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dissertation entitled

QTL ANALYSIS OF GENETIC RESISTANCE TO WHITE
MOLD (*SCLEROTINIA SCLEROTIORUM*) IN COMMON
BEAN (*PHASEOLUS VULGARIS*)

presented by

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has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Plant Breeding and Genetics
Crop and Soil Sciences

James D. Kelly
Major Professor's Signature

07/02/2003
Date

QTL ANALYSIS OF GENETIC RESISTANCE TO WHITE MOLD (*SCLEROTINIA SCLEROTIORUM*) IN COMMON BEAN (*PHASEOLUS VULGARIS*)

By

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A DISSERTATION

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

2003

ABSTRACT

QTL ANALYSIS OF GENETIC RESISTANCE TO WHITE MOLD (*SCLEROTINIA SCLEROTIORUM*) IN COMMON BEAN (*PHASEOLUS VULGARIS*)

By

Marcio Ender

White mold, caused by the necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) De Bary, is a serious disease of common bean (*Phaseolus vulgaris* L.). The use of resistant varieties is a preferred disease control strategy for white mold but has been a difficult management strategy to implement as no major genes for resistance have been identified in common bean. The objectives of this study were to: i) to identify AFLP and RAPD markers linked to quantitative trait loci (QTL) associated with resistance to *S. sclerotiorum* in a Middle American mapping population of common bean; ii) map the putative QTL to the bean integrated map; iii) identify QTL associated with important agronomic traits and their relationship with resistance to white mold; iv) test the potential of marker assisted selection using previously identified markers linked to QTL for resistance to white mold in bean, v) introgress genetic variability from landrace and wild genotypes into adapted genetic background of cultivated common bean.

The Bunsi/Raven RIL population was evaluated for AFLP and RAPD markers linked to QTL for resistance to white mold in the field and greenhouse. Multi-trait bulking segregant analysis including disease severity, yield and days to flowering was used to detect markers linked to QTL for resistance to white mold.

QTL for disease severity index (DSI) using composite interval mapping were detected on linkage groups B2, B5, B7 and B8. The QTL for DSI on B2 and B7 supported results from previous studies, suggesting that QTL for DSI are located in those genomic regions and can be used in marker assisted selection. Important QTL were detected for agronomic traits: days to maturity (R^2=28.5%) on B2, days to flowering (R^2=35.7) on B7, lodging (R^2=29.8) on B7, seed size (R^2=20.1%) on B2 and (R^2=25.7%) on B7 and yield (R^2=9.7%) on B2, and (R^2=12.7%) on B8, and (R^2=21.5%) on B7.

Marker assisted selection (MAS) was performed in a Bunsi/Midland recombinant inbred line (RIL) population using previously identified markers linked to QTL for resistance to white mold, in the cultivar Bunsi. The two groups of RILs (selected and control) resulting from MAS were significantly different for DSI (p=0.03), supporting the potential use of MAS to improve resistance to white mold in common bean.

The inbred backcross method was used to introgress into adapted bean varieties putative resistance to white mold from exotic germplasm including landraces and wild bean types. Two populations were selected, based on visually rated agronomic traits, from seven populations of inbred backcross lines. The two selected populations demonstrated putative resistance to white mold in greenhouse using the straw test. The sources of resistance identified in this study varied in defense response proteins and will be used to further enhance resistance for white mold in common bean.

DEDICATION

To Andrea, Anita, Afonso, Geneci and Georgius.

ACKNOWLEDGMENTS

I would like to express a very special thanks to my major advisor, Dr. James D. Kelly, for giving me the opportunity to pursue the Ph.D. degree in his bean breeding program. His friendship, unsurpassed knowledge regarding common bean and experience in plant breeding made me fortunate for having such an example to follow as a mentor. I wish to thank the committee members Dr. Amy Iezzoni, Dr. David Douches and Dr. Ray Hammerschmidt for their contributions during the development of my research project. I thank the faculty members of Crop and Soil Sciences Department and Plant Breeding and Genetics program, especially Dr. Dechun Wang for all support during the QTL analysis.

I want to thank the bean group, Jerry and Norm for all assistance in the white mold trials, Mark, Veronica, Pat, Halima, Shitaye, Andrea, Emmalea, Belinda, Gerardine, Celeste, Esther, Judy, Maeli, Eveline and Qi for their friendship, help in the field and in the lab and interesting discussions.

Thanks to my friends in the Brazilian and Latin American Communities for being my family in Michigan and all the support.

I am very thankful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Ministry of Education of Brazil for the financial support and to Santa Catarina State University for the opportunity to pursue the Ph.D. Degree.

I thank my family and friends and especially my wonderful wife Andrea for all the love, friendship and support.

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INTRODUCTION

Bean is one of the most important food crops in many countries of Latin America and Africa, where it provides an important source of calories, proteins, dietary fibers, minerals, and vitamins. Among the major food legumes the common bean (*Phaseolus vulgaris* L.) is the third most important worldwide, only surpassed by soybean and peanut. Considering only grain legume food crops that are harvested for dry seeds, the common bean is the most important. Annual per capita consumption of dry bean is as high as 60 kg in Rwanda (ISAR, 2001), and almost 17 kg in Brazil and Mexico (FAO, 2002). Consumption is increasing in the U.S. and Canada (4 kg/person/year), because of diversity in ethnic foods and a greater awareness of the health benefits of bean in the diet.

The common bean is affected by both abiotic and biotic production constraints. Abiotic limitations would include low soil fertility, high temperature and water stress, whereas the most important biotic problems would include weeds, insect pests, and diseases caused by bacteria, virus, and fungal pathogens (Singh, 1999). One of the most destructive diseases of common bean is white mold caused by the necrotrophic pathogenic fungus *Sclerotinia sclerotiorum*. Strategies for controlling white mold in common bean include use of resistant varieties, cultural practices, fungicides, and biological control. Sclerotia produced by *S. sclerotiorum* over winter and remain viable in the soil for many years (Hart and Saettler, 1981).

Field reaction to *S. sclerotiorum* is affected by physiological resistance, agronomic and phenological traits that can act as avoidance mechanisms and are highly influenced by environmental conditions. Avoidance mechanisms can limit the establishment of the fungus and disease development. Avoidance is associated with traits such as plant architecture erectness and porous plant canopy or phenological traits such as early or late flowering. Most of the morphological traits in common bean are easily scored exhibit high heritability and can be effectively selected in the field (Kolkman and Kelly, 2002). Therefore, selection for avoidance traits to white mold is feasible and genetic variability exists in most of the bean commercial classes, especially in small seeded types such as black beans. However in those regions with high humidity during the growing season, conditions can be highly favorable for fungus development and avoidance mechanisms may not be adequate to limit the infection and development of white mold disease. Since white mold is a complex disease, the combination of different approaches to improve resistance that include avoidance mechanisms with physiological resistance seems to be most promising strategy to improve resistance to *S. sclerotiorum* in common bean.

Physiological resistance is controlled by biochemical factors that limit the infection or spread of the pathogen in plant tissue. Possible basis for physiological resistance could include plant defense related enzymes, phytoalexin accumulation, or plant proteins able to inhibit important fungal enzymes. The development of resistant cultivars has had limited success because few sources of physiological resistance to white mold have been identified or used in breeding programs. The detection of physiological resistance is difficult since the reaction to the pathogen under variable field conditions, is affected by plant avoidance mechanisms. Therefore, selection for physiological resistance requires effective screening methods. Most of the screening methods proposed to detect physiological resistance to *S. sclerotiorum* in common bean are based on measuring the ability of the plant to limit fungal growth in controlled inoculation situations. These methods include the limited-term inoculation (Hunter et al., 1981), the excised-stem inoculation technique (Miklas et al., 1992a), growing callus on medium containing pathogen culture filtrate (Miklas et al., 1992b), the straw test (Petzoldt and Dickson, 1996), and the leaf-agar plug assay (Steadman et al., 1997). An indirect method to measure tolerance to oxalic acid (Kolkman and Kelly, 2000) was based on the fact that oxalic acid is the primary pathogenicity factor of *S. sclerotiorum* during the infection process (Godoy et al., 1990). The limitation of using these screening methods is the lack of consistency between methods, and weak associations with field resistance.

Taking in consideration that inheritance of resistance to white mold is complex, highly influenced by the environment, and scoring demands intensive field work, marker assisted selection (MAS) appears to be a promising approach to improve resistance to white mold in bean. MAS might be more important for physiological resistance, since desirable agronomic traits can be efficiently selected through phenotypic selection. The identification of molecular markers tightly linked to quantitative trait loci (QTL) associated with physiological resistance could assist in the development of new varieties with higher levels of resistance to white mold. The detection of QTL involves several important aspects:

1. Identification of sources of resistance to white mold that possess major QTL for resistance that may be useful in plant breeding;

2. Development of adequate mapping populations. Resistance to white mold has exhibited low heritability, and populations such as recombinant inbred lines are more valuable because they can be replicated in different years and locations, resulting in more accurate phenotypic data;

3. Methodologies are available to select candidate markers such as selective genotyping, bulked segregant analysis, and statistical tools to analyze the molecular data and phenotypic data to predict the location and contribution of QTL.

4. When QTL are detected, markers tightly linked to the QTL have to be converted into easy screening robust techniques such as SCAR markers.

MAS goes beyond the identification of markers linked to QTL controlling important quantitative traits. Effective strategies of selection are needed to implement the use of MAS in breeding programs. The generation of adequate population sizes required for selection is an important aspect that needs to be considered. Use of MAS requires large segregating populations to provide sufficient genetic variability for the selection of other important traits.

The identification of markers linked to different QTL for a complexly inherited trait such as resistance to white mold might be a unique opportunity to use MAS and combine QTL to enhance resistance to white mold.

A new approach to overcome the lack of physiological resistance to white mold in cultivated bean could be the identification and introgression of novel sources of resistance from primitive landrace varieties and related wild types of bean. Singh (1999) suggests that as much as 90% of the genetic variability available in the primary gene pool and related species remains underutilized. Thousands of bean accessions, including landraces and wild types, are stored in germplasm collections. Therefore, there is a reservoir of potential sources of novel genes to be identified and introduced in bean breeding programs. To evaluate and utilize efficiently these collections, it is necessary to identify a smaller subset or core collection that likely represents most of the genetic variation available in the entire collection.

Compared to their wild progenitors, cultivated plants display contrasting phenotypic differences that combined are known as domestication syndrome traits that are the consequence of thousands of years of selection for adaptation to cultivated environments (Koinange et al., 1996). Landraces and wild genotypes cannot be tested directly in the field for resistance to white mold due problems of adaptation and especially for the wild bean germplasm, that lack the domestication syndrome traits. Putative sources of physiological resistance to white mold from exotic germplasm are normally detected by greenhouse tests. To verify the reaction to *S. sclerotiorum* in the field it is necessary to introgress the novel genetic variability from the exotic germplasm into adapted cultivars. The inbred backcross method is recognized as an effective method to transfer more complex quantitative traits from diverse and unadapted germplasm into cultivated and productive varieties.

The introgression of physiological resistance to white mold into adapted varieties is the main objective in using exotic germplasm. To understand the different mechanisms involved in plant defense, the evaluation of plant defense related proteins present in exotic germplasm is an important approach to be conducted. To gain insight into physiological resistance and quantitative host-pathogen relationships in general, the activity of plant defense related proteins could be assayed in genotypes with different levels of resistance to *S. sclerotiorum*. β-1,3-Glucanases, chitinases and peroxidases are some of these plant defense related proteins that could be involved in general plant defense mechanisms (Van Loon, 1999).

The objectives of the research were:

1. to identify AFLP and RAPD markers linked to QTL associated with resistance to *S. sclerotiorum* in Middle American mapping population of common bean;
2. to map the putative QTL on the bean integrated map;
3. to compare the mapped QTL in this study with previously identified QTL for resistance to white mold;
4. to identify QTL associated with important agronomic traits and their relationship with resistance to white mold.
5. to test the potential of marker assisted selection using previously identified markers linked to QTL for resistance to white mold;

6. to introgress genetic variability from landrace and wild genotypes into adapted genetic background through the inbred backcross method;
7. to test the reaction to *S. sclerotiorum* and determine the endogenous and induced levels of the defense related proteins in the selected parental varieties and wild and landraces accessions.

The development of a linkage map and identification of QTL associated with resistance to white mold was conducted in a F4 derived RIL population from the cross between two small seeded bean cultivars differing in reaction to white mold, Bunsi and Raven. The putative QTL detected for resistance to white mold and the associations with other agronomic traits are discussed in chapter one.

The potential use of marker assisted selection is discussed in chapter two. AFLP and RAPD markers previously identified to be linked to QTL associated to resistance to white mold were used in a population of 96 RILs derived from a different cross between Bunsi and Midland.

Landraces and wild bean genotypes, identified as putative sources of resistance physiological resistance against *S. sclerotiorum*, were used to introgress novel genetic variability into cultivated bean varieties by developing inbred backcross lines. The greenhouse evaluation of progeny derived from these crosses for white mold resistance, and the determination of the level of endogenous and induced defense related proteins in exotic and cultivated parents are discussed in chapter three. A general discussion of strategies for future improvement of white mold resistance in common bean is presented in the final section.

References

FAO, 2002. Food and Agriculture Organization of the United Nations yearbook: production. Rome.

Godoy, G., J.R Steadman, M.B. Dickman, and R. Dam. 1990. Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris.* Physiological and Molecular Plant Pathology 37:179-191.

Hart, P.H., and A.W. Saettler. 1981. White mold of beans. Cooperative Extension Service, Michigan State University, Extension Bulletin E-892.

Hunter, J.E., M.H. Dickson, and A. Cigna. 1981. Limited-term inoculation: a method to screen bean plants for partial resistance to white mold. Plant Disease 65:414-417.

ISAR, 2001. Bean program 2001: activity review. p 1-8.

Koinange, E. M.K., S.P. Singh, and P. Gepts. 1996. Genetic control of the domestication syndrome in common bean. Crop Sci. 36:1037-1045.

Kolkman, J.M., and J.D. Kelly. 2000. An indirect test using oxalate to determinate physiological resistance to white mold in common bean. Crop Sci. 40:281-285.

Kolkman, J.M. and Kelly, J.D. 2002. Agronomic traits affecting resistance to white mold in common bean. Crop Sci. 42-693-699.

Miklas, P.N., K.F. Grafton, and B.D. Nelson. 1992a. Screening for partial physiological resistance to white mold in dry bean using excised stems. J. Amer. Soc. Hort. Sci. 117:321-327.

Miklas, P.N., K.F. Grafton, G.A. Secor, and P.E. McClean. 1992b. Use of pathogen filtrate to differentiate physiological resistance of dry bean to white mold disease. Crop Sci. 32:310-312.

Petzoldt, R., and M.H. Dickson. 1996. Straw test for resistance to white mold in beans. Annu. Rep. Bean Improv. Coop. 39:142-143.

Singh, P.S. 1999. Common bean improvement in the twenty-first century. S.P. Singh (ed). Kluver Academic Publishers, Dordrecht, The Netherlands.

Steadman, J. R., K. Powers, and B. Higgins. 1997. Screening common bean for white mold resistance using detached leaves. Annu. Rep. Bean Improv. Coop. 40:140-141.

Van Loon, L.C., 1999. Occurrence and properties of plant pathogenesis-related proteins. In: S. K. Datta and S. Muthukrishnan (eds). Pathogenesis-related proteins in plants. CRS Press: New York. p 1-19.

Chapter I

IDENTIFICATION OF QTL ASSOCIATED TO WHITE MOLD RESISTANCE IN COMMON BEAN

Introduction

White mold, caused by the necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a serious disease of common bean (*Phaseolus vulgaris*) world wide, and causes yield loss and reduced seed quality (Steadman, 1979). Strategies for control of white mold in common bean include cultural practices (crop rotation, residue management, irrigation timing), fungicide applications, biological control, and use of resistant cultivars (Steadman, 1979; Ferraz et al., 1999; Gerlagh et al., 1999; Lamey , 1996; Huang et al., 2000). Different microorganisms including fungi and bacteria have been tested as biological control alternatives. One of the most promising fungal biological control agents, *Coniothyrium minitans*, is a mycoparasite of sclerotia of *S. sclerotiorum*, and its potential as a biological control has been reported (Gerlagh et al., 1999; Whipps 2001). The use of resistant varieties is a preferred disease control strategy for white mold but has been a difficult management strategy to implement as no major genes for resistance have been identified in common bean (Steadman et al., 2000). A limited number of resistant bean varieties are available in certain seed type classes, whereas commercial classes such as pinto and great northern are highly susceptible to white mold. Sources of resistance have to be identified in small seeded cultivars and need to be introduced in different bean seed type market classes.

White mold severity in common bean is affected by interaction of environmental conditions such as temperature and soil moisture, plant avoidance mechanisms and physiological resistance. The avoidance mechanisms are associated with plant morphological traits such as upright plant architecture and porous plant canopy which affect the microclimate conditions within the plant canopy that can limit the fungal establishment and development. Upright plants and porous canopy retain less moisture inside the canopy, and this provides less favorable conditions for development of white mold (Schwartz et al., 1978). Soil moisture is a main factor controlling germination of sclerotia from *S. sclerotiorum* (Abawi and Grogan, 1975; Steadman, 1983). The number of apothecia under the porous canopy of the bean genotype Aurora was much lower than under the dense canopy of the genotype GN Tara. As a result there was a reduction in the number of ascospores available to infect senescent blossoms in the plant (Schwartz and Steadman, 1978). Ascospores germination and infection of senescent flowers, stems and pods is favored by high humidity and moisture associated with dense plant canopies, caused by high plant populations, narrow row widths, high fertility growth responses, and excess irrigation or rainfall especially during and after flowering (Schwartz et al., 1987; Lamey, 1996). Phenological traits such as early flowering and early maturity can be important plant avoidance mechanisms, since the flowering period may occur before full canopy closure or before excessive number of ascospores are present. Primary infection of *S. sclerotiorum* is from air-borne ascospores released from apothecia produced by sclerotia that over-winter in the soil. Ascospores dispersed into plant canopy require a nutrient source, such as flower blossoms, for germination (Abawi et al., 1975; Haas and Bolwyn, 1972). Infection of healthy pods, leaves and stems generally results from an infected flower that has fallen and lodged on leaf axis and come into contact with other plant tissues (Hart and Saettler, 1981). These studies indicate that disease severity of *S. sclerotiorum* could be considerably reduced by developing common bean genotypes with an upright porous type of plant architecture (Coyne, 1980).

Stiff-strawed bean genotypes with strong standing ability may have a better chance of escaping initial white mold infection. Bush and upright plant type were tested in various row widths and bush types lodged more than upright types under narrow rows. Lodging resistance of stiff-strawed upright genotypes helped lessen white mold infection. However, other traits such as canopy porosity were also effective in minimizing disease development. For example, Bunsi, an indeterminate type II bean cultivar with an open porous canopy, lodged as much as a short bush type, but had low levels of white mold infection (Park, 1993).

