under irrigation, and continue to function and contribute to the long-term development of the root system in soils infested with soil borne pathogens (Johnson et al., 2000).

Fractal dimension provides a measure of root system size and density of root branching. Since research has shown that ontogeny is closely related to fractal dimension (Berntson et al., 1995), comparisons of genotypes should be at the same state of development. Fractal dimension values observed ranged from 1.40 to 1.55 (Figure 2.1D)

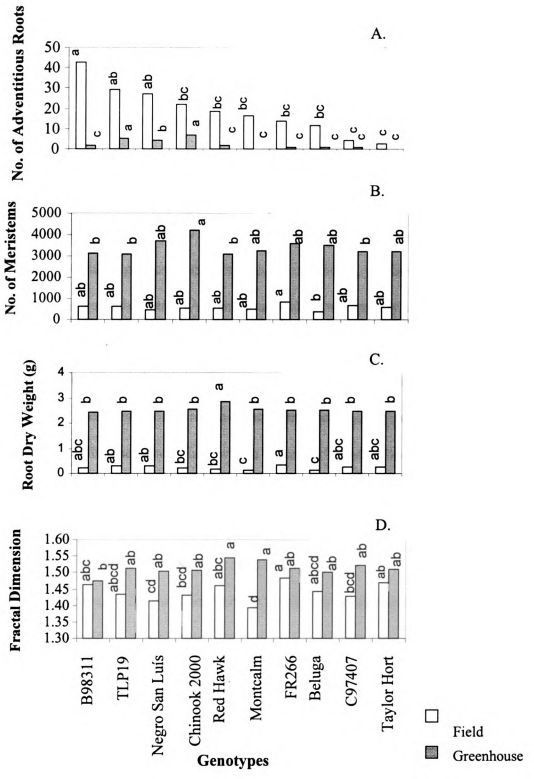

Figure 2.1. Illustration of differences between genotypes 30 days after planting under field and greenhouse conditions for root traits. (Different letters on columns represent statistical differences at $P< 0.05$)

and were similar to the range in fractal dimension reported in an earlier study of bean root systems (Lynch and van Beem, 1993). In our study, the most root rot susceptible genotype, Montcalm, had a notably weak root system as characterized by limited branching which was reflected in the lowest level of fractal dimension observed for the field environment (Figure 2.1D) and the limited amount of dry weight accumulated in the root system (Figure 2.1C).

Plants were evaluated 30 DAP in both the field and greenhouse and developmental stage was similar in both environments, yet the genotype by environment interaction was substantial. Adventitious rooting was greatly enhanced under field conditions compared to greenhouse grown plants (Figure 2.1A), whereas meristem number was high in the greenhouse trial (Figure 2.1B). Chinook 2000 had the largest number of meristems compared to other kidney bean genotypes (Figure2.1B). Chinook 2000 was a plant selection made within 'Chinook' light red kidney cultivar (Kelly et al., 1999a), which was derived from crosses that included a black bean in its genetic background; black bean root systems may be associated with greater branchiness or root system meristem number (Kelly et al., 1992).

The reason for this phenomenon is not well understood, but the large number of lateral adventitious roots in the field may be related to the greater level of stress in the field environment, where nutrient and water supply is dynamic. In the greenhouse screen, root systems had many meristems, which may be related to proliferation of fine roots in low bulk density container systems or the disease stress in the field environment may have reduced the survival of fine roots. Roots in the field interact with indigenous

soil-borne diseases and other soil microorganisms, which could potentially enhance initiation of adventitious roots by attacking the main tap root.

A large number of root meristems have been associated with acquisition of immobile nutrients such as phosphorus (Rubio et al., 2003). In both the greenhouse and field-based screens conducted here no significant difference was observed for number of meristems between Chinook 2000 and the resistant genotype NSL, and between B98311, TLP19, C97407, and Red Hawk (Figure 2.1B). TLP19 was previously selected as tolerant to low phosphorus, thus we hypothesized it would have multiple branched, top-foraging root architecture; however, this was not observed. These traits have been observed in lines resistant to low phosphorus (Bosner et al., 1996; Lynch and Beebe, 1995; Frahm et al., 2003). Other mechanisms such as root plasticity and rhizosphere acidification may contribute to low-phosphorus tolerance in TLP19 (Snapp et al., 1995; Yan et al., 1996).

Root system variation: bean classes

The four bean classes in this study varied significantly in plant growth habit and root system architecture (Table 2.2 and 2.3). Significant differences among bean classes were observed for all traits except root diameter in the field, whereas only root surface area, root volume, root dry weight, and fractal dimension were different in the greenhouse (Appendix A- Table A.1). Large differences between field and greenhouse data were observed for most traits except root diameter, and fractal dimension. Selection for root dry weight would appear to be useful in the greenhouse, but should be delayed to flowering around 45 DAP. The later harvest date would prevent the measurement of

Table 2.2. Bean class means for different root traits measured under greenhouse and field conditions at Entrican, Montcalm County, MI during the summer 2002.

Classes†	Length‡ (cm plant^{-1})	Surface‡ Area (cm^2)	Average‡ Diameter (mm)	Root‡ Volume (cm^3)	Root Dry Weight (g)	Fractal‡ Dimension	Forks ‡	Tips ‡	Crossing ‡
					Greenhouse				
K	1094 a	252.8 a	0.73 a	5.45 a	3.62 a	1.52 a	2984 a	3515 a	398.4 a
S	1127 a	240.6 a	0.68 a	4.12 ab	3.45 ab	1.52 a	3206 a	3584 a	450.1 a
C	1116 a	237.8 ab	0.68 a	4.09 ab	2.47 b	1.51 a	3014 a	3216 a	451.0 a
B	1094 a	228.9 b	0.70 a	4.06 b	2.46 b	1.40 b	2644 a	3258 a	354.0 a
					Field				
K	277 b	72.0 b	0.83 a	1.50 b	0.17 c	1.43 a	411 b	493 a	38.9 b
S	461 a	120.8 a	0.85 a	2.54 a	0.34 a	1.51 b	841 a	816 a	78.2 a
C	377 ab	99.9 ab	0.85 a	2.11 ab	0.26 b	1.45 a	559 ab	613 a	54.0 ab
B	279 b	71.1 b	0.80 a	1.46 b	0.25 b	1.44 a	474 b	558 a	46.7 b

† K= kidney bean class included four genotypes, C= Cranberry bean class included two genotypes, B= Black bean class included three genotypes, and S= Snap bean class included one genotype.

‡ Characteristics were determined using WinRHIZO software, (WinRHIZO, Regents Instruments Inc. (2001) Quebec, Canada).

Different letters on columns represent statistical differences at $P< 0.05$.

Table 2.3. F-values for kidney bean class in contrast with black, snap, and cranberry bean classes for different root traits measured under greenhouse and field conditions at Entrican, Montcalm County, MI during the summer 2002.

Classes†	Length‡ (cm plant^{-1})	Surface‡ Area (cm^2)	Average‡ Diameter (mm)	Root‡ Volume (cm^3)	Root Dry Weight (g)	Fractal‡ Dimension	Forks ‡	Tips ‡	Crossing ‡
					Greenhouse				
K vs. B	0.42	3.10*	1.96	4.29*	5.52*	3.60*	1.43	0.58	0.65
K vs. S	0.13	0.21	1.78	1.46	0.88	0.31	0.28	0.03	0.42
K vs. C	0.10	0.63	2.80	2.74	4.82*	0.24	0.01	0.95	0.73
					Field				
K vs. B	6.98*	8.86*	0.51	8.70*	31.22***	1.50	3.48	1.32	3.95
K vs. S	14.17**	16.32**	0.45	15.08**	46.66***	8.12*	17.64**	5.70*	16.03**
K vs. C	0.01	0.01	0.93	0.05	17.57**	0.11	0.82	0.50	1.34

† K= kidney bean class included four genotypes, C= Cranberry bean class included two genotypes, B= Black bean class included three genotypes, and S= Snap bean class included one genotype.

‡ Characteristics were determined using WinRHIZO software, (WinRHIZO, Regents Instruments Inc. (2001) Quebec, Canada).

*, **, *** Significant at the 0.05, 0.01, 0.001 probability levels respectively.

other root length, area and volume traits for which variability was limited in the greenhouse. Taproots contributed more than 1% of the total root length of kidney bean class in the greenhouse, whereas taproot length was less than 1% in cranberry, snap bean, and black bean classes. Across all bean classes, 95% of the final root length was contributed by the fine roots and ~ 4% by intermediate roots (Appendix A- Table A.2).

Significant major contrasts for root dry weight were observed between kidney bean and all other classes (Table 2.3). Differences by bean market class appeared to be related to shoot growth habit and growth rate, as kidney beans (Type I shoot growth habit) exhibit a relatively shallow root system with lateral roots arising from the taproot at the base of the stem. This is similar to earlier observations of related bean root systems (Kelly, 1998; Stoffella et al., 1979), and the relationship of shoot to root growth habit in bean genotypes studied by Lynch and van Beem (1993). Similar to kidney bean, cranberry bean root systems (Type I shoot growth habit) tended to be shallow (length) and with limited branching (forks, crossing; Tables 2.2 and 2.3). In contrast, root systems of the black bean class (Type II and III shoot growth habit) tended to have many adventitious roots and a dominant tap root system (Figure 2.1A). The black bean class exhibits basal roots similar to those described by Stoffella et al. (1979) in beans and by Zobel (1995) in tomatoes. The black bean B98311 exhibits a deeper root system as described by Frahm et al. (2003) compared to TLP19 and NSL, although the root rot resistant genotype NSL has a highly branched root system similar to B98311.

The snap bean class had only one representative, FR266 genotype, which is a root rot resistant breeding line with a determinate bush growth habit (Silbernagel, 1987) and may not be typical of the level of root rot resistance of this class. Thus no generalizations

can be made about snap beans. It was interesting to note, however, that FR266 exhibits a root system similar to Chinook 2000 at 30 DAP. The root system is highly branched in the upper 12 cm and has a corresponding higher root dry weight (Figure 2.1C). FR266 is also similar to B98311 in possessing deep laterals, but with less secondary root branching in the lower portion of the roots toward the root tips. The source of *Fusarium* root rot resistance in FR266 was derived from PI 203958 (N203), an indeterminate prostrate vine black bean-seeded line from Mexico that was used multiple times as a parent during the crossing process (Sibernagel, 1987). A possible explanation for the similarity in root systems between FR266, Chinook 2000 and B98311, which represent different market classes (Table 2.1), is that FR266 and Chinook 2000 both possess a black bean in their genetic background and B98311 is a black bean genotype.

Similar to the findings of Lynch and van Beem (1993), the indeterminate Type II and type III black bean lines, tended to have more adventitious roots and a deeper, more extensive root system. In our study a larger number of genotypes were compared than in previous research, and it appeared that beyond extensive (deep, many laterals) and intensive (highly branched, shallow) types of root systems we also observed a vigorous, highly branched intensive root system with a large number of extensive laterals. This observation is consistent with it being possible to combine an intensive and extensive root system type from diverse genetic backgrounds. This combination occurred in the black bean class, or in genotypes with black bean parentage.

Root system screening methodology

Substantial differences in adventitious rooting and other root traits were observed at 30 DAP in the field compared to the greenhouse (Figure 2.1). Genotypes with smaller seed size are distinct and appear to generally have a larger number of adventitious roots under field conditions compared to the greenhouse environment. Data collected in our greenhouse studies exhibited no significant differences between bean classes for most of the characteristics studied, perhaps due to root restrictions imposed in a non-stressful environment in the pots, or to differences in growth media (Tables 2.2 and 2.3; Appendix A- Table A.1 and A.2). Laboratory screening systems for root growth response in rice have also found limited correlation with field performance (Clark et al., 2002). Plasticity may be enhanced in a heterogeneous field environment where a range of signals induce branching and root morphological changes (Wraith and Wright, 1998). Root plasticity is an adaptive variable that enhances resource acquisition and anchorage in a heterogeneous environment (Campbell et al., 1991). Investigation of plasticity to a specific constraint may require a controlled environment, whereas testing for general plasticity and ability to overcome realistic stress encountered in the field environment may require field-based evaluations.

Total root dry weight was significantly correlated to fine ($r=0.744$; $P<0.001$) and intermediate ($r=0.657$; $P<0.01$) root classes under field conditions, but was not significantly correlated with average diameter under greenhouse or field conditions (Table 2.4). No significant correlation was observed for average diameter and total root dry weight under greenhouse conditions. The smaller taproot diameter in comparison with the diameter of the intermediate roots is a result of severe disease development and

Table 2.4. Correlation coefficients (r) for root classes grouped in three categories, average diameter, and total root dry weight for greenhouse study and one field trial conducted during the summer, 2002 in Montcalm, MI.

	Average Diameter	Root Diameter Classes †		
		Fine	Intermediate	Taproots
		Greenhouse		
Total root dry weight	0.524	-0.251	0.390	0.465
Average Diameter		-0.361	0.795**	0.809**
		Field		
Total root dry weight	0.292	0.745***	0.657**	-0.009
Average Diameter		0.156	0.605**	0.502*

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

† Root classes were grouped in three major classes: fine roots (included A, B, and C root length in diameter classes) intermediate roots (included D, E, F, and G root length in diameter classes), taproots (included H, I, and J root length in diameter classes).

environmental factors in the field. Average root diameter was highly and significantly correlated with intermediate and taproots root classes both under greenhouse (r=0.795 and r=0.809; $P<0.01$, respectively) and field conditions (r=0.605 and 0.502; $P<0.01$ and $P<0.05$, respectively). The thickening of intermediate roots and taproots appears to be associated with dry weight accumulation of these root classes, for screening conducted in both field and greenhouse environments.

There was a trend towards a greater number of adventitious roots in plants with lower root rot scores in the field environment although variation was high (r=-0.06; $P<0.05$). Root systems with many adventitious roots may avoid some negative effects of disease through replacement of the function of disease-infected roots (Miller, 1986; Stoffella et al., 1979). However, B98311, NSL, and FR266 varied greatly in number of adventitious roots (Figure 2.1A). Our results are consistent with the partial replacement by adventitious roots of roots eliminated by *Fusarium* root rot in susceptible common bean genotypes. A similar association of adventitious rooting with *Fusarium* root rot tolerance was observed in an earlier study Involving inbred bean lines (Snapp et al., 2003). High root plasticity may enhance plant growth and survival through continuous alteration of the root system in response to a varied soil environment (Smucker, 1993). To eliminate the interference of genetic differences among bean classes, a more effective method to study root plasticity would be to generate genetic populations consisting of recombinant inbred lines developed from parents contrasting only in root characteristics. These populations would provide the best opportunity to study root plasticity and the role of adventitious roots and root dry weight in root rot resistance given the wide genetic differences observed between the bean genotypes in this study.

Conclusions

Our results show that genetic variation for root architecture exists among different bean genotypes, and between common bean market classes. Results from this and previous research indicate that root traits such as adventitious roots (Snapp et al., 2003), total root dry weight (Kmiecik and Bliss, 1986), and lateral roots (Burke and Barker, 1966; Schneider and Kelly, 2000) can be quantified as part of a selection process by plant breeders interested in enhanced root rot resistance and over all root health of common bean. Classical methods for studying root systems involve excavation of whole root systems. This poses challenges in keeping root systems intact and extracting fine roots, yet it provides a realistic view of architectural changes that occur as root systems respond to environmental conditions and biotic stress caused by soil borne pathogens. In this study, a field-based screen markedly enhanced adventitious rooting response compared to greenhouse screens. Greenhouse studies can be effective in understanding root system traits, but may be problematic in terms of container restrictions to growth and potentially providing a less stressed, unrealistic environment. Our study indicates that the potential exists to improve rooting characteristics of common bean and despite the genotypic differences between market classes, it would appear that breeders have been effective in introgressing desirable rooting traits from black bean into kidney and snap bean. These introgressed lines such as Chinook 2000 and FR266 would be valuable as parental lines to further enhance the root rot resistance of susceptible commercial kidney and snap bean varieties.

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Chapter III

EFFECT OF DIFFERENT TEMPERATURE AND EXPOSURE TIMES ON MYCELIUM GROWTH OF *FUSARIUM SOLANI* F. SP. *PHASEOLI*

Introduction

Fusarium root rot of common bean (*Phaseolus vulgaris* L.) caused by the soil-borne fungus *Fusarium solani* f. sp. *phaseoli* (refer to as *F. solani* here after) is wide spread throughout bean production areas in Michigan causing severe yield loss. The pathogen can survive in the soil in the form of soil resting spores called chlamydospores for a long period of time. The first symptoms are narrow, long, red to brown lesions on the hypocotyl where fissures often develop lengthwise that in many cases can destroy the taproot.

The severity of root rot will depend on factors such as cropping history, plant spacing, moisture and temperature stresses, and soil compaction (Burke et al., 1972; Singh, 1999; Burke and Miller, 1983; Lockwood, 1986). In general, any factor that contributes to a reduced rate of root growth increases the plants susceptibility to *Fusarium* root rot (Pastor-Corrales and Schwartz, 1994; Singh, 1999). In dry land production areas the disease can be more severe, especially in years of severe drought. High plant populations also increase plants stress and favor infection (Burke, 1965; Dryden and Van Alfen, 1984). Improper cultivation resulting in hard-pan formation, favors other soil borne pathogens such as: *Fusarium oxysporum*, *Pythium* spp., *Rhizoctonia solani*, *Thielaviopsis basicola*, and *Aphanomyces eutiches*. Various

herbicides are also known to induce injury of young roots and aggravate *Fusarium* root rot damage.

Temperature has been reported to have a direct effect on the abundance of *Fusarium* species in soil relative to each other. Many Investigators have studied optimal and lethal temperatures for growth of soil borne pathogens using in vitro and greenhouse soil assays (Zentmyer, 1981; Coelho et al., 2000; Pullman et al., 1981; Campbell and Lin; 1976; Bollen, 1985; Pfender and Hagedorn, 1982; Hargreaves and Fox, 1978; De Boer et al., 1985; Marín et al., 1995). When evaluating kidney and cranberry inbred backcross line populations at the Montcalm Research Farm for *Fusarium* root rot resistance more severe disease symptoms were observed during the 2002 field trial compared to the 2003 field trial (Chapter IV of this manuscript). Moreover while evaluating common bean genotypes for *Fusarium* root rot resistance during the spring of 2003 it was observed that after three weeks of inoculation under greenhouse conditions at 12°C no disease development was observed. After the greenhouse temperature increased to 26°C bean plant roots were covered with lesions. These observations lead us to question if this response was due to an effect of temperature on the pathogen. The objective of this study was to study the effect of different temperatures and exposure times on growth of mycelium of *F. solani* in artificial media (PDA). A retrospective analysis on soil temperature conditions and rainfall for the 2002 and 2003 field trials was conducted.

Materials and Methods

Isolate description

The Hawks 2b isolate of *F. solani* used in this study was collected by Schneider and Kelly (2000) in Presque Isle County, MI. This isolate was recovered from roots of field grown bean plants with *Fusarium* root rot symptoms. The isolate was maintained in cold storage in sterile potting soil at 4°C.

Pathogen isolation and pathogenicity test

For the current study the isolate was re-isolated from the long term cold storage stock and pathogenicity was tested by inoculating several plants (susceptible kidney bean genotypes: 'Red Hawk' and 'Montcalm') with the Hawks 2b isolate and re-isolating the fungus following the procedure of Schneider and Kelly (2000). For this pathogenicity test 72-well greenhouse flats were filled with perlite and a single seed was germinated in each well, using no fewer than 24 seedlings per susceptible genotype and the remaining 24 wells were planted with two resistant genotypes 'Negro San Luís' (small black bean from Mexico) and 'FR266' (*Fusarium* resistant snap bean from ARS/USDA, WA) as checks. Greenhouse temperature ranged from 20 to 25°C. The perlite was saturated with nutrient solution at planting and weekly thereafter. Plants were inoculated with 10 ml of 2×10^5 spore suspension of *F. solani* ten days after planting. The inoculum was applied over the base of the hypocotyl using a 4-liter polyethylene hand sprayer. The inoculum was prepared by scraping PDA plates of the Hawks 2b isolate into distilled water, strained through two layers of cheesecloth, quantified using a hemocytometer and adjusted to the proper spore concentration (2×10^5).

Substrate for growth of F. solani mycelium

The basic medium used in this study was Potato dextrose agar (PDA, 39 g/ L water) at pH 5.7. Inoculum discs of 4 mm diameter were cut with a sterile cork borer from the colony margins of 4-day old cultures of the Hawks-2b isolate grown on PDA (Invitrogen ^{TM}, Life Technologies, MD) and transferred mycelia side down, to the centre of individual Petri plates (15 mm diameter) containing 25 ml of PDA.

Determination of mycelium growth after transfer from parent cultures

To determine the effect of temperature on mycelium growth (mm) and exposure times, the Petri dishes were expose to seven different temperature treatments (5, 10, 15, 20, 25, 30, and 35°C) and radial growth data was collected at five exposure times (24, 48, 72, 96, and 172 hours after the set temperature exposure). Radial growth for all replications was measured after each exposure time along two lines intersecting at right angles at the centre of the inoculum disc, subtracted the inoculum disc radius and averaged over four radial measurements. The experiment was repeated: the first experiment from March 17 to March 24, 2003 and the second experiment from March 29 to April 5, 2004.

Each temperature treatment per exposure time consisted of four replications of inoculated plates and one non-inoculated plate as a control and transferred to a PTC-1 Peltier-effect temperature control chamber (Sable Systems, Henderson, NV). The chamber was set for equilibration 3h prior to the start of the experiment following the procedure of Kirk (2003) with slight modification. Temperature equilibration was

measured after the door of the chamber was opened, and at 5°C set temperature, temperature rose quickly to ~9.2°C but then dropped to 4.9°C in about 1.3 h. At 10, 15, 20, 25, 30, 35°C set temperatures, temperature rose to 14.3, 19.4, 24.3, 35.2, and 39.2°C and recovered in 1.2, 1.2, 1.3, 1.4, and 1.5 h, respectively. After removing the plates from the chamber and measuring radial growth, they were placed under constant light at 20°C temperature for four days. After four days a pathogenicity test was performed using Montcalm as the susceptible genotype and FR266 as the resistant control, following the procedure mentioned above. "Images in this dissertation are presented in color."

Data analyses

All data was analyzed using SAS, (SAS Inst., Cary, N.C., 1995) statistical program. Radial growth was analyzed by two-way analysis of variance so that the effects of two factors (temperature and exposure times) could be assessed for statistically significant differences using PROC GLM. The relation between the exposure time, temperature and radial growth was determined by linear regression analysis using PROC REG.

Field climatic variables at the Montcalm Research Farm (MRF) located at Entrican, MI, were measured with a CR10X measurement and control system (Campbell Scientific Instruments, Logan, Utah) equipped with precipitation, soil (6 cm depth) and air temperature and humidity sensors (45 cm aboce soil).

Results and Discussion

Temperature and exposure time effect on F. solani growth

The effects of temperature and exposure time and their interactions on mycelia growth were significant at $P<0.001$ (Table 3.1). Responses of *Fusarium solani* on PDA to temperature from 5 to 35°C over 24 to 172 exposure hours are shown in Figure 3.1. When contrasting the slope increase between the temperature treatments, significant differences were observed for 10°C vs. all the other exposures (15, 20 25, and 30°C) as well as 15°C vs. 20 and 30°C (Table 3.1). No significant difference was observed for 15°C vs. 25°C. Growth of *F. solani* increased as temperature increased up to 30°C. No growth was observed at 5 and 35°C. Once growth began, however, it continued at a normal rate. Two temperature treatments (5 and 35°) were excluded from further analyses to eliminate skewed data points since these exposures did not contribute to the results on radial growth obtained. When studying lethal temperature of soil borne pathogens, Bollen (1985) reported temperatures in the range of 45 and 50°C as lethal temperatures for *F. solani*. Under the conditions of the current study, *F. solani* grew optimally between 20 and 30°C (Figure 3.1).

Significant time by temperature interaction was observed (Table 3.1). Lower temperature initially caused a greater delay in time before mycelia on agar disk began radial growth at 10°C (Figure 3.1, Table 3.2). The data showed the possibility of the fungi going through acclimation perhaps as a mean of survival. There was no difference in radial growth between 25 and 30°C (Table 3.2). Time within temperatures showed significant difference between exposure times.

Table 3.1. Analyses of variance for the dependent variable mycelia growth with two factors (time and temperature), F-values and their respective level of significance.

Source	DF	Mean Squares	F-values
Time	4	14.12	410.59***
Temperature	6	10.13	294.49***
Contrast 10°C vs. 15°C	1	1.18	93.30***
Contrast 10°C vs. 20°C	1	2.16	170.24***
Contrast 10°C vs. 25°C	1	1.47	116.13***
Contrast 10°C vs. 30°C	1	3.4	268.22***
Contrast 15°C vs. 20°C	1	0.15	11.48**
Contrast 15°C vs. 25°C	1	0.02	1.25
Contrast 15°C vs. 30°C	1	0.57	45.13***
Contrast 20°C vs. 25°C	1	0.07	5.16*
Contrast 20°C vs. 30°C	1	0.14	11.09
Contrast 25°C vs. 30°C	1	0.40	31.37***
Repetition	1	0.12	3.46
Replications	3	0.02	0.65
Replications (Time)	12	0.01	1.14
Time * Temperature	16	0.27	25.29***
Error	187	0.03	
Total	229		

*, **, *** Significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively

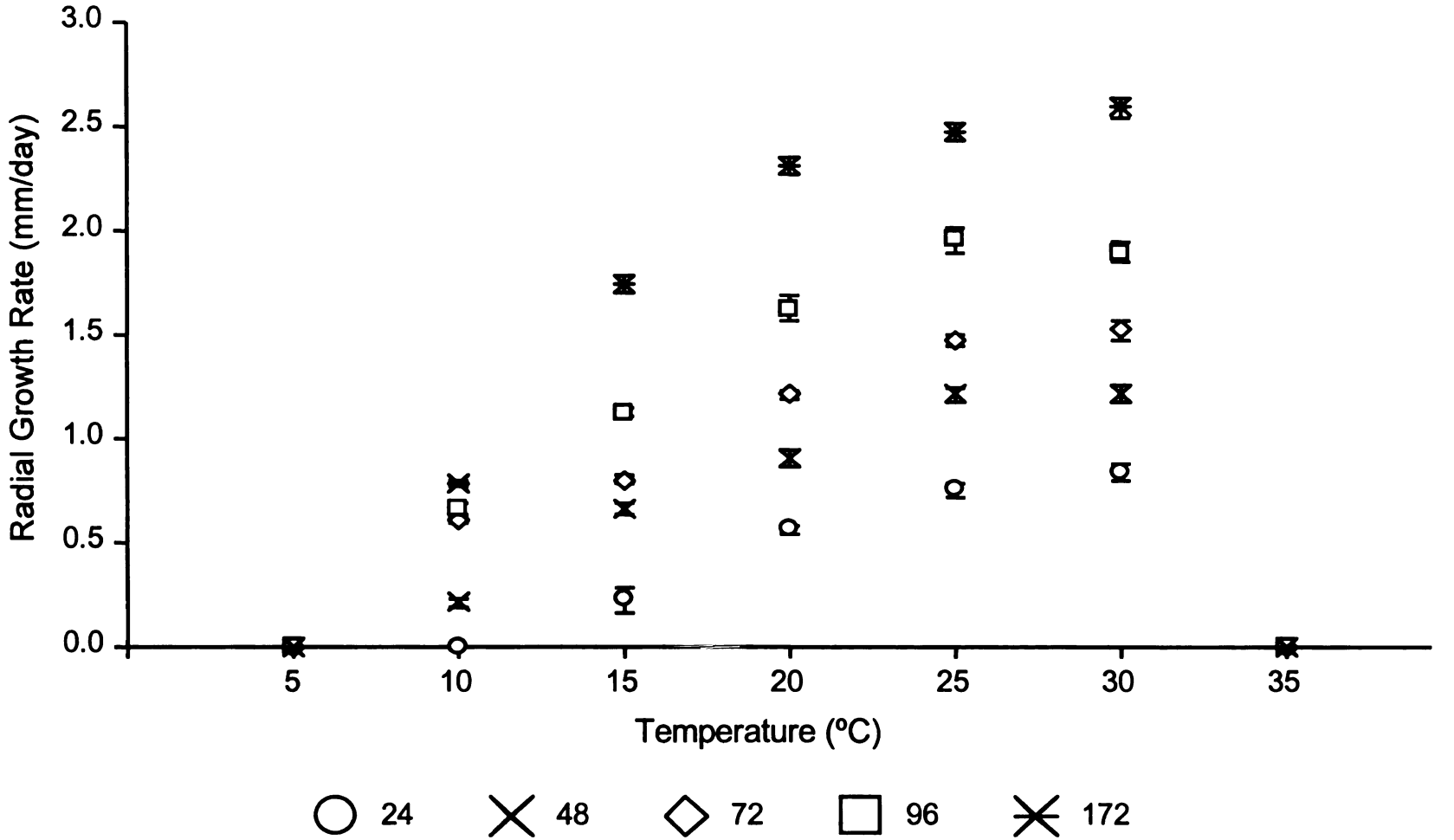


Figure 3.1. Radial growth rate (mm/ day) at 20°C incubation temperature of *Fusarium solani* f. sp. *phaseoli* after exposure to different temperatures for a range of durations; 24, 48, 72, 96 and 172 h. Mean values and standard error for each exposure duration within each temperature were plotted.

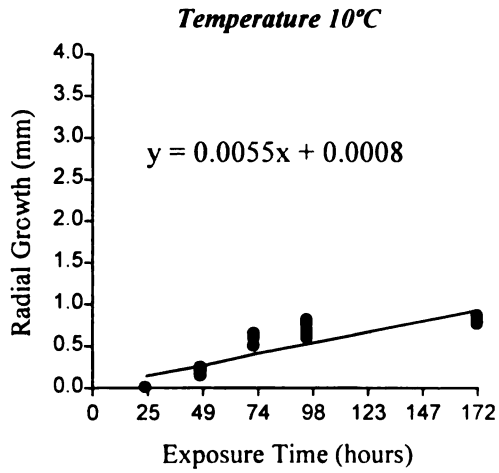
Table 3.2. Radial growth means for *Fusarium solani* f.sp. *phaseoli* within temperature treatments at different exposure times

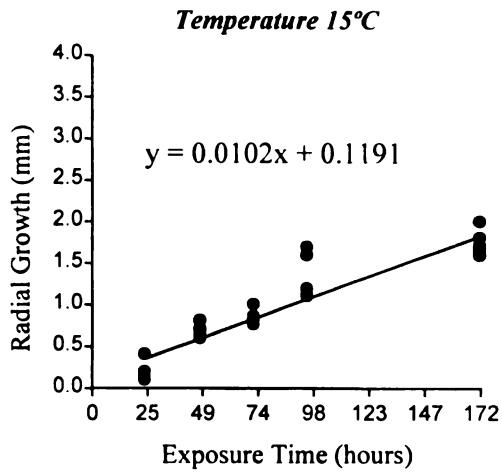
Temperature (°C)	Exposure Time (hours)	Radial Growth
5	24	0.00
	48	0.00
	72	0.00
	96	0.00
	172	0.00
10	24	0.00
	48	0.18
	72	0.59
	96	0.69
	172	0.79
15	24	0.19
	48	0.68
	72	0.83
	96	1.26
	172	1.73
20	24	0.59
	48	0.93
	72	1.28
	96	1.67
	172	2.33
25	24	0.80
	48	1.26
	72	1.61
	96	1.96
	172	2.42
30	24	0.74
	48	1.16
	72	1.57
	96	1.98
	172	2.74
35	24	0.00
	48	0.00
	72	0.00
	96	0.00
	172	0.00
C.V.		11.21
LSD (P<0.05; within temperature)		0.35

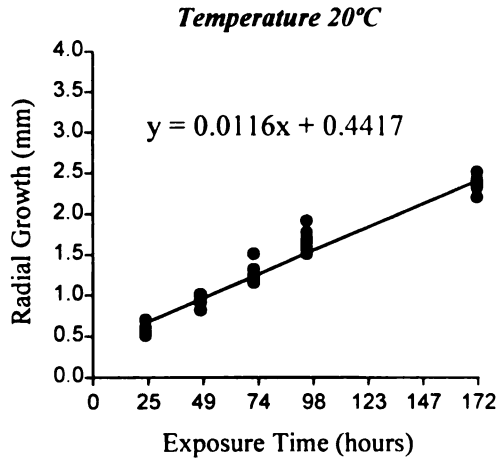
Regression analysis showed that there was a linear response between time and temperature for radial growth at all temperatures (Figure 3.2). The slopes of the regression lines were less than 1, but the intercepts were greater than 0. Regression analysis of the radial growth data points again showed highly significant correlation, r^2 ranged from 0.73 to 0.95, $p<0.001$, which supports that radial growth does measure effects contributed by temperature.