Many studies have attempted to associate differences in growth habit with levels of white mold infection in common bean. Beans are classified in four discrete growth habits known as types I, II, III and IV (Singh, 1982). Type I is strictly determinate, commonly referred to as a bush bean, with few nodes, highly branched and a concentrated flower set. Type II, III, and IV are indeterminate but differ in the length of vine extension, stem strength, and branch number and angle. Type II growth habit, commonly referred to as an upright short vine, has lodging resistance and a limited branching pattern maintained at an acute angle to the dominant main stem. Type III growth habit, commonly referred to as a vine type, has a more prostrate growth habit that lacks adequate stem strength to remain erect during pod fill. Type IV growth habit is a climbing type and is suited to production systems of intercropping with corn (Kelly, 2001).

Determinate navy bean genotypes such as Newport and Midland, were highly susceptible to white mold (Kolkman and Kelly, 2002) when grown under high humidity conditions. The open, porous canopy of larger-seeded Andean determinate beans; however, have been considered a resistant phenotype in semi-arid locations (Miklas et al., 2001; Park et al., 2001). Determinate plant growth habit could be a disease avoidance mechanism based on studies with near isogenic lines differing in growth habit. Near-isogenic determinate genotype GN Nebraska 1 exhibited lower white mold infection than the indeterminate genotype GN Nebraska 1 (Steadman et al., 1973; Coyne et al., 1977), whereas determinate G.N. P 92 was more susceptible than the indeterminate near-isogenic G.N. P 82. Thus, the bush growth habit per se does not decrease the incidence of infection (Schwartz et al., 1978; Coyne et al., 1977; Coyne et al., 1978). Indeterminate plant growth habit commonly has been associated with increased disease incidence and severity. Critical determinants of disease severity were the distribution of the leaf area, especially in the lower canopy levels near the soil (Schwartz et al., 1978). An important aspect that has to be considered is the location where the tests were performed. Studies conducted in the western U.S., under semi-arid conditions and irrigation, support the importance of plant architecture as an avoidance mechanism. Such mechanisms are less effective in high humidity regions such as Michigan, where avoidance has to be combined with physiological resistance to obtain an effective level of field resistance. Avoidance mechanisms may be defeated under favorable conditions for the pathogen, such as high precipitation, humidity and temperatures around 15 to 20°C. An ideal bean resistant ideotype for the Midwest was proposed by Kolkman and Kelly (2002) and should be indeterminate, with type II growth habit, resistant to lodging, medium canopy width and branching pattern, and medium height, days to flowering and maturity, to ensure adequate vegetative growth and yield potential without creating a favorable microclimate within the plant canopy for white mold development.

Growth habit was not a reliable predictor of the ability to escape white mold infection under field conditions (Schwartz et al., 1987). Instead, the plant canopy structure and canopy density associated with the respective growth habits determine whether microclimatic conditions created within the plant canopy are more or less favorable for *S. sclerotiorum* mold colonization and infection (Schwartz et al, 1978, 1987). Park (1993) obtained similar results and concluded that narrow canopy, higher flowering nodes and fewer branches in the upright plant type might have contributed to avoidance of white mold infection. Plants with narrow canopies allow better light penetration and air movement that results in rapid drying of dew on leaves and reduced moisture on the soil surface, which affects sclerotia germination, reducing the number of ascospores available that contribute to disease development (Coyne et. al., 1974; Blad et al. 1978; Schwartz et al., 1987).

Since most of the studies of relationship between plant architecture and white mold were conducted in a limited number of genotypes, it is possible that some conclusions are specific to those genotypes. White mold is a complex disease and is highly affected by environmental conditions, physiological resistance, as well as avoidance mechanisms. However, one can assume that white mold avoidance mechanisms are related to a number of morphological traits that influence the microclimate conditions that can be more or less favorable for the fungus establishment and development. Most of the morphological traits in common bean are highly heritable and are easily scored in the field. Estimates of heritability for days to flowering and days to maturity in a RIL population derived from a cross between two navy beans, Bunsi and Newport, were 0.91 and 0.75 respectively (Kolkman and Kelly, 2002), making early generation selection efficient.

Physiological resistance to white mold in common bean might be associated with endogenous factors that limit the spread of the pathogen in plant tissue. Such factors include increased activities of plant defense-related enzymes (Miklas et al., 1993), phytoalexin accumulation (Sutton and Deverall, 1984), and oxalate tolerance (Tu, 1985). The development of resistant cultivars has had limited success because few sources of physiological resistance to white mold have been found and exploited in bean breeding programs (Miklas et al., 1999). Screening for physiological resistance is highly confounded by morphological avoidance mechanisms in the field; therefore, incorporating physiological resistance into bean requires reliable screening techniques that detect this type of resistance. Many methods based on the fungus growth were proposed to detect physiological resistance to white mold in common bean, including the limited-term inoculation method (Hunter et al., 1981), the excised-stem inoculation technique (Miklas et al., 1992a), growing callus on medium containing pathogen filtrate (Miklas et al., 1992b), the straw test (Petzoldt and Dickson, 1996), and the leaf-agar plug assay (Steadman et al., 1997). An indirect assay to estimate physiological resistance, based on resistance to oxalic acid was developed by Kolkman and Kelly (2000). Since oxalic acid is the primary pathogenicity factor of *S. sclerotiorum* in the infection process (Godoy et al., 1990), genotypes that resist damage due to oxalate may possess a form of physiological resistance.

The tests used to detect physiological resistance to white mold in common bean have produced variable results, are influenced by environmental conditions, and their routine use in breeding programs has been limited. For example, Park et al., (2001) reported low heritability estimates for physiological resistance to white mold, using the straw test that is the most used greenhouse screening method, whereas Miklas et al. (1992; 2001) estimated high heritability for resistance to white mold also using straw test. Heritability estimates for physiological resistance using the oxalate assay were also low (0.19; Kolkman and Kelly, 2000).

Low heritability estimates (0.05 to 0.30) of resistance to white mold under field conditions were reported by Roberts et al., (1982), whereas Miklas et al. (2001) reported high heritability (0.65) for resistance to white mold using a scale from 1 to 9. However, moderate heritability (0.42) for disease severity was obtained by Kolkman and Kelly (2002) under field conditions. Considering the high heritability of avoidance mechanisms compared to low values for physiological resistance, selection based solely on disease severity may improve white mold resistance in the field via avoidance mechanisms, but may limit the opportunity to improve the level of physiological resistance (Kolkman and Kelly, 2002). Undesirable avoidance mechanisms, such as early or late flowering and reduced plant growth, may be not only associated with low levels of white mold, but also may affect negatively the yield potential (Kolkman and Kelly, 2002). Avoidance mechanisms associated with low yield are not useful in plant breeding for resistance to white mold, because they limit the ability of breeders to select for high yield that is always one of the most important objectives. Selection for yield under consistent high white mold pressure could be one of the best approaches to select resistant genotypes with high yield potential.

Combining physiological resistance with morphological avoidance mechanisms is currently the most viable breeding strategy to assure adequate levels of field tolerance to white mold (Miklas et al., 2001). Considering that white mold is a complexly-inherited trait, with low to moderate heritability and highly influenced by environmental factors that demands intensive field work, marker assisted selection (MAS) seems to be a promising approach to improve resistance to white mold in beans. Therefore, the identification of new and novel genetic sources of resistance and detection of quantitative trait loci (QTL) associated with physiological resistance in current and new sources could be a very important breeding strategy.

The combination of disease resistance with agronomically desirable traits has been accomplished by performing a multivariate bulking strategy for QTL detection. The use of multiple traits that are related to the trait of interest was effective in identifying QTL that may not be detected through screening extreme phenotypes (Ronin et al., 1998). Detection of QTL associated with different sources of white mold resistance could provide the opportunity to efficiently combine physiological resistance with avoidance mechanisms. The major potential benefit of multivariate bulking is to identify QTL conditioning resistance in an agronomically acceptable plant type, by avoiding the detection of QTL associated with undesirable avoidance mechanisms such as extreme early flowering or maturity or short dwarf plants that restraint disease development but limit yield potential (Kolkman and Kelly, 2003). Detection of QTL associated with different sources of resistance to white mold could provide the opportunity to combine unique sources of physiological resistance with avoidance mechanisms in common bean, which would not be possible with direct phenotypic selection under white mold pressure in the field.

Mapping of QTL for white mold resistance was undertaken by Park et al. (2001) using both field experiments and the straw test to evaluate resistance to *S. sclerotiorum*. The QTL analysis was conducted on a population of RILs derived from the cross between PC-50 and XAN-159, genotypes differing in reaction to white mold. PC-50 is resistant genotype to white mold and is derived from a single plant selection in the landrace 'Pompadour Checa' from the Dominican Republic (Park et al., 2001). QTL controlling physiological resistance, measured through the straw test, were detected through composite interval mapping on linkage groups B2, B3, B4, B7, B8 and B11 of the integrated bean linkage map, and QTL for field resistance were detected on B4, B7 and B8. Six of the seven candidate QTL for field resistance were found in the same locations as QTL for physiological resistance. Miklas et al., (2001) detected through interval mapping analysis, a QTL on linkage group B7 that explained 38% of the phenotypic variation for disease using the straw test, in a RIL population derived from a cross between G122 and A55. G122 is an upright determinate bean with physiological resistance to white mold. The same QTL detected on B7 were also identified for field resistance along with an additional QTL on B1. The QTL on B1 was mapped near the *fin* gene that is responsible for determinate growth habit in the Andean gene pool. The QTL detected on B7 derived from the Andean gene pool genotypes PC-50 and G122, both mapped near the phaseolin seed protein (*Phs*) gene, suggesting that an important QTL associated with resistance to white mold must reside in this genomic region.

Molecular markers (RAPD and AFLP) linked to white mold resistance were identified by Kolkman and Kelly (2003) using bulked segregant analysis, in a RIL population obtained from a cross between the small seeded cultivars Bunsi and Newport from the Middle American gene pool. QTL for resistance were detected on four linkage groups, B2, B3, B7 and B8. One major QTL for disease incidence identified on linkage group B2, was located near RAPD markers BC20.1800 and O15.1800. The region around BC20.1800 and O15.1800 on the integrated linkage map contains several identified genes that may be important in disease defense responses (Kelly et al., 2003). The largest linkage group associated with white mold resistance was B7. Markers in this linkage group were significantly associated with disease severity index, days to flowering, lodging, and yield and seed size. The QTL identified by Kolkman and Kelly (2003), were found in regions of the genome associated with either plant architecture or general plant defense response genes, such as *PvPR-2* (Walter et al., 1990), and *Pgip* (Toubart et al., 1992) on linkage group B2, the *PvPR-1* gene on B3, and the seed lectins (Brambl and Gada, 1985) on linkage group B7. The QTL on linkage B7, detected in a Middle American genotype by Kolkman and Kelly (2003), did not map near the *Phs*, suggesting the QTL for resistance to white mold was different from that detected in the Andean genotypes by Miklas et al., (2001) and Park et al., (2001),

This study was conducted with the following objectives: 1. to identify AFLP and RAPD markers linked to QTL associated with resistance to *S. sclerotiorum* in a Middle American RIL mapping population of common bean; 2. to map all putative QTL on the bean integrated map; 3. to compare the mapped QTL in this study with previously identified QTL for resistance to white mold; 4. to identify QTL associated with important agronomic traits and their relationship with resistance to white mold.

Materials and Methods

Plant Material

This study was conducted in a RIL population of 98 $F_{4:7}$ derived from the cross between Bunsi and Raven. Both genotypes are classified as small-seeded race Mesoamerica from the Middle American gene pool (Singh, 1999). Bunsi (a.k.a Ex-Rico 23; Tu and Beversdorf, 1985) is an indeterminate navy bean, with physiological resistance and a porous plant canopy (Schwartz et al., 1987; Miklas et al., 1992c; Kolkman and Kelly, 2002). Raven is an indeterminate black bean that despite an upright architecture lacks adequate levels of physiological resistance to *S. sclerotiorum* and is highly susceptible to white mold (Kelly et al., 1994). Ninety-eight F_2 plants were advanced from F_2 to F_4 through single seed descent method in the greenhouse. Seeds of individual F_4 plants were bulked, and advanced in a greenhouse during spring in 2000. Seeds harvested from three $F_{4:5}$ plants were bulked and $F_{4:6}$ lines were increased in the field, in Saginaw, MI, during summer 2000. No selection for agronomic or phenological traits was made during the population development. The 98 $F_{4:7}$ and $F_{4:8}$ lines and the parents, Bunsi and Raven, were evaluated for reaction to white mold in field trials in Entrican, in Montcalm County, MI, in 2001 and 2002, respectively.

Field trials and Traits

The F4 derived lines were evaluated for resistance to white mold in naturally infested field plots in Montcalm County, MI, during 2001 and 2002. Supplemental irrigation was provided to enhance the development of white mold.

he soil type at the Montcalm Research Farm site is a combination of Eutric ilossoboralfs (coarse-loamy, mixed) and Alfic Fragiorthods (coarse-loamy, xed, frigid). Experimental design was a 10 x 10 lattice with four replications. ır row plots were 6 m in length, with 0.5 m row spacing. The two center rows each plot were planted with the RIL, while the two border rows were planted h the highly susceptible cultivar, Midland. Standard agronomic practices for je, fertilization, and insect and weed control were applied to ensure adequate nt growth and development. Plots were irrigated during the growing season ting in the third week after planting with 12 mm of water at least once a week, pending on rainfall, in order to promote uniform disease pressure across the 1. During 2001 plots were irrigated 8 times for a total of 127 mm. In 2002 the lots were irrigated with approximately 180 mm distributed in 11 irrigations.

Plots were rated for disease prior to harvest, when the plants had reached hysiological maturity. Thirty plants per plot were rated from 0 to 4, where 0 = isease present, 1 = 1 to 25 %; 2 = 26 to 50 %; 3 = 51 to 75 %; and 4 = 76% 0 % of the plant with white mold symptoms in order to estimate disease rity index (DSI) and disease incidence (DI) (Hall and Phillips, 1996). Disease erity Index was calculated for each plot on a percentage basis, using the ving formula:

$$\text{DSI (\%)} = \frac{\sum \text{(rating of each plant)} \times 100}{4 \times \text{(number of plants rated)}}$$

Disease incidence as a percentage was calculated as the number of s out of the thirty individuals infected with white mold. The disease was also rated in 2001, based on a scale from 1 to 9 as described by Miklas et al., (2001). The RILs were also evaluated for agronomic and phenological traits, including: yield, seed size, days to flowering, days to maturity, canopy height, architecture and lodging. Days to flowering were characterized by the number of days following planting, when 50% of the plants in a plot have at least one open flower. Four weeks after flowering the plots were evaluated for architecture, using a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate. Lodging was determined at the same time that architecture was rated, based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging. Days to maturity were calculated as the number of days following planting, until 90% of the pods were physiologically mature and the drying down process was initiated. Canopy height was measured at plant maturity on each individual plot, from six measurements per plot. All plots were harvested at maturity, when plots were individually pulled and threshed. Seed yield and weight of 100 seeds were adjusted to 18 % moisture content.

In addition, the 100 RILs and the parents were screened in the greenhouse based on resistance to oxalate (ROX), an indirect assay to determine physiological resistance to white mold (Kolkman and Kelly, 2000). Twenty-one-day old seedlings (2nd trifoliate emerging) were cut at the base of the stem and placed in a 20 mM oxalate solution (pH 4.0). The seedlings were rated for wilting symptoms after 12 to 15 h of exposure to the oxalate solution. A 1 to 6 scale was used to measure wilting, where 1 = no wilting symptoms visible, 2 = 1 leaf with wilting symptoms (the two unifoliate leaves were rated together as one leaf, and the 3 leaflets of a trifoliate leaf were rated together as one leaf), 3 = 2 leaves with wilting symptoms, 4 = 3 or more leaves with wilting symptoms, 5 = petioles collapsing, 6 = main stem (total plant) collapsing. Wilting symptoms ranged from curled leaf tip, to total loss of turgidity in the entire leaf.

AFLP and RAPD Analysis

Plant tissue for DNA extraction was collected from parental genotypes and individual recombinant inbred lines from 10 $F_{4:7}$ plants. DNA was extracted from the plant tissue using a mini-prep procedure (Edwards et al, 1991; Haley et al., 1994).

AFLP molecular markers were detected by performing the DNA digestion with restriction enzymes *EcoRI* and *MseI* (Vos, 1995). Bulked segregant analysis (BSA) approach using AFLP markers with 256 primer pair combinations (*EcoRI* + ANN and *MseI* + CNN) were first tested on the parents and resistant and susceptible bulks to detect useful associated markers. Candidate markers were first tested on the RILs included in the bulks and subsequently on the recombinant population. In addition to the candidate markers, all polymorphic bands were scored and included in the construction of the linkage map. Chi-square test for goodness-of-fit was used to test the deviations of the expected segregation ratio of 9:7 (for F4 derived lines) or 1:1 (RILs).

AFLP procedure

Double digestion, adapter ligation, pre-amplification, and selective amplification were carried out as previously proposed by Vos et al. (1995) with the following modifications described by Hazen et al. (2002). Pre-amplification of 2 µl of restriction ligation product was combined with 25 ng of MseI and EcoRI adapter, 0.5 mM dNTP, 1X PCR buffer (10mM Tris-HCL, pH 7.2 50mM KCl, and 0.1% Triton X-100), 0.5 U Taq polymerase, 1.5 mM MgCl2, total volume 20 µl and amplified in a 96-well PTC-100 Programmable Thermal Controller (MJ Research, Inc) programmed with the following profile [94°C 2 minutes – 26 cycles (94°C 1 min, 56°C 1 min, 72°C 1 min) - 72°C 5 min]. The preamplification PCR product was added to 100 µl of sterile water and 1 µl of diluted product was added to 19 µl of selective amplification cocktail (25 ng EcoRI primer, 30 ng MseI primer, 0.4 mM dNTPs, 1X PCR buffer, 0.4 U Taq polymerase, 1.5 mM MgCl2) and amplified via PCR [94°C 2 min – 12 cycles with annealing temperatures decreasing by 0.7°C/cycle (94°C 30 sec, 65°C 30 sec, 72°C 1 min) – 23 cycles (94°C 30 sec, 56°C 30 sec, 72°C 1 min) - 72°C 2 min].

Polyacrylamide gel electrophoresis

The selective amplification product was combined with 8 µl of formamide loading buffer (98% formamide, 10mm EDTA, pH 8.0, 1.0 mg/ml bromophenol blue; and 1.0 mg/ml xylene cyanol) and electrophoresed for 2hrs 30min at 80W on a 6% polyacrylamide gel cast on a 38 x 50 cm Sequi-Gen GT sequencing cell (BioRad Laboratories Inc., Hercules, CA, USA) to separate the AFLP fragments.

Fragments were visualized using a silver staining procedure (Promega # Q4132). Gels were scored for polymorphism, based on presence or absence of the AFLP, estimating band size in reference to a 100 and a 10 base pair DNA ladder.

RAPD procedure

The parents were also screened with 11 RAPD markers (Williams et al., 1990) mapped by Kolkman and Kelly (2003) on linkage groups B2, B3, B7 and B8. In these linkage groups QTL associated with resistance to white mold were identified. Polymerase Chain Reaction was conducted in a 96-well PTC-100 Programmable Thermal Controller (MJ Research, Inc) programmed for 3 cycles of 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C; 34 cycles of min at 94°C, 1 min at 40°C, and 2 min at 72°C with the final step extended by 1 s for each of the 34 cycles, and a final extension cycle of 5 min at 72°C (Haley et al., 1994). Agarose gel electrophoresis (1.4%) was used to separate RAPD fragments. The band size was estimated in reference to a 100 bp DNA ladder.

The AFLP and RAPD markers were coded in the following way. The letters E and M of the AFLP marker names indicate the restriction enzymes *Eco R* I and *Mse* I, respectively. The three letters following E indicate the *Eco* RI (ANN) selective nucleotides, while the second group of three letters indicate the *Mse* I (CNN) selective nucleotides used in this study. The number following the enzyme/primer combination represents the size of the polymorphic band. RAPD markers were identified by the name of the Operon primer, followed by the size of the polymorphic fragment.

Bulked Segregant Analysis

Bulked segregant analysis (BSA; Michelmore et al., 1991) based on multiple trait DNA pooling procedure as proposed by Kolkman and Kelly (2003), was conducted to create DNA bulks and identify candidate markers significant markers for the Bunsi/Raven RIL population. Low levels of DSI and high yield were combined in the resistant bulk to white mold and high levels of DSI and low yield were combined in the susceptible bulk, within a range from 40 to 46 days to flowering (Table 1.1). Days to flowering was chosen to minimize disease avoidance based on early or late flowering (Kolkman and Kelly, 2002).

Statistical Analysis

The 124 molecular markers (118 AFLP + 6 RAPD) and two phenotypic markers for disease resistance were analyzed and divided in linkage groups by Joinmap software (Stam, 1993). The linkage groups were first anchored through common markers previously mapped on the integrated map. All 28 AFLP primer combinations used in this study were then tested for polymorphism against BAT93 and Jalo EEP558, parents of mapping population used to create the integrated map. Those polymorphic markers were then tested in the BAT93/Jalo EEP558 (BJ) RIL population (Freyre et. al., 1998) and mapped to the integrated bean map using MAPMAKER/EXP (Lander et al., 1987).

Table 1.1. DNA bulking strategy1 used for detecting QTL for white mold resistance in common bean based on disease severity (DSI), yield and days to flowering, in 2001.

Resistant bulk			Susceptible bulk			Population	
Trait	Range	Mean	Trait	Range	Mean	Range	Mean
Low DSI (%)	< 33.6	29.6	High DSI	> 56.1	63.7	23.1 – 72.6	44.6
High Yield (kg/ha)	> 4618	4954	Low Yield	<3329	2881	2130 – 5571	4136
Days to flowering	40 – 46	44.7	Days to flowering	40 – 46	43.4	36.5 – 48.4	43.8

[1] Multi-trait bulking similar to that described by Kolkman and Kelly (2003).

Significant markers associated with resistance to white mold and other traits were identified through single marker analysis (SMA) and composite interval mapping (CIM) analyzed with software QTL Cartographer (Basten et al., 2001) using a window size of 5 cM. Additive effects and coefficient of determination (R^2) were calculated through QTL cartographer. Threshold LOD scores (90 and 95%) for individual traits were determined through a permutation test, with 1000 permutations (Churchill and Doerge, 1994). The effect of multiple QTL located in more than one linkage groups was analyzed using multiple regression (SAS, 1995). The Pearson correlation coefficients (*r*) were calculated by PROC CORR (SAS, 1995). The RIL population was analyzed across the two years as a RCBD, using PROC GLM (SAS, 1995). Estimates of heritability for all traits were calculated on entry mean basis, where $h^2 = \sigma_g^2 / \sigma_e^2 / ry + \sigma_{ge}^2 / y + \sigma_g^2$, (Fehr, 1987). Confidence interval estimates were calculated on entry mean basis (Knapp et al., 1985).

Results and Discussion

Linkage Map

Twenty-eight AFLP primer combinations were identified using selective genotyping and bulked segregant analysis. In total, 124 molecular markers (118 AFLP and 6 RAPD) and two phenotypic markers were evaluated in the Bunsi/Raven (BR) population of 98 F4 derived RILs. Linkage map construction, using Joinmap (Stam, 1993), placed 118 markers on twelve linkage groups for a total of 227.5 cM (Figure 1.1). Linkage groups were compared against the integrated map (Freyre et al., 1998) and seven linkage groups were anchored to the integrated map. B7-a and B7-b were associated on different regions of linkage group B7 of the integrated map. B4-a and B4-b were anchored to linkage group B4 of the integrated map.

Mapping of F_2 or F_3 populations with dominant markers is not ideal, since heterozygous individuals cannot be identified. Mapping RIL populations with dominant markers is much more efficient, since heterozygous genotypes are in very low frequencies in the population (Knapp et al., 1995). DNA fingerprinting an F4-derived population with dominant markers based on a subset of individuals from a more advanced generation, such as the $F_{4:7}$ generation, was adequate to detect QTL and generate a linkage map (Figure 1.1).

QTL associated with resistance to white mold

The two parental genotypes, Bunsi and Raven react differently to white mold and exhibit variation in other agronomic traits in both environments.

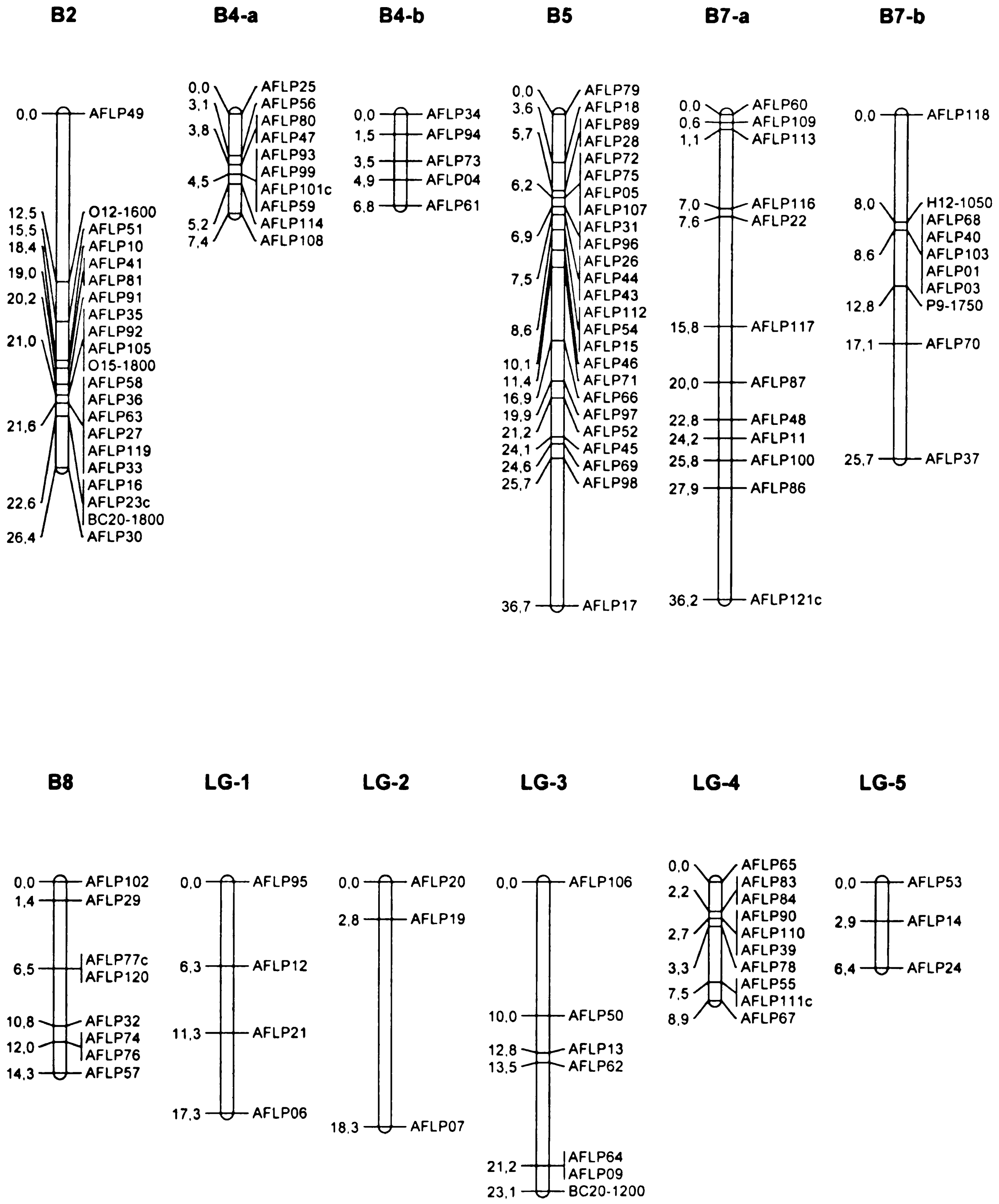

Figure 1.1. Linkage map with 118 markers from 98 F4 derived recombinant inbred lines originated from a cross between Bunsi and Raven. Linkage groups B2 to B8 correspond to the integrated map (Freyre et al., 1998) and linkage groups LG-1 to LG-5 are unassigned linkage groups detected in current study.

Disease pressure was adequate in both years but the environmental variation resulted in different levels of DSI and DI between the two years (Table 1.2). Data for resistance to white mold followed a normal distribution. The agronomic traits, days to flower and yield in individual environments were not always normally distributed. Since combined environments were normally distributed, data was not transformed to correct skewness.

Markers associated with QTL for resistance to white mold and for agronomic traits were detected through single marker analysis and composite interval mapping. The QTL identified with CIM analyses in most of the cases were supported by SMA.

As expected, markers were consistent between DSI and DI measurements since both traits were highly correlated ($r > 0.90$; $P<0.01$). Therefore, only the results for DSI will be discussed, but the considerations are valid for both traits. Different QTL associated with resistance to white mold were identified across the two environments. The different QTL detected can be explained in part by differences in environmental conditions between years 2001 and 2002. In August and September 2001, the cumulative precipitation was 256 mm compared to 105 mm in 2002, whereas the normal precipitation for that period would be 177 mm (Table 1.3). The average maximum temperature in September 2002 was 26°C compared to 21°C in September of 2001. High temperatures combined with reduced precipitation affected disease development in 2002, despite the supplemental irrigation applied to the experiment.

Table 1.2. Means, range and heritability (h^2) of disease severity index (DSI) and agronomic traits in 98 F4 derived (RIL) from the cross Bunsi/Raven, grown in Montcalm, MI, in 2001 and 2002.

		Parental Means		Recombinant inbred lines		
Trait	Year	Bunsi	Raven	Mean	Range	h^2 (90% CI)
DSI (%)	2001	29.9	37.2	44.9	23.1 – 72.6	
	2002	14.6	43.6	27.2	8.4 – 51.2	
	Combined	22.3	40.4	36.1	19.7 – 52.6	0.41(0.18-0.58)
Yield	2001	4046	4719	4136	2130 – 5571	
(kg/ha)	2002	3968	4013	4495	3127– 5627	
	Combined	4013	4371	4315	3015 – 5380	0.56(0.38-0.68)
Days to	2001	40.6	45.9	43.8	36.5 – 48.4	
flower	2002	45.5	47.0	45.3	42.0 – 52.5	
	Combined	43.1	46.5	44.6	39.5 – 50.5	0.79(0.71-0.85)
Days to	2001	104.9	103.9	104.9	103.2 – 111.7	
Maturity	2002	109.5	107.1	105.8	91.8 – 116.5	
	Combined	107.2	105.5	105.4	96.3 – 113.1	0.82(0.75-0.87)
Lodging	2001	3.6	2.4	3.3	2.0 – 4.9	
	2002	3.5	2.9	3.1	1.6 – 4.1	
	Combined	3.6	2.7	3.2	1.8 – 4.5	0.76(0.67-0.83)
Seed size	2001	22.7	21.9	22.2	18.9 – 26.1	
(g.100seed^{-1})	2002	22.8	19.0	21.0	16.7 – 26.2	
	Combined	22.8	20.5	21.6	18.3 – 25.7	0.90(0.85-0.93)

Table 1.3. Monthly precipitation from June to September in 2001 and 2002 in Montcalm, MI.

	Precipitation (mm)			
	June	July	August	September
Observed 2001	71.9	26.9	154.9	101.6
Deviation 2001	-15.2	-36.6	57.4	22.4
Observed 2002	58.9	71.1	81.0	23.9
Deviation 2002	-28.2	7.6	-16.5	-55.4
Normal[1]	87.1	63.5	97.5	79.2

[1] Calculated over 30 year period (1951-1980).

Five linkage groups with QTL for resistance to white mold were detected in 2001 through SMA and three of these genomic regions were confirmed by CIM (Table 1.4 and Table 1.7, 1.8 and 1.9). The first linkage group where a QTL for DSI was identified was B2, located near marker O12.1600. The QTL accounted for 9.9% (R^2) of the phenotypic variability for DSI. Another QTL detected on linkage B2, was located close to the RAPD marker, O15.1800 and explained 9.2% of the DSI (Table 1.7 and Figure 1.2). The QTL for DSI mapped close to marker O15.1800 previously detected by Kolkman and Kelly (2003) in a different RIL population, supporting the existence of a QTL for resistance to white mold in that region on B2. The three AFLP markers AFLP35, AFLP92, AFLP105 mapped to the same position as the marker O15.1800 on B2 (Figure 1.1; Appendix A – Table A.3).

The region around the O15.1800 marker on the integrated linkage map possesses several genes identified in general host defense mechanisms that may play a role in disease resistance. Plant defense-related genes may be triggered as a general resistance response to *S. sclerotiorum* infection. *PvPR-2*, a low molecular weight acidic protein induced during fungal elicitation (Walter et al., 1990), and *Pgip*, a polygalacturonase-inhibiting protein (Toubart et al., 1992), are all located in this region on B2. Differences in *PvPR* gene arrangements were detected between anthracnose (*Colletotrichum. lindemuthianum*) resistant and susceptible bean genotypes indicating that polymorphism between *PvPR* as well as other defense response-related genes may contribute to quantitative resistance (Walter et al., 1990).

Table 1.4. Markers linked to QTL for resistance to white detected through single marker analysis in a 98 derived RIL population from a Bunsi/Raven cross, grown in Montcalm, MI, 2001.

Linkage Group	Marker	LOD[1]	P-value
B5	AFLP66	1.09	0.027
	AFLP97	0.95	0.039
	AFLP52	1.37	0.013
	AFLP98	1.42	0.012
LG-2	AFLP20	1.66	0.006
	AFLP19	1.47	0.010
B2	AFLP10	0.92	0.042
	AFLP91	1.27	0.017
	AFLP35	1.58	0.008
	AFLP92	1.58	0.008
	AFLP105	1.76	0.005
	O15.1800	1.78	0.005
	AFLP58	1.44	0.011
	AFLP36	1.42	0.011
	AFLP63	1.34	0.014
	AFLP27	1.34	0.014
	AFLP119	1.34	0.014
	AFLP33	1.19	0.021
	AFLP16	1.06	0.029
	AFLP23c	1.02	0.032
	BC20.1800	0.99	0.035
B7-a	AFLP121c	1.15	0.023
B8	AFLP102	0.94	0.040
	AFLP29	1.11	0.026
	AFLP77c	1.28	0.016
	AFLP120	1.41	0.012

[1] Log-odds ratio

Table 1.5. Markers linked to QTL for resistance to white detected through single marker analysis in a 98 derived RIL population from a Bunsi/Raven cross, grown in Montcalm, MI, 2002

Linkage Group	Marker	LOD[1]	P-value
B7-b	AFLP118	2.04	0.002
	H12.1050	2.92	<0.001
	AFLP68	3.54	<0.001
	AFLP40	3.58	<0.001
	AFLP103	3.58	<0.001
	AFLP01	3.58	<0.001
	AFLP03	3.43	<0.001
	P9.1750	2.09	0.002
	AFLP70	2.61	0.001
B5	AFLP98	0.94	0.040
LG-2	AFLP07	1.21	0.020
B2	AFLP49	1.03	0.032

[1] Log-odds ratio

Table 1. 6. Markers linked to QTL for resistance to white detected through single marker analysis in a 98 derived RIL population from a Bunsi/Raven cross, grown in Montcalm, MI, 2001 and 2002

Linkage Group	Marker	LOD[1]	P-value
B7-b	AFLP118	1.07	0.028
	H12.1050	1.50	0.009
	AFLP68	1.77	0.005
	AFLP40	1.67	0.006
	AFLP103	1.67	0.006
	AFLP01	1.67	0.006
	AFLP03	1.68	0.006
	P9.1750	0.83	0.054
	AFLP70	1.32	0.015
B5	AFLP66	1.07	0.028
	AFLP97	0.90	0.045
	AFLP52	1.38	0.013
	AFLP98	1.75	0.005
LG-2	AFLP20	1.14	0.024
	AFLP19	1.37	0.013
	AFLP07	1.26	0.017
B2	AFLP35	0.99	0.035
	AFLP92	0.99	0.035
	AFLP105	1.13	0.024
	015.1800	1.26	0.018
B8	AFLP102	1.04	0.031
	AFLP29	0.92	0.042
	AFLP32	0.87	0.048
	AFLP57	0.92	0.043
LG-5	AFLP24	0.95	0.039

[1] Log-odds ratio

Table 1.7. Markers identified on linkage group B2, most closely linked to QTL for resistance to white mold and agronomic traits, including phenotypic variability associated with the QTL and additive effect of the presence of the marker.

	Linkage Group B2				
Trait	Marker	Position of QTL (cM)	Environment	R^2 (%)	Additive Effect[1]
DSI (%)	O12.1600	8.0	Combined	10.1	3.15 (B)[2]
			2001	9.9	3.83
			2002	ns	
	O15.1800	21.0	Combined	8.7	-2.66(B)
			2001	9.2	-3.85
			2002	ns	
Yield (kg/ha)	AFLP49	10.1	Combined	9.7	-171(B)
			2001	ns	
			2002	ns	
Days to flower	AFLP63	21.6	Combined	7.6	0.57(R)
			2001	6.9	0.65
			2002	ns	
Days to maturity	AFLP16	22.6	Combined	28.5	2.06(R)
			2001	28.3	1.58
			2002	25.3	2.61
Lodging	AFLP49	0.01	Combined	8.5	0.17(B)
			2001	7.1	0.19
			2002	ns	
Seed size (g.100seed^{-1})	O15.1800	21.0	Combined	20.1	0.78(B)
			2001	10.3	0.51
			2002	18.8	0.83

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[1] Estimated additive effect of substituting one allele of Raven by one allele of Bunsi.