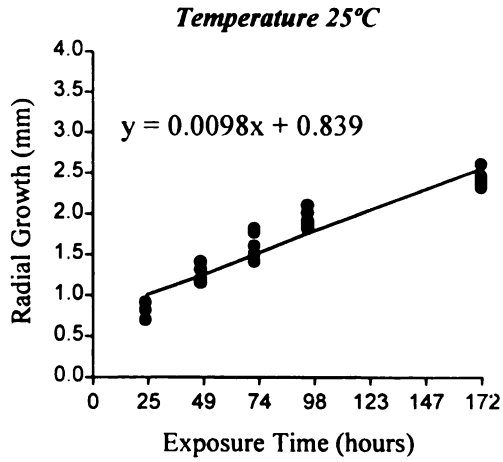
Systematic study requires that independent variables are controlled. It would have been interesting to evaluate a third factor (water or humidity) to see if there is any difference in response or if the relation between temperature and time still holds with this third factor. As Hallsworth and Magan (1996) pointed out, "there is a tendency to treat each factor separately but this approach is doomed because it implies acceptance of independently acting factors... an appreciation of the interaction of such factors is essential for the full understanding of fungal growth". Field observations have shown that *Fusarium* root rot disease incidence on bean (*Phaseolus vulgaris* L) seem to be more severe under drought or flooding conditions implying that water also plays an important role in the field environment.

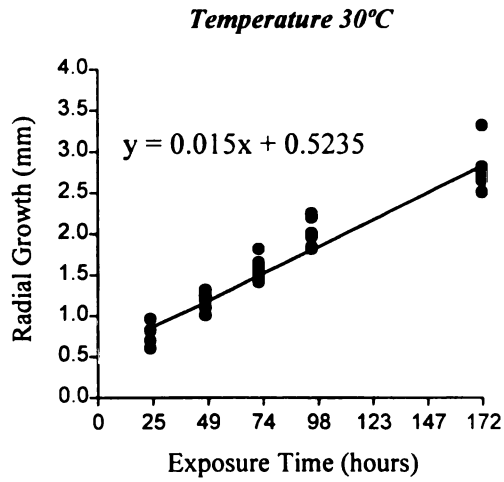
A study conducted by Saremi et al. (1999) indicated that temperature was a major factor affecting structure of communities of *Fusarium* species in soil, where *F. solani* was favored by the highest temperatures (25-30°C). While it was true in this study conducted in Australia and also in studies conducted in South Africa (Sangalang et al., 1995a and 1995b; Jeschke et al., 1990) the opposite effect occurs in the highlands of Mexico where *F. solani* both severity (r=-064) and incidence (r=-084) were negatively related to maximum temperature (Navarrete-Maya et al., 2002). In the same study











Temperature (°C)	F-values	R^2
10	110.73***	0.73
15	300.15***	0.82
20	876.05***	0.95
25	345.81***	0.87
30	521.27***	0.93
*** Significant at $P<0.001$		

Figure 3.2. Relationship between radial growth (mm) and exposure time (24 to 172 h) for temperature treatments (10 to 30°C) on an isolate of *Fusarium solani* f. sp. *phaseoli* on PDA agar. A summary of the regression analysis is shown on the table.

Rhizoctonia solani severity was strongly related to average (r=0.84) and maximum (r=0.92) temperature during the growing cycle, and *F. solani* severity was positively related to rainfall (r=0.76). Therefore care must be taken when interpreting the information given in the current study, since it could change with the interaction of availability of water.

Pathogenicity test

Four days after the removal of culture plates from the temperature control chambers, two-week old bean plants of the susceptible genotype Montcalm and a resistant genotype FR266 were inoculated under greenhouse conditions. The genotypes were planted using perlite and the greenhouse temperature ranged from 20 to 25°C. Characteristic symptoms of *F. solani* were observed, long, red to brown lesions in hypocotyls and discoloration of the root (Figure 3.3). Two weeks after inoculation the susceptible genotype Montcalm was severely diseased (Table 3.3). Interestingly plants inoculated with those culture plates exposed from 15 to 30°C showed more severe root rot disease at 14 days after inoculation compared to plants inoculated with 5 and 10°C temperature exposure culture plates which showed less disease symptoms (Figure 3.3, Table 3.3). Plants inoculated with cultures from plates exposed to 5 and 35°C showed a delay in root rot symptoms of about 5 to 6 days more than the other temperature exposures. Virulence did not seem to be affected at temperatures between 10 and 30°C. After temperature treatments the pathogen seems to have virulence, although disease severity varied between culture plates of different temperature treatments.

Figure 3.3. Illustration of the susceptible genotype Montcalm after two weeks of inoculation with cultures of *Fusarium solani* f. sp. *phaseoli* exposed to 10°C (left), 15 and 20°C (center), and 25 and 30°C (right).

Table 3.3. Results from the pathogenicity test performed using the susceptible genotype Montcalm and the genotype FR266 with partial resistance to *Fusarium* root rot for comparison purpose.

	Root Rot Scoring‡	
Temperature (°C)†	14 - Days after inoculation	20- Days after inoculation
Montcalm		
5	3.5	4.5
10	4.5	5.0
15	5.0	6.0
20	5.5	6.5
25	6.5	7.0
30	6.5	7.0
35	3.5	5.0
FR266		
5	1.0	1.5
10	1.0	2.0
15	1.5	2.0
20	2.0	2.5
25	2.0	2.5
30	2.5	2.5
35	1.0	1.5
Coefficient of Variation (%)	15.75	11.35
LSD	1.26	1.10

‡ Root rot scoring was done using a 1 to 7 scale developed by Schneider and Kelly, 2000 where 1=no disease and 7 severely diseased

† Temperature at which the *F. solani* cultured Petri dishes were exposed

As Pullman et al. (1981) concluded "the longer a propagule was heated and still survived, the longer it required to germinate... indicating that heat damage accumulated gradually to a point beyond which the propagule can not recover... but if heat treatments are stopped before this point, recovery may occur". Since these observations were under controlled conditions, probably under soil environment the propagules would not recover because of surrounding microbial antagonists and additional stress factors (Pullman et al., 1981).

Field studies in Michigan have indicated disease severity in *P. vulgaris* is usually more apparent during flowering and early pod set when the plant and its pod load are most sensitive to stress (Schneider et al., 2001; Román-Avilés et al., 2004; Snapp et al., 2003), although, root rot was observed to occur during the whole cycle and no genotype was immune to the pathogen. In these field studies temperature ranged from 20 to 28°C from the time plants started flowering, July, to the time of pod fill, August. Moreover a significant reduction in root density was observed in severely affected plants. Proportions of *F. solani* in the community of *Fusarium* species increased as the temperature increased, ranging from 12.9 cfu g^{-1} (13-18°C), 21.4 cfu g^{-1} (20-24°C), to 29.0 cfu g^{-1} (25-30°C; Saremi et al., 1999).

Experiments for more precise determination of the effect of temperature, and water potential on *F. solani* in soil environments would probably require experiments that measure the effect of these two factors in specific aspects of the fungi life cycle, over time, as well as its interaction with its legume hosts (e.g. *Glycine max*). It is clear that temperature have an effect on mycelium growth which was observed under controlled

conditions in artificial media (PDA). Observations in this study could vary under a field environment where water can alter the environment for mycelium growth.

Field temperature and rainfall data analysis

When evaluating kidney and cranberry IBL populations for *Fusarium* root rot resistance during the 2002 and 2003 field trials at MRF it was observed that during 2003 lesions developed sooner than in the 2002 summer trail where disease was more severe latter in the season. The analysis of soil temperature conditions and rainfall revealed that in 2003 soil temperatures were cooler over the period when root rot developed sooner, during anthesis where 2002 had warmer soil temperatures (Figure 3.4 A). No major difference in precipitation except for the accumulation rate (Figure 3.4 B), which was evident, and it was hypothesized that cooler soil temperatures might favor expression of *Fusarium* symptoms. Probably the rapid accumulation of rainfall during 2002 compared to 2003 along with the high temperature caused the high disease severity for the 2002 field evaluations of the kidney and cranberry IBL populations.

Conclusions

Temperature has an effect on disease development. Interestingly, the pathogen seems to be acclimating within exposure times between the different temperatures (5°C to 35°C). The pathogenicity test revealed that plants inoculated with those culture plates exposed from 15 to 30°C showed more severe root rot disease at 14 days after inoculation compared to plants inoculated with 5 and 10°C temperature exposure culture plates which showed less disease symptoms. While there was a delay in *Fusarium* root rot symptoms

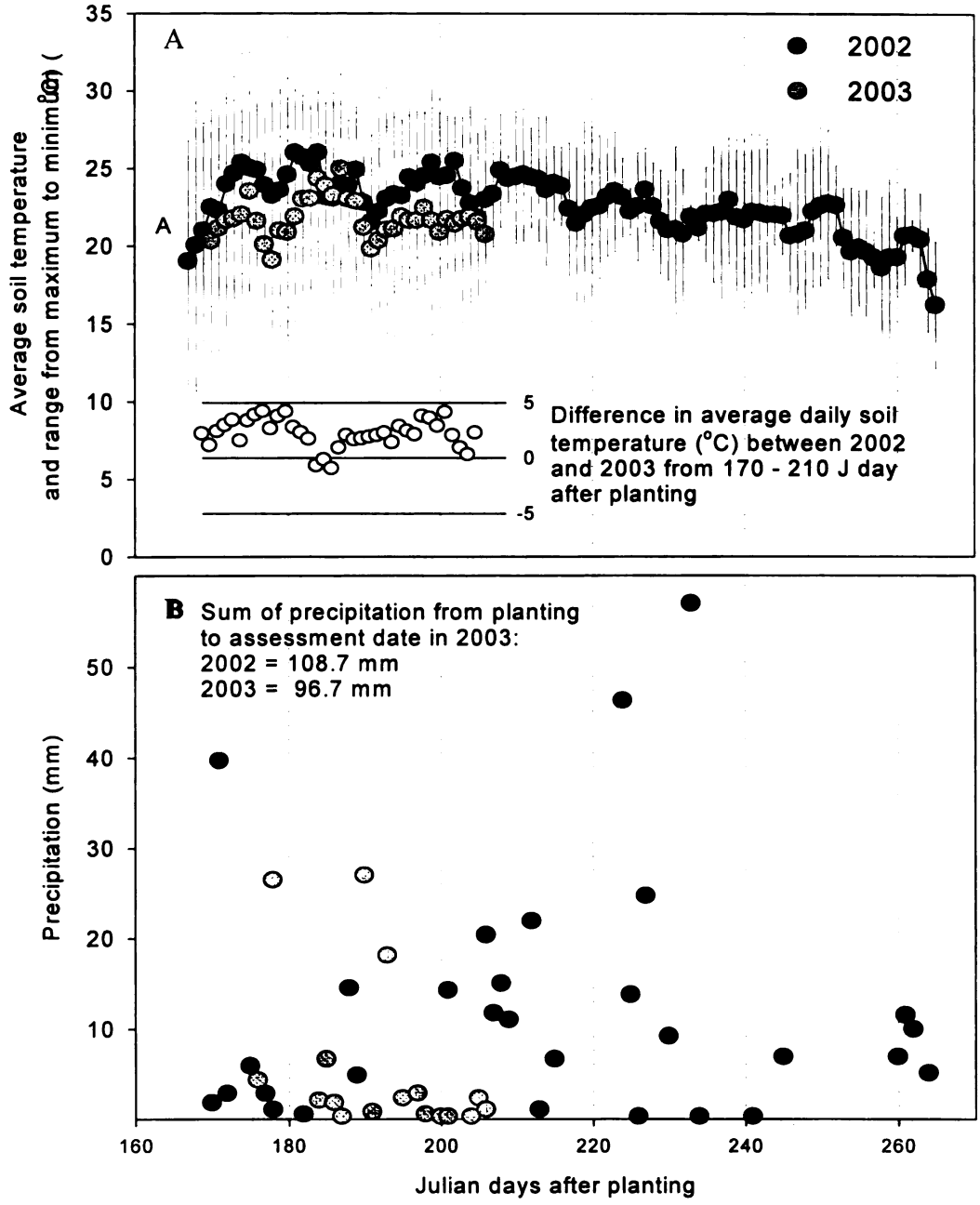


Figure 3.4. The graph shows: (A) the mean and range of daily temperatures over the evaluation periods in 2002 and 2003 field trials at MRF and (B) shows the rainfall. The inset graph is the difference in temperature between 2002 and 2003- 2002 had warmer soil temperatures on equivalent days after planting.

on plants inoculated with cultures from plates exposed to 5 and 35°C of about 5 to 6 days more. Virulence did not seem to be affected at temperatures between 10 and 30°C, although disease severity varied between culture plates of different temperature treatments. It was hypothesized that under field conditions cooler soil temperatures might favor expression of *Fusarium* symptoms.

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Chapter IV

INTROGRESSION OF FUSARIUM ROOT ROT RESISTANCE INTO LARGE SEEDED BEANS AND THE IDENTIFICATION OF QTL ASSOCIATED WITH RESISTANCE

Introduction

Breeding for root rot [caused by *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans.] (*F. solani*) resistance in common bean (*Phaseolus vulgaris* L.) has been a formidable challenge for breeders for many years. Little progress has been made in improving root rot resistance particularly in large seeded Andean genotypes. The polygenic inheritance combined with the strong environmental effects have limited efforts to improve root rot resistance (Beebe et al., 1981; Boomstra et al., 1977; Bravo et al., 1969; Hassan et al., 1971; Schneider and Kelly, 2000; Silbernagel, 1990; Tu and Park, 1993; Wallace and Wilkinson, 1965).

The importance of *Fusarium* root rot disease in bean production was first recognized by Burkholder (1916) in western New York. Having recognized this problem he and his colleagues began a search for a genotype resistant to root rot that could be crossed with cultivars with acceptable seed types. Although Dr. Burkholder and colleagues did find some low levels of root rot resistance, they were unsuccessful in breeding for resistance. Their search continued for many years leading to a uniformly, highly infested seed site resulting in an extremely valuable screening site for root rot resistance breeding programs (Wallace and Wilkinson, 1973).

In Michigan, large seeded beans are highly susceptible to root rot caused by the soil-borne fungus *F. solani* resulting in high yield loss. This fungus is present in all

production areas of Michigan, especially in the central northeast (Presque Isle County), north east (Isabella county) and west (Montcalm county) where sandy or sandy loam soils predominate and where large seeded kidney beans are grown (Schneider et al., 2000). Compounding this problem, stresses such as soil compaction and drought conditions, aggravated by the sandy soils, inhibit root growth and vigor and may ultimately lead to increased susceptibility to root rot disease. An additional problem is that genetic resistance to *Fusarium* root rot has been difficult to incorporate into snap bean and large seeded Andean bean cultivars with acceptable market traits (Estevez de Jensen et al., 1998). An emphasis on quality traits appears to have significantly reduced the genetic variability in these two bean types (Gepts, 1998), which mayhave contributed to severe susceptibility to *F. solani* (Schneider et al., 2001).

Resistance had been identified in tropically adapted small seeded bean lines, however there is a need to characterize and incorporate that resistance into locally adapted red kidney cultivars. Breeders had attempted to transfer the resistance to current commercial kidney bean cultivars, but breeding is time consuming and success is not guaranteed. Gains to date represent partial resistance to *F. solani* in *P. vulgaris*, with large differences in levels of susceptibility (Schneider et al., 2001).

QTL analysis has gained the attention of breeders, since the analysis can overcome some of the common limitations encountered by conventional selection for quantitative traits. A QTL associated with resistance would allow researchers to indirectly select beans for resistance based on the presence of linked markers. Breeders would not have to introduce the disease pathogen and delay evaluation until crop is affected by disease. QTL controlling quantitative traits have been reported for *Fusarium*

root rot in common bean (Schneider et al., 2001; Navarro et al., 2003; Chowdbury et al., 2002). Most of these QTL are located on linkage groups B2 and B3 of the integrated map close to a region where defense response genes *PvPR-1* and *PvPR-2* pathogenicity proteins, *Pgip*, and *ChS* have been identified. Indirect selection for root rot resistance based on markers linked to the resistance QTL may facilitate improvement of disease resistance, while field selection is laborious and destructive sampling is needed to identify resistance.

QTL studies for root rot resistance have used balanced populations such as recombinant inbred lines (RILs), F_2 populations, backcross (BC_1), and double haploid populations in which both parental alleles are present in high frequencies (Butruille, et al., 1999). By using balanced populations, the estimation of the number of QTL and the relative contribution and position of each QTL to the expression of a trait of interest is determined more efficiently. An alternative to the balanced population is the unbalanced population such as the advanced backcross populations where the alleles of one parent are present at a much lower frequency (Tanksley and Nelson, 1996). Unbalanced populations have been used to determine the number of genes controlling a quantitative trait and to introgress desirable QTL from unadapted to more adapted germplasm, in addition to QTL mapping (Fulton et al., 2000; Chetelat and Meglic, 2000; Doganlar et al., 2002; Tanksley and Nelson, 1996). When using unbalanced populations for mapping and identifying QTL there is a loss of resolution and efficiency (Butruille et al., 1999; Tanksley and Nelson, 1996), but unbalanced populations have the advantage of being more genetically and phenotypically similar to the recurrent parent. There is a reduction in power of detection of QTL due to the unequal allele frequency inherited in inbred

backcross populations (Butruille et al., 1999), but inbred backcross lines (IBLs) can provide linkage information to enhance genetic maps (Doganlar et al., 2002). In crosses between Middle-American and Andean gene pools, unbalanced populations are more useful to reduce the frequency of phenotypically inferior genetic material that results from such wide crosses between gene pools. The advantage of using such populations is the recovery of material that resemble the recurrent parent but with the addition of desirable alleles from the donor parent. An example of an unbalanced population is a population of IBLs, also known as backcross RILs (Doganlar et al., 2002). IBLs are generated by backcrossing multiple times without selection to the recurrent parent and the final backcross F_1 lines are advanced by single seed descent to fix any segregating loci and reach the desired level of homozygosity for testing.

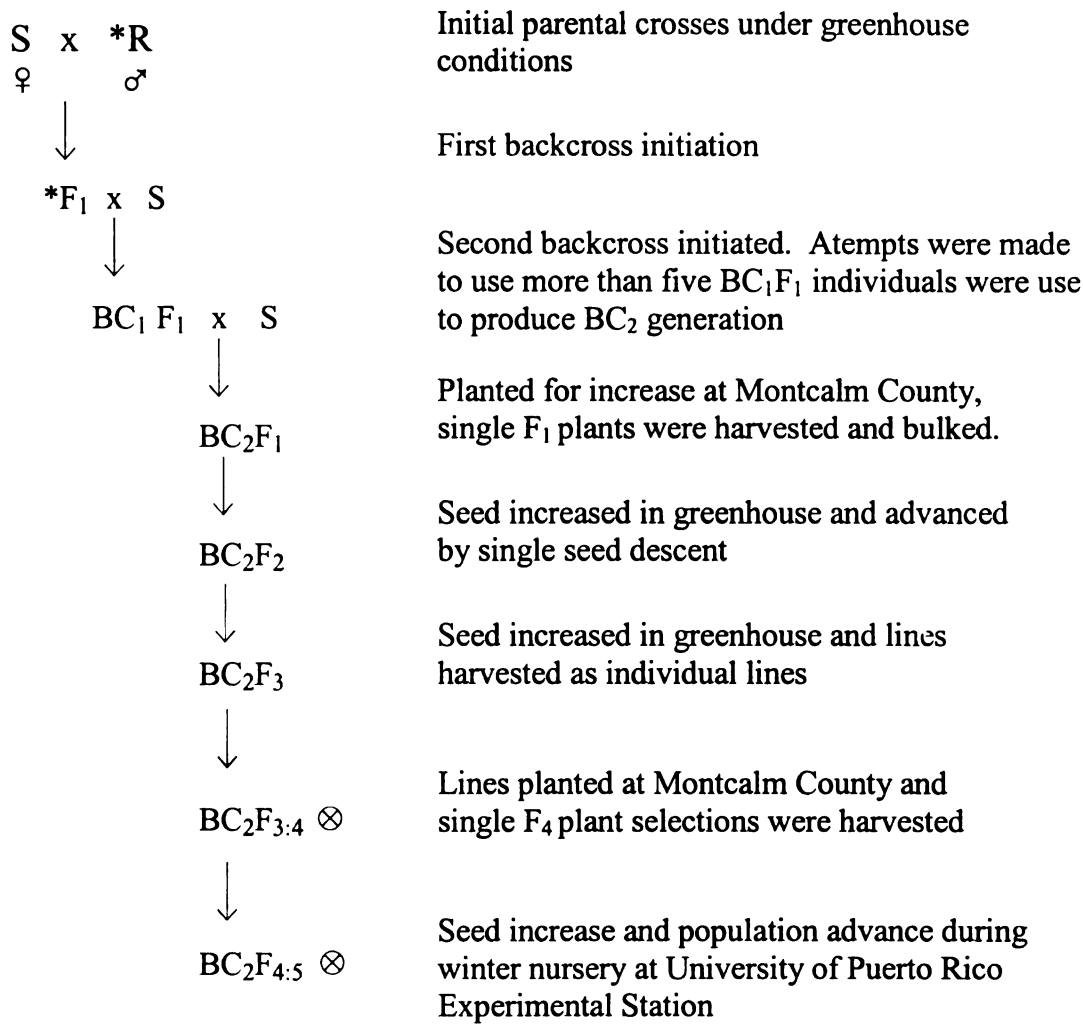
The objectives of this study were to i) introgress *Fusarium* root rot resistance into the large-seeded Andean kidney and cranberry bean populations from a small-seeded black bean from Middle American gene pool; ii) define the inheritance of *Fusarium* root rot resistance in *P. vulgaris*; and iii) identify significant QTL-marker associations which could be used to facilitate indirect selection of *Fusarium* root rot resistance in common bean.

Materials and Methods

Plant Genetic Material

Two populations, one with 91 IBL individuals from a cross of Red Hawk *2/Negro San Luís and the other with 78 IBL individuals from a cross of C97407 *2/Negro San Luís were developed using inbred backcross procedure similar to method

described by Owen et al. (1985a, b) in cucumber (*Cucumis sativa*) and Sullivan and Bliss (1983) in common bean, respectively (Figure 4.1). For this study two backcrosses were made to the recurrent parents Red Hawk and C97407 of Andean origin (Race Nueva Granada). Red Hawk (RH) is a dark red kidney bean developed and released by the Michigan Agricultural Experiment Station and the USDA-ARS as a full season cultivar with excellent processing quality (Kelly et al., 1998). Red Hawk exhibits the Type I upright determinate bush growth habit, is resistant to bean common mosaic virus (BCMV), rust and anthracnose, but RH is highly susceptible to Michigan isolates of root rot, primarily *F. solani*. C97407 is a cranberry bean breeding line (Taylor Hort */ Cardinal) that also exhibits the Type I growth habit but is susceptible to *Fusarium* root rot. This line was derived from Taylor Hort, a commercial cultivar susceptible to BCMV that exhibits good yield. C97407 was derived from a backcross between 'Taylor Hort' and 'Cardinal', which has the single dominant hypersensitive *I* gene resistance to BCMV, but C97407 was never released due to a lack of yield potential. The small-seeded black bean cultivar Negro San Luís (NSL) of Middle American gene pool (Race Durango) commonly planted in the semi-arid highlands of central Mexico was used as the donor parent. Negro San Luís is a late maturing photoperiod sensitive cultivar with an indeterminate type III growth habit and prostrate vine plant structure. Negro San Luís exhibits drought tolerance and because it also exhibits high levels of root rot resistance it was used as the donor parent for root rot resistance in the inter-gene pool crosses.



Symbols description:
* = Flowering was induced because of photoperiod sensitivity,

⊗ = Self-pollination,
S = Susceptible to *Fusarium* root rot (Andean gene pool): Red Hawk and C97407,
R = Resistant to *Fusarium* root rot (Middle American gene pool): Negro San Luís

Figure 4.1. Diagram depicting development of inbred backcross line populations using Red Hawk and C97407 as the recurrent parents and Negro San Luís as the donor parent.

The original crosses were made during spring 1999 between NSL and the Andean cultivars RH and C97407 (Figure 4.1). To initiate the parental crosses, flowering had to be induced in NSL 30 days after planting due to photoperiod sensitivity. The NSL plants were grown under short days using approximately 10 hours of daylight to induce flowering. Flowering was induced within 14 days in all plants. The F_1 generation was planted during fall of 1999 under greenhouse conditions and flowering was also induced since photoperiod sensitivity is expressed as a dominant trait. Because crosses were made between gene pools, a reaction known as dwarf lethality (stunted growth, chlorosis, and formation of adventitious root on the hypocotyl) was expected and observed in the later segregating populations. Dwarf lethality is a temperature dependent interaction (enhanced at temperatures above 30°C) that occurs at early stages of plant development. Deleterious effects of inter-gene pool crossing can be circumvented through careful management of F_1 hybrids following the procedure described by Beaver (1993). F_1 seed, from the kidney and cranberry crosses, was planted in such a way that the tap roots were not allowed to develop since they produce deleterious hormonal effects (results from expression of *DL* genes) on the development of the F_1 plant. Adventitious roots were enhance by adding hormex to form on the hypocotyl and soil was added as the stem elongated to promote the development of more adventitious roots. When the F_1 plants flowered successive backcrosses were then made to RH and C97407, followed by four generations of selfing to produce $BC_2F_{3:4}$ generation IBLs for study and QTL analysis (Figure 4.1).

After the first backcross, eight BC_1F_1 plants were chosen from the kidney IBL population and six BC_1F_1 from the cranberry IBL population to generate the second

backcross. No selection was applied in any generation other than to maintain diversity based on pedigree, and plants were chosen randomly for additional backcrossing. Nevertheless, some lines were lost in subsequent generations due to lethality and photoperiod sensitivity. A total of 91 kidney and 78 cranberry $BC_2 F_{3:4}$ lines were harvested as single plant selections for each population during the summer of 2001 in the field at the Montcalm Research Farm (MRF) located near Entrican, MI (43°20'N; 85°01'W) and the inbred backcross lines were advanced an additional generation ($BC_2 F_{4:5}$) at the Isabela, P.R. Sub-station of the University of Puerto Rico during the winter of 2001/2002. Both IBL populations were treated independently and were considered as two separate experiments in order to reduce error variance.

Population evaluation for Fusarium root rot

a. Field

Inbred backcross line populations, parents and additional checks of known root rot reaction were evaluated for *Fusarium* root rot resistance at flowering stage in four field experiments. Four field experiments were conducted at the MRF, in an alfisol soil, series name Montcalm/McBride loamy sand, which is naturally infected with *F. solani*. In addition to the four experiments at the MRF location a fifth experiment was conducted at the New York State Agricultural Experiment Station (NYSAES) Research Farm on Ontario County near Geneva, NY. The soil type at this farm was a lime silt loam, with fairly rocky and a history of bean production and root rot diseases. The experiment was arranged in a 100 entry randomized block design (91 IBLs from kidney IBL population plus nine checks) with three replications and planting at NYSAES was June 10-16, 2003.

Samples were dug and rated for root rot on July 22 and August 18, 2003 (G. S. Abawi, personal communication).

Two experiments were planted at MRF on June 20, 2002 and both the kidney and cranberry IBL populations were evaluated separately. The kidney population experiment was arranged in a 10 x 10 square lattice design (91 IBL of kidney IBL population plus nine checks) and the cranberry experiment was arranged in a 9 x 9 square lattice design (78 IBL of cranberry IBL population plus three checks). Each experimental unit consisted of 20 plants per row (row length 5.0 m, row width 50 cm and plant spacing 20 cm within rows). The third and fourth experiments were planted at MRF on June 17, 2003 similar to the design of the previous two experiments. The kidney population experiment was arranged in a 10 x 10 square lattice design (91 IBL of kidney IBL population plus nine checks) and the cranberry experiment was arranged in an 8 x 9 rectangular lattice design (64 IBL of cranberry IBL population plus eight checks). The number of IBLs for the cranberry experiment was reduced in the 2003 season due to lost of IBLs to a heavy infection with common bacterial blight (CBB). Each experimental unit of the 2003 trials consisted of 80 plants per row (row length 5.0 m, row width 50 cm and plant spacing 7.6 cm). Standard agronomic practices for tillage, fertilization, insect, and weed control were applied to ensure adequate plant growth and development. All plots received supplemental irrigation. During the 2002 season, plots were irrigated twice, for a total of 41 mm, whereas in the 2003 season the plots were irrigated 3 times for a total of 48 mm of supplemental water.

Three random plants from each row were carefully removed from soil and rated for *Fusarium* root rot symptoms at maturity in the 2002 trial and at flowering stage for the

2003 trial. Plants were rated using the root rating scale of 1 to 7 (Schneider and Kelly, 2000), where 1= healthy root system with no discoloration of root or hypocotyl tissue and no reduction in root mass and 7= pithy or hollow hypocotyl with much extended lesions, root mass is severely reduced and is functionally dead (Figure 4.2). The MRF data was collected by B. Román Avilés from Michigan State University during the 2002 and 2003 field and greenhouse trials and for the NYSAES experiment the data was collected by G. S. Abawi from Cornell University. "Images in this dissertation are presented in color."

Field root rot evaluations were averaged over the two ratings for each genotype, for ANOVA analysis using SAS PROC GLM (SAS, 1995). Pearson correlations were conducted among agronomic traits and root rot scores using SAS PROC CORR. Agronomic data such as days to flower (DTF), days to maturity (DTM), desirability score (DS), height and growth habit was collected. Roots were visually evaluated for root vigor based on a 1 to 5 scale where 1 = strong and vigorous root system and 5 = a root system with some adventitious roots or no roots at all (Table 4.1). To provide objectively to the evaluation, the root vigor rating scale was photographed to provide a permanent record (Figure 4.3). This root vigor scale is different from the root rot evaluation scale (Figure 4.2).

Narrow sense heritability was calculated using the analysis of variance components, of the expected mean squares, corresponding to a lattice design with three replications as the source of variation. The additive genetic variance (σ^2_A) and the estimates of heritability were calculated using the variability between $BC_2F_{4:5}$ lines of the population ($\sigma^2F_{4:5}$). Heritability was adjusted taking into consideration the selfing generation (Hallauer and Miranda, 1988). The σ^2_A estimate between $BC_2F_{4:5}$ were calculated using

Figure 4.2. Visual representation of the *Fusarium* root rot rating scale of 1 to 7, where 1= healthy root system with no discoloration of root or hypocotyl tissue and no reduction in root mass and 7= pithy hypocotyl with much extended lesions, root mass is severely reduced and is functionally dead.

Table 4.1. Phenotypic description of the root vigor scale developed for the evaluation of the genetic populations.

Score	Phenotypic Description
1	Vigorous, deep tap root, with few secondary roots. Roots appear to be resistant to root rot, seem to be healthy, and have high biomass accumulation.
2	Vigorous root, highly branched, with thinner roots compared to No.1 in this scale. Taproot is missing and is being replaced by secondary roots. The root system appears to be slightly affected by root rot but the symptoms are superficial and localized mostly on the hypocotyls. High biomass accumulation is observed.
3	Very shallow root system less branched and with thinner roots compared to No.2 in this scale. Taproot is missing. The root system appears to be susceptible to root rot but the symptoms are superficial and adventitious roots are observed.
4	Weak root system, very shallow, and poor branching pattern. Roots appear highly affected by root rot. Taproot is missing. Root biomass is reduced by around 30% compared to No.3 in this scale and adventitious roots are observed.
5	Very weak root system, highly affected by root rot, root biomass is reduced more than 80% compared to No.1 in this scale and the taproot is replaced by numerous adventitious roots arising from the hypocotyl. Taproot is missing.

Figure 4.3. Visual representation of the root vigor rating scale previously described in Table 4.1.

the formula $\sigma^2F_{4:5}/0.9375$ (Warner, 1952). Narrow sense heritability was calculated as σ^2_A/σ^2_P. Phenotypic variance was determined utilizing the formula $\sigma^2A+(\sigma^2_E/3)$. The standard error for the narrow sense heritability was calculated as follow:

$$SE(h^2_{NS}) = \frac{SE(\sigma^2_A)}{(\frac{\sigma^2 e}{3}) + (\sigma^2_A)} \qquad SE(\sigma^2_A) = \sqrt{\frac{2}{3^2}[\frac{MS^2_{Trt}}{df_{Trt}} + \frac{MS^2_e}{df_e}}$$

b. Greenhouse

Both IBL populations and checks of known disease reaction was evaluated for root rot reaction in the greenhouse using a perlite-based protocol (Schneider and Kelly, 2000). Seventy-two-well greenhouse flats were filled with perlite and a single seed was germinated in each well, using no fewer than three seedlings per line per replication for each individual IBL population. A randomized complete block design with three replications was used and each experiment was evaluated twice. The perlite was saturated with nutrient solution at planting and at weekly intervals. When plants were ten days old, they were inoculated with 10 ml of 2 $x10^5$ spore suspension of *F. solani*. The inoculum was applied over the base of the hypocotyl using a 4-L polyethylene hand sprayer. The inoculum was prepared by scraping PDA plates of *F. solani* into distilled water quantifying using a hemocytometer and adjusting to the spore concentration of 2 x 10^5.