[2] The letter in parenthesis indicates the parent where the marker is present, B=Bunsi; R=Raven.

Table 1.8. Markers identified on linkage group B5, most closely linked to the QTL for resistance to white mold and agronomic traits, including phenotypic variability associated with the QTL and additive effect of the presence of the marker.

	Linkage Group B5				
Trait	Marker	Position of QTL (cM)	Environment	R^2 (%)	Additive Effect[1]
DSI (%)	AFLP98		Combined	ns	
		27.7	2001	10.7	3.16(B)[2]
			2002	ns	
Yield (kg/ha)	AFLP98	25.7	Combined	7.5	-122(B)
Seed (g.100seed^{-1})	AFLP96	6.9	Combined	9.3	-0.49(R)
			2001	8.3	-0.46
			2002	ns	

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[1] Estimated additive effect of substituting one allele of Raven by one allele of Bunsi.

[2] The letter in parenthesis indicates the parent where the marker is present, B=Bunsi; R=Raven.

Table 1.9 . Markers identified on linkage group B8, most closely linked to the QTL for resistance to white mold and agronomic traits, including phenotypic variability associated with the QTL and additive effect of the presence of the marker.

	Linkage Group B8				
Trait	Marker	Position of QTL (cM)	Environment	R^2 (%)	Additive Effect[1]
DSI (%)	AFLP29		Combined	ns	
		1.4	2001	9.2	2.93(B)[2]
			2002	ns	
Yield (kg/ha)	AFLP29	1.4	Combined	12.7	-158(B)
			2001	13.8	-228
			2002	ns	
Lodging	AFLP102	0.1	Combined	6.4	0.15(B)
			2001	8.6	0.21
			2002	11.0	0.19

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[1] Estimated additive effect of substituting one allele of Raven by one allele of Bunsi.

[2] The letter in parenthesis indicates the parent where the marker is present, B=Bunsi; R=Raven.

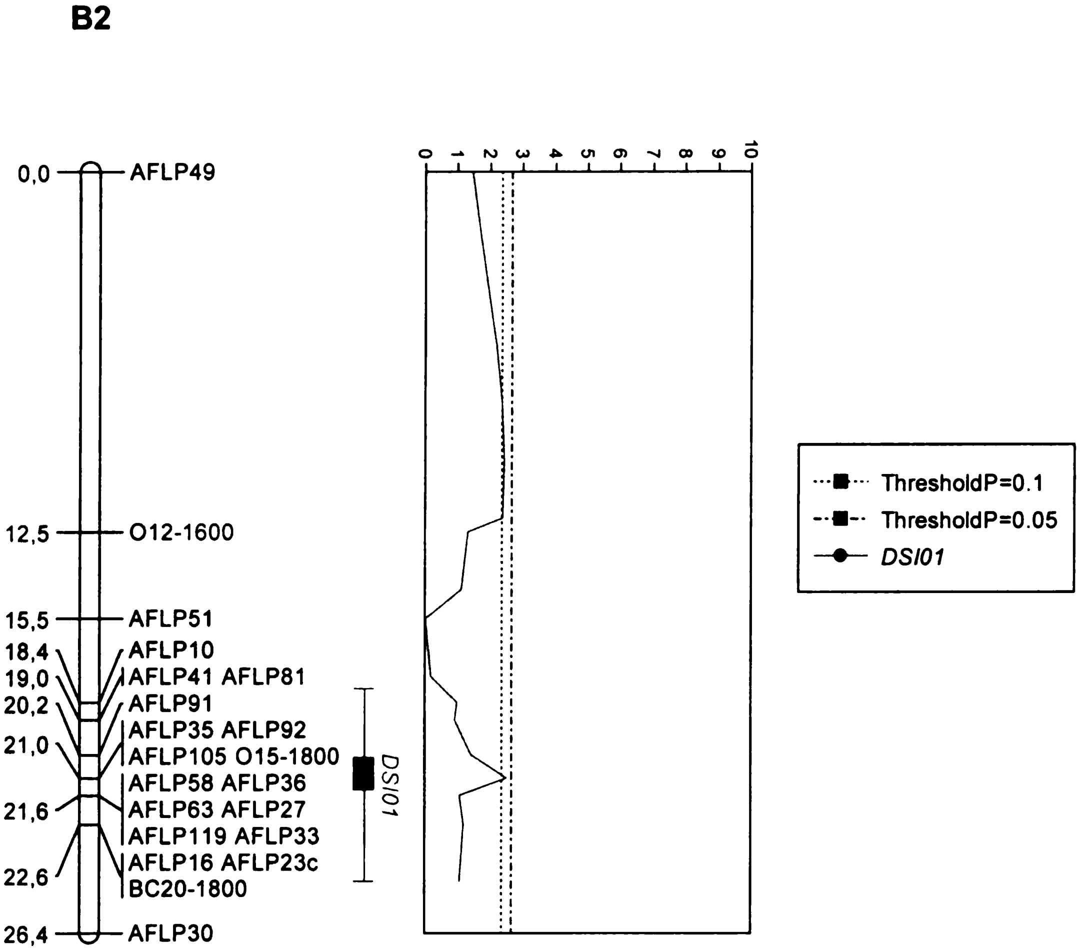

Figure 1.2. QTL for DSI 01 detected on Linkage group B2.

Pgip could also be a very important defense mechanism since polygalacturonases are produced by *S. sclerotiorum* during the infection process. Chalcone synthase, (*ChS*; Ryder et al., 1987), located near O15.1800, is an enzyme required for isoflavonoid phytoalexins biosynthesis, and may be important in general resistance to *S. sclerotiorum*. QTL associated with resistance to white mold (Kolkman and Kelly, 2003), common bacterial blight (CBB) (Nodari et al., 1993), and root rot (Schneider et al., 2001) were identified in this region of linkage group B2.

The second linkage group with a QTL associated with resistance to white mold was mapped to linkage group B5. AFLP98 was the closest marker linked to this QTL that contributed up to 10.7% of phenotypic variation (Table 1.8 and Figure 1.3).

The third linkage group where QTL for resistance to white mold was detected in 2001 was B8. AFLP 29 was the closest marker associated with this QTL that accounted for 9.2% of the phenotypic variability for disease severity (Table 1.9 and Figure 1.4).

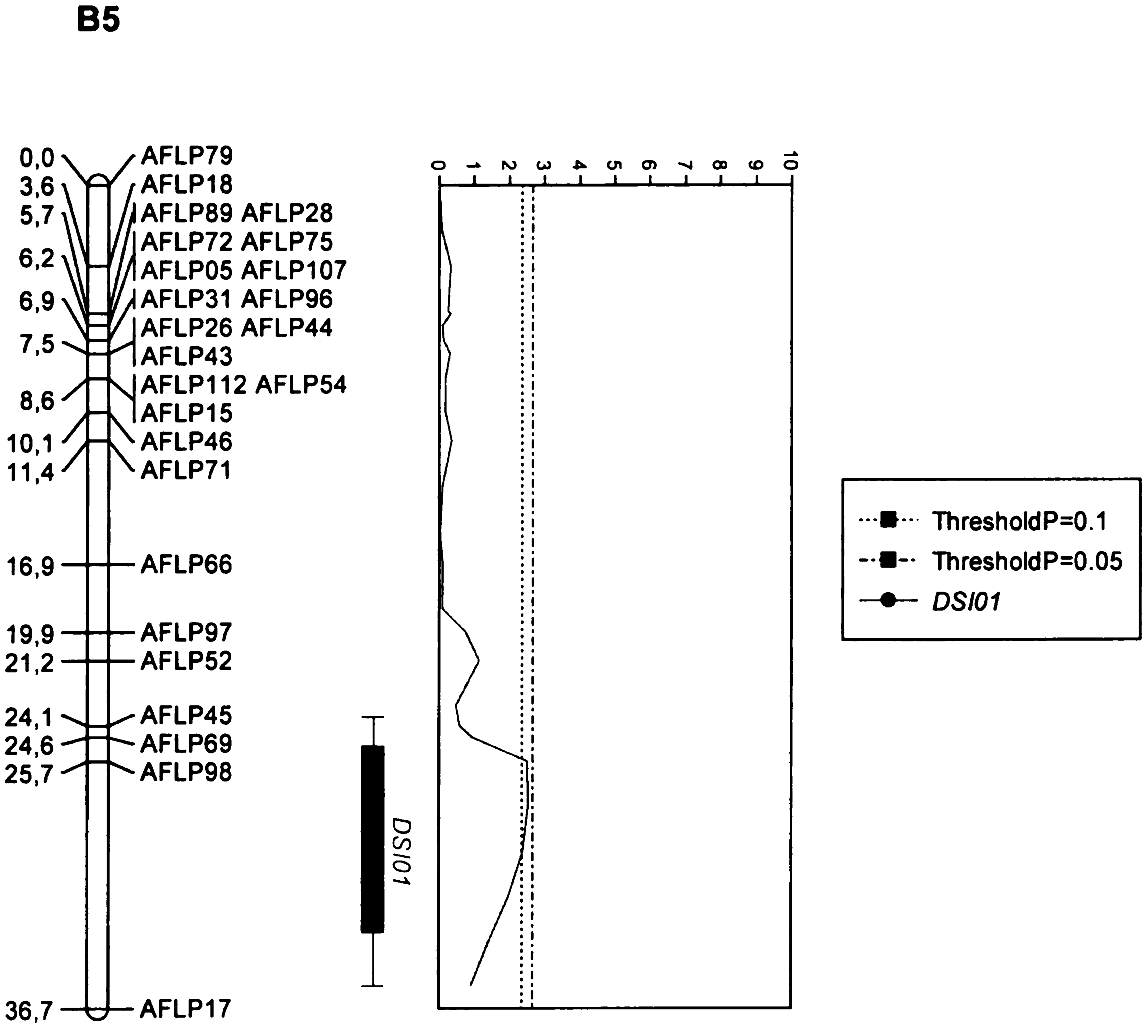

Figure 1.3. QTL for DSI 01 detected on Linkage group B5

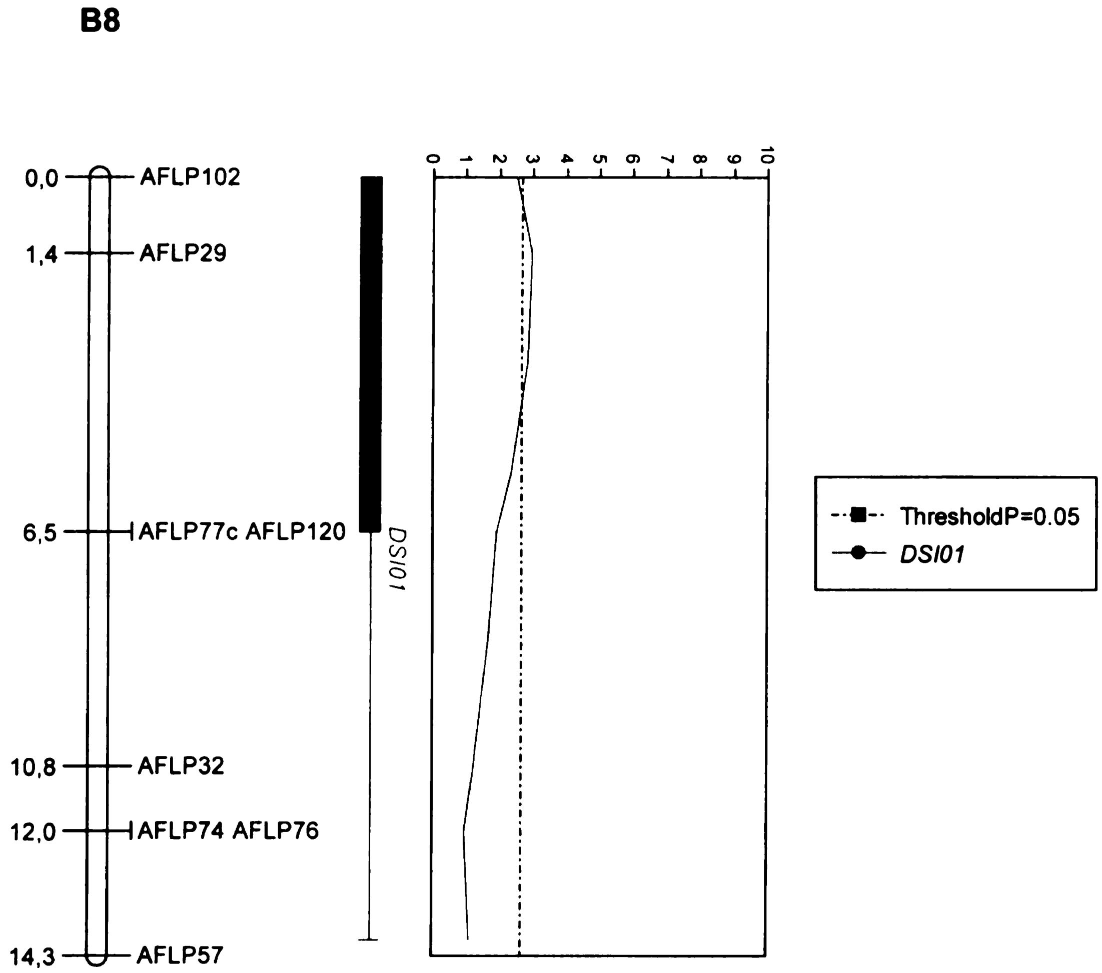

Figure 1.4. QTL for DSI 01 detected on Linkage group B8

In previous studies, a QTL for resistance to white mold was also found on B8, but mapped to a different region on the linkage group (Kolkman and Kelly, 2003). Another QTL on B8 that accounted for 26% of phenotypic variability for disease reaction in the field was detected by Miklas and Delorme (2003) in snap bean. This QTL detected on B8 was mapped between markers AH05.1000 and D1468 on the integrated map and probably is different from that detected in Bunsi/Raven RIL population, since the QTL were mapped more than 30 cM apart.

The genomic region that was associated with DSI in 2002 was mapped on linkage group B7-b, anchored to B7 on the integrated map (Freyre et al., 1998) by marker H12.1050. AFLP03 was the closest marker to this QTL that accounted for 14.7% of the phenotypic variability of DSI (Table 1.5, Table 1.10 and Figure 1.5). In this region of B7, Kolkman and Kelly (2003) identified a major QTL for resistance to white mold along with QTL for other agronomic traits, including days to flowering and maturity, architecture, lodging, seed size and yield. One of the most important markers detected by Kolkman and Kelly (2003) on B7 was $E_{aac}M_{ctt}130$ that corresponded to marker AFLP01 in this study. This QTL accounted for 15.8% of the phenotypic variability for DSI. Seed lectins (phytohaemoglutanins) genes also mapped to this region of B7 and may have a putative role in plant defense (Shewry and Lucas, 1997). Miklas et al., (2001) and Park et al., (2001) also detected QTL for resistance to white mold of B7, but those QTL correspond to B7-a in this study.

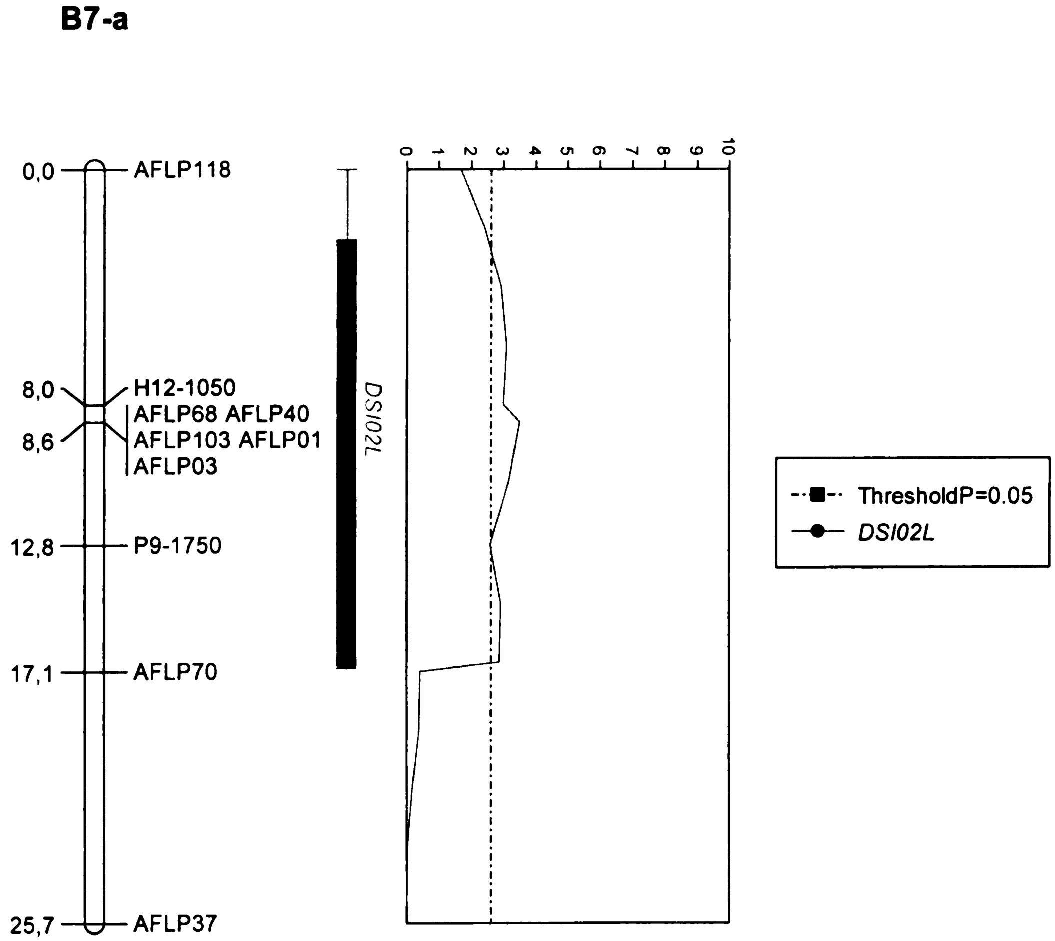

Figure 1.5. QTL for DSI 02 detected on Linkage group B7-a.

Table 1.10. Markers identified on linkage group B7, most closely linked to the QTL for resistance to white mold and agronomic traits, including phenotypic variability associated with the QTL and additive effect of the presence of the marker.