The Hawks 2b isolate of *F. solani* collected by Schneider and Kelly (2000) in Presque Isle County, MI was used for all inoculations. Two weeks after inoculations, seedlings were removed from the flats, and roots were cleaned for excess perlite and

rated using the scale described previously. The fungus was cultured continuously and its pathogenicity was tested by inoculating several susceptible plants of Red Hawk with the Hawks 2b isolate and re-isolating the fungus following the procedure of Schneider and Kelly (2000). Data collected was analyzed as described above for the field experiments.

Marker Analysis

a. DNA extraction and amplification

DNA was extracted using the miniprep procedure proposed by Afanador et al., (1993) with slight modifications. Prior to inoculation with *Fusarium*, tissue from $BC_2F_{4:5}$ IBL populations and parent genotypes was collected for DNA extraction using very young leaves from a trifoliate leaf or 3 discs of leaf tissue (using the lid of a 1.5 ml sterile Eppendorf tube) from greenhouse grown plants, lyophilized, and ground. Ground tissue was stored at -80°C. RAPDs fragments greater in size than 700 bp were amplified using Invitrogen™ brand Taq DNA polymerase (Life Technologies, MD) and those bands smaller in size than 700 bp were amplified by AmpliTaq ® DNA polymerase, Stoffel Fragment (Perkin Elmer, CT).

The extracted DNA was standardized to uniform concentration (10ng μl^{-1}) using DNA fluorometry (Hoefer® DyNA Quant® 200, Hoefer Pharmacia Biotech, San Francisco, CA). DNA was amplified using a Perkin Elmer Cetus DNA Thermal Cycler 480 (Perkin Elmer, Cetus, Norwalk, CT). The Polymerase Chain Reaction (PCR) cycling profile consisted of the following cycles: 3 cycles of 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C, 34 cycles of 1 min at 94 °C, 1 min at 40 °C, and 2 min at 72 °C (final step extended by 1 sec for each of the 34 cycles), and a final extension cycle of 5 min at 72°C.

Approximately 20 μl of amplified DNA from each sample was run on a 1.4% agarose gel containing 0.5 $\mu g \cdot ml^{-1}$, 40 mM Tris-acetate, and 1mM EDTA. DNA was viewed under ultraviolet light and photographed for permanent record. Polymorphisms were recorded as either presence or absence of bands.

b. Identification of RAPD markers linked to resistance gene (s)

The combined selective mapping strategy composed of bulked segregant analysis (BSA) technique (Michelmore et al., 1991) and selective genotyping (Lander and Botstein, 1989) was used to identify RAPD markers linked to the genomic regions conditioning resistance to *Fusarium* root rot. The homozygosity of $BC_2F_{4:5}$ IBL's, to be used in the selected bulks, was determined by inoculating both populations (note: IBL populations will be considered as two separate experiments) under greenhouse environment. Inoculation conditions and symptom evaluation was performed as previously described. Based on these results, a contrasting pair of R and S DNA bulks was developed for each population. One of the bulks represented a pool of DNA from the four most resistant (R) IBLs. Likewise, the second bulk was a pool of DNA from the four most susceptible (S) IBLs. The identification of markers followed a five step process for combined selective mapping used by Miklas et al. (1996) where:

(1)- RAPD markers polymorphic between parents of the mapping populations were identified from a large group of randomly chosen 10-mer primers from selected Operon kits (Operon Technology, CA) and from the University of British Columbia (UBC). A total of 2,500 RAPD primers were screened. A large

number of RAPDs were screened because of the narrow genetic base of the IBL populations developed through backcrossing.

(2)- The DNA bulks were tested with only those primers known to generate polymorphisms between the parents. Among the 2500 RAPD primers only 350 were polymorphic either between parents of the kidney and cranberry populations.

(3)- Only RAPD primers polymorphic between bulks and parents were subsequently screened against the individuals constituting each bulk.

(4)- If the RAPD continued to co segregate with disease reaction in at least seven of the eight (90%) lines composing both DNA bulks, then those primers were used to genotype the entire population.

(5)- Selectively mapped markers and mean disease scores for each IBL were statistically regressed to ascertain RAPD-QTL association.

Additional polymorphic markers previously identified as associated to *Fusarium* root rot resistance (Schneider et al., 2001) were also included in the analysis to establish more genome coverage (Shen et al., 2003) and determine if the same QTL were present in the IBL population derived from different resistant parent.

c. Linkage map construction and QTL analyses

None of the genetic mapping software commonly used would perform multipoint analysis and mapping of the IBL populations. However, mapping programs such as MAPMAKER and JoinMap accept two point information to built genetic maps, and this two point information provides an estimation of recombination frequency and its corresponding LOD score. The mapping program JoinMap was chosen to construct

linkage groups based on a minimum LOD score of 4 (Stam, 1993; van Ooijen and Voorrips, 2001). The graphical presentation of each individual linkage group was developed using MapChart v2.0 (Voorrips, 2001; Voorrips, 2002). The addition of previously identified markers for root rot resistance in F_4 derived RILs served as the required accurate starting point not only for the linkage group construction but also for co-integration with the integrated bean linkage map (Freyre et al., 1998). All RAPD's from each resulting linkage group were analyzed in a multiple regression analysis using SAS's PROC GLM (SAS, 1995) and significance was set at $P< 0.05$. WinQTL-Cartographer v2.0 software package was used to map QTL using composite interval mapping (CIM) which has the power of identifying and positioning individual QTL (Basten et. al., 2003) and single marker analyses (SMA) was used to confirm the QTL identified by CIM. Permutation analysis was performed (1000 permutations) for individual traits in order to identify a significance threshold of the test statistic for individual QTL (Edwards et al., 1987; McMullen et al., 2001; Doerge et al., 1997). MAPMAKER/ EXP 3.0 (Lincoln et al., 1987) was used to anchor the linkage groups identified in this study to the integrated bean map by screening the BAT93 x Jalo EEP558 RIL population (here after refered to as BJ) with those markers that were polymorphic between the BAT and Jalo parents. RAPD markers are identified by the name of the operon primer, followed by the size of the polymorphic fragment.

RAPD data for each IBL was tested for significant associations between root rot resistance and marker genotype by QTL-cartographer SMA and CIM, to detect significant loci and epistatic interactions as described by McMullen et al. (2001). Marker-QTL associations were determined by F-tests with significance at $P<0.05$. SAS

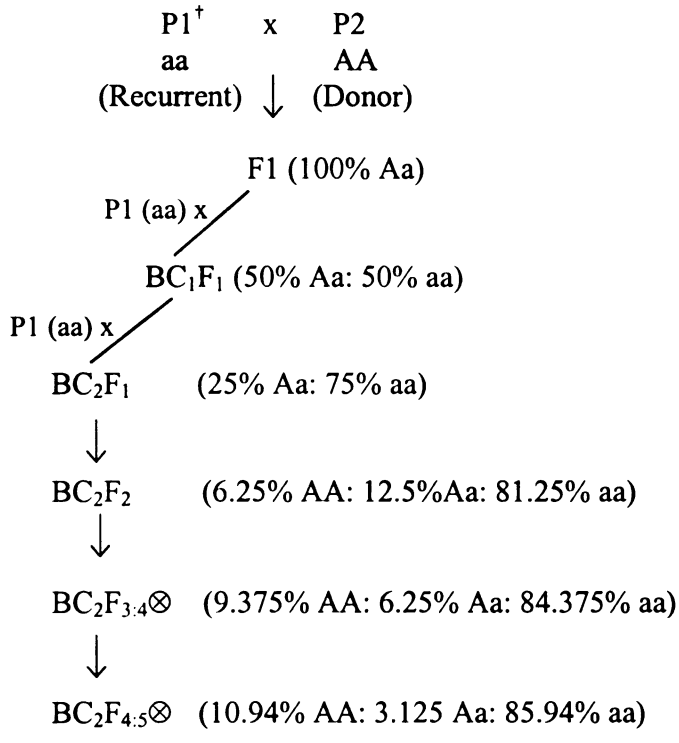
analysis helped complement results from CIM. The presence of a putative QTL was declared significant whenever its LOD-score exceeded the determined threshold level by the permutation analysis. The estimated position of the QTL was the point where the maximum LOD-score was found in the region under consideration. The position of a QTL was always described in relationship to the nearest marker to the left of where the LOD-score peak was located.

Results and Discussion

Inheritance of root rot resistance

The inbred-backcross method of breeding was used to transfer *Fusarium* root rot resistance from NSL to Michigan dark red kidney, Red Hawk and the cranberry breeding line C97407. Using this method to develop the $BC_2F_{4:5}$ populations, the expected mean progeny would be 87% RH or C97407, and 13% donor (NSL) genotype (Figure 4.4) and each line would be expected to be approximately 97% homozygous. The expectation existed that an average donor genotype of 13% was adequate to ensure the infrequent transfer of desired genes to the recurrent parent. The 87% recurrent parent genotype ensures the general similarity of all lines to the recurrent parents for critical agronomic, seed and adaptation traits. The 97% level of homozygosity ensures a homogeneous response of plants within each line to the external factor being tested.

Significant variation for root rot scores and root vigor ratings was observed in all field tests of the kidney IBL and cranberry IBL populations except in the NYSAES field test (Table 4.2, Table 4.3). Significant genetic variation for root rot scores was observed for all four GH experiments (Table 4.2). Genetic variation for combined



Symbols description:
† P1= Red Hawk and C97407; P2= the resistant parent to *Fusarium* root rot: Negro San Luís
⊗= Selfing.

Figure 4.4. Schematic of genetic expectations of inbred backcross line populations. Although root rot is a quantitative character and our interest is to look at whole genome the above diagram is represented as a single locus to illustrate the effect.

Table 4.2. Analysis of variance for root rot ratings for the inbred backcross populations, Red Hawk *2/ Negro San Luís (Kidney inbred backcross line population) and C97407 *2/ Negro San Luís (Cranberry inbred backcross line population).

Source	DF	EMS	F-test	Source	DF	EMS	F-test
	—Kidney IBL GH-1‡—				— Cranberry IBL GH-1—		
Rep †	2	1.104		Rep	2	1.764	
Trt	90	0.750	1.97***	Trt	77	1.063	1.49*
Error	180	0.382		Error	154	0.711	
	— Kidney IBL GH-2—				— Cranberry IBL GH-2—		
Rep	2	0.405		Rep	2	4.855	
Trt	90	0.625	1.73***	Trt	77	1.743	1.98***
Error	180	0.361		Error	154	0.882	
	— Kidney IBL MRF§-2002—				— Cranberry IBL MRF-2002—		
Rep	2	1.789		Rep	2	3.223	
Trt	99	2.818	2.85***	Trt	80	3.483	6.73***
Error	198	0.135		Error	160	0.173	
	— Kidney IBL MRF-2003—				—Cranberry IBL MRF-2003—		
Rep	2	5.730		Rep	2	0.011	
Trt	99	2.530	1.69***	Trt	71	2.326	1.68***
Error	198	1.501		Error	142	1.380	
	— Kidney IBL NYSAES-2003—						
Rep	2	0.601					
Trt	88	0.900	0.94				
Error	176	0.958					
	— Kidney IBL- COMBINED—				— Cranberry IBL -COMBINED—		
Loc	2	190.613		Loc	1	69.176	
Trt	107	3.709	4.36***	Trt	84	3.885	4.21***
Rep	2	1.435		Rep	2	1.333	
Rep(Loc)	4	3.597	4.23**	Rep(Loc)	2	1.713	1.76
Loc * Trt	190	1.349	1.59***	Loc * Trt	67	1.752	1.90***
Error	594	0.850		Error	302	0.923	

† Four experiments are presented for each individual population: a combined analysis over two locations for both of the field experiments at Montcalm County. An additional experiment at NYSAES was conducted for the kidney IBL population during the summer of 2003 and is presented. Rep = replication; Trt = treatments represented by the genotypes; Loc = locations

‡ Kidney IBL GH and Cranberry IBL GH refer to greenhouse experiments in kidney IBL population and cranberry IBL population, respectively.

§ MRF and NYSAES refer to field trial conducted at Michigan State University Research Station located at Montcalm County, MI and New York State Agricultural Experiment Station Geneva, N.Y., respectively.

*, **, *** Significant at the $P<0.05$, 0.01, 0.001 probability levels, respectively

Table 4.3. Analysis of variance for root vigor ratings for the inbred backcross populations, Red Hawk *2/ Negro San Luís (Kidney inbred backcross line population) and C97407 *2/ Negro San Luís (Cranberry inbred backcross line population).

Source	DF	EMS	F-test	Source	DF	EMS	F-test
	— Kidney IBL Root Vigor-02—				— Cranberry IBL Root Vigor-03—		
Rep †	2	0.004		Rep	2	0.276	
Trt	99	1.382	111.91***	Trt	80	1.541	8.52***
Error	198	0.012		Error	160	0.181	
	— Kidney IBL Root Vigor-03—				— Cranberry IBL Root Vigor-03—		
Rep	2	0.014		Rep	2	0.542	
Trt	88	0.885	89.56***	Trt	71	1.191	5.40***
Error	176	0.010		Error	142	0.221	
	— Kidney IBL -COMBINED—				— Cranberry IBL -COMBINED—		
Loc	1	0.015		Loc	1	0.001	
Trt	101	2.381	181.19***	Trt	84	2.475	12.40***
Rep	2	0.014		Rep	2	0.803	
Rep(Loc)	2	0.001	0.06	Rep(Loc)	2	0.310	0.16
Loc * Trt	97	0.336	25.54***	Loc * Trt	67	0.011	0.20**
Error	396	0.013		Error	302	0.199	

†Two experiments are presented for each individual population. A combined analysis over two locations for both of the field experiments at Montcalm County. Rep = replication; Trt = treatments represented by the genotypes; Loc = locations

*, **, *** Significant at the $P<0.05, 0.01, 0.001$ probability levels, respectively

field trials over two environments (summer 2002 and 2003) was significant for both the kidney and cranberry IBL population. Highly significant treatment-by-environment interactions were found for the combined analyses of variance over two environments in the cranberry IBL population. Significant variation for combined field trials over three environments (summer 2002 and 2003 at MRF and 2003 at NYSAES field trials) was highly significant for the kidney IBL population. Means, LSD, and CV (Coefficient of Variation) are presented for agronomic data, root rot and root vigor scores for all experiments conducted with both populations (Appendix B- Tables B.1 and B.2). A summary of the five most resistant and five most susceptible IBLs and checks are presented for comparison purpose in Tables 4.4 and 4.5. The 2003 field trials were chosen to establish the ranked order due to the broader distribution of disease scores. The CVs remained relatively constant for each population and ranged from 6.3 to 23.0% for the kidney IBL population and from 12.3 to 23.6% for the cranberry IBL population (Table 4.4 and 4.5). In general the mean root rot scores for the kidney IBL population was higher than the mean root rot score for the cranberry IBL population, and the cranberry IBL population had a wider distribution of scores (Figure 4.5).

Narrow sense heritability (h^2_N) estimates for root rot rating for the greenhouse trials was intermediate for the kidney and cranberry IBL populations (Table 4.6). H^2_N estimates of field trial for root rot ratings of kidney IBL population was low (10 to 20%) and for cranberry IBL population ranged from intermediate to high (30 to 80%). The intermediate h^2_N estimates indicate that it should be possible to indirectly select for root rot resistance under the greenhouse environment.

Table 4.4. Means of the five lowest and five highest root rot scoring inbred backcross lines for C97407 *2/ NSL population during the summer of 2002 and 2003.

IBL number	MRF‡	Greenhouse
24	2.8 (25)†	5.8 (65)
26	3.3 (3)	5.4 (59)
65	3.6 (9)	5.1 (41)
49	3.6 (61)	6.0 (63)
52	3.8 (39)	6.7 (77)
13	6.2 (70)	5.1 (15)
15	6.3 (22)	5.7 (39)
4	6.4 (64)	3.7 (4)
39	6.7 (74)	4.0 (45)
11	6.8 (32)	6.6 (67)
Negro San Luís	1.1	2.5
C97407	4.1	5.7
Taylor Hort	4.0	5.9
C99833	6.1	-
Montcalm	5.2	6.2
FR266	4.5	3.1
B98311	3.2	-
Test Mean	4.9	5.1
LSD (P=0.05)	1.9	1.5
Coefficient of Variation (%)	23.6	18.3

‡ The field trial from 2003 was used for ranking due to better distribution of *Fusarium* root rot scores compared to the 2002 trial. Numbers in parenthesis represents ranking for the 2002 field trial (ranking was established as 1 to 78). MRF= Montcalm Research Farm

† Root rot score based on a scale 1 to 7 where 1=healthy root system with no discoloration of root or hypocotyls tissue and no reaction in root mass and 7= pithy hypocotyls with much extended root rot lesions, root mass is severely reduced.

Table 4.5. Means of the five lowest and five highest root rot scoring inbred backcross lines for Red Hawk *2/ NSL population during the summer of 2002 and 2003.

IBL number	MRF‡	Greenhouse	NYSAES§
153	3.0 (80) †	7.0 (75)	3.5
169	3.1 (12)	7.0 (70)	4.9
149	3.4 (77)	3.6 (1)	5.9
116	4.0 (17)	6.0 (17)	4.5
138	4.0 (31)	6.1 (21)	5.2
102	6.4 (42)	7.0 (90)	3.7
108	6.6 (64)	7.0 (84)	4.9
164	6.7 (26)	6.9 (41)	2.9
175	6.8 (91)	6.9 (49)	4.5
131	6.9 (73)	6.8 (30)	5.1
Negro San Luís	1.1	2.3	1.9
Red Hawk	5.6	5.8	4.9
FR266	2.4	3.1	3.5
Montcalm	5.8	6.2	6.4
Chinook2000	4.8	5.8	4.3
Beluga	4.9	6.0	-
Taylor Hort	5.8	5.9	-
B98311	3.7	-	-
TLP-19	4.8	-	-
Test Mean	5.3	6.7	4.8
LSD (P=0.05)	1.9	1.0	1.5
Coefficient of Variation (%)	23.0	9.0	4.5

‡ The field trial from 2003 was used for ranking due to better distribution of *Fusarium* root rot scores compared to the 2002 trial. Numbers in parenthesis represents ranking for the 2002 field trial (ranking was established as 1 to 100). MRF= Montcalm Research Farm

† Root rot score based on a scale 1 to 7 where 1=healthy root system with no discoloration of root or hypocotyls tissue and no reaction in root mass and 7= pithy hypocotyls with much extended root rot lesions, root mass is severely reduced.

§ NYSAES= New York State Agricultural Experiment Station

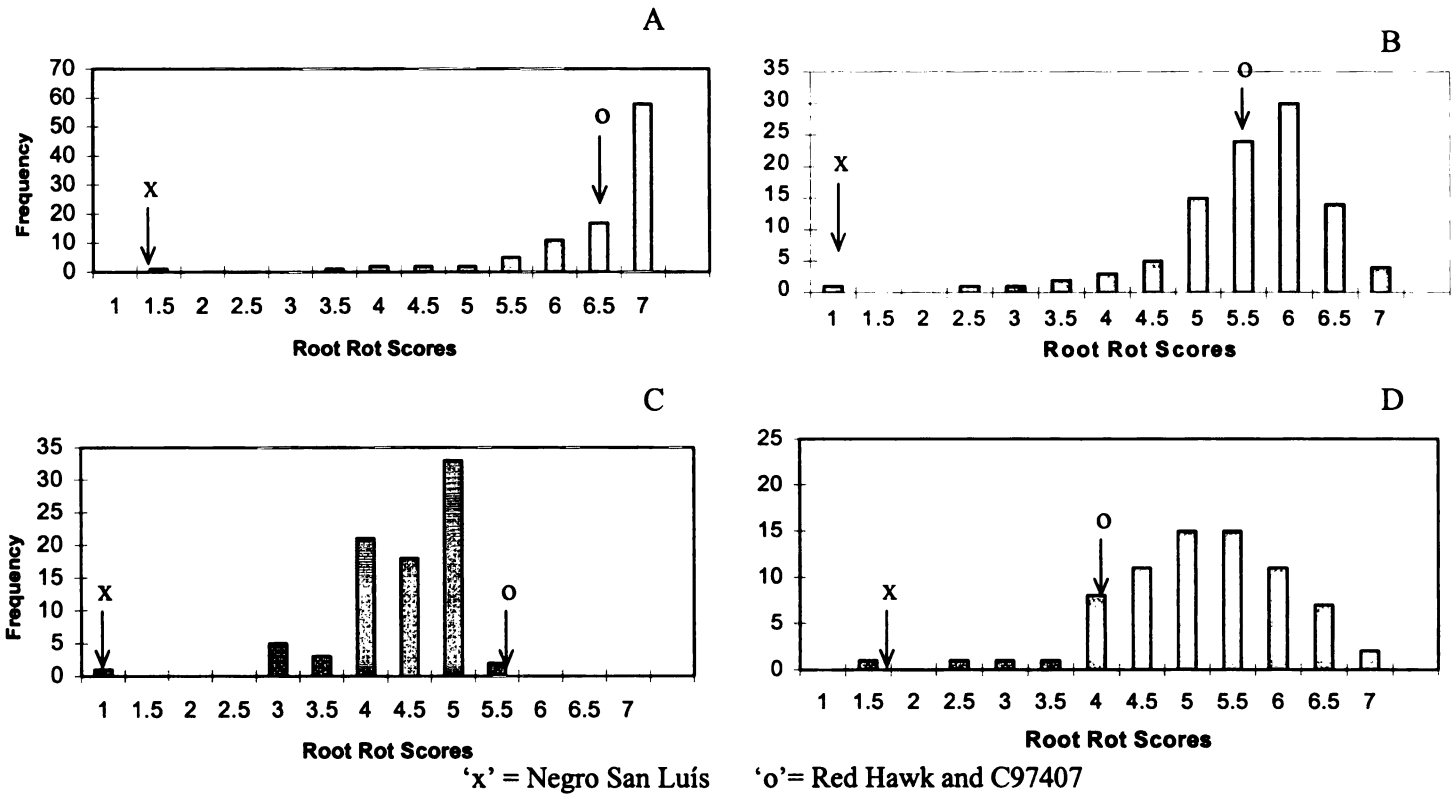


Figure 4.5. *Fusarium* root rot score inbred backcross line population distribution for field experiments conducted at Montcalm County: A. Kidney IBL population 2002, B. Kidney IBL population 2003, C. Cranberry IBL population 2002, D. Cranberry IBL population 2003.

Table 4.6. Heritability estimates ± standard errors of *Fusarium* root rot ratings (scale 1 to 7) and root vigor ratings (scale 1 to 5)for Red Hawk *2/Negro San Luís (Kidney inbred backcross line population) and C97407 *2/Negro San Luís (Cranberry inbred backcross line population) populations evaluated during the 2002 and 2003 field trials .

	$h^2 \pm SE$[§]	
Experiment	Root Rot Ratings	Root Vigor Ratings
	Kidney IBL Population	
GH-1 [†]	0.51 ± 0.20	-
GH-2	0.44 ± 0.22	-
2002 MRF [‡]	0.20 ± 0.28	0.98 ± 0.15
2003 MRF	0.10 ± 0.31	0.99 ± 0.12
2003 NYSAES	0.16 ± 0.30	-
	Cranberry IBL Population	
GH-1	0.51 ± 0.24	-
GH-2	0.35 ± 0.28	-
2002 MRF	0.82 ± 0.17	0.85 ± 0.16
2003 MRF	0.30 ± 0.29	0.83 ± 0.18

† GH refer to greenhouse experiments in Kidney inbred backcross line and Cranberry inbred backcross line populations, respectively.

‡ MRF and NYSAES refer to field trial conducted at Michigan State University Research Station located at Montcalm County, MI and New York State Agricultural Experiment Station Geneva, N.Y., respectively.

§ SE = standard errors

Low h^2_N estimates requires selection based on progeny performance in advanced generations evaluated in replicated trials, in different locations and/or environments (Fehr, 1993). Similar intermediate to high h^2_N estimates (48 to 71%) for root rot ratings were observed when evaluating $F_{4:5}$ RILs of the Montcalm x FR266 and Isles x FR266 populations (Schneider et al., 2001).

In the current study the h^2_N of 30% and 82% observed in the cranberry IBL population are high (82%) enough estimates to indicate that selection can be effective but low (30%) enough to imply that effective selection requires progeny testing, and evaluation of root rot as a quantitative character. The higher h^2_N observed in the 2002 trials is believed to be due to the difficulty in classifying plants late in the season. The roots and hypocotyls, regardless of the degree of resistance, were covered with lesions and it was necessary to score on the depth of *Fusarium* root rot lession rather than percentage of infected surface area. The difficulty in classifying plants late in the season in terms of degree of resistance is observed in the increase of root rot rating mean score for 2002 compared to the 2003 trials (Table 4.4; Table 4.5). When evaluating F_2 progenies from crosses between Redkote x 2114-12 (R2) and Redkote x N203 (RN203), high h^2_N for cross R2 (44.3%) compared with RN203 (25.9%) were observed, indicating a higher probability of selecting true breeding root rot resistant segregants from the R2 cross (Hassan et al., 1971). Evaluating for root rot at 13-week old field grown plants, Hassan et al. (1971) obtained high heritability estimates (77.9% for R2 and 79.7% for RN203) compared to 5-week old field grown plants which had much lower and dissimilar heritability estimates (33.6 and 10.0%). Due to the differences in root rot evaluations, results obtained in the current study and previous studies suggest that the inheritance

pattern for root rot was influenced by the testing procedures employed, age of plants, and the parents involved (Boomstra and Bliss, 1977; Hassan et al., 1971). Time course changes (e.g. anthesis, pod fill, and maturity) in plant development should be considered in evaluating *Fusarium* root rot resistance of common bean (Hall and Phillips, 2004b). Cultivars that appeared to have similar levels of resistance at week four differed dramatically at week six and eight indicating that resistance of seedlings may not reflect resistance of older plants (Hall and Phillips, 2004b). Evaluating earlier in the season during anthesis would make the evaluation for root rot resistance more effective in differentiating between lines in a population. Moreover, evaluating all lines in the population late in the season would be affected by disease to some degree making it difficult to evaluate and would most probably result in an increase in experimental error.

The low to high heritability estimates for root rot resistance obtained in the greenhouse and field evaluations, indicate that improvement of genetic resistance to *Fusarium* root rot is possible although it may be time consuming due to the time needed to evaluate for root rot resistance (Table 4.6). However quantitative inheritance is highly influenced by the environment and breeding for improved resistance to *Fusarium* root rot could protect the bean plant by providing certain levels of general resistance against other soil borne pathogens. This level of general resistance can cease to function with stress caused by an inadequate oxygen supply to the host due to flooding or even drought stress, which can aggravate severe disease pressure.

Heritability estimates for root vigor rating scores were high for both populations evaluated at MRF during 2002 and 2003. Estimates ranged from 83 to 85% in the cranberry IBL population and from 98 to 99% in the kidney IBL population. The

development of IBLs by using genotypes contrasting only in root characteristics would be the most effective method to study root plasticity (Román Avilés et al., 2004).

Narrow sense heritability estimates for root traits in other crops are similar to those obtained in the current study. Intermediate heritability estimates (43 to 52%) based progeny means values were observed for number of seminal roots on F_3 progeny of wheat (Richards and Passioura, 1981). In breeding rain-fed drought resistant cultivars of rice (*Oryza sativa* L.) using F_1 plants, h^2_N estimates ranged from intermediate to high (33 to 77%) for traits such as root length, root thickness, root volume, and root dry weight (Ekanayake et al., 1985). Selection should be successful in early segregating generations if selection is practiced for these root characters. Only limited heritability values for these types of traits are found in the literature due mainly to the root by environment interaction or root plasticity and root harvesting which makes the evaluation of large populations challenging.

Representative frequency distributions for root rot ratings are illustrated in Figure 4.5. Continuous variation was observed for both populations, but there was a broader range of root rot scores for the cranberry IBL population. The susceptible parents C97407 and RH always scored above 4.0 which is a significantly higher score than that values observed for NSL, the resistant parent. Transgressive segregation towards susceptibility was observed in both populations which is not unexpected given the population structure and the susceptible recurrent parents used in the development of the IBL populations.

Under severe disease pressure root rot resistance can be overcome as it is evident by the range in root rot ratings (2.4 to 6.2) for the resistant check, FR266, and a small, but

less significant increase in root rot rating (1.1 to 2.5) for the resistant parent NSL (Table 4.4 and 4.5). However, the susceptible genotypes (e.g. Red Hawk, Montcalm, and C97407) were consistently higher with scores above five (5.6 to 6.2). This supports the conclusion that the resistant genotypes have a substantial reduction of disease incidence and that the environment plays a vital role in disease severity as observed in Figure 4.5. This same pattern of results was observed by Schneider et al. (2001) when evaluating root rot resistance in Montcalm x FR266 F_4 derived population, where FR266 ranged from 2.0 to 4.5 and the susceptible parent Montcalm scored 1 to 2 points more than FR266 in all experiments. *F. solani* has the ability to detoxify phaseolin, a phytoalexin produced by bean plants in response to infection by this fungus (Van Etten and Smith, 1975). This detoxification which involves conversion of phaseolin to 1a-hydroxyphaseollone may explain why this fungus is not being affected by phaseolin. Such a reaction is enzymatic and therefore under genetic control, but no genetic study of this system has been made to confirm the reaction and gain a fuller understanding of the effect of this reaction on disease development in common bean.

Pearson correlations were performed between the experimental trials in the field and greenhouse among root rot ratings and DTF and root vigor ratings (Table 4.7; Table 4.8). Greenhouse evaluations for root rot resistance were positively and significantly correlated with the field evaluations and correlation coefficients (r) ranged from 0.36 ($P<0.001$) to 0.40 ($P<0.001$). A significant but small correlation was observed for the kidney IBL population evaluated at MRF and NYSAES for root rot scores ($r= 0.14$; $P<0.01$). Root vigor evaluations were positively and significantly correlated with the kidney IBL population at MRF evaluations during 2002 and 2003 ($r=0.82$; $P<0.001$ and

0.06; $P<0.05$) and also with the kidney IBL population in the greenhouse evaluation (r=-0.26; $P<0.001$ and 0.05; $P<0.05$). Negative but significant correlations were observed between DTF and root rot ratings for the kidney population at MRF during 2002 and 2003 (r=-0.19; $P<0.001$ and -0.11; $P<0.05$) and between DTF and root vigor evaluation for MRF 2003 (r= -0.12; $P<0.05$).

A negative correlation between root rot ratings with DTF is more or less expected especially when considering genotypes with a more determinate growth habit. Vegetative and root growth of determinate genotypes usually ceases at flowering and the plant partitions all resources to seed development which results in less available energy for host defense response. Furthermore, root death may tend to stimulate damage caused by the pathogen through the release of nitrogenous compounds which can stimulate pathogen growth (Toussoun, 1970). Damage caused by severe root rot disease could result in negative correlations with maturity and as a consequence reduced yield. Root vigor evaluations were significantly and positively correlated with root rot ratings of the cranberry IBL population at MRF during both years, r values ranged from 0.24 ($P<0.001$) to 0.81 ($P<0.001$; Table 4.7 and 4.8). Similar results were observed for the kidney IBL population. Significant correlations in the cranberry IBL population were not observed between the greenhouse, root vigor evaluations and DTF. Greenhouse root rot evaluations for cranberry IBL population was positively and significantly correlated with field evaluations during 2002 (r=0.21; $P<0.01$). Negative correlations were observed between root vigor and DTF for both years and r values ranged from -0.09 (NS) to -0.15 ($P<0.05$). Similar results for correlations values between DTF and greenhouse and field

Table 4.7. Pearson rank correlation coefficients for means of *Fusarium* root rot ratings for the kidney inbred backcross line population for field (MRF and NYSAES) and greenhouse experiments, days to flower, and root architecture ratings. The 2002 r values are presented in normal print in the upper right-hand diagonal whereas the 2003 r values are printed in bold in the lower left-hand diagonal.

	Days to flower	Root vigor ratings	Greenhouse	MRF
Days to flower	-	-0.07	-0.03	-0.19***
Root vigor ratings	**-0.12***	-	-0.26***	0.82***
Greenhouse	**0.15****	**0.05***	-	0.40***
MRF†	**-0.11***	**0.06***	**0.36*****	-
NYSAES	**0.11**	**0.20*****	**0.01**	**-0.14****

† MRF, and NYSAES refers field trial conducted at Michigan State University Montcalm Research Farm during 2002 and 2003, and New York State Agricultural Experiment Station Geneva, NY during 2003, respectively.

*, **, *** Significant at the P<0.5, 0.01, 0.001 significance level, respectively.

Table 4.8. Pearson rank correlation coefficients between means of *Fusarium* root rot ratings for cranberry inbred backcross line population for field (MRF) and greenhouse experiments, days to flower, and root architecture ratings. The 2002 r values are presented in normal print in the upper right-hand diagonal whereas the 2003 r values are printed in bold in the lower left-hand diagonal.

	Days to flower	Root vigor rating	Greenhouse	MRF
Days to flower	-	-0.15*	-0.05	-0.15
Root vigor rating	**-0.09**	-	0.11	0.81***
Greenhouse	**-0.01**	**-0.03**	-	0.21**
MRF†	**-0.14***	**0.24*****	**-0.07**	-

† MRF refer to field trial conducted at Michigan State University Montcalm Research Farm during 2002 and 2003.
*, **, *** Significant at the P<0.5, 0.01, 0.001 significance level, respectively.

evaluations were observed by Schneider et al. (2001) where r values ranged from -0.11 to -0.13 (NS) between GH experiment 2 and 3 and DTF; -0.27 ($P<0.05$) from GH experiment 1 and DTF; between -0.40 ($P<0.01$) to -0.53 ($P<0.001$) between field experiments and DTF.

Linkage map construction

Twenty-five hundred decamer primers (Operon Technology, CA) were screened against the parents of both genetic populations. A total of 350 primers generated polymorphisms between both sets of parents and were analyzed against the four individuals constituting the R and S bulks. A total of 85 primers produced polymorphism between the parental and bulks and were subsequently analyzed against the entire populations. Linkage map construction, using JoinMap (Stam, 1993), placed 33 RAPD markers on ten partial linkage groups (LG) for a total of 183 cM (Appendix A, Table A3), LG 1 to 6 were generated for the cranberry IBL population and LG7 to 10 corresponded to the kidney IBL population. The remaining 52 RAPD markers remained unassigned and could not be used for the QTL analysis. The partial LGs were compared against the integrated bean map (Freyre et al., 1998) and five LGs possessing QTL associated with root rot resistance co-integrated with B2 and B5 linkage groups of the integrated map (Figure 4.6 and 4.7). The partial LGs were anchored to the integrated bean map by genotyping the BJ RIL population with markers identified as linked to QTL for root rot resistance that were previously mapped to the integrated map. In addition, there were two partial LGs possessing QTL associated with root rot resistance that remained unassigned (Figure 4.8).

Fewer QTL were expected to be detected in the IBL populations because at least 87% of the loci were fixed for either RH or C97407 alleles and the population was segregating for fewer loci compared to a single cross F_2 population. Thus, most QTL detected in previous studies were not identified in the current work probably due to the different genetic material and thus different QTL were identitified. The inability to detect QTL previously identified by Schneider et al. (2001) could also be due to the unbalanced population structure or to missing markers flanking the QTL region. The low level of polymorphism identified in this study is mainly due to the population structure. IBL populations are skewed towards the recurrent parent where undesirable variability is eliminated in addition to potential desirable variability from the donor parent. The DNA bulks further reduce variability as they are developed to eliminate all background differences not associated with the trait of interest. In a QTL mapping study using IBLs of *Lycopersicon pimpinellifolium*, more QTL than expected were detected among the IBL populations but most QTL identified in previous studies with tomato (*L. esculentum*) were not identified because they were not segregating in the IBLs (Doganlar et al., 2002). These same IBL populations with *L. pimpinellifolium* were segregating for fewer loci than the advanced backcross-QTL populations (AB-QTL) from the same parental cross. Despite the drawbacks of IBLs, the unbalanced population structure seems to be as polymorphic as the AB-QTL population and can be considered a permanent mapping resource where new markers can be added to the map as they become available and used for molecular analyses of quantitative traits (Doganlar et al., 2002).

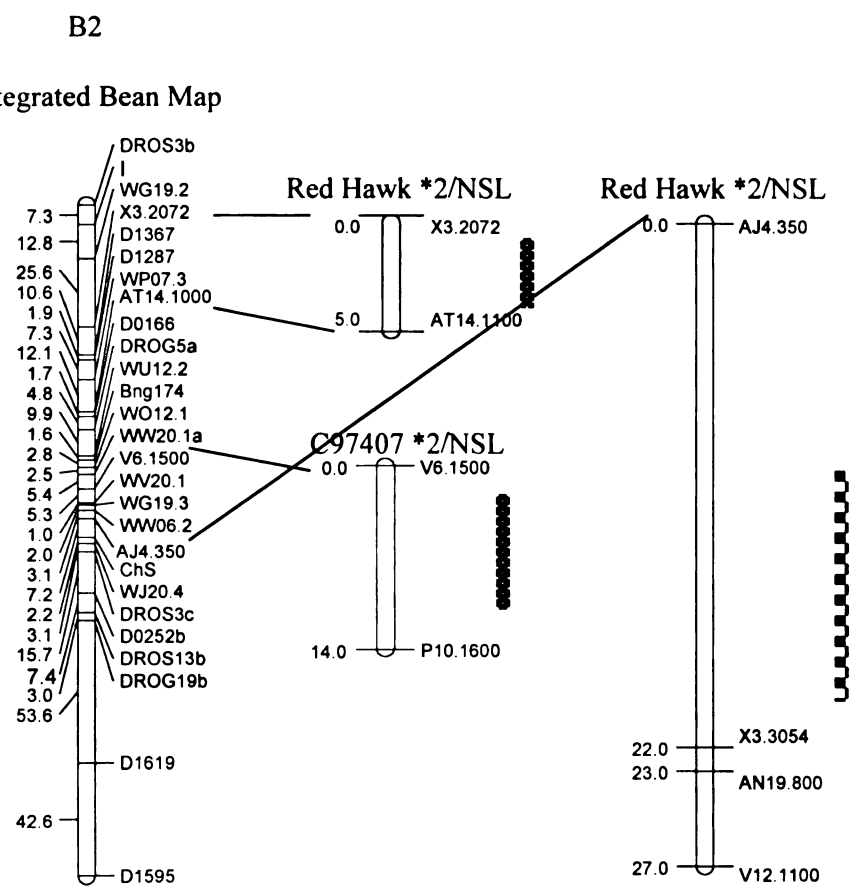


Figure 4.6. Illustration of partial linkage groups possessing selectively mapped QTL conditioning resistance to *Fusarium* root rot for the Red Hawk *2/NSL and C97407 *2/NSL inbred backcross line populations; with partial linkage groups co-integrated with the integrated bean map.

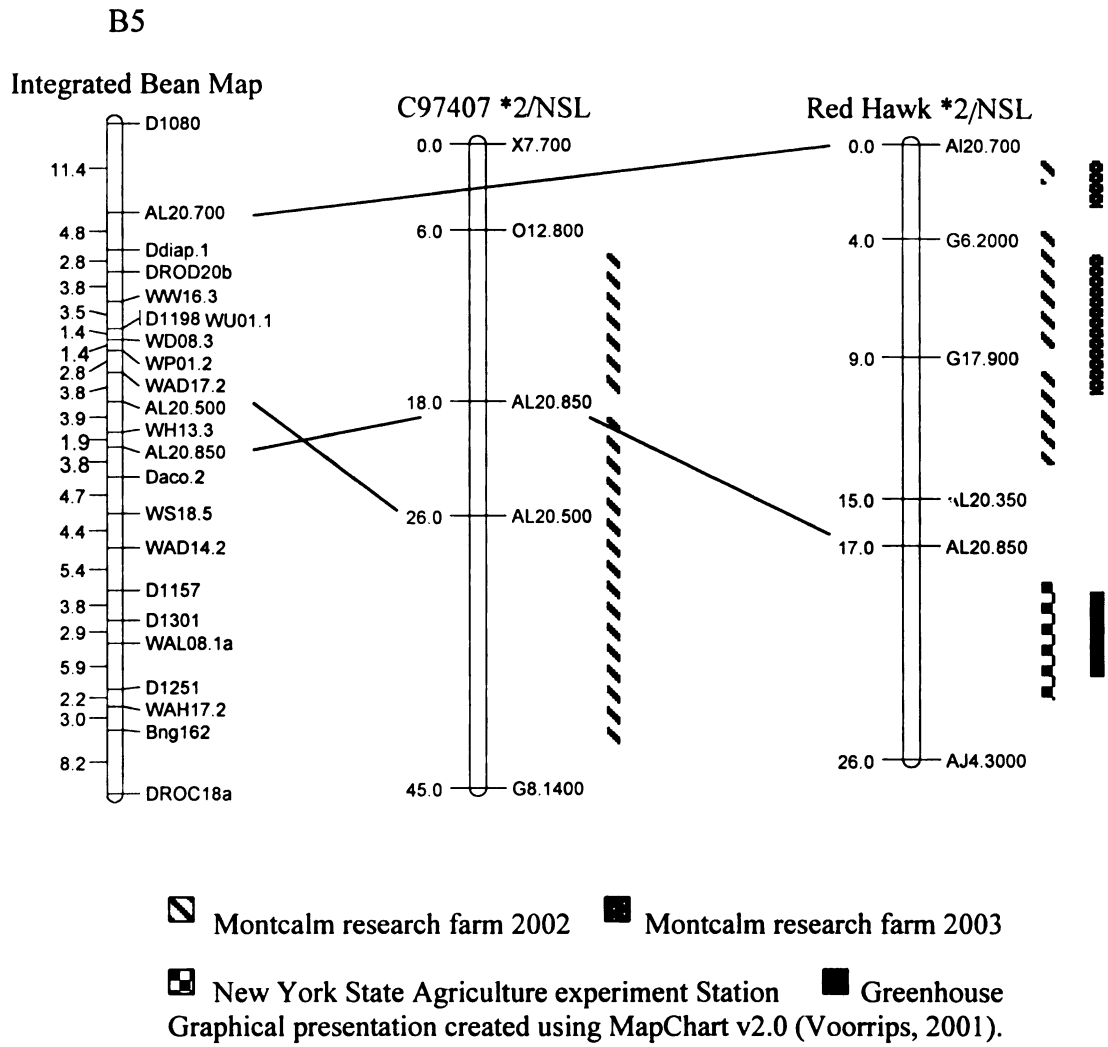


Figure 4.7. Illustration of partial linkage groups possessing selectively mapped QTL conditioning resistance to *Fusarium* root rot for the Red Hawk *2/NSL and C97407 *2/NSL inbred backcross line populations; with partial linkage groups co-integrated with the integrated bean map.

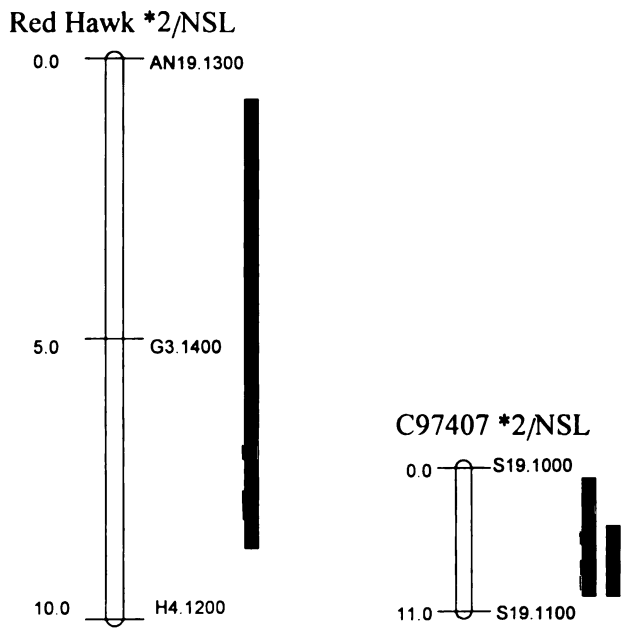


Figure 4.8. Illustration of partial linkage groups possessing selectively mapped QTL conditioning resistance to *Fusarium* root rot for the Red Hawk *2/NSL and C97407 *2/NSL inbred backcross line populations; both partial linkage groups remained unassigned to the integrated bean map.

QTL analysis for root rot resistance

In the current study, QTL associated with *Fusarium* root rot resistance in bean were identified using a combination of selective genotyping and BSA. The parental genotypes Red Hawk, C97407, and NSL react differently to *Fusarium* root rot and exhibit variation in other agronomic traits in the environments tested. Disease pressure was adequate to separate genotypic differences in both years, but the environmental variation resulted in variation in the level of root rot detected between the two years and locations (Figures 4.5). QTL associated with root rot resistance and agronomic traits were detected through CIM and SMA. QTL analysis was conducted for each year of the study separately due to the high genotype by environment interaction obtained when combining data for both 2002 and 2003 field trials. The different QTL detected across two environments (2002 and 2003) can be explained in part by differences in environmental conditions between the two years of study. During the 2002 planting season higher soil temperatures (24°C/ 30°C min/max) were recorded compared to the 2003 planting season (20°C/25°C min/max). Temperature has proved to be an important factor in mycelia growth of the pathogen where higher mycelium growth was observed as temperature increased (see Chapter III of this manuscript). Precipitation during the 2003 planting season was constant and accumulated slower (347 mm), compared to 2002 which accumulated rapidly and was not as constant (354 mm). The two factors (temperature and precipitation) could have caused sufficient stress to the plant, resulting in an increase in disease severity for the 2002 field season.

Coefficients of determination (R^2) which reflect the amount of variation explained by a given marker ranged from 1.2 to 53.3% for root rot resistance detected in different environments (Table 4.9, 4.10). One QTL between AL20.850 and AJ4.3000 markers was identified on B5 in the kidney IBL population that explained 27% (Figure 4.9 A) and 9.8% (Figure 4.9 B) of the phenotypic variation for resistance to *Fusarium* root rot in the NYSAES and greenhouse trials, respectively. The same QTL on B5 was identified in the cranberry IBL population which explained 53.3% (AL20.850-G8.1400; Figure 4.11 A) of the phenotypic variability for root rot resistance. The possibility exists that the QTL on B5 could be the same QTL detected in the kidney IBL population, since both QTL had a common flanking marker (AL20.850). The QTL appears to belong to the same linkage group, and could have remained separated in the analysis due to low marker density of the bean map. QTL in the current study identified on B5, mapped close to a previously identified QTL for root rot resistance from a different resistant source (Navarro et al., 2003). One QTL was identified in the kidney IBL population which explained 1.2% (AL20.700-G6.2000; Figure 4.10 A) of the variation for root rot resistance in the 2002 field trial and the same QTL explained 33.0% (Figure 4.10 B) of the phenotypic variation in the 2003 field trial. The QTL with the very small effect (1.2%) was not significant but was highly significant in 2003. Most of the QTL discussed above could be considered major QTL due to their large effect supported by the high LOD values (relative to the QTL peak) greater than the threshold values determined by the permutation analyses. A very significant effect is shown for a QTL for resistance in the cranberry IBL population, spanning a distance of ~25 cM on B5 between AL20.850 and G8.1400 (R^2=53.3%; Figure 4.11 A) with a LOD score of over 15 (a LOD

Table 4.9. QTL for resistance to root rot and agronomic traits, including phenotypic variability associated with the QTL, marker interval, LOD score, and R^2 values identified on the Red Hawk *2/NSL population.

Trait	Linkage[§] Group	Marker Interval	Parent donating the allele	Position of[†] QTL (cM)	Environment[‡]	R^2 (%)[¶]	LOD
Root Rot Rating	7 (B5)	AL20.850- AJ4.3000	NSL	21.0	GH2	9.8*	7.89
			NSL	21.0	NYSAES03	27.0**	6.70
	7 (B5)	AL20.700- G6.2000	NSL	2.0	MRF02	1.2	7.57
			NSL	0.0	MRF03	33.0***	7.81
	7 [¥]	G17.900- AL20.350	NSL	11.0	MRF02	30.0**	8.31
	7	G6.2000- G17.900	NSL	9.0	MRF03	19.0***	4.02
	7	G6.2000- AL20.350	NSL	6.0	MRF02	29.0*	8.40
	8	AN19.1300- H4.1200	NSL	7.1	GH2	39.0***	9.95
	9 (B2)	AJ4.350- X3.3054	RH	20.0	NYSAES03	5.0****	10.50
Yield (kg/ha)	10 (B2)	X3.2072- AT14.1100	RH	2.0	MRF03	22.0***	3.61
Seed Size ($g.100seeds^{-1}$)	8	AN19.1300- G3.1400	NSL	7.0	MRF03	29.0*	6.70
Growth Habit	9 (B2)	AJ4.350- X3.3054	RH	10.0	MRF02	49.0***	37.01
			RH	20.0	MRF02	3.0**	26.75
			RH	10.0	MRF03	52.0****	36.02
Days to Maturity	9 (B2)	AJ4.350- X3.3054	RH	14.0	MRF03	1.5*	11.31

§ Linkage groups detected in current study. Parenthesis corresponds to location of marker on the integrated map (Freyre et al., 1998).
† Position of the QTL peak to the right of the left side marker; ¶ *, **, ***, and **** Significance at the 5%, 1%, 0.1% and 0.01% levels, respectively.
‡ MRF02 and MRF03 refer to Montcalm County evaluations during the summer 2002 and 2003, respectively; GH1 and GH2 refer to greenhouse evaluations one and two respectively; NYSAES03 refers to field evaluation at N.Y. State Agricultural Experiment Station Geneva, N.Y.; RH= Red Hawk, NSL= Negro San Luís
¥ G17.900 is a RAPD markers previously identified by Schneider et al., 2001 as linked to QTL associated with root rot resistance.

Table 4.10. QTL for resistance to root rot and agronomic traits, including phenotypic variability associated with the QTL, marker interval, LOD score, and R^2 values identified on the C97407 *2/NSL population.

Trait	Linkage§ Group	Marker Interval	Parent donating the allele	Position of† QTL (cM)	Environment‡	R^2 (%)¶	LOD
Root Rot Rating	1 ¥	O12.800- AL20.850	NSL	12.01	MRF02	7.3***	6.98
	1 (B5)	AL20.850- G8.1400	NSL	28.01	MRF02	53.3****	15.72
	5	S19.1000- S19.1100	C97407	10.00	GH1	10.7***	2.35
			C97407	6.01	GH2	33.6***	4.02
Root Vigor Rating	4 (B2)	V6.1500- P10.1600	C97407	8.01	MRF03	1.8*	7.78
Growth Habit	1 (B5)	AL20.500- G8.1400	NSL	44.01	MRF02	16.5*	1.00
	2 (B8)	S13.1700- M10.900	NSL	0.20	MRF02	1.4	1.00
	2	S13.1700- G17.800	NSL	12.01	MRF02	5.4**	1.32
		S13.1700- M10.900	NSL	8.01	MRF03	19.2*	33.21
	2	G17.800- M10.850	NSL	20.01	MRF02	6.4*	1.59
	3 (B9)	AL20.2000- G8.500	C97407	12.01	MRF02	13.2**	2.25

§ Linkage groups detected in current study. Parenthesis corresponds to location of marker on the integrated map (Freyre et al., 1998). .

† Position of the QTL peak to the right of the left side marker; ¶ *, **, ***, and **** , Significance at the 5%, 1%, 0.1% and 0.01% levels, respectively.

‡ MRF02 and MRF03 refer to Montcalm County evaluations during the summer 2002 and 2003, respectively; GH1 and GH2 refer to greenhouse evaluations one and two respectively.

¥ O12.800 is a marker previously identified by Schneider, 2001 as linked to QTL associated with root rot resistance. C=C97407, NSL= Negro San Luís

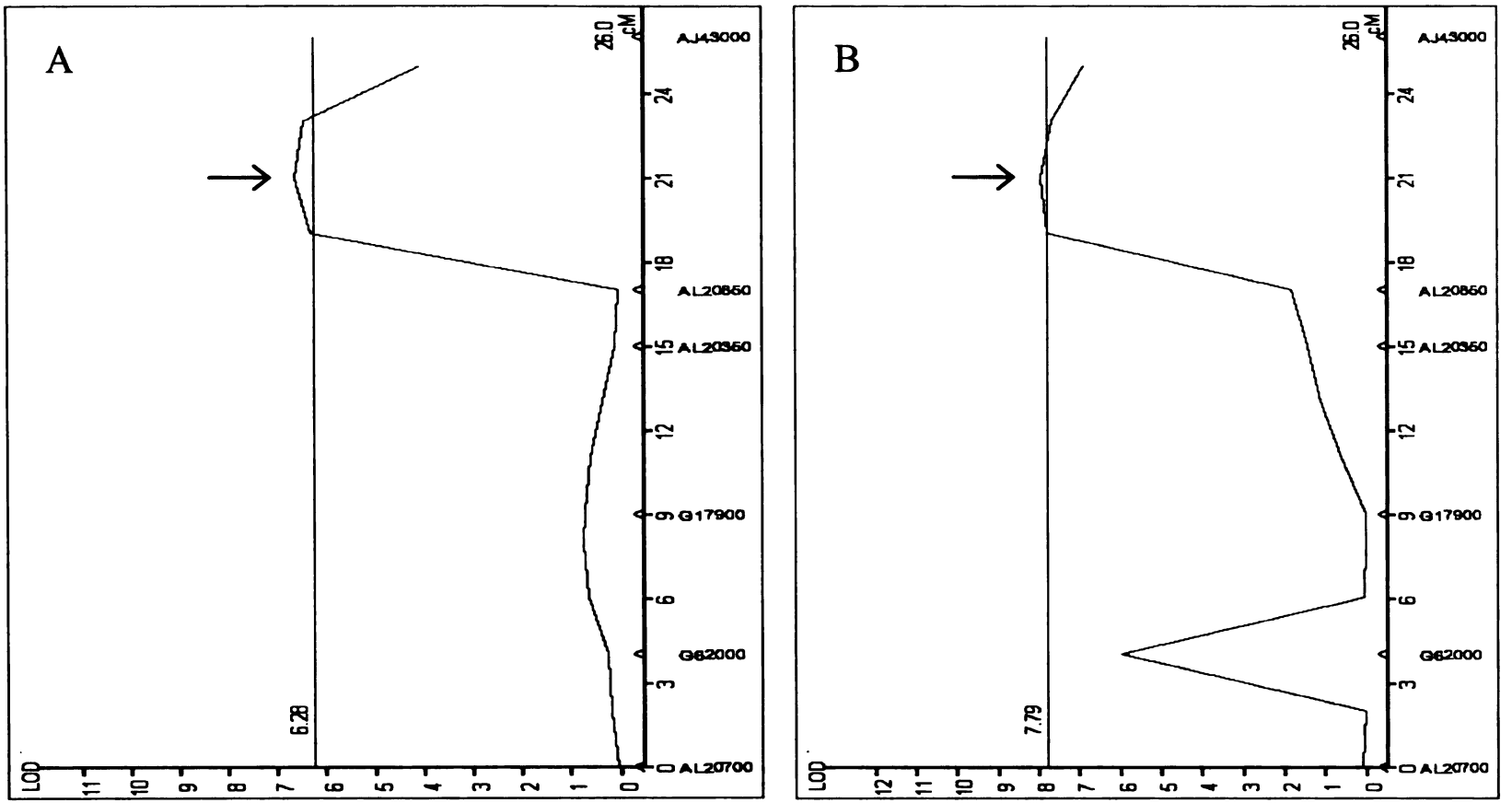


Figure 4.9. QTL identified using composite interval mapping on linkage group B5 for *Fusarium* root rot resistance in the kidney IBL population found in the A)-NYSAES2003 and B)-Greenhouse-1 environments. QTL position is shown with an arrow.

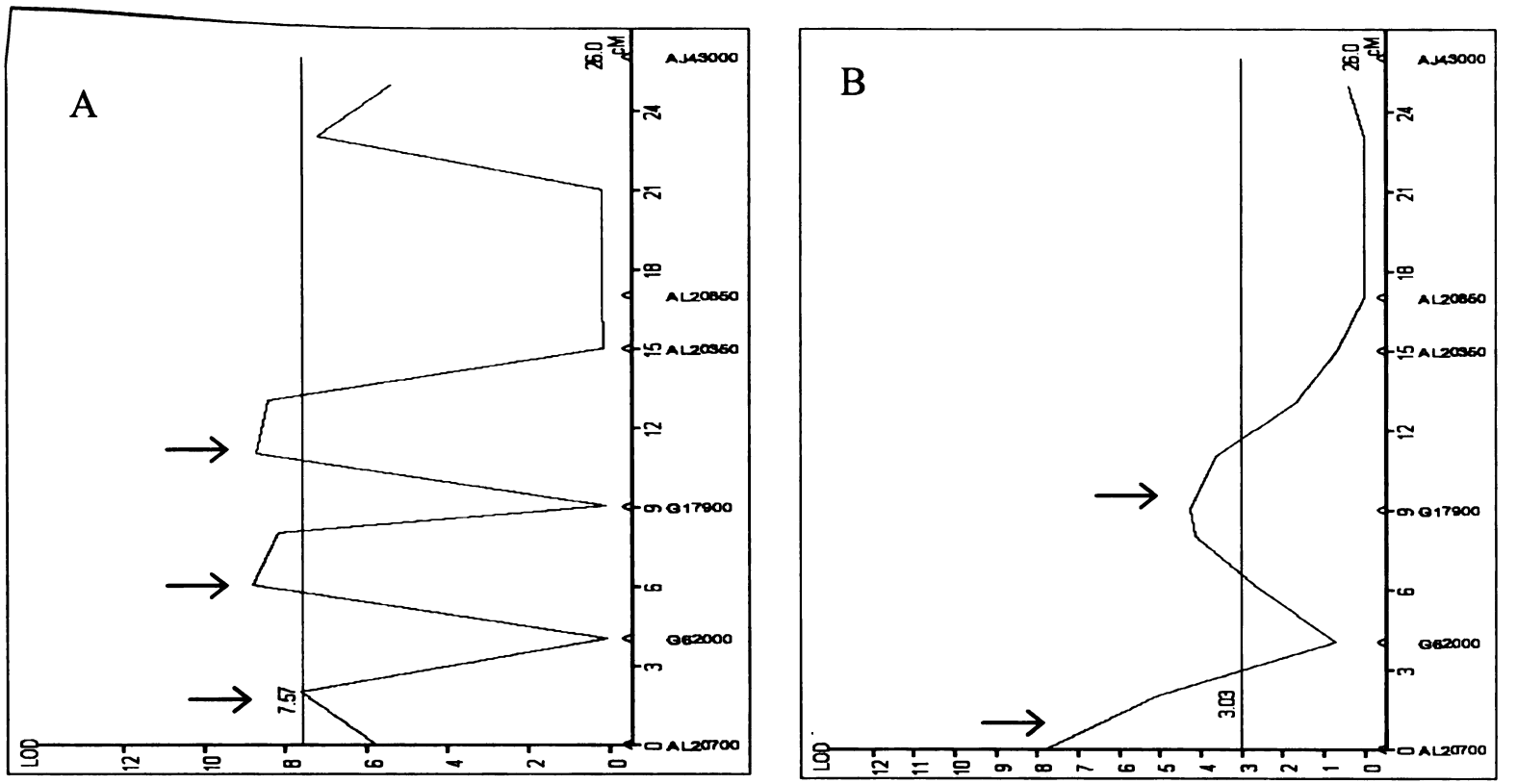


Figure 4.10. QTL identified using composite interval mapping on linkage group B5 for *Fusarium* root rot resistance in the kidney IBL population found in the A)-MRF2002 and B)-MRF2003 environments. QTL position is shown with an arrow.

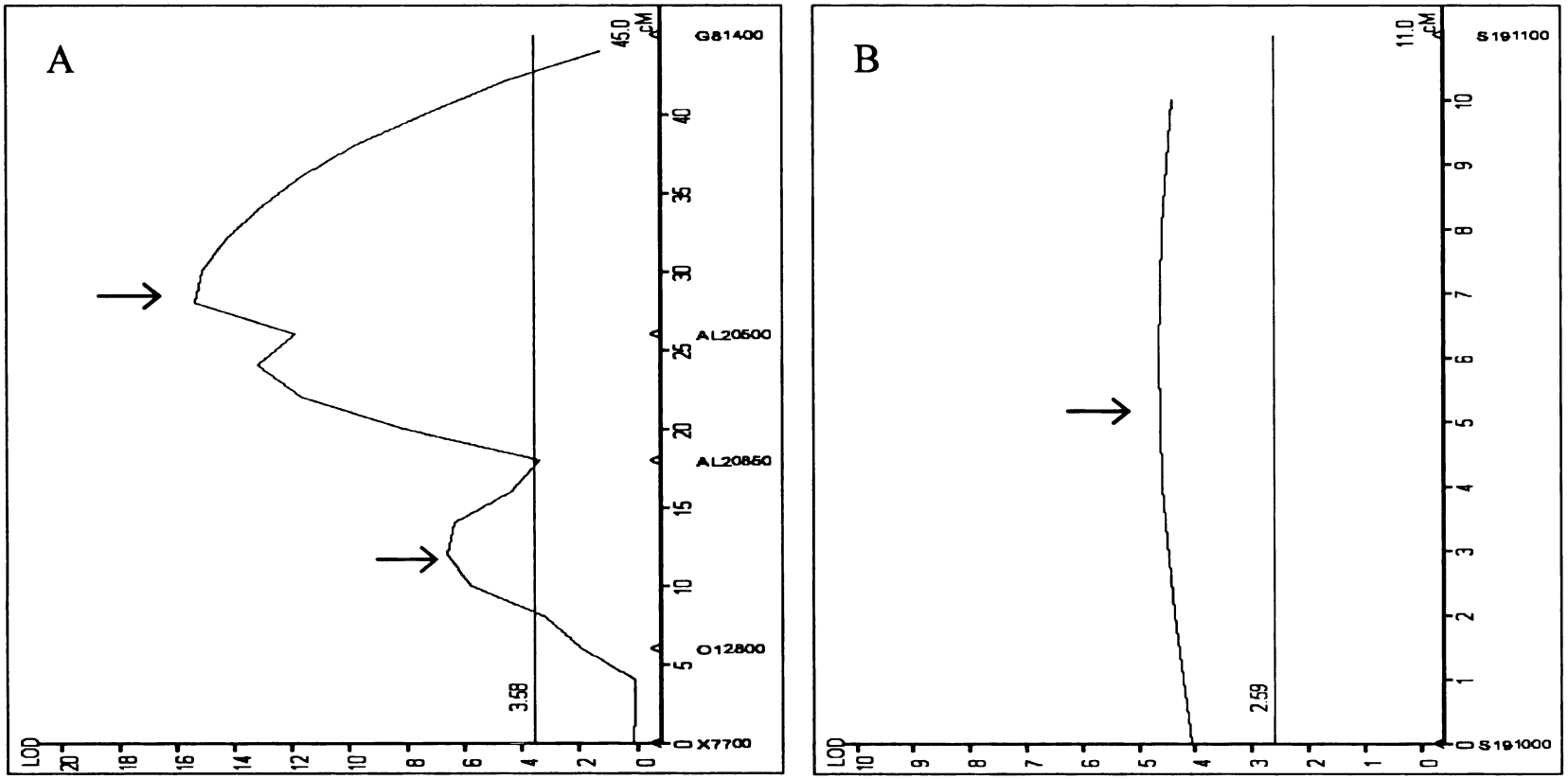


Figure 4.11. QTL identified using composite interval mapping for *Fusarium* root rot resistance in the cranberry IBL population found in the A)-MRF2002 (linkage group B5) and B)-Greenhouse-2 (unassigned partial linkage group) environments. QTL position is shown with an arrow.

value ≥ 3.58 is considered highly significant for this particular QTL). A large-effect QTL might be due to a series of linked QTL, each of small effect (Flint and Mott, 2001). When two QTL are very close together, there is little chance that recombination will separate them, so at a larger window distance in the analysis e.g. 100cM (less stringent), their effects on the LOD score accumulate, appearing as one large effect, whereas at shorter window distance e.g. 20 cM, the effects cancel out and no QTL is detected. WinQTL Cartographer blocks out a region of the many centimorgans (cM) on either side of the markers flanking the test position when picking background markers. This region is called the window size and it is set at 10 cM by default. Window size is important to consider, if the size is set too stringent it becomes hard to detect a QTL, especially when there is low marker saturation of the linkage group. The direction of linked QTL effects and the mapping method (e.g. CIM) are important factors to consider when chosing the window size.

Two QTL identified in the kidney IBL population in 2002 and 2003 trials, explained 19.0% (G6.2000-G17.900; Figure 4.10 B) and 30.0 % (G17.900-AL20.350; Figure 4.10 A) of the phenotypic variation for root rot resistance, respectively. Both of these QTL have a common flanking marker suggesting that they may be sharing a common region on the genome. A QTL for root rot resistance was previously a identified linked to the G17.900 marker by Schneider et al. (2001). A QTL for root rot resistance in the kidney IBL population was detected in the same general region spanning ~13.0 cM between G6.2000 and AL20.350 on B5 that explained 29% of the phenotypic variability in the 2002 experiment (Figure 4.10 A). This QTL also shares a common marker (G6.2000) with the previously described QTL between G6.2000 and G17.900

(19.0%; Figure 4.10 B). The QTL displayed large effects (R^2=29 and 30%) and was highly significant supported by the LOD value of ~9.0 (relative to the QTL peak) that is greater than the threshold value (LOD= 7.57). The G6.2000 marker is 4.0 cM from G17.900 anchored to B5, confirming linkage of these two markers and increasing the possibility of being the same QTL identified between the different environments or at least the same genome regions associated with G6.2000.