Trait	Marker	Position of QTL (cM)	Environment	R^2 (%)	Additive Effect[1]
	Linkage Group B7-b				
DSI (%)	AFLP03	8.6	Combined	ns	
			2001	ns	
			2002	14.7	-4.17(B)[2]
	P9.1750	14.8	Combined	ns	
			2001	ns	
			2002	14.2	-4.01(B)
Days to flower	AFLP03	8.6	Combined	35.7	-1.21(B)
			2001	24.5	-1.21
			2002	38.3	-1.24
Days to Maturity	AFLP70	17.1	Combined	9.8	-1.21(R)
			2001	8.2	-0.84
			2002	8.5	-1.51
Lodging	AFLP03	8.6	Combined	ns	
			2001	ns	
			2002	9.6	-0.17(B)
Seed size (g.100seed^{-1})	AFLP03	8.6	Combined	9.4	0.49(B)
			2001	7.6	0.44
			2002	11.4	0.59
	Linkage Group B7-a				
Yield (kg/ha)	AFLP121c[3]	33.9	Combined	21.5	-158
			2001	ns	
			2002	11.3	-148
Days to flower	AFLP113	1.1	Combined	8.4	-0.60(B)
	AFLP22	13.6	2001	14.8	-0.96(R)
Lodging	AFLP117	15.8	Combined	29.8	0.33(B)
			2001	30.2	0.39
			2002	19.9	0.26
Seed size (g.100seed^{-1})	AFLP22	9.6	Combined	25.7	-0.83(R)
			2001	27.3	-0.87
			2002	19.6	-0.81

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[1] Estimated additive effect of substituting one allele of Raven by one allele of Bunsi.

[2] The letter in parenthesis indicates the parent where the marker is present, B=Bunsi; R=Raven.

[3] Codominant marker.

The major QTL identified in this study would appear to be different from the QTL detected on B7 in Andean genotypes G122 and PC-50 (Miklas et al., 2001; Park et al., 2001). QTL for DSI were detected on B7-a and LG2 using SMA, but the QTL were not confirmed by CIM. The lack of consistency between methods does not mean necessarily that these QTL are false positives but were more easily detected by SMA than by CIM. The power of QTL detection is different between methods and is influenced by population size and also by environmental conditions, resulting in the lack of expression and detection of a QTL at a significant level of probability.

The QTL for resistance to white mold identified in the Bunsi/Raven population on B2 and B7 supported the results of previous studies (Kolkman and Kelly, 2003). The fact that different QTL were identified in different years suggest that depending on the environmental conditions, different genes may play a more important role for resistance to white mold, including genes associated with general defense or those associated with avoidance traits.

Resistance to oxalate was tested in the 98 RILs and three QTL were detected through SMA at 5% level of probability, but were not confirmed by CIM. The QTL were detected near markers AFLP69 on B5, AFLP100 on B7-a, and AFLP42 that was not linked to any of the12 linkage groups identified in this study. On B5 AFLP 69 was placed near AFLP 98 where a QTL for DSI was mapped. Additional support for QTL for DSI on B5 comes from the detection of QTL for ROX in the same genomic region.

Resistance to white mold and agronomic traits

Understanding the associations between DSI and phenological/agronomic traits should contribute to the development of more efficient strategies to select for resistance to white mold.

DSI was significantly correlated with yield in both years, however the correlation coefficient was lower in 2002 (r = - 0.45**) compared to 2001 (r = - 0.77**) (Table 1.11). The lower correlation coefficient could be a consequence of slower disease development in 2002. White mold did not develop to the same level observed in 2001 due the high temperatures and low precipitation that occurred in September 2002 (Table 1.3). Lodging was correlated with DSI in both years. In 2001 high lodging probably was a consequence of the disease, since the disease developed early after flowering and the highly susceptible lines lodged due to disease. In contrast in 2002 more prostrate plants contributed to high DSI by promoting a more favorable microclimate for the disease development under the decumbent plant canopy. Architecture was not associated with DSI in 2001, whereas more upright lines had less white mold (r=0.42**) in 2002 (Table 1.11). Plant architecture likely played an important role in avoiding disease as a result of the higher temperatures and lower precipitation in 2002. The environmental conditions were more favorable for white mold development in 2001 and plant structure was not detected as an important factor in disease development. Under more favorable conditions for *S. sclerotiorum*, avoidance mechanisms may not be sufficient to restrict disease development

Table 1.11. Phenotypic correlations between Disease Severity index and phenological and agronomic traits in a 98 F4 derived RIL population from a Bunsi/Raven cross grown in Montcalm in 2001 and 2002.

	DSI 01[1]	DSI 02	DSI Combined
DSI 01	1	0.33**	0.79**
DSI 02	0.33**	1	0.84**
Yield 01	-0.77**	-0.23*	-0.60**
Yield 02	-0.31**	-0.45**	-0.47**
Combined Yield	-0.69**	-0.38**	-0.64**
Days to flower 01	ns	0.42**	0.29**
Days to flower 02	ns	0.43**	0.30**
Combined flower	ns	0.46**	0.33**
Days to maturity01	ns	0.27**	ns
Days to maturity02	ns	0.26**	ns
Combined maturity	ns	0.28**	ns
Lodging 01	0.51**	0.23*	0.44**
Lodging 02	0.27**	0.65**	0.58**
Combined Lodging	0.45**	0.46**	0.56**
Architecture 01	ns	0.29**	0.27**
Architecture 02	ns	0.42**	0.36**
Combined Architecture	ns	0.38**	0.35**
100 seed weight 01	-0.21*	ns	ns
100 seed weight 02	ns	-0.26**	-0.24*
Combined seed	ns	ns	-0.22*
Height 02	-0.22*	-0.31**	-0.33**
Resistance to oxalate	ns	ns	ns

[1] 01=2001; 02=2002.

and physiological resistance would play an increasingly important role in field resistance. The identification of QTL on B2 instead of on B7 suggests that physiological resistance was more important in 2001 compared to 2002. More upright RILs that were very susceptible to white mold in 2001 developed lower levels of disease in 2002.

The linkage groups B2 and B7 were the most important for white mold resistance in 2001 and 2002, respectively. QTL associated with phenological and agronomic traits were detected on B2 and B7. Significant QTL for days to flowering (AFLP 63; R^2 = 7.6%), days to maturity (AFLP16; R^2 = 28.5%), and lodging (AFLP 49; R^2 = 8.5%) across environments were detected on B2 (Table 1.7). The association of the marker AFLP03 with a QTL for DSI in 2002 in the same region of B7-b where QTL were detected for days to flowering (R^2 = 35.7), and seed size (R^2 = 9.4) across the environments and for lodging (R^2 = 9.6) in 2002. Another QTL close to marker AFLP70 (R^2 = 9.8), associated with days to maturity was detected in the same linkage group. On B7-a, QTL associated with yield, days to flower, lodging and seed size were identified. A QTL explaining 21.5% of phenotypic variation for yield across the two years was detected near marker AFLP121c on B7-a. Marker AFLP116 from B7-a mapped to the *Phs* gene region on B7 in BJ population and might explain the effect on traits such as seed size and yield.

The QTL detected in Bunsi/Raven population for DSI accounted individually for up to 15% of the phenotypic variability. In other crops similar results were reported for QTL for resistance to white mold and associations with agro

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agronomic traits. In soybean, three QTL, explaining 8, 9 and 10% of the variability for DSI were detected in a 152 F3-derived line population in four environments (Kim and Diers, 2000). Two of these QTL were also significantly associated with disease avoidance mechanisms such as plant height, lodging, and date of flowering. The other QTL was not related to agronomic traits and may be involved in physiological resistance to white mold. Another study with soybean was conducted in five RIL populations using the laboratory detached leaf method to measure lesion area on leaves inoculated with mycelium plugs (Arahana et al., 2001). Seven, out of 28 putative QTL for resistance to *S. sclerotiorum*, were identified in multiple populations. Some of the QTL regions mapped near resistance genes and resistance gene analogs. The level of phenotypic variation explained by individual QTL ranged from 4 to 10%.

QTL for branching pattern, seed weight, oil content and flowering date were also found to be associated with resistance to white mold in sunflower (Mestries et al., 1998). Colocalization of QTL affecting resistance to both *S. sclerotiorum* and *Diaphorte helianthi* in sunflower was found by Bert et al., (2002), suggesting a common component in the mechanism of resistance to these two pathogens.

The Bunsi/Raven RIL population was known to be segregating for two disease resistance genes, *Co-1* and *bc-3*. *Co-1* confers resistance to race 73 of *C. lindemuthianum* and *bc-3* conditions resistance to bean common mosaic necrotic virus (BCMNV) strain NL-3 in common bean. The 98 RILs were tested for the reaction to race 73 of *C. lindemuthianum* and BCMNV strain NL-3 and both traits were considered phenotypic markers in Bunsi/Raven RIL population. Each phenotypic marker was linked with one molecular marker that was not included in the twelve linkage groups. The *Co-1* gene was linked to AFLP 42 at 7.2cM and gene *bc-3* was mapped 16.0 cM from marker AFLP85. These phenotypic markers were not studied further as *Co-1* is located on B1 and *bc-3* is located on B6 (Kelly et al., 2003), genomic regions where QTL for white mold were not detected in this study.

Factors affecting Mapping and QTL detection

The use of bulked segregant analysis (BSA) to detect candidate markers using only field data from 2001 may have resulted in the clustering of markers on three linkage groups, B2, B4 and B5. BSA resulted in detection of many polymorphic bands that mapped in regions associated with resistance to white mold. This hypothesis is supported by the fact that QTL for DSI in 2001 were detected on two linkage groups where markers clustered, B2 and B5.

A second reason for the clustering could be the use of restriction enzymes *EcoRI* and *MseI* to digest the DNA in the AFLP analysis. *EcoRI* is non-sensitive to cytosine methylation compared to the alternative restriction enzyme *PstI* that is notably sensitive to methylation. In soybean, AFLP markers generated using *EcoRI/MseI* deviated from a random distribution when compared to markers generated by *PstI/MseI* (Young et al., 1999). The presence of one *EcoRI/MseI* cluster per linkage group and the infrequent presence of markers derived from *PstI* suggested that those clusters could be mapped to heterochromatic regions close to centromers. However, in the rice genome the clustering of markers in only a few chromosomes could in part be explained due the small size of the rice genome (Harushima et al., 1998).

In the Bunsi/Raven population the detection of two linkage groups B4-a and B4-b that are mapped to B4 on the integrated map did not support the hypothesis of one cluster per chromosome in the centromere region. Further studies could be conducted in Bunsi/Raven population by including AFLP markers using *PstI/MseI* restriction enzymes.

The detection of QTL for complexly-inherited traits is highly dependent on the environment where the trait is measured. Environmental conditions or even microclimate variations may affect the expression of QTL for resistance to white mold in common bean. The detection and location of the QTL is also affected by the population size used in the study (Miklas et al., 2001).

The population used in this study was developed from a cross between Bunsi and Raven that are adapted to Michigan environmental conditions. A population derived from parents with a narrow genetic background is considered more useful for detection of QTL with minor effects. If a population from a wide cross is tested, QTL are more likely identified, but may be related with broad differences in gene pools, instead of minor differences associated with an agronomically important complexly-inherited trait. Based on the narrow genetic background of the Bunsi/Raven RIL population used in this study, a broad coverage of the genome was not expected. Genetic maps to characterize a crop species are constructed between more diverse parents, for instance, from different gene pools in common bean. The map generated in Bunsi/Raven population, covered 227.5 cM of the genome compared to 1226 cM of the integrated map from BJ population, indicating that Bunsi and Raven have a similar genetic background, despite differences for resistance to white mold. Only genetic differences can result in polymorphic bands that are used to establish a linkage map. In the Bunsi/Raven population, the similar genetic background increases the probability of detecting those genomic regions associated with the trait of interest. Both parents display an indeterminate growth habit, which should be considered a useful aspect to detect minor QTL, because growth habit is eliminated as a factor that might interfere with resistance to white mold. Differences in growth habit have been shown to be associated with different levels of white mold in common bean. Determinate navy bean genotypes such as Newport and Midland, are very susceptible to white mold (Kolkman and Kelly, 2000), whereas the open, porous canopy of larger-seeded Andean determinate beans have been considered a resistant phenotype (Miklas et al., 2001; Park et al., 2001) in semi-arid regions. Bunsi and Raven exhibit contrasting architecture, and the effect of upright plant type on resistance to white mold was evaluated in different years with different environmental conditions. Further studies to detect more minor QTL associated with physiological resistance should be carried out in populations derived from parents with similar architecture and growth habit.

Different genomic regions detected in this study in different years can be used to improve resistance to white mold in common bean. The QTL detected on B5 may represent a new source of physiological resistance for white mold. The identification of these different QTL is crucial because it allows one to enhance resistance by selecting more than one QTL from different linkage group regardless of the type of resistance mechanism.

References

Abawi, G.S., and R.G. Grogan. 1975. Source of primary inoculum and effects of temperature and moisture on infection of beans by *Sclerotinia sclerotiorum*. Phytopathology 65:300-309.

Abawi, G.S., F.J. Polach, and W.T. Molin. 1975. Infection of bean by ascospores of *Whetzelinia sclertoiorum*. Phytopathology 65:673-678.

Arahana, V.S., G.L. Graef, J.E. Specht, J.R. Steadman, and K.M. Eskridge. 2001. Identification of QTL for resistance to *Sclerotinia sclerotiorum* in soybean. Crop Sci. 41:180-188.

Basten, CJ, BS Weir, Z-B Zeng. 2001. *QTL Cartographer*, Version 1.15. Dept. of Statistics, Norht Caroline State University, Raleigh, NC.

Bert, P.F., I. Jouan, D. Tourvieille de Labrouhe, F. Serre, P. Nicolas, and F. Vear. 2002. Comparative genetic analysis of quantitative traits in sunflower (*Helianthus annuus* L.) 1. QTL involved in resistance to *Sclerotinia sclerotiorum* and *Diaporthe helianthi*. Theor. Appl. Genet. 105:985-993.

Blad, B.L.; J.R. Steadman, and A. Weiss. 1978. Canopy structure and irrigation influence white mold disease and microclimate of dry edible beans. Phytopathology 68:1431-1437.

Brambl, R., and W. Gada. 1985. Plant seed lectins disrupt growth of germinating fungal spores. Physiol. Plant 64:402-408.

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

Coyne, D.P., J.R. Steadman, and F.N. Anderson. 1974. Effect of modified plant architecture of Great Northern dry bean varieties (*Phaseolus vulgaris*) on white mold severity and components of yield. Plant Disease Reporter 58:379-382.

Coyne, D.P., J.R. Steadman, and H.F. Schwartz. 1977. Reaction of *Phaseolus* dry bean germoplasm to *Sclerotinia sclerotiorum*. Plant Disease Reporter 61:226-230.

Coyne, D.P., J.R. Steadman, and H.F. Schwartz. 1978. Effect of genetic blends of dry beans (*Phaseolus vulgaris*) of different plant architecture on apothecia production of *Sclerotinia sclerotiorum* and white mold infection. Euphytica 27:225-231.

Coyne, D.P. 1980. Modification of plant architecture and crop yield by breeding. HortScience 15:244-247.

Edwards, K.C., C. Johnstone and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nuc. Acids Res. 19:1349.

Fehr, W. 1987. Heritability. pp. 95-105. In: Principles of cultivar development, theory and technique, v.1. Iowa State University, Ames, U.S.A.

Ferraz, L.C.L., A.C. Café Filho, L.C.B. Nasser. 1999. Effects of soil moisture, organic matter and grass mulching on the carpogenic germination of sclerotia and infection of bean by *Sclerotinia sclerotiorum*. Plant Pathology 48:77-82.

Freyre, R., P.W. Skroch, V. Gelfroy, AF. Adam-Blondon, A Shirmohamadali, W.C. Johnson, V. Llaca, R.O. Nodari, P.A. Pereira, S.M. Tsai, J. Tohme, M. Dron, J. Nienhuis, C.E. Vallejos. P. Gepts. 1998. Towards and integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theor. Appl. Genet. 97:847-856.

Gerlagh, M., H.M. Goossen-van de Geijn, N.J. Fokkema, and P.F.G. Vereijken. 1999. Long-term biosanitation by application of *Coniothryrium minitans* on *Sclerotinia sclerotiorum*-infected crops. Phytopathology 89:141-147.

Godoy, G., J.R Steadman, M.B. Dickman, and R. Dam. 1990. Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris.* Physiological and Molecular Plant Pathology 37:179-191.

Haas, J.H., and B. Bolwyn. 1972. Ecology and epidemiology of *Sclerotinia wilt* of white beans in Ontario. Can. J. Plant Sci. 52: 525-533.

Haley, S.D., P.N. Miklas, L. Afanador, and J.D. Kelly. 1994. Random amplified polymorphic DNA marker variability between and within gene pools of common bean. J. Am. Soc. Hort. Sci. 119:122-125.

Hall, R., and L.G. Phillips. 1996. Evaluation of parameters to assess resistance of white bean to white mold. Annu. Rep. Bean Improv. Coop. 39:306-307.

Hart, P.H., and A.W. Saettler. 1981. White mold of beans. Cooperative Extension Service, Michigan State University, Extension Bulletin E-892.

Harushima, Y., M. Yano, A. Shomura. 1998. A high-density rice genetic linkage map with 2275 markers using a single F2 population. Genetics 148:479-494.

Hazen, SP, P Leroy, R Ward. 2002. AFLP in Triticum aestivum L.: patterns of genetic diversity and genome distribution. *Euphytica* 125:89-102.

Huang, H.C., and R.S. Erickson. 2000. Biocontrol of apothecial production of *Sclerotinia sclerotiorum* in pulse and oiseed crops. Annual Report of the Bean Improvement Cooperative 43:90-91.

Hunter, J.E., M.H. Dickson, and A. Cigna. 1981. Limited-term inoculation: a method to screen bean plants for partial resistance to white mold. Plant Disease 65:414-417.

Kelly, J. D. 2001. Remarking bean plant architecture for efficient production. Advances in Agronomy. 71:109-144.

Kelly, J.D., G.L. Hosfield, G.V. Varner, M.A. Uebersax, S.D. Haley, and J. Taylor. 1994. Registration of Raven black bean. Crop Sci. 34:1406-1407.

Kelly, J.D., P. Gepts, P.N. Miklas, and D.P. Coyne. 2003. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. Field Crops Res. 82:135-154.

Kim, H.S., and B.W. Diers. 2000. Inheritance of partial resistance to *Sclerotinia* stem rot in soybean. Crop Sci. 40:55-61.

Knapp, S.J., W.W. Stroup, and W.M. Ross. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 36:1037-1045.

Kolkman, J.M., and J.D. Kelly. 1999. Inheritance to white mold in common bean. Annual Report of the Improvement Cooperative 42:47-48.

Kolkman J.M. 2000. Genetic dissection of resistance to white mold (*Sclerotinia sclerotiorum*) in common bean (*Phaseolus vulgaris*) Thesis (Ph.D.) Michigan State University. Plant Breeding and Genetics Program, Dept. of Crop and Soil Sciences.

Kolkman, J.M., and J.D. Kelly. 2000. An indirect test using oxalate to determinate physiological resistance to white mold in common bean. Crop Sci. 40:281-285.

Kolkman, J.M. and Kelly, J.D. 2002. Agronomic traits affecting resistance to white mold in common bean. Crop Sci. 42-693-699.