A QTL (AJ4.350- X3.3054) significantly associated with NYSAES03 explained 15% (R^2) of variation for root rot resistance (Figure 4.12 A). This marker (AJ4.350) was anchored to B2 of the integrated map, and was, located in the vicinity of the locus for chalcone synthase (*ChS*), polygalacturonase-inhibiting protein (*Pgip*), and the pathogenicity related protein (*PvPR-2*). *ChS* is an enzyme required for isoflavonoid phytoalexins biosynthesis (Ryder et al. 1987) and may be associated with general resistance to *F. solani*. The expression of plant defense response-related genes, such as chitinase genes, and genes of the phytoalexin biosynthesis pathway (*ChS*) have been demonstrated, where the pathogen rapidly induce plant defense responses at the mRNA level when bean plants were inoculated with *F. solani* (Mohr et al., 1998). *In vitro* studies with *F. solani* showed that chitinases are necessary to inhibit fungal growth, but upon prolonged exposure to these enzymes, the fungus was able to resume growth (Ludwig and Boller, 1990). The proteolyc processing of chitinase may be part of the pathogen's strategy to inactivate the plant's defense. Another suggested way of counteracting plant defense responses is the inactivation of phytoalexins by *F. solani*. Very virulent strains of the pathogen constitutively produced kievitone hydratase, an enzyme that converts phytoalexin kievitone to a less toxic metabolite.

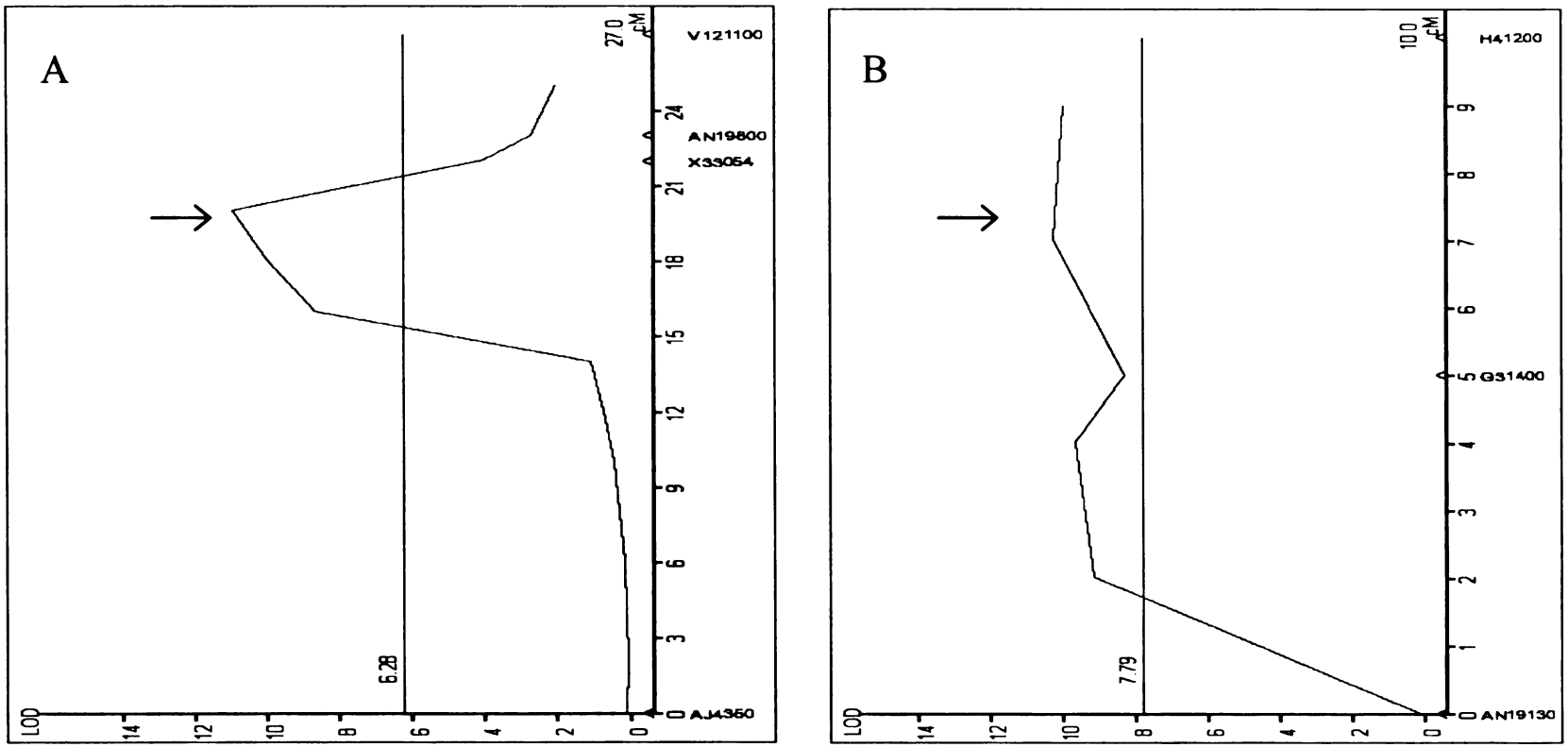


Figure 4.12. QTL identified using composite interval mapping for *Fusarium* root rot resistance in the kidney IBL population found in the A)-NYSAES2003 (linkage group B2) and B)-Greenhouse-2 (unassigned partial linkage group) environments. QTL position is shown with an arrow..

The *PvPR-2* is a low molecular weight acidic protein induced during fungal elicitation (Walter et al., 1990) and may imply a role in root rot resistance in common bean. The *Pgip* is a polygalacturonase-inhibiting protein (Toubart et al., 1992; De Lorenzo et al., 2002) that has previously been reported as associated with resistance to anthracnose (caused by *Colletotrichum lindemuthianum*). Plant defense response is a very complex mechanism that is triggered by pathogen attack. Several defense response genes co-localize with QTL reflecting a functional relationship between the QTL and the defense response genes (Geffroy et al., 2000). Other QTL for root rot resistance and for white mold have been previously mapped to regions close to *ChS*, *Pgip*, and the *PV-PR-2* on B2 and B3 of the common bean integrated map suggesting that physiological resistance to *Fusarium* root rot and white mold is associated with a generalized host defense response (Schneider et al., 2001; Kolkman and Kelly, 2003). A study of the biochemical response of soybean to *F. solani* f. sp. *glycine* infection showed that inoculation of soybean roots in soil induced PAL the first enzyme in the phenylpropanoid biosynthetic pathway, the phytoalexin glyceollin, and lignin, indicating that these defense response compounds may be involved in the partial resistance response to *Fusarium* (Lozovaya et al., 2004).

One QTL (V6.1500-P10.1600) was detected that explained only 1.8% of the phenotypic variation for root vigor evaluation of the cranberry IBL population for 2003 (Figure 4.6). The small effect QTL was significant as it was supported by a maximum LOD value of 7.78 above the LOD threshold value (6.5) determined for CIM. Root vigor was significantly and positively correlated with root rot scores for MRF during 2003 for both the kidney IBL ($r=0.06$; $P<0.05$) and cranberry IBL ($r=0.24$; $P<0.001$) populations

(Table 4.7 and 4.8). A QTL (X3.2072-AT14.1100) was identified as significantly associated with yield in 2003 and explained 22.0% of the phenotypic variation (Figure 4.6). Both QTL are also located in the vicinity of the locus for the pathogenicity related protein *PvPR-2* protein on B2. All the markers anchored to B2 of the integrated map were located close to markers previously identified by Schneider et al. (2001) and Navarro et al. (2003) for root rot resistance in common bean. Both researchers used different root rot resistance sources N203 and FR266 (Schneider et al., 2001) and Puebla 152 (Navarro et al., 2003). There is the possibility that different root rot resistance sources carry the same QTL which could explain resistance due to a more generalized response than that conditioned by a major resistance gene which is a more specific specialized response.

A QTL in the cranberry IBL population explaining 10.7% (Figure 4.13) of the phenotypic variability for root rot resistance in the first greenhouse evaluation and 33.6% (Figure 4.11 B) of phenotypic variability in the second greenhouse evaluation was detected in the same region of an unassigned linkage group (Figure 4.8). The QTL spanned a distance of ~11cM between S19.1000 and S19.1100; however C97407 not NSL parent contributed to the favorable allele for this QTL. One last QTL was identified in the kidney IBL population between AN19.1300-H4.1200 that explaining up to 39.0% of the phenotypic variability for the second greenhouse evaluation (Figure 4.12 B). Insufficient polymorphisms in BJ RIL population prevented mapping of markers of the unassigned partial linkage groups on Figure 4.8 to the *P. vulgaris* integrated map. These QTL can still be use in MAS breeding and can later be mapped in a more saturated map

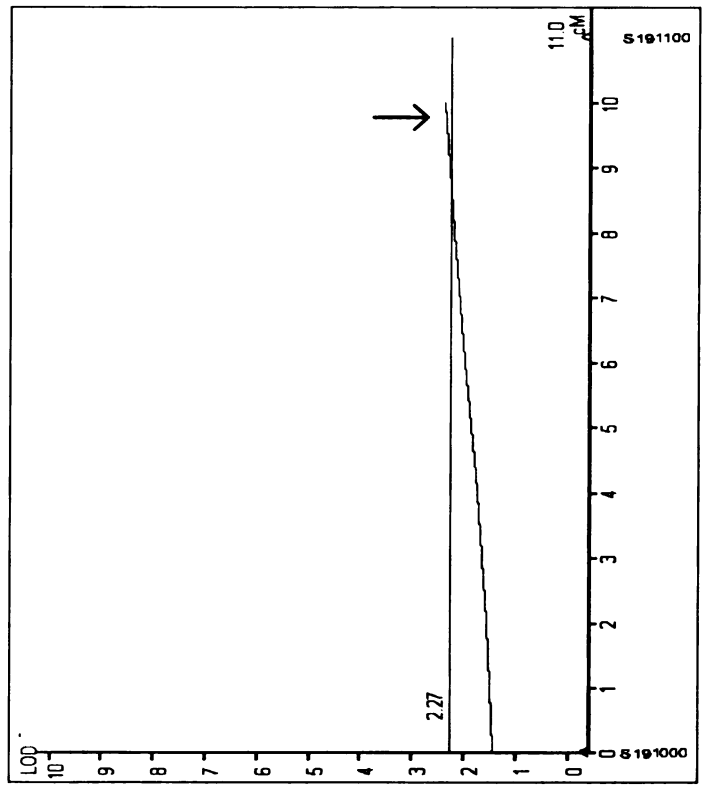


Figure 4.13. QTL identified using composite interval mapping for *Fusarium* root rot resistance in the cranberry IBL population found in the Greenhouse-1 (unassigned partial LG) environment. QTL position is shown with an arrow.

when the marker has a greater possibility of being assigned to a specific linkage group of the common bean integrated map.

Multiple regression analysis using combinations of significant markers linked to root rot QTL and their interactions revealed that epistatic interactions were not significant. Up to 7.6% and 73.8% of phenotypic variation for greenhouse and field ratings, respectively, was explained by a set of QTL flanking markers that included AL20.850, G17.900, AJ4.350, O12.800, and V6.1500. The differences in size of the QTL in the greenhouse compared to the field most probably due to the environment effect which may have affected the full expression of the QTL. In another study conducted for root rot resistance in common bean, total phenotypic variation explained by two QTL was about 50% indicating major effect of these two QTL on root rot resistance (Chowdbury et al., 2002). Only 29% of the phenotypic variation for root rot ratings was accounted by a subset of four markers in a study conducted by Schneider et al. (2001). Large effect QTL (those QTL that explained 15% of phenotypic variability or more) associated with resistance to root rot are useful starting points for marker assisted selection. The regions to which a QTL is localized can be quite large and such regions may contain more than one minor QTL.

Soil borne pathogens such as *F. solani* seem to have a life cycle highly influenced by the environment. The large amount of environmental variation that affects growth of *F. solani* and other soil borne pathogens makes the quantification of root rot diseases difficult and may be a reason why QTL associated with these traits are difficult to identify. In the current and previous root rot studies, markers significantly associated with field root rot ratings were not significantly associated with greenhouse root rot

ratings and *vice versa*, with the exception of AL20.850 which was significantly associated with the second greenhouse screening and field. It was not surprising that the QTL associated with field evaluations were not the same QTL associated with greenhouse evaluations. This anomaly could result from masking of genetic variation for physiological resistance present in the field by strong environmental factors such as temperature and moisture and other root rot pathogens in the field environment, which are absent in the greenhouse environment.

Differences in environmental conditions may have made the identification of QTL associated with environmental factors (temperature and moisture) difficult to identify under the greenhouse environment. An example of a trait affected by the environment present in the field and not in the greenhouse is DTF. The greenhouse evaluations were conducted prior to anthesis while the field evaluations were conducted post-anthesis. This same observation could explain the negative but significant correlation observed between DTF and field and greenhouse evaluations which could correspond to the different developmental stages at which plants were rated for disease reaction (Tables 4.7, 4.8). No QTL was identified for DTF but a small effect QTL for DTM was identified between AJ4.350-X3.3054 that only explained 1.5% of the variability. All the IBLs in the populations were evaluated in the field at the same time regardless of the developmental stage so those plants that where more advanced in maturity also exhibited more severe symptoms or more disease. Similar associations of DTF and other pathogens have been previously observed where the effect of DTF may be a result of the timing of disease initiation and the subsequent severity of infection through the entire season (Abawi, 1989; Miklas et al., 1996; Pilet et al. 1998; Kolkman and Kelly, 2002 and

2003). In the case of root rot this association is also valid due to the significant correlations between DTF and field root rot ratings observed in this and other studies (Schneider et al., 2001). Bean plants seem to be more affected by root rot during flowering and later stages of development when the plant isundergoing reproductive growth and is more susceptible to abiotic and biotic stresses.

Alternatively, QTL associated with resistance to root rot can be used to select progeny possessing complementary QTL for intermating. The G17.900 and O12.800 markers are linked to QTL previously identified by Schneider et al. (2001) and the same QTL was confirmed in the cranberry IBL populations of the current study on B5. The previous QTL have proven to be a large effect QTL that were identified in both the 2002 and 2003 environment and could be combined in one genotype (Figure 4.7). No corresponding specific regions represented by these markers were found on the integrated map in the current study or in the study conducted by Schneider et al. (2001). Based on their proximity to other markers that co-integrated with the bean-integrated map these QTL appear to reside on linkage group B5. Although a QTL between O12.800 and G8.1400 was not identified in the kidney IBL population, the RAPD marker showed the desired size polymorphic fragment between RH and NSL and segregated in the IBL population but these markers remained unassigned when creating partial linkage groups for the kidney IBL population. The QTL on linkage group B5 between G6.2000 and G17.900 explained up to 19% of the phenotypic variability for resistance to root rot in the kidney IBL in the 2003 environment and up to 30% (G17.900-AL20.350) of the variability in the 2002 environment.

QTL linked to AL20.850 and AL20.500 could be combined with those linked to G17.900 and O12.800. AL20.850 is on B5 and was linked to a large effect QTL (R^2= 27 and 53.3) in both the kidney and cranberry IBL populations as well as in the greenhouse environment (R^2=9.8). The QTL linked to AL20.500 in the cranberry IBL population was also polymorphic in the kidney IBL population. Although the later QTL were not identified in both populations the markers linked to this QTL associated with resistance can be utilized to enhance conventional breeding approaches by providing information that the breeder can use to make educated decisions and choices of which putatively linked resistance loci, with large effect to combine into a single genotype (Figure 4.6 and 4.7).

Growth habit QTL

In this study AJ4.350 and AL20.500 were closely linked to QTL associated with root rot resistance and determinate growth habit in the kidney and cranberry IBL population, respectively. The QTL between AL20.500 and G8.1400 explained up to 16.5% of the phenotypic variability while the QTL between AJ4.350-X3.3054 explained up to 52% of the phenotypic variability for *Fusarium* root rot resistance. Both of these QTL seem to have a large effect on determinate growth habit. Growth habit in the IBL populations was scored as a phenotypic marker since the progeny were either determinate (Type I) or indeterminate (Type II and Type III). Growth habit was unexpectedly, significantly associated with two markers that mapped to two different linkage groups B2 and B5 implying that two genes may control this trait.

The growth habit QTL with the largest effect was located at the end of partial LG9 (B2 on the integrated bean map) between the AJ4.350 and X3.3054 markers separated by 22cM (Figure 4.6); Red Hawk contributed the determinate allele for this QTL. The gene for determinate growth habit, *fin*, had previously been mapped to linkage group B1 (Freyre et al., 1998; linkage group D1, Koinange et al., 1996). The *fin* gene occurs at high frequency in the Andean gene pool of common bean compared with the Middle American gene pool. The discrepancy on the location of determinate gene on B2 vs. B1 may be explained by specific technical difficulties associated with RAPD markers. The fragment amplified by AJ4.350 in the BJ mapping population may not be the same fragment originally shown to be linked to growth habit in the kidney IBL population leading to an error locating this QTL on B2. Amplification of a DNA fragment in RAPD reactions will occur only if the primer binds to both ends of the fragment on opposite DNA strands. Because of the short primers used (only 10 bp) the reannealing temperature in the PCR must be low (35-40°C) for the primer to bind. At such low temperatures, however, binding is not very specific, which means that primers will bind also to sequences, which are not completely complementary, and a mismatch will occur.

Wide crosses, using the Andean gene pool as the source for the determinate growth habit, were previously used to map *fin* gene, which is a prominent single gene trait in Andean germplasm (Koinange et al., 1996). Similar findings have been reported where QTL associated with determinate growth were identified in different linkage groups other than B1. For example, Bassett, (1997) showed tight linkage between the Z locus controlling partly colored seedcoats in common bean and the *Fin* gene, but the Z locus was later mapped to B3 (McClean et al., 2002). The Z locus does not appear to be

linked to the *Fin* gene, but linked to another determinate factor not previously known. The disagreement may have resulted from the assumption made by Bassett (1997) that he was working with the *Fin* and now it would appear that he was working with another determinancy factor linked to *Z*. Once *Z* and *Fin* were mapped it became clear that there must be at least two different loci affecting growth habit since *Fin* is on B1 and *Z* is on B3. The locus controlling growth habit linked to the z^{sel} allele from 'Steuben Yellow Eye' (Race Nueva Granada from Andean gene pool) was linked at 1.03 cM to a dominant gene conferring indeterminate growth habit (Bassett, 1997). In crosses between seed coat pattern testers and the dark red kidney cultivar Red Hawk (used as recurrent parent in the kidney IBL population), using the marker closely linked to *Z* (OAM10.490; Brady et al., 1998) showed that Red Hawk carries the recessive *z* allele (Emmalea Ernest, personal communication). It is believed that Red Hawk carries a recessive allele conferring determinancy at the unnamed locus linked to z, since Ernest crossed Red Hawk and Yellow Eye and observed linkage between indeterminancy and the Yellow Eye seed coat pattern (Ernest and Kelly, 2004). These results may suggest the possibility that the AJ4.350 marker in the current study may be mapped to the wrong linkage group (B2), since Red Hawk carries the recessive z allele indicating that Red Hawk could also carry the unnamed growth habit locus tightly linked to *Z* on B3; or alternatively Z was incorrectly mapped to B3 by McClean et al., 2002.

In contrast to the source of indeterminancy used by Bassett (1997) to find the linkage between Z and growth habit, G12873 was used by Koinange et al. (1996) as a source of indeterminancy to map *Fin* to B1. The G12873 is a small seeded wild bean from the Middle American gene pool. Both sources of indeterminancy were homozygous

dominant at only one growth habit locus. These results suggest that if growth habit evolved separately in each gene pool as suggested by Singh et al. (1991), the two determinate growth habit loci mapped in these two distinctly different crosses would not necessarily be located on the same linkage group (Bassett, 1997).

The determinate growth habit in genotypes of the Middle American gene pool exists mainly in cultivated germplasm of the navy bean market class (Kelly, 2000), which was introduced by mutagenesis since the transfer of determinancy from the Andean gene pool through breeding was unsuccessful. The first determinate navy bean cultivar, Sanilac (released in 1956) was the most widely used source of the determinate growth habit in navy been breeding programs worldwide (Kelly, 2000). It is possible that the determinate growth habit trait in the navy bean germplasm is controlled by a different locus than that of the *fin* gene in Andean gene pool. Support of the possible unique genes for determinate growth habit in Middle American gene pool is based on mapping studies conducted by Tar'ran et al. (2002) and Kolkman and Kelly (2003). A second gene for determinate growth habit mapped to B9 of the integrated map and was identified in a population developed from a cross between navy bean cultivars OAC Seaforth and OAC95-4 from Middle American gene pool (Tar'ran et al., 2002). The determinancy growth habit gene was placed on a distal end of the linkage group, 32.3 cM from the nearest marker. More recently from a population developed from a cross between Bunsi and Newport, two small seeded navy bean cultivars from Middle American gene pool, the determinate growth habit mapped to B7, and represented a novel source of determinancy in navy bean (Kolkman and Kelly, 2003). The possibility of two loci conditioning determinancy in navy bean is unlikely due to the origins of determinancy in

navy bean introducde through mutation breeding. Given the extensive distance (32.3 cM) between marker and growth habit on B9 (Tar'ran et al., 2002), data on actual location of the determinancy gene in the Middle American gene pool needs additional confirmation.

In the current study a QTL (AL20.2000-G8.1400) associated with determinate growth habit (R^2=16.5, P<0.05) in the cranberry IBL population also mapped to B9 of the integrated bean map, with the difference that this growth habit QTL is in a large seeded background (Andean) and not in a Middle American background. In addition to the two QTL associated with growth habit, other small effect QTL for growth habit were identified such as: QTL between S13.1700 and M10.900 (MRF02; R^2=1.4; *P*<0.01), and the same QTL at MRF03 explained up to 19.2% of variability, and G17.800-M10.1500 (R^2=6.4; *P*<0.05).

An expected pleiotropic effect or linked QTL was observed between growth habit and DTM. QTL for both of these traits were identified between the same marker interval (AJ4.350-X3.3054) on B2, although the QTL for DTM was small (R^2=1.5) compared to 3.0% to 52.0% for growth habit (Table 4.9). The determinancy gene is known to exhibit multiple pleiotropic effects on growth habit and life cycle as it affects DTF, DTM, number of nodes, and number of pods (Koinange et al., 1996) indicating that the effect observed in the current study may be due to pleiotropic effect and not linked QTL.

Conclusions

One of the most important traits to consider in plant breeding programs is resistance to pathogens. Pedigree selection, single cross, and backcross breeding have

been used extensively to transfer disease resistance into desirable cultivars (Boomstra and Bliss, 1977; Schneider et al., 2001; Kolkman and Kelly, 2003). However, complex inheritance combined with low heritability have limited attempts to incorporate *Fusarium* root rot resistance into bean cultivars, despite that known sources of resistance have existed for more than 60 years (Wallace and Wilkinson, 1975). Root rot from N203 and NY2114-12 have been shown to be quantitatively inherited (Wallace and Wilkinson, 1965; Schneider et al., 2001). Complexly inherited traits are highly affected by the environment and controlled by many minor genes, as resistance is usually characterized by a continuous distribution, that is under polygenic control (Kearsey, 1998, Parlevliet, 2002).

Some traits such as root rot resistance are genetically very complex and difficult to evaluate, and therefore the efficiency of phenotypic selection is low. The inability to classify root rot scores from either greenhouse or field evaluations into discrete categories suggests that root rot resistance in bean is under polygenic control and as a result should be treated as a quantitative trait (Figure 4.5).

There are some aspects of the plant-pathogen interaction that complicate the dissection of resistance to certain diseases into QTL. One example is that root rot severity is rated on an ordinal scale (scale 1 to 7 by Schneider and Kelly, 2000), rather than a truly quantitative evaluation that would have a normal distribution required for most if not all-statistical analyses. Genetic differences between isolates of the pathogen could also result in different QTL profiles (Asíns, 2002). In addition the time and method of inoculation can alter the detection of QTL, as well as the root rot complex in the field and make the evaluation more challenging.

Several regions and QTL significantly associated with field and greenhouse root rot evaluations were identified in the current study. These QTL explained from 1.2 to 53% of the phenotypic variability for root rot trait (Table 4.9; Table 4.10). A single QTL between AL20.850 and G8.1400 on B5 accounted for up to 53% of the phenotypic variation for *Fusarium* root rot resistance in the field environment. Interestingly a QTL that explained up to 30% of the phenotypic variation for root rot, between G17.900 and AL20.350, was linked to G17.900 marker previously identified as associated to root rot resistance (Schneider et al. 2001). Alternatively, genotypes possessing the G17.900 marker associated with QTL controlling *Fusarium* root rot resistance could be recombined with other genotypes possessing the O12.800, AL20.850, and AL20.500 linked markers with the ultimate goal of combining different resistance QTL into a single genotype that should exhibit higher levels of resistance than either genotype alone. When conducted multiple regression analyses, up to 73.3% of the phenotypic variation could be accounted by a combination of five markers that fit the linear regression model.

QTL effects may be environmentally sensitive (Gurganus et al., 1998; Asíns, 2002). QTL analyses have revealed that the gene effects are not equally distributed among QTL. Often a substantial portion of the genetic variation in a population can be explained by a few QTL of moderately large effect or several QTL with small effect. Another important observation from QTL analyses is that 'useful' alleles that enhance the trait value are present not only in the 'resistant' parent but also in the 'susceptible' parent (referred to as transgressive segregation). Therefore, using phenotypic evaluation to determine the breeding value of the resistant breeding germplasm is likely to be misleading with respect to quantitative traits.

In general, QTL analyses have some limitations in estimating the number of important genomic regions controlling any particular trait. First, the ability to detect small effect QTL may be restricted by the number of progeny evaluated, and if the progeny number is low, false negatives are likely (Beavis, 1998). Second, recombination between molecular markers and the genomic region of interest may limit the ability to detect QTL, particularly in a situation where portions of the genome remain unmapped (also applies to unbalanced populations such as the IBL populations). Third, several closely linked QTL still cannot be distinguished from a single factor with large effect (Lynch and Walsh, 1998), even using the CIM approach. Fourth the use of unbalanced populations, such as IBL populations, is less efficient for mapping and precisely estimating QTL effects (Doganlar et al., 2002). Overall, these limitations would result in an under estimation of the number of genomic regions affecting any trait. Therefore, it is fair to assume that the number of QTL detected in the current study represents a minimal estimate of the true number of factors affecting a given trait. Nine QTL were identified in the current study which are directly associated with root rot and around eight QTL associated with agronomic traits such as growth habit, seed size, maturity, and yield. The current study shows that molecular marker data for IBL populations can be used to perform linkage analysis and test marker-trait associations for the identification of QTL. Moreover mapping QTL and at the same time introgressing them into genotypic background with a desirable phenotype, such as that of the recurrent parent, makes IBLs attractive for the study of quantitative traits such as root rot resistance and to enhance the breeding of quantitatively inherited traits by regenerating the recurrent parent more quickly.

Future Research

Absolute field resistance to root rot does not exist in common bean but genotypes with high levels of resistance, from Middle-American gene pool are available that can be used to introgress resistance into large seeded Andean bean. The goal of this research was to enhance resistance of Michigan dark red kidney (Red Hawk) and cranberry bean (C97407) by introgressing resistance from a small seeded black bean from Mexico which is highly resistant and identify QTL associated with resistance for use in MAS. QTL associated with resistance were identified in linkage group B2, close to *Pv*PR-2 (pathogenicity related protein), *Pgip*, and *ChS* which may be associated with general resistance to root rot caused by *F. solani*.

Resistance to *Fusarium* root rot is a quantitatively inherited trait, challenging to measure, and highly influenced by the environment. Due to the complexity of this trait (root rot resistance) exhibiting low to moderate heritability make MAS an important approach to enhance resistance to root rot. By identifying different QTL the breeder would have the opportunity to combine through MAS those QTL identified in different genotypes regardless of their function in root rot resistance. Pyramiding of different QTL is necessary to significantly improve genetic gain in complexly inherited traits such as root rot reistance.

The introgression of *Fusarium* root rot resistance into large seeded bean germplasm will require the utilization of resistance sources from other gene pools. Obviously previous studies have demonstrated that root rot resistance and agronomically acceptable dark red kidney genotypes will not be achieved through single cross breeding approaches and most likely a modified backcross breeding method is a better alternative.

Using molecular markers and greenhouse screening evaluations could be a useful approach when making educated decisions about which progeny to be intermated or backcrossed before performing the actual crosses thus saving time and resources. The markers closely linked to QTL associated with resistance to *Fusarium* root rot identified in this study and previous studies provide a method to genotypically select for resistant progeny that can later be used for backcrossing experiments. The resistance levels of the backcross progeny can than be tested in the greenhouse before the lines are taken to the field and for more laborious field trials and testing.

Future work on root rot resistance would involve the selection of IBL from both populations with the best marker combination associated with resistance to *Fusarium* root rot and test in different environments to verify their performance. Once the best marker combination associated with resistance is tested, proved efficient, and are consistent in different environments, an approach can be made to convert those markers into SCAR markers. The reproducibility of RAPD markers is a problem and SCARs are more specific and easier to score. In addition a SCAR marker for AJ4.350 which is associated with the determinate growth habit QTL could assist in determining the correct location of this QTL on the integrated bean map.

One strategy to transfer resistance to root rot caused by *F. solani* would be to backcross QTL with large effect into susceptible kidney and cranberry genotypes. Researchers can simultaneously backcross QTL associated with resistance by MAS and obtain genotypes which are similar to the recurrent parent but with the desired QTL introgressed. The researcher can identify genotypes that have vigorous roots combined with root rot resistance and use these genotypes as resistance donor parents. By

combining both QTL associated with resistance and a vigorous root system one can study in depth the relation between resistance to root rot and root traits in a RIL population. Genotypes that combine both traits exist (e.g. Chinook 2000) and could be used in a backcross program to improve root rot resistance of large seeded beans in combination with MAS using markers linked to QTL that are associated with resistance in the other gene pool.

To overcome problems associated with mapping RAPD markers, parents of the IBL populations may be screened with microsatellites (SSR markers) previously mapped on linkage groups where QTL have been located (B2, B3, B5, and B7). If polymorphic SSR markers exist between the parents of the populations the entire populations can be screened with the polymorphic SSRs. Once IBL populations are screened withthose SSRs linked to root rot resistance the mapping position of the previously identified markers linked to QTL associated to root rot resistance in the BJ population could be confiremed.

It is important to develop other root rot mapping populations with different resistance sources to continue the search for new QTL associated with resistance that could be transfered into large seeded bean germplasm. N203 is the most resistant source available and perhaps could be used in a backcross program similar to the one used in this study with NSL resistance source. Both NSL and N203 might be related since they both are small black seeded beans from Mexico with root rot resistance. Both of these resistance source result to have some QTL in common. Despite the genetic similarity, N203 may still contribute novel QTL for root rot resistance due to its high level of resistance that has been maintained for many years in many locations. Whether all QTL

in NSSL were identified is still in question since the size of the IBL populations used in the current study were not sufficiently large for a QTL study but they were large enough for a breeding populations. Very important the BC_1 population used as parents was probably large enough to transfer sufficient genetic variability from the donor NSL.

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GENERAL SUMMARY

- Genetic variation exists among different bean genotypes, and between market classes for root traits such as root length, root diameter, root surface area, fractal dimension, root dry weight, number of meristem, and adventitious roots.

- Selection for root dry weight appears to be useful in the greenhouse, but should be delayed to 45 days after planting (anthesis) to allow greater expression of root traits.

- Plant breeders interested in enhancing root rot resistance and overall root health under field conditions should focus on evaluating adventitious roots, root dry weight, and lateral roots in a breeding population.

- The potential for improving root characteristics in common bean exists and breeders have been successful in transferring root traits from the black Middle American bean into the large seeded Andean such as the light red kidney Chinook 2000 and the snap bean breeding line FR266. Both of these large seeded bean genotypes can serve as parents to enhance root traits of future commercial kidney and snap bean varieties.

- It is clear that temperature has an effect on *F. solani* mycelium growth and interestingly, the pathogen seems to be acclimating as a means of surviving the

exposure times (24, 48, 72, 96, 172 hours) to the different temperatures (5°C to 35°C).

- It was hypothesized that under field conditions cooler soil temperatures might favor expression of *Fusarium* symptoms.

- Using kidney and cranberry inbred backcross line (IBL) populations nine QTL significantly associated with *Fusarium* root rot resistance in field and greenhouse were identified that explained from 5 to 53% of the total phenotypic variability. QTL sharing flanking markers (G6.2000, G17.900, AL20.850 and AL20.350) were identified in multiple locations and in both the kidney and cranberry IBL populations.

- QTL associated with *Fusarium* root rot resistance identified in the current study were located on B2 and B5 of the integrated bean map close to previously identified QTL for other resistant sources.

- Using a linear regression model a combination of five markers accounted for 73% of the phenotypic variation.

- Large effect QTL (R^2>50) identified on different linkage groups could be combine to enhance genetic resistance to *Fusarium* root rot in common bean for

root rot resistance and provide the opportunity for marker assisted backcrossing to avoid problems with inter-gene pool crosses.