Kolkman, J.M. and Kelly, J.D. 2003. QTL conferring resistance and avoidance to white mold in common bean. Crop Sci. 43:539-548.

Lamey, A. 1996. Disease control update. Northarvest Bean Grower spring:22.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.

Mestries, E., L. Gentzbittel, D. Tourvieille de Labrouhe, P. Nicolas, and F. Vear. 1998. Analyses of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflowers (*Helianthus annuus* L.) using molecular markers. Mol. Breed. 4:215-226.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

Miklas, P.N., K.F. Grafton, and B.D. Nelson. 1992a. Screening for partial physiological resistance to white mold in dry bean using excised stems. J. Amer. Soc. Hort. Sci. 117:321-327.

Miklas, P.N., K.F. Grafton, G.A. Secor, and P.E. Mcclean. 1992b. Use of pathogen filtrate to differentiate physiological resistance of dry bean to white mold disease. Crop Sci. 32:310-312.

Miklas, P.N., and K.F. Grafton. 1992c. Inheritance of partial resistance to white mold in inbred populations of dry bean. Crop Sci. 32:943-948.

Miklas, P.N., K.F. Grafton, and P. E. McClean. 1993. Estimating phenylalanine ammonia-lyase activity in common beans inoculated with *Sclerotinia sclerotiorum*. HortScience 28:937-938.

Miklas, P.N., R. Delorme, R. Hannan, and M.H. Dickson. 1999. Using a subsample of the core collection to identify new sources of resistance to white mold in common bean. Crop Sci. 39:569-573.

Miklas P.N., R. Delorme, W.C. Johnson, and P. Gepts. 2001. QTL conditioning physiological resistance and avoidance to white mold in dry bean. Crop Sci. 41:309-315.

Miklas, P.N., and R. Delorme. 2003. Identification of QTL conditioning resistance to white mold in snap bean. J. Amer. Soc. Hort. Sci. 128:564-570.

Nodari, R.O., S.M. Tsai, P. Guzman, R.L. Gilbertson, and P. Gepts. 1993. Towards an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. Genetics 134:341-350.

Park, S.J. 1993. Response of bush and upright plant type selections to white mold and seed yield of common beans grown in various row widths in southern Ontario. Can. J. Plant Sci. 73:265-272.

Park S.O., D.P. Coyne, J.R. Steadman, and P.W. Shroch. 2001. Mapping of QTL for resistance to white mold disease in common bean. Crop Sci. 41:1253-1262.

Petzoldt, R., and M.H. Dickson. 1996. Straw test for resistance to white mold in beans. Annu. Rep. Bean Improv. Coop. 39:142-143.

Ronin , Y.I., A.B. Korol, and J.I. Weller. 1998. Selective genotyping to detect quantitative trait loci affecting multiple traits: interval mapping analysis. Theor. Appl. Genet. 97:1169-1178.

Roberts, M.E., M.H. Dickson, and J.E. Hunter. 1982. Heritability of white mold resistance. Annu. Rep. Bean Improv. Coop. 25:104.

Ryder, T.B., S.A. Hedrick, J.N. Bell, X. Liang, S.D. Clouse, and C.J. Lamb. 1987. Organization and differential activation of a gene family encoding the plant defense enzyme chalcone synthase in *Phaseolus vulgaris*. Mol. Gen. Genet. 210:219-233.

SAS Institute. 1995. The SAS system for Windows. Release 6.12. SAS Inst., Cary, NC.

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

Schwartz, H.F., J.R. Steadman, and D.P. Coyne. 1978. Influence of Phaseolus vulgaris blossoming characteristics and canopy structure upon reaction to *Sclerotinia sclerotiorum*. Phytopathology 68:465-470.

Schwartz, H.F., D.H. Casciano, J.A. Asenga, and D.R. Wood. 1987. Field measurement of white mold effects upon dry beans with genetic resistance or upright plant architecture. Crop Sci. 27:699-702.

Shewry, P.R., J.A. Lucas. 1997. Plant proteins that confer resistance to pests and pathogens. Adv. Bot. Res. 26:135-192.

Singh, S.P. 1982. A key for identification of different growth habits of Phaseolus vulgaris L. Annu. Rep. Bean Improv. Coop. 25:92-95.

Singh, P.S. 1999. Integrated Genetic Improvement. In:Common bean improvement in the twenty-first century. S.P. Singh (ed). Kluver Academic Publishers, Dordrecht, The Netherlands.

Stam, P. 1993. Construction of integrated genetic linkage maps by means of new computer package: Joinmap. Plant J. 3:739-744.

Steadman, J.R., D.P. Coyne, and G.E. Cook. 1973. Reduction of severity of white mold disease on great northern beans by wider row spacing and determinate plant growth habit. Plant Disease Reporter 57:1070-1071.

Steadman. J.R. 1983. White mold- a serious yield-limiting disease of bean. Plant Disease 67:346-50.

Steadman, J.R. 1979. Control of plant diseases caused by *Sclerotinia* species. Phytopathology 69:904-907.

Steadman, J. R., K. Powers, and B. Higgins. 1997. Screening common bean for white mold resistance using detached leaves. Annu. Rep. Bean Improv. Coop. 40:140-141.

Steadman, J.R., R. Jung, M.S. Adams, K. Powers, and B Higgins. 2000. Random amplified polymorphic DNA (RAPD) distinguishes three species of *Sclerotinia sclerotiorum* but not pathogenic variability in *S. sclerotiorum* isolates from diverse host and geographic origin. Annu. Rep. Bean Improv. Coop. 43:158-159.

Sutton, D.C., and B.J. Deverall. 1984. Phytoalexin accumulation during infection of bean and soybean by ascospores and mycelium of *Sclerotinia sclerotiorum*. Plant Pathology 33:377-383.

Tar'an, B., T.E. Michaels, and K.P. Pauls. 2001. Mapping genetic factors affecting the reaction to *Xanthomonas axonopodis* pv *phaseoli* in *Phaseolus vulgaris* L. under field conditions. Genome 44:1046-1056.

Toubart, P., A Desiderio, G. Salvi, F. Cervone, L. Daroda, and G. De Lorenzo. 1992. Cloning and characterization of the gene encoding the endopolygalacturonase-inhibiting protein (Pgip) of *Phaseolus vulgaris* L. Plant J. 2:367-373.

Tu, J.C. 1985. Tolerance of white bean (*Phaseolus vulgaris*) to white mold (*Sclerotinia sclerotiorum*) associated with tolerance to oxalic acid. Physiological Plant Pathology 26:111-117.

Tu, J.C., and W.D. Beversdorf. 1982. Tolerance to white mold (*Sclerotinia sclerotiorum* (Lib.) De Bary) in Ex Rico 23, a cultivar of white bean (*Phaseolus vulgaris* L.). Can. J. Plant Sci. 62:65-69.

Vos, P., R. Hogers, M. Bleeker, M. Rijans, T. van der Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nuc. Acid Res. 23:4407-4414.

Walter, M.H., J. Liu, C. Grand, C.J. Lamb, and D. Hess. 1990. Bean-pathogenesis-related proteins deduced from elicitor-induced transcripts are members of a ubiquitous new class of conserved PR proteins including pollen allergens. Mol. Gen. Genet. 222:353-360.

Whipps, J.M. 2001. Developments in the use of *Coniothyrum minitans* for the biocontrol of *Sclerotinia sclerotiorum*. The XI International *Sclerotinia* Workshop. York, UK.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc. Acids Res. 18:6531-6535.

Young, W.P., J.M. Schupp, and P. Keim. 1999. DNA methylation and AFLP marker distribution in the soybean genome. Theor. Appl. Genet. 99:785-790.

Chapter II

MARKER-ASSISTED SELECTION FOR WHITE MOLD RESISTANCE IN COMMON BEAN.

Introduction

Complexly inherited traits such as resistance to white mold (*Sclerotinia sclerotiorum*) seem to have the most potential for marker assisted selection (MAS), because environmental effects confound direct selection. Since the cost to evaluate these complex traits with low to moderate heritability is high, MAS would be more likely to show the greatest gain. However, for many reasons including complex inheritance, low heritability, and environmental effects, markers associated with QTL have been difficult to find, account for small amount of actual genetic variability, and when available have exhibited limited usefulness across a range of genetic backgrounds or environments (Kelly and Miklas, 1999).

Application of MAS in plant breeding was analyzed by Staub and Serquen (1996) where they considered that the potential utility of MAS is limited by: 1) the number of molecular marker loci required to detect all significant associations; 2) population sizes required to detect QTL for traits with low heritability; 3) the sampling errors associated with the weighting of indices when combining molecular marker loci; and 4) phenotypic information and cost per unit of information gained.

Greater genetic gain can be made when flanking markers tightly linked to QTL are used as compared to single markers loosely linked to a QTL. The trade off is that it requires the characterization of twice as many markers as compared to selection using markers.

To improve the effectiveness of MAS, markers closely linked to the QTL need to be detected. Initially, the QTL needs to be precisely located, which is dependent on the population size, environmental conditions where the population is being tested and accuracy in the rating process. Another important aspect is to have available an adequately saturated linkage map at least in these areas were the QTL are mapped. The effectiveness of any MAS procedure will depend on the accuracy of the phenotypic classification of the complex trait of interest and the degree of linkage between marker(s) and traits of interest.

Various strategies for plant breeding were tested by Lande and Thompson (1990) using computer simulations to characterize MAS. Gain from selection of quantitative traits based on estimated additive effects could be greater for MAS than for phenotypic selection. The relative worth of MAS was greatest for characters with low heritability when additive genetic variance was associated with the marker. Later Gimelfarb and Lande (1994) demonstrated that MAS could also be effective for non-additive characters when compared to phenotypic selection.

Another limitation of using molecular markers to assist in selection of quantitative traits is the level of phenotypic variability explained by the QTL. The detection of QTL or combination of QTL that account for a significant amount of phenotypic variation is always desired, otherwise the MAS may not be cost-effective to be used in regular basis by breeders. Markers close to genomic regions with QTL linked to complexly inherited traits that explain only a small fraction of the phenotypic variability may not be considered useful by plant breeders in a selection process.

Detection of QTL for complexly inherited traits requires larger populations than those commonly used in simple genetic studies. Simulation studies including different population sizes showed that with a population of 100 individuals, only from 3 to 6 of 10 theoretical simulated QTL controlling 63% of the phenotypic variability were detected (Young, 1999), suggesting that in order to obtain more reliable and comprehensive data about quantitative traits, populations of at least 500 individuals are needed. In terms of practical application, large populations are not only more difficult to develop but are also more difficult to evaluate for complex traits. In addition, complexly inherited traits are highly influenced by the environment and require increased replication that results in very large field experiments. The increased number of years and locations necessary to effectively evaluate a population for a quantitative trait represents very high costs to plant breeding programs.

The effectiveness of large populations can quickly shrink if many data points are missing. QTL mapping presumes accurate phenotypic scoring methods, something that can be difficult to optimize in traits that are difficult to score. Just a few individuals incorrectly scored can totally confound QTL detection and mapping. High quality data from both field and the laboratory combined with the latest biometric approaches should reduce the problem of false positives in the identification of QTL (Young, 1999).

Methodologies of identification of QTL involve different aspects, including the similarity of the genetic background of the parents used to develop the mapping population, approaches to select candidate markers such as selective genotyping (Lander and Botstein, 1989), bulked segregant analysis (BSA) (Michelmore et al., 1991), and statistical methods to analyze the molecular data and phenotypic data in order to predict QTL. More powerful software to detect QTL was initiated with the development of Mapmaker-QTL (Lander et al., 1987) and advances with computer programs based on algorithms like composite interval mapping (CIM), analysis and permutation tests, such as QTL cartographer (Basten et al., 2001; Churchill and Doerge, 1994). QTL identification can be strongly affected by environmental factors. Methods, such as CIM, allow the evaluation of QTL x E by combining field data from different environments. Even when a promising QTL is identified, further work is required to test QTL in a different genetic background or environments. Repetition over different years and locations, tests in genetically unrelated populations and analyses of near isogenic lines to isolate the effects of individual QTL, are additional consideration in analyzing QTL.

Efforts to utilize MAS for the improvement of quantitative traits have been limited, but may improve as better quantitative data is generated and denser linkage maps become available from map co-integration across laboratories (Freyre et al., 1998).

Resistance to white mold is affected by physiological responses, more related with general plant defense mechanisms, than by avoidance mechanisms that include phenological and morphological traits. Architectural traits are normally easier to select in the field due the higher heritability. MAS probably would not be cost-effective for agronomic traits, but could be very important for the indirect selection of physiological resistance. However, agronomic traits could be included with other traits in the screening process of MAS. Often, qualitative traits are easier, faster, and more cost effective to select for directly rather than indirectly. In the case of agronomic traits such as plant growth habit, height, flowering, and maturity, indirect selection will likely not improve efficiency over regular field selection.

Non-specific resistance for white mold is not easily identified in the bean progeny when compared to major genes for race specific resistance to other bean diseases, because it is a quantitative trait and highly influenced by the environment conditions. The identification of markers linked to different QTL for a complexly inherited trait could represent a unique opportunity to bring together different plant defense mechanisms and improve non-specific resistance or horizontal resistance. The genotypes possessing most of these QTL associated with general plant defense mechanisms could represent important progress towards improving physiological resistance to white mold.

Use of MAS requires large segregating populations in order to have sufficient genetic variability for other important traits. MAS can be very efficient in early generations, but is also challenging if the goal is to select favorable alleles at specific loci without excessively reducing genetic variability in the rest of the genome. Considering the selection pressure required to fix alleles at several specific loci of interest, a single MAS step would require working with large segregating populations (Ribaut and Bertrán, 1999).

MAS goes beyond the identification of markers linked to QTL controlling important quantitative or qualitative traits. An effective strategy is needed in order to use MAS in a selection process. The appropriate generation and population size required for MAS, are some of the questions that need resolution before using MAS technology in a breeding program. Simulation studies have demonstrated the advantage of using DNA markers as an efficient complementary tool to conventional selection, especially when the MAS is applied in early generations of selection (Edwards and Page, 1994).

While many studies have been conducted to map QTL of important traits in maize (Bernardo, 1999), very few empirical applications of MAS in breeding programs have been reported. Most studies employing MAS have been theoretical, using computer simulations (Knapp, 1998; Van Berloo and Stam, 2001; Ribaut et al., 2002). The results of such simulation studies suggest that MAS can be used to improve quantitative traits in plant breeding programs.

In theory, MAS is proposed to be more efficient than phenotypic selection when heritability of a trait is low, where tight linkage exists between QTL and DNA markers (Knapp, 1998), particularly in earlier generations of selection before recombinational erosion of marker-QTL associations interfere with indirect selection (Lee, 1995). Comparing MAS and phenotypic selection for quantitative traits in sweet corn, Yousef and Juvik (2001) observed that the average MAS and phenotypic selection gain across different populations and selected traits was

10.9% and 6.1%, respectively. Use of MAS is most appropriate when traits are difficult and costly to measure. They concluded that incorporating DNA markers into traditional breeding programs could expedite the selection progress and be cost-effective.

Molecular markers (RAPD and AFLP) linked to white mold resistance were identified by Kolkman and Kelly (2003) using selective genotyping and BSA, in a population of recombinant inbred lines obtained from a cross between Bunsi (R) and Newport (S). The identified markers mapped to four linkage groups, B2, B3, B7 and B8 on the integrated bean map. One major QTL for disease incidence (DI) and disease severity index (DSI) was identified on linkage group B2, and was located near RAPD markers BC20.1800 and O15.1800. The region around BC20.1800 and O15.1800 on the integrated linkage map contains several identified genes that may be important in disease defense responses. The largest linkage group associated with white mold resistance was B7 (Kolkman and Kelly, 2003). Markers $E_{AAC}M_{CTT}130$ and $E_{AGG}M_{CTT}85$ that mapped in this linkage group were significantly associated with disease severity index, days to flowering, lodging, yield and seed size. The combination of RAPD marker BC20.1800 from B2 and AFLP markers, $E_{AAC}M_{CTT}130$ and $E_{AGG}M_{CTT}85$ from B7 contributed to 30% of variability for DSI and 27% for DI. The use of these markers in a strategy for selecting resistance to white mold was suggested by Kolkman and Kelly (2003).

The goal of this study was to evaluate the potential of MAS to enhance resistance to white mold, based on selection for RAPD marker BC20.1800 and

AFLP markers, $E_{AAC}M_{CTT}130$ and $E_{AGG}M_{CTT}85$ in a RIL mapping population of Bunsi (B) / Midland (S).

Materials and Methods

The MAS strategy was based on RAPD and AFLP markers linked to QTL for resistance to white mold and identified in a F_3-derived family population, developed from a cross between two navy bean cultivars, Bunsi and Newport (Kolkman and Kelly, 2003). MAS strategy was conducted on a 96 $F_{4:7}$ RIL population developed from a cross between Bunsi and Midland. Bunsi, with an indeterminate (type II) growth habit, has physiological resistance and a porous canopy for avoidance to white mold (Miklas and Grafton, 1992; Park et al., 2001). Midland is a susceptible cultivar with a determinate (type I) growth habit. Ninety-six F_2 plants were advanced from F_2 to F_4 through single seed descent method in the greenhouse. Seeds of individual F_4 plants were bulked, and advanced in a greenhouse. Seeds harvested from three $F_{4:5}$ plants were bulked and $F_{4:6}$ lines were increased in the field, in Saginaw, MI, during summer 2000. No selection for agronomic or phenological traits was made during the population development.

Plant tissue for DNA extraction was collected from parental genotypes and RIL from $F_{4:5}$ plants cultivated in the greenhouse. DNA was extracted from the plant tissue using a mini-prep procedure (Edwards et at., 1991; Haley et al., 1994).

The 96 RILs were scored based on one RAPD marker, BC20.1800 (R2=12.6% for DSI), mapped on linkage group B2 and two AFLP markers $E_{AAC}M_{CTT}130$ (R2=15.8% for DSI) and $E_{AGG}M_{CTT}85$ (R2=16.8% for DSI) located on linkage group B7. These three markers combined accounted for 30% of the phenotypic variability for DSI in Bunsi/Newport RIL across four environments (Kolkman, 2000). BC20.1800 and $E_{AGG}M_{CTT}85$ are markers in repulsion phase and marker $E_{AAC}M_{CTT}130$ was detected in coupling phase with resistance to white mold. The source of the three markers was Bunsi, the resistant parent from the RIL population where the three markers linked to QTL for resistance to white mold were identified.

Polymerase chain reaction for the RAPD marker was conducted in a 96-well PTC-100 programmable thermal controller (MJ Research, Inc) programmed for 3 cycles of 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C; 34 cycles of min at 94 °C, 1 min at 40 °C, and 2 min at 72 °C with the final step extended by 1 s for each of the 34 cycles, and a final extension cycle of 5 min at 72 °C (Haley et al., 1994). RAPD markers are identified by the name of the Operon primer, followed by the size of the polymorphic fragment, in reference to a 100 base pair DNA ladder (Invitrogen). The AFLP analysis was carried out as described by Hazen et al. (2002). Polyacrylamide gel electrophoresis was used to separate AFLP fragments. Fragments were visualized using a silver staining procedure (Promega). Gels were scored for band polymorphism, estimating band size in reference to a 100 and a 10 bp DNA ladder.

Two groups (selected=S and control=C) of 10 RILs each were selected based on the MAS strategy used. The S group was selected based on the presence of three markers in the linkage phase (coupling or repulsion) associated to the QTL conferring resistance to white mold. The lines include in S group had present the AFLP marker $E_{AAC}M_{CTT}130$ and absence of RAPD marker BC20.1800 and AFLP marker $E_{AGG}M_{CTT}85$. The control group was selected based on the absence of marker $E_{AAC}M_{CTT}130$ and presence of markers BC20.1800 and $E_{AGG}M_{CTT}85$. To investigate the association of growth habit with resistance to white mold, five lines selected in each group were either determinate or indeterminate. The 20 RILs and the parents, Bunsi and Midland, were planted in Montcalm County, MI, in June of 2001 and 2002. Plot rows were 6 m in length, with 0.5 m row spacing. The two center rows of each plot were planted with the RIL, while the two border rows were planted with the susceptible parent, Midland.

The soil type at the Montcalm Research Farm sites is a combination of Eutric Glossoboralfs (coarse-loamy, mixed) and Alfic Fragiorthods (coarse-loamy, mixed, frigid). Standard agronomic practices for tillage, fertilization, insect and weed control were applied to ensure adequate plant growth and development. Plots were irrigated during the whole growing season starting in the third week after planting with 12 mm of water at least once a week, depending on rainfall, in order to promote uniform disease pressure across the field. During 2001 plots were irrigated 8 times, totalizing 127 mm. In 2002 the plots were irrigated 11 times with approximately 140 mm of water.

Description of the traits:

Plots were rated for disease prior to harvest, when the plants had reached the physiological maturity. Thirty plants per plot were rated from 0 to 4, where 0 = no disease present, 1 = 1 to 25 %; 2 = 26 to 50 %; 3 = 51 to 75 %; and 4 = 76%

to 100 % of the plant with white mold symptoms in order to estimate DSI and DI (Hall and Phillips, 1996). DSI was calculated for each plot on a percentage basis, using the following formula:

$$\text{DSI (\%)} = \frac{\sum \text{(rating of each plant)} \times 100}{4 \times \text{(number of plants rated)}}$$

Disease incidence was calculated as the number of plants out of thirty evaluated individuals with white mold infection, based as a percentage. The RILs were also evaluated for agronomic and phenological traits, including: yield, seed size, days to flowering, and days to maturity, canopy height, architecture and lodging. Days to flowering were characterized by the number of days following planting, when 50% of the plants in a plot have at least one open flower. Around four weeks after flowering the plots were evaluated for architecture, using a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate. Lodging was determined at the same time that architecture was rated, based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging. Days to maturity were calculated as the number of days following planting, to when 90% of the pods were physiologically mature and drying down. Canopy height was measured at plant maturity on each individual plot, from six measurements per plot. All plots were harvested at maturity, when plots were individually pulled and threshed. Seed yield and weight of 100 seeds were adjusted to 18 % moisture content. In addition, the 20 RILs and the parents were screened in the greenhouse based on resistance to oxalate (ROX), an indirect assay to determine physiological resistance to white mold (Kolkman and Kelly, 2000). Twenty-one-day old seedlings (2nd trifoliate emerging) were cut at the base of the stem and placed in a 20 mM oxalate solution (pH 4.0). The seedlings were rated for wilting symptoms after 12 to 15 h of exposure to the oxalate solution (approximately 6 to 9 h of daylight). A 1 to 6 scale was used to measure wilting, where 1 = no wilting symptoms and 6 = total plant collapsing, as described by Kolkman and Kelly (2000).

The experiment was analyzed as a mixed model, using PROC MIXED (SAS, 1995) considering the groups (selected and control) and growth habit (determinate and indeterminate) as fixed factors and the genotypes as a random factor. The Pearson correlation coefficients (*r*) were calculated by PROC CORR (SAS, 1995).

Results and Discussion

The potential of MAS based on RAPD marker BC20.1800 and AFLP markers, $E_{AAC}M_{CTT}130$ and $E_{AGG}M_{CTT}85$ linked to QTL for resistance to white mold, identified previously in the cultivar Bunsi (Kolkman and Kelly, 2003), was confirmed in the F4 derived RILs originated from a cross between Bunsi and Midland. Based on two years of field evaluation under white mold pressure, the RILs included in the selected (S) group developed less disease than the control (C) group (Table 2.1). The two groups of RILs resulting from MAS used in this study were significantly different for DSI (p=0.034) and DI (p=0.057). The absolute differences either for DSI or DI were around 10%, what is questionable in terms of efficiency of MAS based on these three markers. However, the combination of these three markers in the original population where they were detected, contributed to 30% for DSI and 27% for DI, suggesting that although significant, the genetic gains would not be very large. There was no significant interaction between groups and growth habit or between groups and years, indicating the consistency and importance of the QTL associated with the markers used in this MAS study. The selected group compared to the control group was more resistant to oxalate (Table 2.1). Growth habit was not associated with resistance to white mold in both groups, contradicting previous studies that suggested that determinate growth habit was associated with higher susceptibility to white mold (Kolkman and Kelly, 2002). The lack of association between growth habit and resistance to white mold may have been the consequence of the reduced number of lines evaluated in this study.

The two groups resulting from the MAS were significantly different (p=0.046) in yield (Table 2.2). Higher yield could be a consequence of less disease and could also be a direct effect of the QTL close to marker $E_{AAC}M_{CTT}130$ that mapped to B7 (Kolkman and Kelly, 2003). QTL linked to marker $E_{AAC}M_{CTT}130$ marker accounted for 34.4% of phenotypic variability for yield in the Bunsi/Newport RIL population and was also associated with QTL for days to flowering (R^2=13.9%) and days to maturity (R^2= 6.0%).

Table 3.1 Dis

Table 2.1. Disease severity index (DSI), disease incidence (DI), resistance to oxalate (ROX), of the two MAS groups of Bunsi/Midland RILs grown under white mold pressure at Montcalm, MI, in 2001 and 2002.

		DSI(%)			DI(%)			ROX[1]
		2001	2002	Combined	2001	2002	Combined	
MAS group	Selected[2]	35.9	31.8	33.9	61.6	62.7	62.1	2.5
	Control[3]	42.2	40.6	41.4	72.3	72.5	72.4	3.4
	P-value			*0.034*			*0.057*	*0.001*
Growth habit	Type I	36.4	34.3	35.4	63.7	65.2	64.5	3.1
	Type II	41.7	38.1	39.9	70.2	69.9	70.1	2.8
	P-value			ns			ns	ns
Parents	Bunsi	23.9	19.2	21.5	45.6	46.7	46.1	1.4
	Midland	51.4	53.9	52.6	80.0	85.6	82.8	3.9
Mean (20)		39.1	36.2	37.6	66.9	67.6	67.3	2.9
CV (%)		32.8	36.8	34.1	21.5	27.1	24.2	18.3

[1] Resistance to oxalate was based on a scale from 1(resistant to oxalate) to 6(sensitive to oxalate) (Kolkman and Kelly, 2000) was conducted in a greenhouse.

[2] Selected group: presence of AFLP marker $E_{AAC}M_{CTT}130$ and absence of RAPD marker BC20.1800 and AFLP marker $E_{AGG}M_{CTT}85$, linked to QTL for resistance to white mold.

[3] Control group: absence of AFLP marker $E_{AAC}M_{CTT}130$ and presence of RAPD marker BC20.1800 and AFLP marker $E_{AGG}M_{CTT}85$, linked to QTL for resistance to white mold.

Although MAS did not significantly affect days to flowering and days to maturity (Table 2.2), both traits were associated with DI (Table 2.3), suggesting that earliness could function as an avoidance mechanism. However, selection for early genotypes can result in reduction of yield potential, since days to maturity was positively associated with yield (r=0.39**). Combining acceptable agronomic traits with resistance to white mold is crucial to select for useful genotypes in a breeding program.

The selected group exhibited more upright plants compared to the control group (Table 2.2). The architecture ratings for the selected and control groups were 2.8 and 3.3 (p=0.043), respectively. In addition, architecture was associated with DSI (r=0.37**) and DI (r=0.30**) (Table 2.3). Architecture may be part of the plant avoidance mechanisms that in combination with physiological resistance could result in higher levels of resistance to white mold. DSI was negatively associated with yield and seed size, indicating the effect of white mold disease on those traits. Consequently yield and seed size were positively correlated. ROX presumed to be associated with physiological resistance, was only associated with seed size. A biological association between seed size and resistance to oxalate is not known. The correlation coefficient between ROX and DSI was 0.38 (p =0.10), suggesting that both traits might be associated. ROX could play a role in the physiological resistance against *S. sclerotiorum* and consequently would affect the field resistance that is measured by DSI. The correlation for ROX was calculated based on genotype means (N=20), compared to other traits where the correlation was estimated on a plot basis (N=120).

Table 2.2. Combined yield, seed size, days to flowering, days to maturity, plant architecture and lodging of the two MAS groups of Bunsi/Midland RILs (selected[1] and control[2]), grown under white mold pressure in Montcalm, MI, in 2001 and 2002.

		Yield (kg/ha)	Seed size (g.100 seed^{-1})	Days to Flowering	Days to Maturity	Architecture[3] (1 to 5)	Lodging[4] (1 to 5)
MAS Group	Selected	3793	20.5	42.3	101.5	2.8	3.0
	Control	3371	18.8	44.0	100.2	3.3	3.2
	P-value[5]	*0.046*	*0.004*	*0.076*	ns	*0.043*	ns
Growth habit	Type I	3516	19.3	43.0	100.2	2.7	2.6
	Type II	3648	20.0	43.3	101.5	3.4	3.6
	P-value	ns	ns	ns	ns	*0.003*	*0.001*
Parents	Bunsi	4131	19.3	42.5	102.5	4.0	3.4
	Midland	3391	17.9	41.7	98.2	2.8	2.7
Mean (20 RILs)		3582	19.7	43.4	100.8	3.1	3.05
CV (%)		15.7	5.5	3.3	2.4	18.1	16.6

[1] Selected group: presence of AFLP marker $E_{AAC}M_{CTT}130$ and absence of RAPD marker BC20.1800 and AFLP marker $E_{AGG}M_{CTT}85$, linked to QTL for resistance to white mold.

[2] Control group: absence of AFLP marker $E_{AAC}M_{CTT}130$ and presence of RAPD marker BC20.1800 and AFLP marker $E_{AGG}M_{CTT}85$, linked to QTL for resistance to white mold.

[3] Scale 1 to 5, where 1 = fully upright, 3 = bush, and 5 = prostrate.

[4] Scale 1 to 5, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging.

[5] P-value > 0.10.

Table 2.3. Phenotypic correlation[1] of disease severity index (DSI), disease incidence (DI), resistance to oxalate (ROX), yield, seed size, days to flowering, days to maturity, plant architecture and lodging for the 20 Bunsi/Midland RILs included in the two groups (selected[2] and control[3]), grown under white mold pressure, in Montcalm, MI, in 2001 and 2002.

	DSI	DI	ROX	Yield	Seed size	Flowering	Maturity	Architecture	Lodging
DSI	-	0.83**	ns	-0.44**	-0.30**	0.25*	ns	0.30**	0.22*
DI		-	ns	-0.21*	-0.23*	0.33**	0.26**	0.37**	0.22*
ROX			-	ns	-0.46*	ns	ns	ns	ns
Yield				-	0.48**	ns	0.39**	0.22*	0.18*
Seed size					-	ns	0.27**	ns	ns
Days to flowering						-	0.42**	0.48**	0.30**
Days to maturity							-	0.48**	0.25**
Architecture									0.64**

[1] Correlation estimated based on individual plot (N=120), whereas r was estimated for resistance to oxalate based on genotype mean (N=20).

[2] Selected group: presence of AFLP marker $E_{AAC}M_{CTT}130$ and absence of RAPD marker BC20.1800 and AFLP marker $E_{AGG}M_{CTT}85$, linked to QTL for resistance to white mold.

[3] Control group: absence of AFLP marker $E_{AAC}M_{CTT}130$ and presence of RAPD marker BC20.1800 and AFLP marker $E_{AGG}M_{CTT}85$, linked to QTL for resistance to white mold.

Thus the power of detecting associations is reduced with smaller numbers evaluated for ROX.

This study was conducted to determine the effectiveness of MAS for complexly inherited traits such as resistance to white mold in common bean. With the detection of QTL and discovery of markers linked to these traits and the application of MAS for white mold resistance should become more routine in bean breeding programs. MAS can be performed in the absence of the pathogen and in early generations when genetic resistance is not clearly expressed due to environmental variation in the field. Usually the contribution of each QTL to a quantitative trait is small, which makes pyramiding different QTL crucial to significantly improve the genetic gain for a complexly inherited trait.

A strategy that could be used to improve resistance to white mold would combine phenotypic selection for favorable agronomic traits with molecular markers linked to QTL associated with resistance to *S. sclerotiorum*. Considering that most of the agronomic traits have a high heritability, selection could effectively be conducted in early generations and in preliminary yield trials. Environments with less favorable conditions for white mold could be used to avoid selection problems regarding cause/effect that frequently occur with traits such as architecture and lodging that can significantly affect and/or be influenced by disease occurrence or severity. Scoring agronomic traits without the occurrence of disease would allow a more efficient selection for these architectural traits that could be combined with molecular markers associated with physiological resistance. During the field season, the lines could be screened for the markers included in the MAS strategy and during the final phenotypic selection procedure; the breeder could select those lines possessing favorable agronomic traits and the desirable QTL linked to resistance to white mold.

The MAS study performed in Bunsi/Midland population was based only on molecular markers without including any agronomic traits related with avoidance mechanisms or yield. This approach could result in selection of lines that are not commercially acceptable. The inclusion of agronomic traits in the selection process is important in a plant breeding program and likely would be the most effective way to combine resistance to white mold with desirable agronomic traits. Combining different QTL for white mold resistance and agronomic traits would require large populations in order to select lines that display the desired combination of markers and agronomic traits.

A multiple regression analysis for DSI, including agronomic traits and markers linked to QTL located on B2 and B7, was conducted by Kolkman and Kelly (2003). The model proposed with the highest R^2 (59.4%) was $Y = -109.5 + 1.3$ (canopy width) + 5 ($E_{AAC}M_{CTT}130$) – 11.0 (BC20.1800) + 0.9 (days to flowering). The same two markers included in this model were used in addition to $E_{AGG}M_{CTT}85$. A simulated selection using marker and agronomic variables of this model was applied to another 98 entry RIL population derived from the cross between Bunsi and Raven, using ratings for architecture that are related to canopy width. From those traits measured in the Bunsi/Raven RIL population, architecture is most similar to canopy width used in the model.

The effectiveness of MAS in a population of RIL derived from a cross between Bunsi and Raven was tested. The selection was initially based only on markers (Group M) $E_{AAC}M_{CTT}130$ (presence) and BC20.1800 (absence), resulting in 28 selected lines. The second step in the selection process was to include the architecture canopy width trait (Group M+A). Lines with score for architecture < 2.5 and > 3.0 were excluded. These values were considered equivalent to a medium canopy width and branching pattern, in a white mold resistant plant ideotype proposed for U.S. Midwest environmental conditions (Kolkman and Kelly, 2002). When yield was also included as a selection criterion, ten lines with high yield were selected (Group M+A+Y). The control group (Group C) was formed by lines with $E_{AAC}M_{CTT}130$ (absence) and BC20.1800 (presence), regardless of phenological and agronomic traits.

The group M (based on markers) was compared to the control group and was significantly different for DSI and DI, suggesting that the MAS strategy based on markers would be effective to select lines with higher levels of resistance to white mold (Table 2.4). MAS was also effective in improving yield and seed size, which could be a direct effect of the QTL on those traits or a consequence of lower levels of white mold disease in the M group. Days to flowering and days to maturity were affected by the selection strategy, however, in practical sense the selected lines still fell in an acceptable commercial range. Including architecture (group M+A) as a selection criterion did not result in RILs with higher levels of DSI and DI, which was unexpected, but could be explained because some of the 10 lines excluded from group M to form group M+A, with

Table 2. 4. Disease severity index (DSI), disease incidence (DI), yield (Y), seed size, days to flowering, days to maturity, lodging, and architecture (A) of MAS groups derived from 98 entry RIL population of Bunsi/Raven, grown under white mold pressure at Montcalm, MI, in 2001 and 2002.

Trait	Population mean(98)[5]	GroupM[1] (28)	GroupM+A[2] (18)	GroupM+A+Y[3] (10)	Control[4] (14)
DSI (%)	36.1	33.2**[6]	33.0**	27.7**	40.4
DI (%)	63.9	59.9**	59.5**	52.5**	72.5
Yield(kg/ha)	4315	4484**	4517**	4775**	4192
Seed size (g.100seed^{-1})	21.6	22.4**	22.7**	22.6**	20.7
Days to Flowering	44.5	44.0**	43.9**	43.9**	45.7
Days to Maturity	105.4	106.9**	107.0**	107.5**	104.7
Lodging[7]	3.2	3.2ns	3.1ns	2.9ns	3.1
Architecture[8]	2.7	2.8ns	2.8ns	2.8ns	2.7

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[1] Selection based on presence of AFLP marker $E_{AAC}M_{CTT}130$ and absence of RAPD marker BC20.1800, linked to QTL for resistance to white mold.

[2] Selection based on markers (Group M) and architecture ratings between 2.5 and 3.0 were included.

[3] Selection based on markers, architecture (GroupM+A) and yield (> 4370 kg/ha).

[4] Selection based on absence of AFLP marker $E_{AAC}M_{CTT}130$ and presence of RAPD marker BC20.1800, linked to QTL for resistance to white mold.

[5] Number of selected individuals included in each MAS group.

[6] Comparisons against Group control, where **, * and *ns* indicates $p < 0.01$, 0.05 and non significant, respectively.

[7] Scale 1 to 5, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging.

[8] Scale 1 to 5, where 1 = fully upright, 3 = bush, and 5 = prostrate.

architecture ratings of 2.1 developed lower levels of disease probably due avoidance. Progress for resistance to white mold was detected when yield was included in the multivariate selection process. Therefore yield is an important trait that should be considered when selecting for resistance to white mold. Selection for yield under high white mold pressure would be an effective approach to improve resistance to white mold. Selection only based on disease severity or incidence can result in lines with limited potential use in breeding due to combinations of undesirable agronomic traits.