APPENDICES

APPENDIX A

Table A1. Root characteristics of bean genotypes harvested during the vegetative stage of development.

Root Characteristics	Genotypes†				
	Red Hawk	Montcalm	Beluga	Chinook 2000	FR266
Total Root Length (cm plant^{-1})	978.9 (ab)	1089.0 (ab)	1079.3 (ab)	1227.3 (a)	1127.4 (ab)
Fine (A-C)	902.8	1017.4	1018.3	1164.6	1066.1
Intermediate (D-G)	67.6	66.7	56.7	58.5	56.8
Taproots (H-J)	8.5	4.87	4.30	4.12	4.4
Surface Area (cm^2)	241.95 (ab)	251.8 (a)	242.7 (ab)	258.6 (a)	240.6 (ab)
Projected Area (cm^2)	77.0 (ab)	80.1 (a)	77.2 (ab)	82.3 (a)	76.6 (ab)
Average Diameter (mm)	0.8 (a)	0.7 (ab)	0.7 (ab)	0.6 (b)	0.6 (b)
Total Root Volume (cm^3)	4.9 (a)	4.7 (a)	4.4 (ab)	4.3 (ab)	4.1 (ab)
Density (g/cm^3)	7.9 (a)	6.8 (ab)	5.4 (b)	5.6 (b)	5.9 (ab)
Length Per Volume (cm/m^3)	979.0 (ab)	1089.0 (ab)	1079.4 (ab)	1227.3 (a)	1127.4 (ab)
Fractal Dimension	1.54 (a)	1.54 (a)	1.50 (ab)	1.51 (ab)	1.51 (ab)
No. of Meristems or Tips	3101.4 (b)	3244.8 (ab)	3486.8 (ab)	4226.2 (a)	3583.6 (ab)
Forks	2759.6 (a)	2996.8 (a)	2741.3 (a)	3438.1 (a)	3205.5(a)
Crossings	342.3 (a)	393.3 (a)	363.3 (a)	494.8 (a)	450.1 (a)
Vegetative Weight (g)					
Fresh	6.87 (a)	5.1 (bc)	4.6 (cd)	4.8 (bcd)	3.8 (ef)
Dry	1.1 (a)	0.7 (b)	0.6 (b)	0.7 (b)	0.5 (bc)
Root Weight (g)					
Fresh	7.8 (a)	7.5 (a)	5.8 (ab)	6.7 (ab)	6.5 (ab)
Dry	2.9 (a)	2.5 (b)	2.5 (b)	2.5 (b)	2.5 (b)

Continuation of Table A1.

Root Characteristics	Genotypes†				
	C97407	Taylor Hort	Negro San Luís	TLP19	B98311
Total Root Length (cm plant^{-1})	1174.1 (ab)	1057.9 (ab)	1106.7 (ab)	1117.6 (ab)	906.0 (b)
Fine (A-C)	1115.3	1003.4	1048.4	1053.2	855.4
Intermediate (D-G)	55.5	51.2	54.7	60.2	47.1
Taproots (H-J)	3.3	3.3	3.6	4.2	3.5
Surface Area (cm^2)	246.5 (ab)	229.4 (ab)	237.4 (ab)	255.2 (a)	198.7 (b)
Projected Area (cm^2)	78.4 (ab)	73.0 (ab)	75.5 (ab)	81.2 (a)	63.2 (b)
Average Diameter (mm)	0.6 (b)	0.7 (ab)	0.6 (b)	0.7 (ab)	0.7 (ab)
Total Root Volume (cm^3)	4.1 (ab)	4.0 (ab)	4.2 (ab)	4.8 (a)	3.6 (b)
Density (g/cm^3)	4.9 (b)	5.8 (b)	6.1 (ab)	5.9 (ab)	5.3 (b)
Length Per Volume (cm/m^3)	1174.1(ab)	1057.9 (ab)	1106.8 (ab)	1117.6 (ab)	906.1 (b)
Fractal Dimension	1.5 (ab)	1.5 (ab)	1.5 (ab)	1.5 (ab)	1.3 (b)
No. of Meristems or Tips	3212.6 (ab)	3218.8 (ab)	3721.4 (ab)	3066.4 (b)	2871.4 (b)
Forks	3301.5 (a)	2726.9 (a)	2907.1(a)	2633.2 (a)	2353.5 (a)
Crossings	513.4 (a)	388.6 (a)	390.3 (a)	347.0 (a)	308.3 (a)
Vegetative Weight (g)					
Fresh	5.6 (b)	5.2 (bc)	4.3 (de)	3.1 (f)	2.2 (g)
Dry	0.7 (b)	0.6 (b)	0.7 (b)	0.2 (d)	0.3 (cd)
Root Weight (g)					
Fresh	5.8 (ab)	5.9 (ab)	6.5 (ab)	6.7 (ab)	4.9 (b)
Dry	2.5 (b)	2.5 (b)	2.5 (b)	2.5 (b)	2.4 (b)

† Each value is the mean of three replications (three pots per genotype per replication). Pair-wise comparisons are shown in parenthesis.

Table A2. Mean values of length of root classes obtained under greenhouse environment.

Bean Classes †	Genotypes	Root Classes (cm)‡									
		Fine			Intermediate				Taproots		
		A	B	C	D	E	F	G	H	I	J
K	Beluga	463.8	450.0	104.5	29.7	16.3	7.4	3.4	1.9	1.1	1.3
	Chinook 2000	594.3	474.6	95.7	29.7	17.1	8.1	3.7	1.9	0.9	1.3
	Montcalm	465.4	445.1	106.9	33.6	19.7	9.0	4.5	2.4	1.0	1.5
	Red Hawk	392.5	410.7	99.6	31.8	19.9	10.1	5.9	3.3	2.2	3.0
C	C97407	569.1	445.5	100.7	30.0	15.6	6.8	3.1	1.6	0.8	0.9
	Taylor Hort	477.8	428.9	96.8	27.0	14.7	6.5	3.0	1.6	0.8	0.9
S	FR266	542.3	428.9	95.0	28.9	16.2	7.9	3.9	2.1	1.1	1.2
B	B98311	404.9	375.7	74.8	24.8	13.3	6.0	3.0	1.5	0.9	1.1
	Negro San Luís	517.3	441.0	90.2	28.8	15.5	6.8	3.6	1.8	0.8	1.0
	TLP19	449.1	496.8	107.3	32.0	17.4	7.5	3.4	1.9	0.9	1.3
Grand Mean		487.7	439.8	97.1	29.6	16.6	7.6	3.8	1.9	1.1	1.4
LSD (0.05)		191.7	78.9	20.1	8.5	5.6	3.1	1.9	1.2	0.8	1.3
Coefficient of Variation		33.2	15.1	17.5	24.1	28.5	34.2	42.8	50.5	66.2	83.3

† K= Kidney, C= Cranberry, S= Snap, and B= Black beans

‡ Roots were divided into ten classes, based upon root length and diameter [class A (0-0.5 cm), class B (0.51-1.0 cm), class C (1.01-1.5 cm), class D (1.51-2.0 cm), class E (2.01-2.5 cm), class F (2.51-3.0 cm), class G (3.01-3.5 cm), class H (3.51-4.0 cm), class I (4.01-4.5 cm), and class J (> 4.5 cm)]

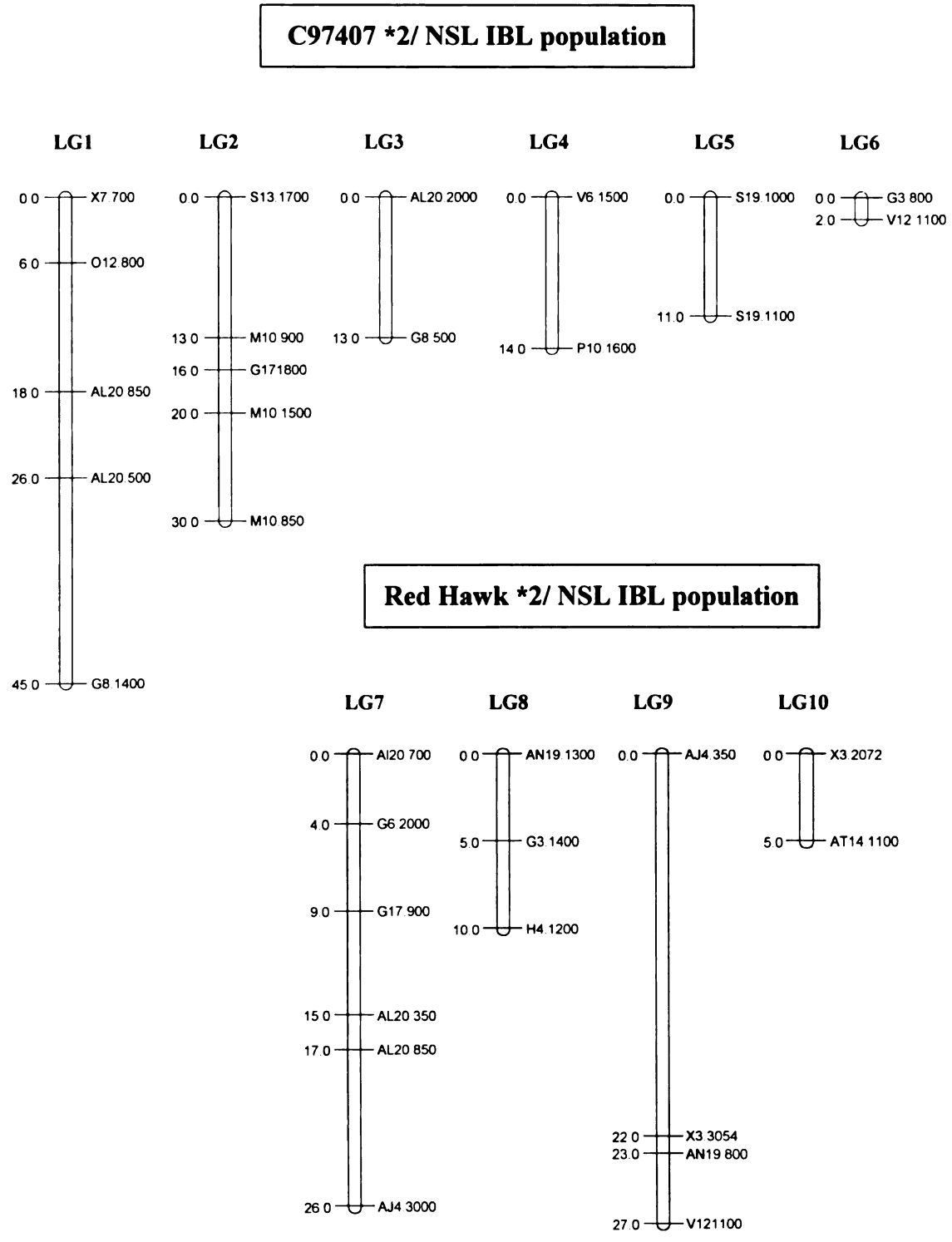

Figure A1. Illustration of partial linkage groups generated in the current genetic study for the cranberry and the kidney IBL populations for the QTL analysis.

APPENDIX B

Table B1. Root rot scoring and agronomic traits means of BC_2F_4 derived IBLs for the C97407 *2/ NSL population at Montcalm, MI, during the summer of 2002.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
NSL	Negro San Luís	1.0	2.0	-	-	-	3.0	48.0	-	3
70	C97407*2/NSL	2.6	2.6	45.6	39.3	104.0	3.3	47.3	3	3
26	C97407*2/NSL	2.7	3.0	50.7	40.0	99.0	2.7	43.7	2	1
64	C97407*2/NSL	2.7	4.8	37.0	38.0	94.3	1.3	45.0	4	1
32	C97407*2/NSL	3.0	4.7	39.8	38.0	103.0	2.0	43.7	3	1
36	C97407*2/NSL	3.0	4.6	53.0	38.7	95.7	3.3	43.0	5	1
28	C97407*2/NSL	3.3	3.1	66.5	39.0	97.0	2.3	43.7	3	1,3
5	C97407*2/NSL	3.4	4.2	46.1	38.7	94.3	2.0	43.7	4	1
65	C97407*2/NSL	3.4	4.8	52.7	40.3	95.0	1.3	45.7	4	1
3	C97407*2/NSL	3.6	5.1	46.9	38.0	92.0	2.0	43.0	4	1
53	C97407*2/NSL	3.6	5.2	43.6	38.7	92.0	2.0	44.0	3	1
74	C97407*2/NSL	3.6	6.1	49.8	38.0	92.0	2.0	46.0	5	1
50	C97407*2/NSL	3.7	6.3	-	39.7	103.0	-	-	4	1
58	C97407*2/NSL	3.7	5.3	41.7	38.0	99.0	2.0	45.0	3	3
59	C97407*2/NSL	3.7	4.8	37.1	40.0	103.0	3.7	44.7	4	3
60	C97407*2/NSL	3.7	5.3	47.4	39.3	106.0	3.0	46.0	3	3
61	C97407*2/NSL	3.7	4.0	53.5	39.3	106.0	3.3	42.0	3	1
75	C97407*2/NSL	3.8	5.3	40.8	38.0	92.0	2.0	45.0	5	1
10	C97407*2/NSL	3.9	5.8	47.8	38.0	104.0	2.3	45.3	5	1
18	C97407*2/NSL	3.9	5.1	42.3	38.0	88.7	1.3	43.7	4	1
27	C97407*2/NSL	3.9	5.6	43.6	38.0	94.7	2.0	43.0	3	1

Continuation of Table B1.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
76	C97407*2/NSL	3.9	6.1	46.3	38.0	91.7	2.0	44.3	4	1
19	C97407*2/NSL	4.0	5.9	51.4	39.3	92.0	2.0	45.0	4	1
21	C97407*2/NSL	4.0	6.7	-	38.0	92.0	-	-	4	1
24	C97407*2/NSL	4.0	5.7	52.7	40.0	105.0	2.7	47.7	3	3
34	C97407*2/NSL	4.0	4.2	51.2	39.0	100.0	2.0	46.0	3	1
35	C97407*2/NSL	4.0	4.9	40.7	38.0	94.3	2.0	42.0	4	1
38	C97407*2/NSL	4.0	5.6	45.5	39.3	99.0	2.0	44.0	4	1
47	C97407*2/NSL	4.0	5.3	41.7	38.7	93.3	1.3	44.0	3	1
71	C97407*2/NSL	4.0	5.0	37.9	38.0	103.0	3.3	45.0	3	3
67	C97407*2/NSL	4.1	5.9	51.8	40.0	94.0	3.0	43.3	3	3
11	C97407*2/NSL	4.1	5.7	57.9	38.0	103.0	2.0	46.0	3	1
16	C97407*2/NSL	4.3	6.6	34.8	38.7	91.3	1.7	42.3	3	1
20	C97407*2/NSL	4.3	6.3	-	38.0	92.0	-	-	4	1
23	C97407*2/NSL	4.3	6.7	-	38.7	91.7	-	-	3	1
31	C97407*2/NSL	4.3	5.7	43.8	38.0	93.3	2.0	44.3	3	1
41	C97407*2/NSL	4.3	7.0	53.5	39.3	106.0	2.0	43.7	3	1
46	C97407*2/NSL	4.3	5.7	41.3	38.0	99.7	2.0	43.7	3	1
52	C97407*2/NSL	4.3	6.7	51.8	38.0	89.3	2.7	43.3	3	1
57	C97407*2/NSL	4.3	6.7	38.0	38.7	106.0	3.0	45.0	3	3
62	C97407*2/NSL	4.3	5.7	44.3	38.0	98.7	2.7	42.7	4	1
63	C97407*2/NSL	4.3	6.4	46.1	40.7	98.3	3.0	46.3	3	3
66	C97407*2/NSL	4.3	5.3	41.7	39.3	104.0	3.3	45.0	3	3

Continuation of Table B1.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
68	C97407*2/NSL	4.3	5.7	57.8	38.7	105.0	3.7	42.3	3	3
69	C97407*2/NSL	4.3	6.2	45.2	38.0	93.3	2.3	42.7	4	1
77	C97407*2/NSL	4.3	5.3	-	38.0	102.0	-	-	3	1
C97407	C92167/THORT	4.3	6.8	41.9	38.0	92.0	1.0	45.0	4	1
C81008	TAYLOR HORT	4.4	5.3	51.6	38.0	92.0	1.7	45.0	4	1
29	C97407*2/NSL	4.6	5.8	57.1	38.0	92.3	2.0	44.3	3	1
44	C97407*2/NSL	4.6	6.3	-	38.0	100.7	2.0	43.0	2	1
1	C97407*2/NSL	4.7	6.6	50.4	39.0	92.0	2.0	41.0	3	1
12	C97407*2/NSL	4.7	6.7	42.1	38.0	95.3	2.0	43.0	3	1
17	C97407*2/NSL	4.7	5.7	49.5	38.0	92.0	1.7	43.0	4	1
22	C97407*2/NSL	4.7	7.0	-	38.0	96.3	-	-	4	1
25	C97407*2/NSL	4.7	6.3	41.4	38.0	92.0	1.7	44.0	5	1
33	C97407*2/NSL	4.7	7.0	42.0	38.0	94.0	2.0	42.7	4	1
37	C97407*2/NSL	4.7	7.0	57.0	38.7	104.0	2.7	43.3	4	1
40	C97407*2/NSL	4.7	7.0	56.3	38.0	92.0	1.3	44.7	3	1
42	C97407*2/NSL	4.7	5.3	44.2	38.0	94.7	2.0	43.0	3	1
48	C97407*2/NSL	4.7	6.7	47.2	38.0	104.0	2.0	43.3	3	1
49	C97407*2/NSL	4.7	6.7	50.7	38.0	92.0	2.0	43.0	3	1
78	C97407*2/NSL	4.7	7.0	-	38.0	105.0	-	-	6	1
8	C97407*2/NSL	4.8	6.6	46.0	38.0	104.0	2.0	45.0	5	1
4	C97407*2/NSL	4.9	6.8	51.1	38.7	92.0	2.0	43.7	3	1
55	C97407*2/NSL	4.9	6.4	51.8	38.0	89.3	2.0	44.7	4	1

Continuation of Table B1.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
2	C97407*2/NSL	5.0	7.0	-	40.0	92.3	-	-	3	1
6	C97407*2/NSL	5.0	6.6	37.2	38.7	92.0	2.7	42.3	3	1
7	C97407*2/NSL	5.0	6.3	53.9	38.3	104.0	3.3	41.3	3	1
9	C97407*2/NSL	5.0	7.0	52.5	37.7	92.7	2.0	44.0	2	1
13	C97407*2/NSL	5.0	7.0	40.9	38.0	93.0	2.3	40.3	2	1
14	C97407*2/NSL	5.0	6.3	45.2	38.0	99.7	2.7	43.0	3	1
15	C97407*2/NSL	5.0	6.3	43.2	38.7	92.0	2.0	43.3	4	1
30	C97407*2/NSL	5.0	6.7	47.8	40.3	98.7	2.0	42.0	4	1
39	C97407*2/NSL	5.0	7.0	40.5	38.0	103.3	2.0	42.0	3	1
43	C97407*2/NSL	5.0	7.0	-	38.0	92.0	-	-	4	1
45	C97407*2/NSL	5.0	6.3	53.4	38.0	92.0	2.0	42.0	3	1
51	C97407*2/NSL	5.0	7.0	-	39.0	106.0	-	-	3	1
54	C97407*2/NSL	5.0	6.3	38.7	37.7	102.0	2.0	44.0	3	1
72	C97407*2/NSL	5.0	6.7	-	39.3	92.7	-	-	4	1
73	C97407*2/NSL	5.0	6.7	-	38.3	103.0	-	-	2	1
56	C97407*2/NSL	5.3	6.7	51.6	39.0	106.0	2.7	45.7	3	3
	Means	5.9	4.2	46.9	38.5	97.1	2.3	43.9	3.4	1.3
	LSD (P=0.05)	1.2	0.7	13.6	1.1	2.8	0.7	1.7	1.9	1.9
	Coefficient Variation (%)	12.3	10.0	17.9	1.8	1.8	19.7	2.5	7.9	8.2

[1] Scale 1 to 7 where 1=no disease and 7=severely diseased. [2] Scale 1 to 5 where 1=vigorous root and 5=weak root. [3] Measured as g.100seed^{-1}. DTF= days to flower and DTM= days to maturity. [4] Scale 1 to5 where 1=no lodging and 5=excessive lodging. [5] DS= Desirability score 1 to 9 given at maturity takes into consideration traits such as height, lodging resistance, pod load, favorable pod to ground distance, uniformity of maturity, and absence of disease, the higher the score more desirable. [6] 1=determinate growth habit, 2 and 3 = indeterminate growth habit (Singh, 1982). '-' refers to missing data due to plants that were not harvested because of severe common bacterial blight disease infection. No yield data was collected for the 2002 field trial.

Table B2. Root rot scoring and agronomic traits means of BC_2F_4 derived IBLs for the C97407 *2/ NSL population at Montcalm, MI, during the summer of 2003.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Yield (kg/ha)	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
B98311	X98102/RAVEN	2.2	3.8	3130	31.2	44.0	85.0	2.0	50.0	5.0	2
24	C97407*2/NSL	2.8	4.0	2549	50.3	41.0	88.0	3.0	47.5	5.0	3
26	C97407*2/NSL	3.3	2.7	2081	52.5	41.0	86.0	2.4	44.2	4.0	1
65	C97407*2/NSL	3.6	3.4	2114	50.2	39.0	85.0	1.6	45.4	3.5	1
49	C97407*2/NSL	3.6	4.7	1923	50.6	42.0	84.5	2.0	43.4	3.5	1
52	C97407*2/NSL	3.8	4.3	2309	51.5	38.0	82.5	2.5	43.6	3.5	1
35	C97407*2/NSL	3.8	4.0	1866	45.1	38.0	82.0	1.9	42.0	3.0	1
68	C97407*2/NSL	3.9	4.3	2232	56.4	39.0	90.0	3.5	43.4	4.5	3
58	C97407*2/NSL	3.9	3.7	2155	42.8	38.0	81.0	2.0	44.4	5.0	3
C81008	TAYLOR HORT	4.0	4.4	2395	53.5	38.0	82.5	1.4	44.9	5.0	1
71	C97407*2/NSL	4.0	4.0	2339	38.3	38.0	90.0	3.5	45.2	4.5	3
C97407	C92167/THORT	4.1	4.3	1993	44.7	38.0	81.0	1.1	44.8	4.0	1
37	C97407*2/NSL	4.1	4.7	1747	34.0	39.0	86.0	1.6	44.4	4.0	1
57	C97407*2/NSL	4.2	4.3	2676	43.0	46.0	85.0	3.0	45.2	4.5	3
34	C97407*2/NSL	4.2	4.0	2428	54.8	39.0	82.0	2.1	45.9	5.0	1
75	C97407*2/NSL	4.2	3.8	2372	42.3	38.0	90.0	2.0	45.6	5.0	1
5	C97407*2/NSL	4.2	3.4	2031	50.2	39.0	82.0	2.0	43.6	5.0	1
61	C97407*2/NSL	4.3	3.7	2788	51.4	42.0	90.0	3.4	42.1	5.0	3
53	C97407*2/NSL	4.3	3.6	2380	50.6	40.0	82.0	1.9	43.6	4.5	1
29	C97407*2/NSL	4.4	4.6	2159	58.4	38.0	81.0	2.0	44.1	4.5	1

Continuation of Table B2.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Yield (kg/ha)	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
30	C97407*2/NSL	4.4	5.0	2076	45.2	40.0	86.1	2.0	42.0	3.5	1
18	C97407*2/NSL	4.4	3.9	2059	46.0	38.0	79.0	1.6	43.2	3.5	1
66	C97407*2/NSL	4.6	4.3	2335	45.4	47.0	89.0	3.5	44.5	4.5	3
63	C97407*2/NSL	4.7	4.3	3061	49.0	40.0	90.0	2.9	46.5	5.5	3
60	C97407*2/NSL	4.7	3.7	2599	50.7	47.0	90.1	3.1	45.9	5.0	3
74	C97407*2/NSL	4.7	3.6	1717	53.4	40.0	84.9	2.0	45.5	4.0	1
76	C97407*2/NSL	4.8	3.9	2129	48.3	38.0	82.0	2.0	44.5	5.0	1
10	C97407*2/NSL	4.8	3.9	1952	48.1	40.0	86.0	2.5	44.9	3.5	1
42	C97407*2/NSL	4.9	4.7	2046	47.3	39.0	81.0	1.9	43.1	4.0	1
69	C97407*2/NSL	4.9	4.3	1709	50.6	39.0	83.0	2.5	42.5	3.0	1
67	C97407*2/NSL	5.0	4.1	2655	55.8	44.0	86.0	3.0	44.0	4.0	3
56	C97407*2/NSL	5.0	5.3	2539	50.6	42.0	86.0	2.9	45.1	5.0	3
47	C97407*2/NSL	5.0	4.0	2382	38.2	38.0	84.0	1.5	44.1	3.5	1
9	C97407*2/NSL	5.0	5.0	2126	51.1	38.0	85.0	2.0	44.0	4.5	1
17	C97407*2/NSL	5.0	4.7	2055	49.2	38.0	80.9	1.6	43.0	4.0	1
36	C97407*2/NSL	5.0	3.0	1981	52.8	39.0	85.0	3.0	43.1	3.0	1
45	C97407*2/NSL	5.0	5.0	1893	55.3	38.0	84.0	2.0	42.0	3.5	1
59	C97407*2/NSL	5.1	3.7	2386	36.2	42.0	90.0	3.5	45.5	5.0	3
25	C97407*2/NSL	5.1	4.7	1997	41.7	40.0	86.0	1.5	44.1	4.0	1
27	C97407*2/NSL	5.1	3.9	1698	47.6	39.0	85.0	2.0	43.6	4.5	1

Continuation of Table B2.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Yield (kg/ha)	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
28	C97407*2/NSL	5.2	3.3	2104	67.7	39.0	85.4	2.6	43.4	3.0	1,3
14	C97407*2/NSL	5.2	5.0	2065	49.0	39.0	83.0	2.5	43.0	3.0	1
7	C97407*2/NSL	5.2	5.0	1943	51.3	38.0	86.0	3.0	41.4	3.0	1
K74002	MONTCALM	5.2	3.6	1921	49.4	41.0	85.0	2.0	46.6	4.5	1
41	C97407*2/NSL	5.2	4.3	1633	36.9	38.0	79.5	1.5	42.0	3.5	1
40	C97407*2/NSL	5.2	4.7	1606	41.0	41.0	83.0	2.0	42.9	3.5	1
55	C97407*2/NSL	5.3	4.9	2621	50.5	39.0	82.5	2.0	45.0	5.0	1
32	C97407*2/NSL	5.3	3.0	2039	44.5	39.0	83.0	2.1	43.4	4.0	1
31	C97407*2/NSL	5.3	4.3	1965	48.5	38.0	83.0	2.0	44.0	3.5	1
12	C97407*2/NSL	5.3	4.7	1825	48.5	38.0	83.5	2.0	43.1	3.5	1
6	C97407*2/NSL	5.3	5.0	1682	51.9	40.0	86.0	2.0	43.4	2.5	1
54	C97407*2/NSL	5.4	5.0	2439	39.5	38.0	82.6	2.0	44.0	3.5	1
62	C97407*2/NSL	5.6	4.3	2463	45.6	38.0	83.0	2.6	42.9	3.5	1
3	C97407*2/NSL	5.6	3.6	2412	48.6	38.0	86.0	1.9	43.0	4.5	1
8	C97407*2/NSL	5.6	4.8	1944	42.2	38.0	83.1	2.0	45.0	4.0	1
33	C97407*2/NSL	5.6	4.7	1794	45.2	42.0	81.1	2.0	43.1	3.5	1
44	C97407*2/NSL	5.6	4.6	1719	49.2	38.0	82.0	2.0	42.9	4.0	1
48	C97407*2/NSL	5.6	4.7	1518	42.4	40.0	85.9	2.5	46.1	2.5	1
19	C97407*2/NSL	5.7	4.0	2049	50.4	39.0	81.0	2.0	45.1	5.0	1
64	C97407*2/NSL	5.7	2.7	1681	55.7	38.0	80.9	1.6	44.4	4.0	1

Continuation of Table B2.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Yield (kg/ha)	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
70	C97407*2/NSL	5.8	2.6	2514	47.9	41.0	88.0	3.6	46.9	4.5	3
46	C97407*2/NSL	5.8	4.3	2038	40.7	39.0	84.6	2.0	43.4	3.5	1
16	C97407*2/NSL	6.0	4.3	1491	44.3	40.0	82.0	2.0	42.1	2.5	1
C99833	CARDINAL/K94803	6.1	4.0	2364	58.6	38.0	83.0	1.5	46.6	5.0	1
38	C97407*2/NSL	6.2	4.0	1993	48.4	38.0	83.0	2.0	44.0	4.5	1
I86216	FR266	6.2	3.4	1465	53.7	40.0	86.0	2.0	41.2	2.0	1
1	C97407*2/NSL	6.2	4.7	1150	44.4	40.0	82.0	2.5	40.5	2.0	1
13	C97407*2/NSL	6.2	5.0	1051	44.4	40.0	82.0	2.5	40.5	2.0	1
15	C97407*2/NSL	6.3	5.0	1695	42.0	38.0	83.1	2.5	42.5	3.0	1
4	C97407*2/NSL	6.4	4.9	2136	56.1	39.0	81.0	2.0	43.3	4.0	1
39	C97407*2/NSL	6.7	5.0	1519	49.6	40.0	82.9	2.0	43.0	4.5	1
11	C97407*2/NSL	6.8	4.1	2049	57.8	38.0	86.0	2.0	46.1	4.5	1
	Means	4.9	4.2	2083	48.3	39.6	84.3	2.2	44.1	4.0	1.4
	LSD (P=0.05)	1.9	0.8	4.6	13.6	0.4	1.2	0.7	1.7	1.0	1.9
	Coefficient of variation (%)	23.6	11.2	14.9	15.3	0.6	0.7	17.1	2.1	13.8	14.1

[1] Scale 1 to 7 where 1=no disease and 7=severely diseased. [2] Scale 1 to 5 where 1=vigorous root and 5=weak root. [3] Measured as g.100seed^{-1}. DTF= days to flower and DTM= days to maturity. [4] Scale 1 to5 where 1=no lodging and 5=excessive lodging. [5] Desirability score 1 to 9 given at maturity takes into consideration traits such as height, lodging resistance, pod load, favorable pod to ground distance, uniformity of maturity, and absence of disease, the higher the score more desirable. [6] 1=determinate growth habit, 2 and 3 = indeterminate growth habit (Singh, 1982). '-' refers to missing data due to plants that were not harvested because of severe common bacterial blight disease infection.

Table B3. Root rot scoring and agronomic traits means of BC_2F_4 derived IBLs for the Red Hawk *2/ NSL population at Montcalm, MI, during the summer of 2002.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
I98531	NSL	1.1	1.2	-	-	-	3.0	48.7	-	3
B98311	X98102/RAVEN	3.2	2.2	19.8	44.3	94.0	2.0	47.0	3.0	2
I86216	FR266 (SNP)	3.7	3.8	28.5	43.0	96.3	2.0	42.7	5.0	1
I98501	TLP-19 (CIAT)	4.0	3.2	19.2	45.0	96.7	2.7	48.0	3.0	3
180	RED HAWK *2/NSL	4.1	2.7	42.3	40.0	85.7	2.0	43.3	5.0	1
178	RED HAWK *2/NSL	4.2	2.8	51.3	42.7	96.0	2.7	44.7	3.0	1
K94602	CHINOOK2000	4.8	3.7	51.4	39.3	83.3	2.0	47.0	3.7	1
173	RED HAWK *2/NSL	4.9	3.3	41.4	43.0	92.0	2.0	44.7	3.0	1
154	RED HAWK *2/NSL	5.1	3.7	55.1	42.3	93.0	3.0	43.0	3.0	3
K90101	RED HAWK	5.2	5.0	54.8	39.0	93.0	2.0	45.0	3.7	1
147	RED HAWK *2/NSL	5.3	3.3	49.8	39.3	88.7	2.0	46.3	4.0	1
169	RED HAWK *2/NSL	5.3	3.3	37.1	42.0	94.0	1.3	44.0	3.0	1
170	RED HAWK *2/NSL	5.3	3.7	54.3	39.3	93.0	2.0	46.7	4.0	1
158	RED HAWK *2/NSL	5.6	3.7	48.3	39.0	94.0	2.7	43.7	3.0	1,3
110	RED HAWK *2/NSL	5.7	4.7	48.2	45.0	94.0	3.0	46.0	4.0	3
113	RED HAWK *2/NSL	5.7	3.7	42.3	43.3	91.3	3.0	47.0	3.0	3
116	RED HAWK *2/NSL	5.7	4.7	51.0	43.7	96.7	4.7	43.3	4.0	3
117	RED HAWK *2/NSL	5.7	3.7	48.8	40.0	92.0	2.0	45.0	4.0	1
185	RED HAWK *2/NSL	5.7	4.3	43.6	38.7	82.0	2.0	43.3	3.0	1
K90902	BEA/50B1807//LASSEN, BELUGA	5.7	4.3	42.1	43.7	90.0	2.0	46.3	4.3	1

Continuation of Table B3.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
161	RED HAWK *2/NSL	5.8	3.7	54.2	38.7	94.0	2.0	43.7	4.0	1
106	RED HAWK *2/NSL	6.0	3.7	49.4	39.3	93.0	2.0	45.7	4.3	1
139	RED HAWK *2/NSL	6.0	4.7	39.9	43.3	94.0	1.7	44.7	3.0	1
148	RED HAWK *2/NSL	6.0	4.3	53.6	39.7	94.0	3.0	44.0	3.0	1
101	RED HAWK *2/NSL	6.1	4.3	53.1	42.0	93.0	3.0	46.3	3.0	3
164	RED HAWK *2/NSL	6.1	4.0	52.0	42.3	94.0	1.3	43.0	3.0	1
166	RED HAWK *2/NSL	6.1	4.0	47.0	39.3	82.0	1.7	43.3	3.0	1
130	RED HAWK *2/NSL	6.2	4.3	32.5	42.7	94.0	2.0	44.3	3.0	1
176	RED HAWK *2/NSL	6.2	4.0	53.4	39.3	86.0	2.0	44.0	4.3	1
121	RED HAWK *2/NSL	6.3	4.7	56.0	41.0	92.0	2.0	46.0	4.0	1
126	RED HAWK *2/NSL	6.3	4.3	46.4	39.0	93.0	2.0	45.0	4.3	1
135	RED HAWK *2/NSL	6.3	4.7	49.4	40.7	93.0	2.0	45.0	5.0	1
136	RED HAWK *2/NSL	6.3	4.7	50.9	42.0	96.7	2.0	44.0	4.0	1
138	RED HAWK *2/NSL	6.3	4.0	39.4	45.0	96.7	3.7	42.3	3.0	1
142	RED HAWK *2/NSL	6.3	4.3	47.5	39.3	94.0	2.0	44.7	3.3	1
150	RED HAWK *2/NSL	6.3	4.3	44.0	41.0	93.0	2.0	42.3	2.0	1
179	RED HAWK *2/NSL	6.3	4.7	42.1	43.3	94.0	2.0	43.7	3.0	1
189	RED HAWK *2/NSL	6.3	5.0	51.6	43.0	93.0	2.0	44.7	3.0	1
K74002	MONTCALM	6.3	4.7	47.7	38.3	93.0	2.7	47.7	4.0	1
137	RED HAWK *2/NSL	6.4	4.3	42.3	38.0	83.0	2.0	44.3	3.0	1
141	RED HAWK *2/NSL	6.4	5.0	54.5	39.7	93.0	2.7	44.7	4.0	1
102	RED HAWK *2/NSL	6.6	4.3	51.2	39.7	85.7	2.0	44.7	3.7	1

Continuation of Table B3.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
112	RED HAWK *2/NSL	6.6	4.3	52.5	38.7	82.0	3.0	46.0	3.0	3
122	RED HAWK *2/NSL	6.6	3.7	48.2	40.7	94.0	2.0	45.0	3.0	1
132	RED HAWK *2/NSL	6.6	4.7	50.9	42.0	82.0	2.3	42.0	3.0	1
145	RED HAWK *2/NSL	6.6	5.0	44.0	44.0	96.0	2.0	41.3	2.0	1
156	RED HAWK *2/NSL	6.6	5.0	48.7	40.7	94.0	2.0	41.3	3.0	3
119	RED HAWK *2/NSL	6.7	4.7	55.2	41.0	93.0	2.0	44.7	3.0	1
123	RED HAWK *2/NSL	6.7	4.7	52.1	39.3	82.0	2.0	44.0	4.0	1
127	RED HAWK *2/NSL	6.7	5.0	48.6	38.7	82.0	2.0	42.3	3.7	1
143	RED HAWK *2/NSL	6.7	5.0	49.8	39.0	94.0	2.0	45.7	3.0	1
144	RED HAWK *2/NSL	6.7	4.3	52.5	39.0	93.0	2.0	45.7	4.0	1
146	RED HAWK *2/NSL	6.7	4.7	39.6	39.7	92.3	2.0	41.0	3.3	1
159	RED HAWK *2/NSL	6.7	4.7	46.2	38.3	86.0	2.0	44.7	3.0	1
171	RED HAWK *2/NSL	6.7	4.7	45.8	41.3	96.0	2.0	45.7	4.0	1
104	RED HAWK *2/NSL	6.8	5.0	50.5	42.0	96.7	3.0	43.7	3.0	3
181	RED HAWK *2/NSL	6.8	4.7	55.5	39.3	93.0	2.0	45.7	4.0	1
118	RED HAWK *2/NSL	6.9	4.9	54.0	40.0	82.0	1.3	43.0	3.3	1
129	RED HAWK *2/NSL	6.9	5.0	46.2	38.7	90.0	2.0	43.7	3.3	1
184	RED HAWK *2/NSL	6.9	4.7	57.3	42.0	82.0	2.0	46.0	3.0	1
103	RED HAWK *2/NSL	7.0	4.7	56.5	43.3	93.0	3.0	45.0	3.0	1
105	RED HAWK *2/NSL	7.0	4.7	51.6	43.3	94.0	3.0	45.7	3.0	3
107	RED HAWK *2/NSL	7.0	5.0	57.3	43.3	94.0	2.7	46.7	2.0	3
108	RED HAWK *2/NSL	7.0	5.0	57.7	42.0	83.0	3.0	44.7	3.0	3

Continuation of Table B3.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
109	RED HAWK *2/NSL	7.0	5.0	54.7	44.3	93.0	2.0	44.0	3.0	1
111	RED HAWK *2/NSL	7.0	4.7	50.0	43.3	94.7	2.3	45.3	3.0	3
114	RED HAWK *2/NSL	7.0	4.7	53.3	39.0	94.0	3.7	44.7	2.7	3
115	RED HAWK *2/NSL	7.0	5.0	51.2	38.3	86.0	1.7	43.0	3.0	1
120	RED HAWK *2/NSL	7.0	4.7	45.1	38.7	83.0	2.0	44.7	3.0	1
124	RED HAWK *2/NSL	7.0	5.0	46.7	38.7	94.0	2.0	44.3	3.3	1
125	RED HAWK *2/NSL	7.0	5.0	52.1	39.3	93.0	2.3	44.0	3.7	1
128	RED HAWK *2/NSL	7.0	4.0	48.6	38.7	83.3	2.0	43.3	3.0	1
131	RED HAWK *2/NSL	7.0	4.3	51.3	38.7	82.0	1.7	43.3	4.3	1
133	RED HAWK *2/NSL	7.0	5.0	50.0	38.7	85.0	1.0	43.7	4.0	1
134	RED HAWK *2/NSL	7.0	5.0	51.9	41.7	93.7	2.0	45.7	3.0	1
140	RED HAWK *2/NSL	7.0	5.0	53.8	38.3	83.0	2.0	45.3	4.0	1
149	RED HAWK *2/NSL	7.0	4.7	37.3	41.3	87.0	1.3	46.3	4.0	1
151	RED HAWK *2/NSL	7.0	4.7	51.2	38.3	93.0	2.3	42.7	3.0	1
152	RED HAWK *2/NSL	7.0	4.7	43.8	43.0	94.7	2.3	42.0	2.7	1
153	RED HAWK *2/NSL	7.0	5.0	55.7	41.0	94.0	3.0	46.0	3.0	3
155	RED HAWK *2/NSL	7.0	4.0	46.8	39.3	87.7	2.0	45.0	2.7	3
157	RED HAWK *2/NSL	7.0	4.7	51.4	38.7	93.0	2.0	45.0	3.0	1,3
160	RED HAWK *2/NSL	7.0	5.0	42.4	39.0	93.0	1.7	44.7	5.0	1
162	RED HAWK *2/NSL	7.0	4.7	51.4	41.3	92.0	1.3	42.0	4.0	1,3
163	RED HAWK *2/NSL	7.0	5.0	50.3	42.7	94.0	2.0	43.7	3.0	1
165	RED HAWK *2/NSL	7.0	5.0	45.9	38.3	85.7	1.7	44.0	3.0	1

Continuation of Table B3.

IBLs	Pedigree, Name	Root[1] Rot Score	Root[2] Vigor Score	Seed[3] Size (g)	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
167	RED HAWK *2/NSL	7.0	5.0	33.8	42.0	84.0	1.0	45.3	3.7	1
168	RED HAWK *2/NSL	7.0	5.0	42.1	41.7	94.0	1.7	43.7	4.0	1
172	RED HAWK *2/NSL	7.0	5.0	41.8	42.0	94.0	2.0	44.0	4.0	1,3
174	RED HAWK *2/NSL	7.0	5.0	39.3	42.0	96.7	2.0	45.0	4.0	1
175	RED HAWK *2/NSL	7.0	4.7	50.9	38.7	90.7	1.0	42.0	3.0	1
177	RED HAWK *2/NSL	7.0	5.0	38.8	45.0	94.0	2.0	47.7	3.0	1
182	RED HAWK *2/NSL	7.0	4.7	52.1	42.0	93.0	2.7	46.7	3.7	1
183	RED HAWK *2/NSL	7.0	5.0	49.9	39.3	92.0	2.0	44.7	3.0	1
186	RED HAWK *2/NSL	7.0	5.0	41.8	42.7	94.0	2.0	43.0	3.0	1
187	RED HAWK *2/NSL	7.0	4.7	56.3	38.0	84.0	2.0	45.7	3.0	1
188	RED HAWK *2/NSL	7.0	4.7	58.7	39.0	93.0	2.0	44.0	5.0	1
190	RED HAWK *2/NSL	7.0	5.0	52.0	42.7	94.0	2.0	46.0	3.0	1
191	RED HAWK *2/NSL	7.0	4.7	49.9	41.7	96.0	2.7	43.0	3.0	1
	Means	6.3	4.4	48.0	40.8	91.1	2.2	44.6	3.4	1.4
	LSD (P=0.05)	0.6	0.2	2.8	2.5	2.7	0.5	2.0	0.5	1.9
	Coefficient of Variation (%)	5.8	2.5	3.6	3.8	1.9	14.9	2.8	8.7	18.9

[1] Scale 1 to 7 where 1=no disease and 7=severely diseased. [2] Scale 1 to 5 where 1=vigorous root and 5=weak root. [3] Measured as g.100seed^{-1}. DTF= days to flower and DTM= days to maturity. [4] Scale 1 to5 where 1=no lodging and 5=excessive lodging. [5] desirability score 1 to 9 given at maturity takes into consideration traits such as height, lodging resistance, pod load, favorable pod to ground distance, uniformity of maturity, and absence of disease, the higher the score more desirable. [6] 1=determinate growth habit, 2 and 3 = indeterminate growth habit (Singh, 1982). '-' refers to missing data due to plants that were not harvested because of severe common bacterial blight disease infection. No yield data was collected for the 2002 field trial.

Table B4. Root rot scoring and agronomic traits means of BC_2F_4 derived IBLs for the Red Hawk *2/ NSL population at Montcalm, MI, during the summer of 2003. Root rot data collected at Geneva, N.Y. for the kidney IBL is also included for comparison.

IBLs	Pedigree, Name	Root[1] Rot MRF	Root[2] Vigor Score	Root[1] Rot N.Y.	Yield (kg/ha)	Seed[3] Size	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
I98531	NSL	1.1	3.2	1.8	-	-	-	-	2.7	48.0	4.0	3
I86216	FR266 (SNP)	2.4	4.7	3.1	1210	27.2	42.0	85.0	2.7	47.7	2.3	1
153	RED HAWK *2/NSL	3.0	5.0	4.3	2515	43.2	40.0	84.0	3.0	46.0	4.0	3
169	RED HAWK *2/NSL	3.1	3.3	5.0	1420	27.5	40.0	83.7	1.3	44.0	3.7	1
149	RED HAWK *2/NSL	3.4	4.7	6.0	2378	27.4	42.0	83.7	1.3	46.3	4.0	1
B98311	X98102/RAVEN	3.7	4.8	-	3396	20.1	44.0	86.0	2.0	50.0	5.0	2
116	RED HAWK *2/NSL	4.0	4.7	4.5	1757	33.0	44.0	87.3	4.7	43.3	2.3	3
138	RED HAWK *2/NSL	4.0	4.0	5.2	1839	30.4	42.0	87.3	3.7	42.3	3.0	1
148	RED HAWK *2/NSL	4.1	4.3	5.5	2390	43.9	40.0	84.0	3.0	44.0	3.3	1
140	RED HAWK *2/NSL	4.1	5.0	4.9	2269	39.1	38.0	76.3	2.0	45.3	4.0	1
185	RED HAWK *2/NSL	4.2	4.3	4.4	1585	33.6	38.0	79.7	2.0	43.3	3.3	1
143	RED HAWK *2/NSL	4.3	5.0	4.5	2351	38.5	38.0	84.7	2.0	45.7	4.7	1
189	RED HAWK *2/NSL	4.4	5.0	4.9	2176	38.9	39.0	83.0	2.0	44.7	4.0	1
171	RED HAWK *2/NSL	4.6	4.7	4.9	2034	39.2	38.0	86.0	2.0	45.7	5.3	1
152	RED HAWK *2/NSL	4.6	4.7	-	1765	35.8	41.0	84.7	2.3	42.0	3.7	1
156	RED HAWK *2/NSL	4.6	5.0	5.2	1993	37.5	38.0	86.0	2.0	41.3	2.7	3
157	RED HAWK *2/NSL	4.6	4.7	4.0	2204	41.7	38.0	83.0	2.0	45.0	3.3	1,3
174	RED HAWK *2/NSL	4.7	5.0	5.2	1279	29.0	38.0	86.3	2.0	45.0	4.3	1
119	RED HAWK *2/NSL	4.8	4.7	4.5	1868	34.2	38.0	84.7	2.0	44.7	4.0	1
187	RED HAWK *2/NSL	4.8	4.7	4.6	2396	41.6	38.0	75.3	2.0	45.7	5.3	1
C81008	TAYLOR HORT	4.8	3.8	-	1776	35.6	38.0	85.7	2.0	42.7	4.0	1

Continuation of Table B4.

IBLs	Pedigree, Name	Root[1] Rot MRF	Root[2] Vigor Score	Root[1] Rot N.Y.	Yield (kg/ha)	Seed[3] Size	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
I98501	TLP-19(CIAT)	4.8	1.2	-	3052	16.4	45.0	88.0	3.0	48.7	4.7	3
186	RED HAWK *2/NSL	4.9	5.0	4.7	2169	33.1	40.0	85.3	2.0	43.0	3.7	1
113	RED HAWK *2/NSL	4.9	3.7	5.0	2111	38.2	46.0	80.7	3.0	47.0	4.0	3
163	RED HAWK *2/NSL	4.9	5.0	5.9	2041	34.7	41.0	84.0	2.0	43.7	3.7	1
172	RED HAWK *2/NSL	4.9	5.0	5.2	1906	31.8	38.0	84.0	2.0	44.0	4.0	1,3
K90902	BELUGA	4.9	4.3	-	1499	37.0	41.0	81.3	2.0	46.3	5.0	1
158	RED HAWK *2/NSL	5.0	3.7	5.4	1838	38.6	39.0	84.0	2.7	43.7	3.7	1,3
136	RED HAWK *2/NSL	5.1	4.7	5.2	1793	33.4	39.0	83.3	2.0	44.0	3.7	1
145	RED HAWK *2/NSL	5.2	5.0	4.7	1617	27.7	38.0	86.0	2.0	41.3	3.3	1
105	RED HAWK *2/NSL	5.2	4.7	5.3	2023	44.0	46.0	84.0	3.0	45.7	4.3	3
128	RED HAWK *2/NSL	5.2	4.0	5.6	2087	37.2	39.0	83.0	2.0	43.3	4.3	1
150	RED HAWK *2/NSL	5.2	4.3	5.0	1567	34.4	38.0	83.0	2.0	42.3	3.0	1
168	RED HAWK *2/NSL	5.2	5.0	4.7	1678	31.5	38.0	84.0	1.7	43.7	4.0	1
182	RED HAWK *2/NSL	5.2	4.7	5.1	2297	36.9	42.0	83.0	2.7	46.7	4.3	1
183	RED HAWK *2/NSL	5.2	5.0	4.9	1950	38.5	40.0	83.3	2.0	44.7	3.3	1
103	RED HAWK *2/NSL	5.3	4.7	5.0	2048	37.8	43.0	83.0	3.0	45.0	4.3	1
106	RED HAWK *2/NSL	5.3	3.7	5.3	2143	41.4	39.0	83.3	2.0	45.7	5.0	1
109	RED HAWK *2/NSL	5.3	5.0	5.1	1814	38.4	39.0	84.7	2.0	44.0	4.0	1
114	RED HAWK *2/NSL	5.3	4.7	5.0	2294	37.6	42.0	83.0	3.7	44.7	4.0	3
123	RED HAWK *2/NSL	5.3	4.7	5.6	2082	39.1	40.0	77.3	2.0	44.0	4.0	1
134	RED HAWK *2/NSL	5.3	5.0	4.8	1737	40.6	38.0	80.0	2.0	45.7	4.3	1
147	RED HAWK *2/NSL	5.3	3.3	4.8	1417	35.0	41.0	86.3	2.0	46.3	4.0	1

Continuation of Table B4.

IBLs	Pedigree, Name	Root[1] Rot MRF	Root[2] Vigor Score	Root[1] Rot N.Y.	Yield (kg/ha)	Seed[3] Size	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
162	RED HAWK *2/NSL	5.3	4.7	4.3	2398	44.0	38.0	80.7	1.3	42.0	2.0	1,3
179	RED HAWK *2/NSL	5.3	4.7	4.1	1137	26.6	41.0	84.0	2.0	43.7	3.3	1
180	RED HAWK *2/NSL	5.3	2.7	4.9	1978	37.1	38.0	84.0	2.0	43.3	3.3	1
181	RED HAWK *2/NSL	5.3	4.7	4.8	2395	34.0	38.0	83.0	2.0	45.7	5.3	1
111	RED HAWK *2/NSL	5.4	4.7	4.2	1605	36.7	38.0	83.3	2.3	45.3	3.7	3
132	RED HAWK *2/NSL	5.4	4.7	3.9	1476	56.6	40.3	74.0	2.3	42.0	2.3	1
155	RED HAWK *2/NSL	5.4	4.0	5.1	2027	38.4	39.0	80.7	2.0	45.0	3.0	3
184	RED HAWK *2/NSL	5.4	4.7	5.2	2328	37.9	38.0	78.0	2.0	46.0	4.7	1
154	RED HAWK *2/NSL	5.4	3.7	5.4	1979	40.2	41.0	84.0	3.0	43.0	3.7	3
126	RED HAWK *2/NSL	5.6	4.3	4.9	1987	36.8	38.0	83.3	2.0	45.0	4.7	1
146	RED HAWK *2/NSL	5.6	4.7	4.2	1614	31.8	41.0	85.0	2.0	41.0	3.0	1
K90101	RED HAWK	5.6	5.0	4.5	2042	40.3	38.0	83.0	2.0	45.0	4.7	1
133	RED HAWK *2/NSL	5.6	5.0	5.0	1793	37.1	38.0	73.7	1.0	43.7	4.3	1
137	RED HAWK *2/NSL	5.6	4.3	4.8	1999	36.9	38.0	83.0	2.0	44.3	5.0	1
165	RED HAWK *2/NSL	5.6	5.0	4.5	1759	36.0	38.0	83.7	1.7	44.0	5.0	1
167	RED HAWK *2/NSL	5.6	5.0	4.4	2035	27.1	41.0	78.0	1.0	45.3	5.0	1
173	RED HAWK *2/NSL	5.6	3.3	3.9	1599	30.9	42.0	82.0	2.0	44.7	4.0	1
115	RED HAWK *2/NSL	5.7	5.0	4.4	1658	38.2	38.0	83.3	1.7	43.0	3.7	1
141	RED HAWK *2/NSL	5.7	5.0	4.8	2331	38.6	38.0	84.7	2.7	44.7	4.3	1
144	RED HAWK *2/NSL	5.7	4.3	4.7	2424	38.9	38.0	84.0	2.0	45.7	5.3	1
178	RED HAWK *2/NSL	5.7	2.8	5.1	2293	41.0	38.0	85.3	2.7	44.7	4.7	1
107	RED HAWK *2/NSL	5.8	5.0	4.8	1735	47.1	40.0	84.0	2.7	46.7	4.0	3
117	RED HAWK *2/NSL	5.8	3.7	3.8	1859	35.4	41.0	82.3	2.0	45.0	5.0	1

Continuation of Table B4.

IBLs	Pedigree, Name	Root[1] Rot MRF	Root[2] Vigor Score	Root[1] Rot N.Y.	Yield (kg/ha)	Seed[3] Size	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
121	RED HAWK *2/NSL	5.8	4.7	3.7	2078	38.1	38.0	82.0	2.0	46.0	4.0	1
124	RED HAWK *2/NSL	5.8	5.0	5.5	2181	34.0	38.0	84.0	2.0	44.3	5.0	1
K74002	MONTCALM	5.8	2.2	-	1919	43.1	39.0	84.3	2.0	47.0	5.0	1
118	RED HAWK *2/NSL	5.8	4.9	4.8	1616	38.5	38.0	73.3	1.3	43.0	3.3	1
135	RED HAWK *2/NSL	5.8	4.7	5.5	2456	37.4	39.0	83.3	2.0	45.0	5.0	1
142	RED HAWK *2/NSL	5.8	4.3	4.8	2162	36.7	40.0	84.0	2.0	44.7	5.0	1
151	RED HAWK *2/NSL	5.8	4.7	4.6	1256	38.6	38.3	83.0	2.3	42.7	2.7	1
188	RED HAWK *2/NSL	5.8	4.7	5.6	2458	42.9	38.0	84.0	2.0	44.0	4.0	1
104	RED HAWK *2/NSL	5.9	5.0	5.5	2083	43.4	38.0	87.3	3.0	43.7	3.3	3
130	RED HAWK *2/NSL	5.9	4.3	4.4	2132	26.9	39.0	84.0	2.0	44.3	5.7	1
170	RED HAWK *2/NSL	5.9	3.7	4.7	2065	42.5	38.7	84.7	2.0	46.7	5.0	1
191	RED HAWK *2/NSL	5.9	4.7	5.2	1454	35.3	38.0	87.3	2.7	43.0	2.3	1
120	RED HAWK *2/NSL	6.0	4.7	5.4	2082	34.0	40.0	79.7	2.0	44.7	5.0	1
122	RED HAWK *2/NSL	6.0	3.7	5.2	2607	39.4	38.0	83.3	2.0	45.0	4.3	1
127	RED HAWK *2/NSL	6.0	5.0	5.4	1833	36.9	38.0	74.0	2.0	42.3	4.0	1
166	RED HAWK *2/NSL	6.0	4.0	4.7	2119	36.0	38.0	79.3	1.7	43.3	4.0	1
101	RED HAWK *2/NSL	6.1	4.3	5.8	2152	45.6	41.0	84.7	3.0	46.3	4.3	3
161	RED HAWK *2/NSL	6.1	3.7	5.2	2251	41.7	39.0	84.0	2.0	43.7	4.3	1
112	RED HAWK *2/NSL	6.1	4.3	4.8	2189	38.2	39.0	76.7	3.0	46.0	4.0	3
110	RED HAWK *2/NSL	6.2	4.7	5.3	2040	35.7	44.0	84.0	3.0	46.0	3.7	3

Continuation of Table B4.

IBLs	Pedigree, Name	Root[1] Rot MRF	Root[2] Vigor Score	Root[1] Rot N.Y.	Yield (kg/ha)	Seed[3] Size	DTF	DTM	Lodging[4]	Height (cm)	DS[5]	Growth[6] Habit
7599-8	RED HAWK *2/NSL	6.2	3.7	4.8	1446	29.6	42.0	82.0	2.0	47.0	5.0	1
139	RED HAWK *2/NSL	6.2	4.7	4.2	2136	32.7	41.0	85.3	1.7	44.7	4.3	1
160	RED HAWK *2/NSL	6.2	5.0	4.8	1796	33.3	38.0	83.0	1.7	44.7	4.7	1
176	RED HAWK *2/NSL	6.2	4.0	4.5	1595	37.9	38.0	84.0	2.0	44.0	3.7	1
125	RED HAWK *2/NSL	6.3	5.0	3.9	2130	36.3	38.0	83.0	2.3	44.0	3.7	1
129	RED HAWK *2/NSL	6.3	5.0	5.7	1849	32.4	39.0	80.3	2.0	43.7	4.0	1
159	RED HAWK *2/NSL	6.3	4.7	5.5	1789	35.9	38.0	83.3	2.0	44.7	4.0	1
177	RED HAWK *2/NSL	6.3	5.0	4.5	2174	32.8	42.0	84.0	2.0	47.7	5.0	1
190	RED HAWK *2/NSL	6.3	5.0	4.7	1889	39.0	42.0	85.3	2.0	46.0	5.3	1
102	RED HAWK *2/NSL	6.4	4.3	3.6	2342	46.2	38.0	82.7	2.0	44.7	4.3	1
108	RED HAWK *2/NSL	6.6	5.0	5.0	2066	39.6	48.0	74.7	3.0	44.7	3.7	3
164	RED HAWK *2/NSL	6.7	4.0	2.9	1606	39.7	32.7	83.7	1.3	43.0	3.3	1
175	RED HAWK *2/NSL	6.8	4.7	4.5	1875	39.4	38.0	83.0	1.0	42.0	2.7	1
131	RED HAWK *2/NSL	6.9	4.3	5.1	1699	35.8	38.0	80.7	1.7	43.3	3.7	1
Average Means		5.3	4.4	5.8	1977	37.8	39.6	83.0	2.2	44.6	4.1	1.4
LSD (P=0.05)		1.9	0.2	2.1	5.6	9.3	0.7	3.1	0.4	1.8	0.8	1.9
Coefficient of Variation (%)		23.0	2.7	24.2	18.4	15.0	1.1	2.3	12.4	2.4	12.5	17.6

[1] Scale 1 to 7 where 1=no disease and 7=severely diseased; MRF= Montcalm Research Farm and N.Y.= New York State Agriculture Experimental Station 2003 trial. [2] Scale 1 to 5 where 1=vigorous root and 5=weak root.[3] Measured as g.100seed^{-1}. DTF= days to flower and DTM= days to maturity. [4] Scale 1 to5 where 1=no lodging and 5=excessive lodging.[5] Score 1 to 9 given at maturity takes into consideration traits such as height, lodging resistance, pod load, favorable pod to ground distance, uniformity of maturity, and absence of disease, the higher the score more desirable. [6] 1=determinate growth habit, 2 and 3 = indeterminate growth habit (Singh, 1982). '-' refers to missing data due to plants that were not harvested because of severe common bacterial blight disease infection.

APPENDIX C

THE POSSIBLE ASSOCIATION BETWEEN A VIGOROUS ROOT SYSTEM AND GROWTH HABIT IN BEAN (*PHASEOLUS VULGARIS* L.) AS AN INDICATOR OF *FUSARIUM* ROOT ROT RESISTANCE

To better understand the relationship of vigorous root system to growth habit described in this discussion it is important to understand the concept of growth habit in common bean. Bean architecture is a complex character whose importance is evident due to its association with productivity. Although the architecture of bean plants has been extensively studied precise information about the genetics underlying the different phenotypes is lacking.

Growth habit in common bean is known to be genetically controlled (Koinange et al., 1996). Architecture could be divided in different characters; the presence or absence of terminal flower is a qualitative character with two alternatives (determinate growth habit vs. indeterminate growth habit). For example in *P. vulgaris*, a single gene difference separates the determinate *(fin fin)* from the indeterminate growth habit, *Fin Fin* and *Fin_* (Rudorf, 1958; Lamprecht, 1935). *Fin* is a dominant gene and the expression of a recessive *fin* produces a determinate growth habit (Norton, 1915). Determinancy, which is defined as the early transition from a vegetative terminal meristem to a reproductive one, and has multiple effects on growth habit and the life cycle as it causes the appearance of a terminal inflorescence, a reduction in the number of nodes and pods on the main stem and branches, and a shortening of the life cycle (Koinange et al., 1996). The *fin* gene has pleiotropic effects on the number of nodes on the main stem, the number of pods, and the number of days to flower and maturity. Control of twining has been

attributed previously to the gene *Tor*, distinct from the *fin* gene (Norton, 1915). Twining was correlated later with *fin* suggesting that either *fin* had a pleiotropic effect on twining or that *Tor* was tightly linked to *fin* (Koinange et al., 1996). Other characters related with architecture of the bean plant are: the ability to climb or the rotation capacity of the stem controlled by the *Tor* gene (Norton, 1915; Kretchmer and Wallace, 1978; Lamprecht, 1947); number and length of internodes, controlled by *Ico* gene (Lamprecht, 1961); presence of vines; plant height and length; number of branching, and others (Coyne, 1980).

The *Z* locus controlling partly colored seedcoats in common bean was previously thought to be tightly linked to the *Fin* gene on B1, based on a tight linkage with a determinancy gene in an Andean population (Bassett, 1997). Later the *Z* locus was mapped by McClean et al. (2002) to linkage group B3 of the integrated bean map, invalidating the tight linkage between the *Z* locus with the *Fin* gene and suggesting the existence of multiple growth habit genes in the Andean gene pool. In general results from these previous studies suggest that a second gene for determinate growth habit tightly linked to the *Z* locus (different than the *Fin*) exists in common bean. This gene has not yet been named. Tar'an et al. (2002) using a population of Middle American origin suggested that a second gene for determinate growth habit (*GH* unnamed locus) exists in bean, and the second gene is located in a different linkage group (B9) than the *fin* gene (B1). More recently from a population developed from a cross between Bunsi and Newport, two small seeded navy bean cultivars from Middle American gene pool, the determinate growth habit mapped to B7, and represented a novel source of determinancy in navy bean (Kolkman and Kelly, 2003). The likelihood of two loci

conditioning determinate growth habit in navy bean is unlikely. Given the extensive distance (32.3 cM) between marker and growth habit on B9 (Tar'an et al., 2002), data on actual location needs additional confirmation in Middle American germplasm.

There are also examples of environment dependent changes of the growth habit in some plant species. Determinate cowpea (*Vigna unguiculata*) elongated and became indeterminate under night temperatures of 24°C under a 12h photoperiod (Summerfield and Wien, 1980). This same phenomenon was observed in lines of *P. vulgaris*, which were found to be unstable for plant growth habit, and changed from an indeterminate bush to climbing type in some specific environments (Kretchmer et al., 1977). The instability of plant growth habit was due to a photoperiod response, which was due to a photomorphic response under the control of the phytochrome. Photoperiod sensitivity (*Ppd*) is linked to the *fin* locus in common bean (*Ppd*; Wallace et al., 1993; Koinange et al., 1996). The major gene controlling the photoperiod response in common bean was different from the gene controlling the change from indeterminate bush to climbing growth habit (Kretchmer et al., 1977). Judging from the plasticity observed one could hypothesize that the branching of indeterminate plants is in fact a determinate type from which lateral shoot grows vigorously assuming a terminal position and the initially terminal reproductive meristem appears in a lateral position. Therefore the indeterminate growth is only an outward appearance due to strong vigor of lateral growth and is only a morphological variation derived from the determinate growth.

Dry bean breeding has focused on modifying architecture to increase yield, standability, pest resistance (e.g. modification of trichomes of leaves to reduce population of leafhoppers), and disease avoidance associated with white mold resistance (Coyne,

1980; Kelly, 2000). Breeders have focused for many years on the vegetative portion of the bean plant when breeding for disease resistance. Limited attention, however, has been given to the root system. Plants vary enormously in the morphology and physiology of their roots and these roots are directly affected by the environment. Researchers have only begun to understand the diversity in root form and function as well as the major role they play when a plant is under stress (Eissenstat et al., 2000). Casson and Lindsey (2003) stated that "plants are polar structures....the shoot apical meristem represents a source of undifferentiated cells that divide and contribute to the new leaf primordial during vegetative growth, and to influorescence and floral meristem during the reproductive phase....the root also produces lateral structures, but these are not the direct products of the primary root meristem". So, when breeding for resistance to root rot caused by *F. solani*, breeders should examine more closely the possibility of breeding for specificroot characters or overall root health.

Field studies have demonstrated that there are differences between bean classes for root characters that contribute to root rot resistance (Snapp et al., 2003; Román-Avilés et al., 2004). Many of these characters appear to act as a mechanism of survival when the pathogens take over the root system, e.g. adventitious root formation thought to be for anchoring, water, and nutrient acquisition. Other characters such as root dry weight have proved to be good indicators of root rot resistance and more reliable (quantitative measure) than the conventional rating scale commonly used in the field evaluations (Kmiecik and Bliss, 1986; Román- Avilés et al., 2004; Hall and Phillips, 2004).

Dry beans with a determinate growth habit exhibit a shallower root system (Kelly, 1998), whereas plants with an indeterminate growth habit exhibit a more dominant deeper taproot. Similarly the indeterminate type II and type III black bean lines, tended to have more adventitious roots and a deeper, more extensive root system (Lynch and van Beem, 1993). Also a tight relationship between shoot and root growth habit in bean genotypes has been identified by Lynch and van Beem (1993). Experiments using grafted bean plants have shown that differences in seed yield have been associated with the genotypes of rootstock, but not with that of the shoot (White and Castillo, 1989). These results agree with the extensive grafting work conducted to improve root system of fruits, nuts, and ornamental plants (Hartman and Kester, 1968). More recently, White and Castillo (1992) found that among bean genotypes of similar growth habit and phenology, variation exist in shoot characteristics that affected harvest index, phenology, and yield under water deficit stress. However, this variation appeared to be much less important than variation in characteristics of the root system.

Field observations on the light red kidney bean genotype Chinook 2000 (Kelly et al., 1992; Kelly et al., 1999) showed that this large seeded bean had variability for a very vigorous root system. Plants of Chinook 2000 with a vigorous root showed a significant higher level of root rot resistance, although they exhibited a determinate growth habit (Figure C.1). This observation lead to re-selection of Chinook 2000 for root rot resistance based on the vigorous root system observed during the summer of 2002 and 2003 and the selected plants are currently being evaluated for root rot resistance and yield. This same observation was observed in the genotype FR266 with a

Figure C1. Chinook 2000 is a light red kidney bean genotype released developed at Michigan State University. This genotype was segregating for a very vigorous root system and had a significant higher level of root rot resistance.

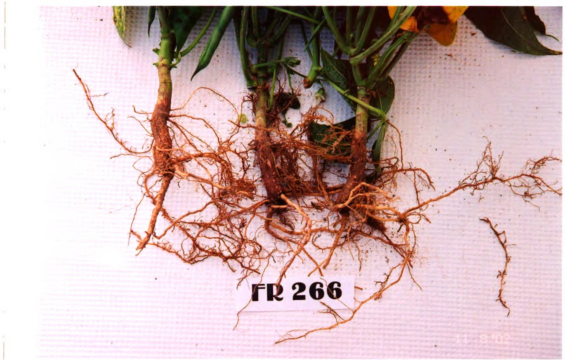

Figure C2. FR266 is a bush snap bean developed by USDA/ARS. This genotype was breed for root rot resistance with a determinate growth habit.

determinate growth habit (Silbernagel, 1987) which was breed as a root rot resistant genotype (Figure C2). Interestingly both of these genotypes have black bean parents in their genetic background and it is thought that the vigorous root system came from their respective black bean parents. CN49-242 which is an indeterminate black bean that is used as an anthracnose [caused by *Colletotrichum lindemuthianum*] differential was used as a parent of Chinook (Kelly et al., 1992). PI 203958, which is a wild, small-seeded vine black bean from Mexico, was used multiple times as a donor in crossings that lead to the development of FR266 (Silbernagel, 1987). The two bean genotypes with determinate growth habit and vigorous root system are examples that the tight relationship between shoot and root suggested by Lynch and vanBeem (1993) was broken.

The uncoupling of these two characters suggests the occurrence of pleiotropic effect. A gene can influence many phenotypes and when one gene affects several aspects of the phenotype, it is said to be pleiotropic. There is clear pleiotrophic effect of *Fin* gene on shoot characteristics other than roots (Koinange et al., 1996), but these two determinate growth habit genotypes possess a vigorous root system, a characteristic normally found in plants with the indeterminate growth habit.

Although Chinook 2000 and FR266 have a very vigorous root system similar to a root system of genotypes with the indeterminate growth habit, these genotypes still lacks root vigor of an indeterminate plant. It appears that the vigorous root system character was not fully transferred to these determinate growth habit genotypes. A possible explanation for this observation could be the same possible pleiotropic effect of

determinate growth on other characters related with the architecture system of the bean plant. Other characters affected by pleiotrophic *Fin* gene are number and length of internodes, presence of vines, plant height and length, and number of branches. The growth habit genes may also have an effect on the development of the root system, but the association might not be as direct as that proposed by Lynch and van Beem (1993). Although this vigorous root system is composed of many other characters such as primary root, secondary and tertiary roots, root hairs, angles and radii, all of these characters will have an effect on overall root architecture.

A second possibility is that there may be a gene(s) in the indeterminate growth habit that is responsible for the full development of the vigorous root system and that the determinate growth habit is either lacking this gene(s) or if present it is not being fully expressed. This gene might require another gene for its full expression (e.g. complementary gene(s) or a non-specific gene(s)) that is only present in the indeterminate growth habit, or this gene(s) may be masked by another gene present in the determinate growth habit. An example would be that intergene pool crosses may result in dwarfing which results from the dosage dependent effect of the dominant alleles of two complementary genes, *Dl*-1 and *Dl*-2 (White et al., 1992). Grafting studies by Shii et al. (1981) demonstrated that *Dl*-1 acts in the root system while *Dl*-2 acts in the shoot, making the *Dl* system a unique example of where two genes have been demonstrated to control a strong root-shoot interaction. When a plant is heterozygous at both loci, growth is severely reduced, and when a plant is homozygous dominant, lethal dwarfing occurs. *Dl*-1 is found in germplasm of Mesoamerican origin, while *Dl*-2 occurs mainly in Andean germplasm (Singh, 1989), and has a strong influence on growth. There still needs to be

additional research to determine whether additional alleles or genes affect the dwarfing response. When studying seedling roots of common bean some association of shoot habit and root architecture was observed, as the climbing genotype HAB 229 had a more dominant tap-root system whereas the prostrate genotype Carioca had a large number of root meristems (Lynch and van Beem, 1993).

Regardless of whether the vigorous root from the indeterminate growth habit bean was or not fully transferred to the determinate growth habit bean, 'Chinook 2000', exhibits partial resistance to *Fusarium* root rot. Breeders have been successful in transferring root traits that lead to a vigorous root in 'Chinook 2000'. In the attempt to transfer resistance to root rot, breeders have indirectly selected for root traits. An extensive breeding effort would therefore, be required to introgress the desired root developmental or morphological characteristics from a small seeded cultivars of Middle American gene pool to the large seeded cultivars of the Andean gene pool, in such a way that agronomically satisfactory breeding material is obtained. This introgression has been to be proven partially possible with 'Chinook 2000' and 'FR266'. Transfering the vigorous root system found in these large seeded bean into other large seeded market classes by using these two genotypes as donor parents for the vigorous root system, should hasten efforts in breeding of other root rot resistant genotypes. Root genetic studies have received limited attention, but has much promise when it comes to improving root rot resistance in common bean.

In an attempt to introgress the desired root vigor from the small seeded bean to the large seeded bean class, breeders could employ an inbred-backcross method of breeding or by single cross using either one of these two large seeded bean genotypes as

parents and selecting for the desirable phenotype. The advantage of using the inbred backcross method is that no selection is conducted during the breeding process and breeders are able to recover the desired phenotype and seed size of the recurrent parent faster than by single crosses. Usually three backcrosses to the recurrent parent should be enough to obtain IBLs, which are agronomically acceptable with the desired seed size, desired root vigor, and a determinate growth habit and can be advanced by using single seed descent. The inbred-backcross method of breeding was used successfully to transfer the beneficial root growth of an exotic bean for phosphorus efficiency, into the adapted cultivar Sanilac (Gabelman et al., 1986).

Another alternative would be the phenotypic recurrent selection scheme used by Brothers and Kelly (1993) and Kelly and Adams (1987) to combine the architectured characteristics of the small navy bean ideotype with seed size, shape, and color of the pinto bean. Breeders could employ phenotypic recurrent selection to combine the determinate growth habit of the large seeded with the vigorous root architecture of the indeterminate genotype. This alternative would require more work than single crosses and backcross methods, but it have been successfully used to combine traits from distant genetic backgrounds (e.g. different gene pools). This breeding system may not be the most adequate for root rot resistance since it involves single selection. Breeding for quantitative traits involve replicated trials in different years and locations (multisite testing) that should be perform during the breeding process until the desired level of homozygosity is obtained not only to prevent loss of desirable alleles with selection but also to fix the desired alleles in a more homozygous background.

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APPENDIX D

DEVELOPMENT OF A SCAR MARKER LINKED TO A QTL ASSOCIATED WITH DETERMINATE GROWTH HABIT

Introduction

Growth habit is an important trait in common bean controlled by the *fin* gene (Lamprecht, 1935). The *fin* gene, which conditions the early transformation of terminal meristems from vegetative to a reproductive state (determinate growth habit is a prominent gene trait present in the Andean gene pool) has been mapped to B1 of the integrated bean map (Koinange et al., 1996; Freyre et al., 1998). Moreover, the *fin* gene has pleitropic effects on the number of nodes on the main stem, the number of pods, and the number of days to flower and maturity (Koinange et al., 1996). The probable existence of multiple genes controlling growth habit in common bean has been previously discussed (McClean et al., 2002; Bassett, 1997).

Additional controversy regarding the determinacy gene in Andean bean germplasm, centers on the *Z* locus controlling partly colored seedcoats in common bean. The Z locus is tightly linked to a determinancy gene, previously thought to be *Fin* (Bassett, 1997). The *Z* locus was mapped by McClean et al. (2002) to linkage group B3 of the integrated bean map, disputing the tight linkage of the *Z* locus with the *Fin* gene and suggesting the existence of multiple genes controlling growth habit. Results from these previous studies suggest that an second unknown gene for determinate growth habit in Andean germplasm tightly linked to the *Z* locus (different than the *Fin*) exists in common bean.

Red Hawk, a dark red kidney bean highly susceptible to *Fusarium* root rot was used as the recurrent parent in the development of kidney inbred backcross lines (IBL) population to study the QTL for resistance to root rot. Resistance was being introgressed from an indeterminate small black bean (Negro San Luís) from the Middle American gene pool, highly resistant to *Fusarium* root rot, into the large seeded determinate Red Hawk from Andean gene pool. Red Hawk was screened with the OAM10.490 RAPD marker tightly linked to the *Z* locus (Brady et al., 1998) and crossed with seed coat pattern testers which reveal segregation for the recessive *z* allele (Emmalea Ernest, personal communication). It is believed that Red Hawk carries a recessive allele conferring determinancy at the unnamed locus linked to *z*, since crosses between Red Hawk and the partially-colored Yellow Eye revealed tight linkage between indeterminancy and the Yellow Eye seed coat pattern (Ernest and Kelly, 2004).

The AJ4.350 RAPD marker was found to be linked to a QTL associated with growth habit that accounted for up to 52% ($P<0.0001$) of phenotypic variability in the current study (see Chapter IV of current manuscript). Interestingly the QTL was located on linkage group B2 of the integrated bean map. Results from crosses between Red Hawk and Yellow Eye suggested the possibility that the AJ4.350 marker may not reside on B2 since Red Hawk carries the recessive z allele indicating that Red Hawk could also carry the unnamed growth habit locus tightly linked to *Z* locus on B3. It is possible that the DNA fragment amplified in the BAT93 x Jalo EEP558 (BJ) mapping population was not the same fragment tightly linked to a QTL associated with growth habit originally shown to be present in the kidney IBL population.

The use of RAPD markers in mapping is restricted because they are very sensitive to variations in reaction conditions making them non reproducible across laboratories. A precise match between the RAPD and the template DNA depends on the choice of the heat-stable DNA polymerase and thermal cycler, the concentration of magnesium, and the annealing temperature (Gu et al., 1995; Melotto et al., 1996). To confirm the mapping position of the AJ4.350 on B2 the RAPD marker was converted into a sequence characterized amplified region (SCAR) DNA marker to determine its true location in comparison with determinate growth habit genes that have been previously mapped. SCAR markers are relatively easy to score (appear as a single easy to score band) and are designed for specific PCR amplifications of polymorphic fragments. These markers are longer than RAPD markers (over 20 nucleotides) and require a highly stringent annealing temperature that prevents mismatching in an the priming site during DNA amplification (Paran and Michelmore, 1993).

The objectives of this study were i) to clone and sequence the DNA fragment amplified by RAPD marker AJ4.350, ii) to develop a SCAR marker with more specificity than the RAPD AJ4.350 and iii) to map the SCAR marker in the kidney IBL and the BJ RIL populations.

Materials and Methods

RAPD analysis

A QTL associated with determinate growth habit was identified by the combined mapping strategy composed of BSA technique (Michelmore et al., 1991) and selective genotyping (Lander and Botstein, 1989) in inbred backcross line ($BC_2F_{4:5}$) kidney

population. Development of this population and its evaluation for resistance to root rot and growth habit have been described previously in Chapter IV of the current manuscript and complete data collected for the kidney IBL population can be found in Appendix B3 and B4.

Cloning and Sequencing of the RAPD fragment

The AJ4.350 RAPD amplification product (Figure D1) linked to the QTL associated with growth habit was purified by using the QIAquick™ Gel Extraction Kit for cutting and purifying the DNA fragment (Quigen, Chatsworth, Calif.), cloned by means of the TOPO TA Cloning® Kit (Invitrogen Carlsbad, CA) and the Wizard® Plus SV Minipreps DNA Purification System from Promega for plasmid extraction. Cloned amplification products were sequenced by the Michigan State University sequencing facilities following the procedure described by Melotto et al., (1996). The plasmid vector used was pCR®4-TOPO® which was transformed into the competent *E. coli* cells. Double stranded DNA sequencing was carried out using M13 fluorescently labeled primer.

SCAR analysis

SCAR marker for the AJ4.350 RAPD marker were generated and tested initially in the parents of the kidney IBL population (Red Hawk and Negro San Luís) and BAT93 and JaloEEP558 RIL population. Amplification of the DNA fragment by the SCAR primer was carried out utilizing the PCR procedures on Table D1. Various Mg^{++} magnesium concentrations and annealing temperatures were tested. The presence or absence of DNA amplification in the parental genotypes and in selected samples from the

IBL population was confirmed for the AJ4.350 RAPD and its derived SCAR marker SAJ4 using *Taq* DNA polymerase (Invitrogen™ Life Technologies, MD).

Table D1. Illustration of the PCR amplification conditions for the DNA fragment of the SCAR marker developed.

Amplification conditions	Standard and initial† PCR conditions	Modified PCR conditions‡
Reaction	$MgCl_2$ 4 mM, dNTPs mix 0.2 mM each, 30ng of primer mix, 30ng of template DNA, 1.25 units of *Taq* DNA polymerase, and PCR buffer minus Mg 1X; total of 30µl which were completed with water	The following concentrations were tested 4.8mM, 4.0mM, 3.75mM, 3.5mM, 2.5mM, 2.0mM and 1.5mM; the difference in Mg^{+2} was added to the final water volume
Traditional 3-step PCR procedure	34 cycles 2 min at 94°C/ 30s at 94°C/ 30s at 65°C/ 30s at 72°C, and 4°C soak	(34 cycles) 30s at 59°C (34 cycles) 30s at 64°C (34 cycles) 30s at 65°C (34 cycles) 30s at 66°C (34 cycles) 30s at 67°C (34 cycles) 30s at 69°C (40 cycles) 30s at 66°C
Touch down§ PCR procedure	11 cycles 2 min at 94°C/ 30s at 94°C / 30s at 64°C (1°/ cycle)/ 30s at 72°C/ then 17 cycles 30s at 94°C/ 30s at 52°C/ 30s at 72°C and 4°C soak	(11 cycles) 30s at 64°C; (17cycles) 30s at 52°C (12 cycles) 30s at 67°C; (22 cycles) 30s at 54°C

† Underlined sections correspond to the portion of the reaction that was changed.
‡ Modified PCR reactions and annealing temperatures and cycles
§ Touchdown PCR involves decreasing the annealing temperature by 1°C/ cycle to a 'touchdown' annealing temperature which is then used for 10 or so cycles

Results and Discussion

The specific SCAR primers were synthesized based on the DNA fragment sequence amplified by the RAPD marker and contained the original 10 bp of the RAPD marker plus an additional internal 14 bp (Table D2).

The SCAR marker SAJ4 amplified DNA fragment of the expected size (350 bp) and appeared as a single monomorphic band in agarose gel (Figure D2). It is considered monomorphic due to the presence of the expected fragment size in both the determinate (Red Hawk) and the indeterminate (Negro San Luís) genotypes. Due to the monomorphic amplification several different approaches were taken to produce the desired polymorphism. Modified PCR conditions, including various Mg^{++} concentrations and annealing temperatures (Table D1), did not produce polymorphic SCAR products between the respective genotypes. An additional SCAR primer was developed but no primer produced a clear polymorphic fragment with the expected size as the one shown in Table D2. The SCAR primer on the table below gave the best amplification with the correct fragment size 350 bp of the original RAPD marker.

Table D2. SCAR marker developed from the AJ4 RAPD marker.

SCAR Marker	Primer	Sequence†
SAJ4	SAJ4.3FP	5'-CAC CAT TCC AAC CAC TTA CGC TGG -3'
	SAJ4.3RP	5'- GAA TGC GAC CGA AGA TAT TTA TC -3'

† Underlined sequence correspond to the original RAPD marker sequence.

RAPD polymorphisms presumably result from differences in nucleotide sequence at priming sites. In converting RAPD to SCARs markers, Paran and Michelmore (1993) found that six of nine RAPD polymorphisms were derived from mismatches in one or a few nucleotides in the priming sites. These nucleotides mismatches in the priming site were tolerated by extended SCAR primers producing undifferentiated amplification

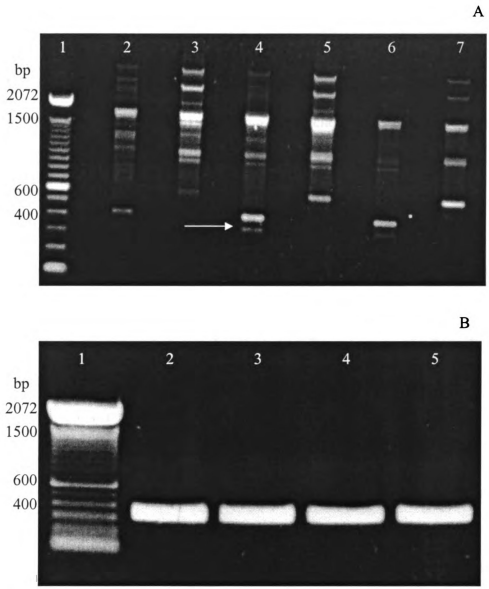

Figure D1. Illustration of: (A). RAPD fragment amplification for the AJ4.350 that was used for cloning, (numbers on columns indicate: 1= DNA 100bp ladder, 2 and 3 are lines from the kidney inbred backcross line population, 4=Red Hawk, 5=Negro San Luís, 6=JaloEEP558; and 7=BAT93; white arrow shows the DNA fragment that was cloned from Red Hawk). (B). The amplified fragment using the specific SCAR primer developed from the AJ4.350 RAPD, (numbers on columns indicate: 1= DNA 100bp ladder; 2, 3, 4, and 5 correspond to Red Hawk, Negro San Luís, JaloEEP558, and BAT93 respectively).

products from both parents. Likewise, the original SCAR primers for the growth habit QTL amplified a single monomorphic PCR product. When optimizing PCR parameters, modification of annealing temperatures and Mg^{++} concentrations are generally considered to be most effective (Horejsi et al., 1999; Paran and Michelmore, 1993; Zhang and Stommel, 2001). Increased annealing temperature most of the times can eliminate amplification of the allele from one of the parents by taking advantage of differing amounts of mismatches within a primer sequence.

In the present study, a number of PCR conditions including various annealing temperatures and Mg^{++} concentrations were tested but failed to differentiate parental genotypes with these primers tested (Table D.1). SCAR primers can also be redesigned to generate polymorphism but in this case they also failed to produce polymorphic fragments in the present study.

SCARs have several advantages over RAPD markers in marker-assisted selection (MAS). Because more stringent reaction conditions are used, the SCAR markers are generally more allele specific. The SCAR amplifications are more stable and reliable, and more easily reproduced in different laboratories with various thermal cyclers. It was unfortunate that the desired polymorphic fragment was not amplified with the SCAR primer since the information provided could have helped in confirming the precise location of the growth habit QTL which unexpectedly mapped to B2 instead of B1 or B3 where the Andean determinancy growth habit genes (*fin* and un named determinancy gene tightly linked to the *Z* locus) were previously mapped (Freyre et al., 1998; McClean et al., 2002).

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APPENDIX E

MARKER ASSISTED BACKCROSS AS A STRATEGY FOR THE SIMULTANEOUS INTROGRESSION OF *I* AND *CO-4*^{2} RESISTANCE GENES INTO "TEBO"

Introduction

'Tebo' is a specialty bean (*Phaseolus vulgaris* L.) class from Japan with small to medium white seed (30 g/100seed) and a determinate (Type I) growth habit. This bean class is highly susceptible to bean common mosaic virus (BCMV) and anthracnose (caused by the fungus *Colletotrichum lindemuthianum*). In 2001 all production fields of 'Tebo' in Michigan were infected with BCMV. The virus observed in 'Tebo' seed imported from the state of Washington appears to be a new strain, NL-4 type of BCMV. The virus problem could threaten bean production in Michigan and developing virus resistant cultivars of Tebo would provide a solution.

Anthracnose and BCMV are two of the most widespread and economically important diseases of common bean. Anthracnose is a seed-transmitted pathogen that exhibits extensive physiological variability, which causes genetic resistance to be less durable (Balardin and Kelly, 1998). Although once a severe problem for Michigan bean growers, development of resistance, improved seed production, and certification programs has reduced the threat of anthracnose. However, the disease still exists and is present in susceptible varieties every year and occasionally causes severe losses. Resistance provided by *I* gene is being used frequently in the development of new bean varieties because it provides resistance to all strains of BCMV. The systemic necrosis symptom (black root reaction) caused by BCMNV and top necrosis caused by BCMV

occurs in bean varieties with the *I* gene. One of the main efforts of the dry bean breeding program is the immediate incorporation of the *I* gene into all susceptible varieties. The second step would be protection of the *I* gene resistance to BCMNV and pyramiding of the *I* gene and recessive resistance genes to provide durable resistance in 'Tebo'. Seeds infected with anthracnose and BCMV, when planted, serve as the source of infection for succeeding crops. Even a low percentage of infected seed can result in the introduction and buildup of new strains of anthracnose and BCMV in Michigan. Genetic resistance is the only economically and effective strategy to control these two seed borne diseases.

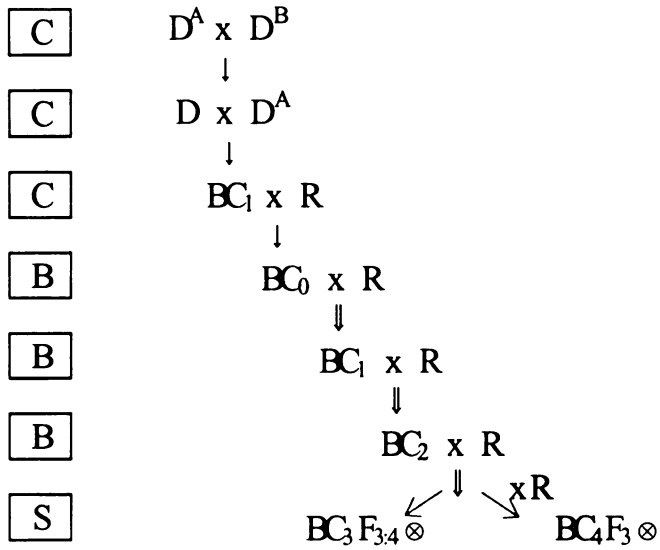
The development of molecular markers linked to major disease resistance genes provide an opportunity to indirectly select for resistance. SCAR-markers tightly linked to the *I* gene and *Co-4*^{2} gene have been developed (Melotto et al., 1996; Young et al., 1998) and shown to be effective across a wide range of germplasm in both gene pools (Middle American and Andean). These markers are increasingly valuable for indirect selection of resistance genes when introgressing these two major genes into susceptible genotypes. Introgression is the process of incorporating a small amount of genetic material from one species to another or among same species. This can be accomplished by repeated backcrossing of the interspecific F_1 to one of the parents, in this case 'Tebo'. The backcrossing is followed by marker assisted selection (MAS) which will advance the selection process for the genes of interest during early generations of the breeding process (Knapp, 1998; Kelly and Miklas, 1998). The *Co-4*^{2} gene exhibit the broadest resistance to anthracnose in common bean, whereas the *I* gene has been employed to provide effective resistance to all strains of BCMV.

For the development of the population, parents that possessed the *Co-4*^{2} and *I* genes were used. The major objective was to introduce by MAS-backcrossing the *I* gene into 'Tebo' but since there is a possibility to transfer resistance to anthracnose (at the same time) an attempt was made to transfer both genes simultaneously using MAS. We hypothesized that with effective genetic sources of resistance for these two diseases, the marker-assisted backcrossing technique would enable us to transfer resistance to 'Tebo' in a relatively short time frame.

Materials and Methods

Genetic Material

The genetic material was developed following a breeding plan similar to the Breeding Plan B presented by Frisch and Melchinger (2001), with some modifications (Figure E.1). Initial crosses were made between 'Matterhorn' and SEL 1308 and G99750 to merge the two target genes into one individual. SEL 1308 obtained from the International Center for Tropical Agriculture (CIAT) carries the *Co-4*^{2} gene and was used as the source of resistance to anthracnose (Melotto and Kelly, 2001). 'Matterhorn' and the Great Northern breeding line, G99750 possess the *I* gene and *bc-3* gene for BCMV resistance. Three BC_1F_1 lines from a backcross to 'Matterhorn' were selected and crossed to 'Tebo' during fall, 2001. The first backcross to 'Tebo' was made during winter of 2001 and the second backcross was made during spring of 2002 (Tebo//G99750/A810, Tebo//Matterhorn*2/ A815, Tebo//Matterhorn*2/ A814 where:



Symbol description:
D^A and D^B are 'Matterhorn' and SEL 1308 respectively with the target genes
D = donor lines with the target genes (A814, A815, A818)
R = recipient line or genotype ('Tebo')
↓ = no selection
⇓ = marker-assisted selection only for the presence of the target genes
x = crossing
⊗ = selfing
C,B,S inside boxes= generations with crossing, backcrossing, and selfing, respectively

Figure E1. Breeding Plan for the marker-assisted backcrossing of *Co-4*^{2} and *I* genes into 'Tebo'.

A810=Red Hawk/ SEL 1308, A814= Matterhorn/ SEL 1308, and A815= Matterhorn/ SEL 1308). There was a possibility of transferring *bc-3* gene to 'Tebo' since the gene was present in the G99750 parent. Kelly (1997) explained that plants carrying the *I* and *bc-3* suggested gene combination, when inoculated with NL-3 strain of BCMNV shows no reaction or symptoms and no virus can be recovered from these plants after back inoculation. The *bc-3* gene is very effective in preventing the virus replication in the plant tissue so the *I* gene is not induced to cause a hypersensitive resistance reaction. Since there is no effective marker for *bc-3* gene we proceeded to inoculate with the NL-3 strain those plants that resulted from the Tebo//G99750/A810 initial crosses. Seeds free of anthracnose and BCMV will prevent the spread of both diseases; reduce yield loss and maturity delay due to BCMV, and loss of seed quality due to anthracnose.

Marker assisted selection (MAS) using SCAR markers

SCAR markers (DNA molecular markers) tightly linked to the *I* gene and *Co-4*^{2} gene have been identified and were used after each backcross to indirectly select for resistance to BCMV and anthracnose (Melotto et al., 1996; Young et al., 1998). SCAR-marker assisted selection was employed starting in BC_1 to Tebo and subsequent backcrosses for selection only for the target genes (*I* gene and *Co-4*^{2} gene). DNA extraction was performed following the mini-prep procedure proposed by Afanador et al., (1993). SAS13 and SW13 SCAR markers linked to *Co-4*^{2} and *I* gene, respectively, were used to screen the segregating populations assumed to carry the target genes. The PCR profiles were as follows (Young et al., 1998; Melotto et al., 1996): (1). SW13 (expected size 690-bp) - 34 cycles of 10s at 94°C/ 40s at 67°C and 2 min at 72°C/ followed by one

cycle of 5 min at 72°C; (2). SAS13 (expected size 950-bp) - 34 cycles of 10s at 94°C/ 2.4 min at 72°C/ followed by one cycle of 5 min at 72°C

The presence or absence of DNA amplification in SEL1308, Black Magic, RWK10, and the individuals of the population were confirmed using *Taq* DNA polymerase (Invitrogen ®, Life Technologies). Those lines that showed the amplification of the single band of the predicted size (Young et al., 1998; Melotto et al., 1996; Melotto and Kelly, 2001) for both SCAR markers were used for further backcrossing to Tebo.

Results and Discussion

Transfer of resistance genes by screening during each successive backcross

Screening of the Tebo//G99750/A810 crosses for the presence of the *bc-3* gene by inoculating with NL-3 strain resulted in plants being affected by mosaic. These plants were all eliminated and only the Tebo //Matterhorn*2/ A815 and Tebo //Matterhorn*2/ A814 crosses were used in this study. When screening the BC_1F_1 individuals only one plant out of 70 BC_1F_1 individuals showed a clear distinct band for both the SAS13 and SW13 markers. This single plant (Tebo //Matterhorn*2/ A814-4) was used as male parent for producing the BC_2F_1 individuals. Backcrossing selection was performed only for the presence of the target genes. The SW13 and SAS13 marker amplified DNA fragments of the expected size and appeared as a single polymorphic band in agarose gel electrophoresis (Melotto et al., 1996; Young et al., 1998).

When screening the resulting 75 BC_2F_1 individuals, from 25 different crosses, 14 plants showed a clear band of the expected size similar to the positive control, RWK10 for the SW13 marker and 18 plants showed a clear band of the expected size similar to

the positive control SEL1308 for SAS13 marker. Twelve plants that showed a clear band for the *I* gene and a weak band for the *Co-4*^{2} gene originated from nine different crosses. The marker band for the *Co-4*^{2} gene was lost after producing the BC_2 generation. All BC_2F_1 individuals that exhibited the SW13 marker band were considered as donor parents of the *I* gene and used to produce the BC_3F_1 individuals.

After the third backcross several selfing generations (advanced to the $BC_3F_{2:3}$) were performed during fall, winter, and spring, under greenhouse conditions, in order to create homozygous lines with the target genes. A fourth backcross was made using three random individuals from the BC_3 population. Both populations were taken to the field during the summer of 2003 to advance to BC_3 $F_{3:4}$ and BC_4F_3 generations. It is important that both target genes are recovered in each cross and backcross generation, and that a homozygous individual with both target genes (*I* gene and *Co-4*^{2} gene) is present in the progeny of the selfing generation.

MAS was used to rapidly advance the selection process of backcross work during early generations of the backcross breeding. Marker-assisted backcrossing for disease resistance will most likely have little or no effect in reducing the need for replicated field trials and testing (Yu et al., 2000). This means that plant breeding expertise and decision making ability will remain important for future genetic improvement. For this reason final testing of breeding lines for disease resistance, by greenhouse screening and field evaluation, is always required regardless of how tightly a marker might be linked to a gene. For example, in the current work SAS13 is tightly linked to *Co-4*^{2} but the fragment amplified by this SCAR marker was lost. This doesn't necessarily mean that the resistance gene is not there but will need to be verified through direct inoculation.

Alternatively, the absence of SAS13 fragment during the development of the population could have resulted from the loss of the marker due to a recombination (cross-over) between the marker and the gene. Loss of the marker due to recombination could be possible since the SAS13 marker was found to be 3.5 cM from the *Co-4*^{2} gene (Young et al., 1998), although 4.5cM is considered a very tight linkage for MAS.

Greenhouse population screening with BCMV

Direct greenhouse screening of 385 BC_3 $F_{3:4}$ and 46 BC_4F_3 individuals for BCMN was performed using the NL-3 strain of BCMV. The susceptible class comprises plants with mosaic indicating the absence of resistance genes while those plants that exhibit top necrosis suggested the presence of the single *I* gene. In the BC3 (Tebo/3/Matterhorn *2/ A814) and BC_4 (Tebo/4/Matterhorn *2/ A814) populations, the cross was successful in producing plants that showed top necrosis indicating that the *I* gene was inherited and was present in successive generations. After the greenhouse screening, the resistant plants were sent to the Puerto Rico winter nursery for seed increase and generation advance. Resistant lines carrying the *I* gene will be inoculated with Race 7 of *C. lindemuthianum* cultures, since 'Tebo' was susceptible to this race and there is still a chance of identifying progeny carrying the *Co-4*^{2} gene for anthracnose resistance. These resistant 'Tebo' plants will be included in preliminary yield trials during the summer of 2004. The final step will be to combine *I* gene with other strain specific recessive resistance genes to give complete resistance against BCMV and provide more durable virus resistance in 'Tebo'. If resistant individuals in the population are not identified after screening with Race 7 for anthracnose resistance the *Co-4*^{2} gene will have to be re-

introduced into new virus resistant 'Tebo' cultivars with the *I* gene using other markers linked to the *Co-4*^{2} gene such as SH18 and SBB14 (Awale and Kelly, 2001).

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