Progress towards the effective use of MAS for quantitative traits in plant breeding programs depends on the detection of QTL associated with those complexly inherited traits in different genetic germplasm sources. The identification of QTL is only one step in MAS. Detection of markers tightly linked to the QTL and when possible, combinations of markers flanking the genomic regions are important aspects to develop an effective strategy for MAS. Pyramiding multiple QTL with individual minor effects is a challenging task, but the tools are available and it seems that MAS will play a more important role in breeding for quantitative traits in the future.

Growth habit was not a reliable predictor of the ability to escape white mold infection under field conditions (Schwartz et al.,1987). Instead, the plant canopy structure and canopy density associated with the respective growth habits influence microclimatic conditions within the plant canopy that are more or less favorable for *S. sclerotiorum* colonization, infection and spread (Schwartz et al, 1978; Schwartz et al., 1987). Park (1993) obtained similar results and concluded that narrow canopy, higher flowering nodes, and fewer branches in the upright plant type might contribute to avoid white mold infection. Narrow canopy plants allow better light penetration and air movement that results in rapid drying of dew on leaves and reduced moisture on the soil surface, resulting in a less favorable microclimate for white mold development (Coyne et. al., 1974; Blad et al. 1978; Schwartz et al., 1987).

Kolkman and Kelly (2002) proposed that the small-seeded ideotype resistant to white mold for the Midwest should possess physiological resistance to white mold and be indeterminate (Type II), resistant lodging, medium canopy width and branching pattern, and medium height, days to flower and maturity, to ensure adequate vegetative growth and yield potential without creating a favorable microclimate within the plant canopy for disease development.

Agronomic and phenological traits should be combined with MAS to select commercially acceptable lines with a desired architecture, phenological cycle, and yield potential, in addition to improved resistance to white mold. The phenotypic selection for all these traits with high heritability could be conducted in environments where the screening for the disease was not possible. The marker data in addition to the phenotypic ratings should allow the breeder to more efficiently select the best lines to be tested for white mold resistance in the next generation.

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References

Basten, CJ, BS Weir, Z-B Zeng. 2001. QTL Cartographer, Version 1.15. Dep. of Statistics, Norht Caroline State University, Raleigh, NC.

Bernardo, R. 1999. Selection response with marker-based assortative mating. Crop Sci. 39:69-73.

Blad, B.L.; J.R. Steadman, and A. Weiss. 1978. Canopy structure and irrigation influence white mold disease and microclimate of dry edible beans. Phytopathology 68:1431-1437.

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

Coyne, D.P., J.R. Steadman, and F.N. Anderson. 1974. Effect of modified plant architecture of Great Northern dry bean varieties (*Phaseolus vulgaris*) on white mold severity and components of yield. Plant Disease Report 58:379-382.

Edwards, K.C., C. Johnstone and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nuc. Acids Res. 19:1349.

Edwards, M.D., and N.J. Page. 1994. Evaluation of marker-assisted selection through computer simulation. Theor. Appl. Genet. 88:376-382.

Freyre, R., P.W. Skroch, V. Gelfroy, AF. Adam-Blondon, A Shirmohamadali, W.C. Johnson, V. Llaca, R.O Nodari, P.A Pereira, S.M. Tsai, J. Tohme, M. Dron, J. Nienhuis, C.E. Vallejos. P. Gepts. 1998. Towards and integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theor. Appl. Genet. 97:847-856.

Gimelfarb, A., and R. Lande. 1994. Simulation of marker assisted selection in hybrid populations. Genet. Res. 63:39-47.

Haley, S.D., P.N. Miklas, L. Afanador, and J.D. Kelly. 1994. Random amplified polymorphic DNA marker variability between and within gene pools of common bean. J. Am. Soc. Hort. Sci. 119:122-125.

Hall, R., and L.G. Phillips. 1996. Evaluation of parameters to assess resistance of white bean to white mold. Annu. Rep. Bean Improv. Coop. 39:306-307.

Hazen, S.P., P. Leroy, and R. Ward. 2002. AFLP in *Triticum aestivum* L.: patterns of genetic diversity and genome distribution. Euphytica. 125:89-102.

Kelly, J.D. and P.N. Miklas. 1999. Marker-assisted selection. pp 93-132. In: Singh, S.P. (ed). Common bean improvement in the twenty-first century. Kluver Acad. Pub. Norwell, U.S.A.

Knapp, S.J. 1998. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci. 38:1164-1174.

Kolkman JM. 2000. Genetic dissection of resistance to white mold (*Sclerotinia sclerotiorum*) in common bean (*Phaseolus vulgaris*) Thesis (Ph.D.) Michigan State University. Plant Breeding and Genetics Program, Dept. of Crop and Soil Sciences.

Kolkman, J.M., and J.D. Kelly. 2000. An indirect test using oxalate to determinate physiological resistance to white mold in common bean. Crop Sci. 40:281-285.

Kolkman, J.M. and Kelly, J.D. 2002. Agronomic traits affecting resistance to white mold in common bean. Crop Sci. 42-693-699.

Kolkman, J.M. and Kelly, J.D. 2003. QTL conferring resistance and avoidance to white mold in common bean. Crop Sci. 43:539-548.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S.E. Lincoln, and L. Newburg. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.

Lande, R., and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743-756.

Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Lee, M. 1995. DNA markers and plant breeding programs. Adv. Agron. 55:265-344.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

Miklas, P.N., and K.F. Grafton. 1992. Inheritance of partial resistance to white mold in inbred populations of dry bean. Crop Sci. 32:943-948.

Park, S.J. 1993. Response of bush and upright plant type selections to white mold and seed yield of common beans grown in various row widths in southern Ontario. Can. J. Plant Sci. 73:265-272.

Park S.O., D.P. Coyne, JR Steadman, and P.W. Shroch. 2001. Mapping of QTL for resistance to white mold disease in common bean. Crop Sci. 41:1253-1262.

Ribaut, J-M., and J. Bertran. 1999. Single large-scale marker-assisted selection (SLS-MAS). Molecular Breeding 5:531-541.

Ribaut, J-M, C. Jiang, and D. Hoisington. 2002. Simulation experiments on efficiencies of gene introgression by backcrossing. Crop Sci. 42:557-565.

SAS Institute. 1995. The SAS system for Windows. Release 6.12. SAS Inst., Cary, NC.

Schwartz, H.F., J.R. Steadman, and D.P. Coyne. 1978. Influence of *Phaseolus vulgaris* blossoming characteristics and canopy structure upon reaction to *Sclerotinia sclerotiorum*. Phytopathology 68:465-470.

Schwartz, H.F., D.H. Casciano, J.A. Asenga, and D.R. Wood. 1987. Field measurement of white mold effects upon dry beans with genetic resistance or upright plant architecture. Crop Sci. 27:699-702.

Staub, J.E., and F.C. Serquen. 1996. Genetic markers, map construction, and their application in plant breeding. HortScience 31:729-741.

Van Berloo, R., P. Stam. 2001. Simultaneous marker-assisted selection for multiple traits in autogamous crops. Theor. Appl. Genet. 102:1107-1112.

Young, N.D. 1999. A cautiously optimistic vision for marker-assisted breeding. Molecular Breeding 5:505-510.

Yousef, G.G. and A. Juvik. 2001. Comparison on phenotypic and marker-assisted selection for quantitative traits in sweet corn. Crop Sci. 41:645:655.

Chapter III

USE OF INBRED BACKCROSS METHOD TO INTRODUCE RESISTANCE TO WHITE MOLD FROM EXOTIC GERMPLASM INTO COMMON BEAN

Introduction

White mold, caused by *Sclerotinia sclerotiorum*, is a serious disease of common bean (*Phaseolus vulgaris*) that results in substantial yield loss and reduced seed quality (Steadman, 1983). Genetic resistance to *S. sclerotiorum* in cultivated bean is partial, highly affected by climate conditions, and confounded by plant avoidance mechanisms (Schwartz et al., 1987; Miklas et al., 1993). Different approaches to breeding for white mold resistance are needed due to major differences in climate in different bean growing regions of the world where the disease occurs. Upright plant architecture seems to be effective in avoiding the disease in more arid western production areas of the U.S., but offers less advantage in the more humid Midwest states, including Michigan. Adequate air movement through the canopy is critical to help reduce white mold infection, and architectural avoidance needs to be combined with sources of physiological resistance to enhance overall resistance in bean (Webster and Kelly, 2000).

A strategy to increase the genetic variability for physiological resistance to white mold in cultivated bean varieties is to introgress genes from primitive landrace varieties and related wild types of common bean. Moreover, the potential of wild beans as a source of unique germplasm to improve resistance and other traits including yield is receiving increased attention by breeders.

Exotic germplasm should be considered as source of novel variability for physiological resistance to white mold. Improved architecture and other traits considered as avoidance mechanisms are already available in cultivated commercial bean genotypes. Adequate levels of physiological resistance is lacking in most of the cultivated bean classes and is the most likely type of resistance that would be expected to be introduced from exotic bean germplasm.

The genetic base of commercial cultivars within specific market classes is narrow (McClean et al., 1993).The average world yield of common bean remains low (<900 kg ha^{-1}), and bean production suffers from a wide range of abiotic and biotic constraints. Improving resistance to abiotic stresses, such as drought and soil mineral deficiency and toxicity, simultaneously with resistance to biotic stresses, especially white mold, in high yielding cultivars for North America is a challenging goal. In order to accomplish this task, breeders should consider that as much as 90% of the genetic variability available in the primary gene pool of *P. vulgaris* and related species remains under utilized (Singh, 1999).

An example of effective use of exotic bean germplasm is the introgression of resistance to bruchid (*Zabrotis subfasciatus*) from wild Mexican bean genotypes. The protein arcelin, a plant-resistance factor that inhibits *Z. subfasciatus* growth, was identified in wild Mexican bean populations (Osborn et al., 1988) and has been successfully bred into cultivated beans (Cardona et al., 1990) using recurrent backcrossing. This protein is inherited as a single dominant gene and at least seven co-dominant allelic variants are available for breeding (Acosta-Gallegos et al., 1998; Cardona and Kornegay, 1999).

Compared with their wild progenitors, cultivated plants often show drastic phenotypic differences in traits such as seed dormancy, seed dispersal mechanisms, cycle (e.g. earliness), growth habit, photoperiod sensitivity and size of the harvested parts (Koinange et al., 1996). The combined phenotypic differences are called the domestication syndrome traits that result from selection during several thousands of years for adaptation to cultivated environments. The most important traits of the domestication syndrome in common bean are the loss of seed dispersal ability conditioned by the presence of fibers in the pods, and dormancy, conditioned by impermeability of the seed coat. The major genes controlling the domestication syndrome in common bean were mapped to three of the eleven linkage groups, B1, B2, and B8 (Freyre et al., 1998) of the bean genome (Koinange et al., 1996). The major phenotypic effect of individual factors and the high heritability of individual traits may reflect the domestication process. Useful mutations with a major effect would have had a higher likelihood of being detected and selected (Koinange et al., 1996). The simple genetic control of the domestication syndrome traits suggests that the introgression of diversity from the wild to the cultivated *P. vulgaris* should be feasible and not involve special difficulties. Small genetic changes on B1, B2 and B8 in the wild types should allow breeders to return to cultivated bean and therefore would permit the evaluation of novel traits in a cultivated background. The potential offered by landraces and wild types as sources of quantitative traits can only be tested when QTL associated with the trait of interest are transferred into adapted germplasm.

In terms of resistance to *S. sclerotiorum*, field resistance is the final goal of breeders. Putative sources of resistance are detected by greenhouse tests but need to be tested under high disease pressure in field conditions to verify the reaction to *S. sclerotiorum*. However, before field screening can be conducted, germplasm conversion may be needed to adapt many of these accessions to temperate North American conditions where photoperiod sensitivity, late maturity, and climbing growth habit interfere with field evaluation (Webster and Kelly, 2000).

Germplasm conversion consists of backcrossing insensitivity to photoperiod and other agronomic traits from an adapted donor parent to the unadapted accessions having putative resistance to *S. sclerotiorum*. Germplasm conversion is a long term project. The level of adaptation of the resulting lines can affect the breeders ability to identify superior sources for quantitative traits, such as resistance to white mold. Lack of complete adaptation may result in avoidance to white mold, but may be associated with undesired agronomic and/or phenological traits. Alternatively, plant introductions can be backcrossed into adapted cultivars and BC_nF_n progenies developed without selection can be tested for resistance under field conditions. The inbred backcross method has recently received attention as an effective method to transfer more complex quantitative traits from unadapted or diverse germplasm into otherwise adapted, productive cultivars (Bliss, 1993; Tanskley and McCouch, 1997; Hartman and St. Clair, 1998). Different percentages of the donor parent can be incorporated into the inbred backcross line population. The percentage of plant introduction germplasm that should be used in a breeding population was studied in soybean by Thorpe and Fehr (1970) using landraces and adapted varieties. The results indicated that the use of 25% unadapted germplasm was more successful than 50% unadapted germplasm in soybean crosses.

Very large collections of germplasm from around the world have been assembled in efforts to conserve the extant genetic variation of numerous species (Miklas et al., 1999). To evaluate and utilize effectively these collections, it is first necessary to identify a smaller subset or core collection for testing that represents most of the genetic variation in the entire collection (Skinner et al., 1999). Establishment of core collections, by including a representative sample from the total accessions maintained in germplasm banks, facilitates the evaluation of a wide array of genetic diversity for resistance to a complex pathogen such as *S. sclerotiorum*. Once a core accession is identified with potential resistance to certain pathogen, an expanded search for resistance can be conducted among accessions with similar passport data in the active collection (Miklas et al., 1999). On the basis of several statistical models, Brown (1989) suggested that at least 70% of the alleles presented in the entire collection will be represented in a core collection comprised of at least 10% of the total accessions. The importance of core collections is recognized, but the core accessions still need to be evaluated to detect useful genes. Since most of the core accessions cannot be tested directly in the field due problems with adaptation, it is necessary to use indirect screening methods to select for resistance to *S. sclerotiorum*. Considerable efforts have been made to develop greenhouse and laboratory screening methods in order to identify physiological resistance to *S. sclerotiorum* (Hunter et al., 1981; Miklas et al., 1992a; Miklas et al., 1992b; Petzoldt and Dickson, 1996; Steadman et al., 1997; Kolkman and Kelly, 2000). The identification of putative sources of resistance is the step to effectively exploit exotic germplasm in breeding programs. Only those selected core accessions with putative resistance to *S. sclerotiorum*, based on different screening methods, would be used in developing inbred backcross lines.

Crosses between cultivated and wild bean genotypes that contrast for important traits could generate mapping populations that may permit more detailed analysis about the genetic control of these traits. As the results accumulate from molecular mapping studies in crop plants, patterns are likely to emerge concerning the chromosomal positions of key loci controlling yield and other important traits. Once this information is available, exotic germplasm could be sampled for targeted areas of the genome through backcross breeding and the generation of allelic series in near isogenic lines that would then be evaluated agronomically (Tanskley and McCouch, 1997).

Attempts to use exotic germplasm have been conducted in different plant species. Studies conducted in tomato and rice indicated that exotic germplasm contains many new and useful genes that can significantly enhance agricultural production, even for complex traits like yield (Tanskley and Nelson, 1996; Tanksley and McCouch, 1997). The same studies also demonstrated that the phenotype of exotic germplasm is a poor predictor of its genetic potential. Combining molecular mapping techniques with inbred backcross was proposed by Tanskley and Nelson (1996) as a method for simultaneously discover and transfer valuable QTL from unadapted germplasm into elite breeding lines. Use of molecular mapping techniques like the advanced backcross QTL method could be crucial to identify important QTL and explore the genetic resources available.

The introgression of a QTL for yield from *Glycine soja* into commercial soybean cultivars was tested by Concibido et al. (2003). The detection of a QTL from the exotic germplasm accounting for 9% of phenotypic variability for yield demonstrated the potential of using exotic germplasm to improve yield in soybean. The availability of a number of SSR markers around the QTL region makes them good candidates for MAS. Thompson and Nelson (1998) evaluated the genetic diversity and agronomic performance of experimental lines derived from plant introductions (PIs) maintained in the USDA Soybean Germplasm Collection. Experimental lines possessed 25, 50, 75 or 100% of PI germplasm (based on pedigrees). The increased genetic diversity and yield provided evidence that exotic germplasm can contribute genes with the potential to increase yield.

Most of the important and unique alleles are masked by the preponderance of undesirable alleles in the wild species which restricts the use of the wild species for the genetic improvement of quantitative traits. Initially, wild germplasm from within the same gene pool of *P. vulgaris* should be used to increase genetic variability of quantitative traits, prior to using the wild germplasm from the other gene pool. After successfully introducing resistance to *S.*

sclerotiorum from wild germplasm to adapted cultivars within the same gene pool, the next step would be to transfer the sources of resistance between gene pools. The use of exotic germplasm to introduce novel genetic variability for important qualitative and quantitative traits is supported by plant breeders (Ininda et al., 1996; Concibido et al., 2003). The identification of sources of physiological resistance to white mold in bean is the main goal where use is made of landraces and wild genotypes as novel sources of genetic resistance to *S. sclerotiorum*. A better understanding of the different mechanisms involved in plant defense against *S. sclerotiorum*, and how they can affect the spread of fungus throughout the plant are important aspects that could help to identify sources of physiological resistance to *S. sclerotiorum*.

Physiological Resistance and Plant Defense Related Proteins

Physiological resistance is controlled by factors such as increased activities of plant defense-related enzymes (Miklas et al., 1993) and phytoalexin accumulation (Sutton and Deverall, 1984) that limit spread of the pathogen in plant tissue. Miklas et al., (1993) measured the phenylalanine ammonia-lyase (PAL) in detached stems inoculated with growing mycelium of *S. sclerotiorum* in five bean cultivars with differing levels of resistance to *S. sclerotiorum*. Greater PAL activity was observed in the resistant cultivar when compared to the susceptible cultivar, suggesting that PAL activity may be involved in physiological resistance of common bean to *S. sclerotiorum*.

In order to gain insight into physiological resistance and quantitative host-pathogen relationships in general, the activity of plant defense related proteins should be assayed in different genotypes with different levels of resistance to *S. sclerotiorum*. β-1,3-Glucanases, chitinases and peroxidases are some of these plant defense related proteins that might be involved in general plant defense mechanisms (Van Loon, 1999).

β-1,3-Glucanases are capable of degrading β-1,3-glucans (Leubner-Metzger and Meins, 1999) and are implicated in various functions since the substrate is widespread in fungal cell wall (Gooday, 1994). β-1,3-Glucanases may be directly involved in fungal resistance by inhibiting fungal growth through the degradation of fungal cell wall. β-1,3-Glucanases may also be involved indirectly in fungal resistance by producing β-glucan elicitors by fungal cell wall degradation. β-glucan elicitors have been reported to induce resistance against fungal pathogens by inducing plant defenses (Bhandal and Paxton, 1991).

Chitin is a linear homopolymer of β-1,4-linked N-acetyl-D-glucosamine residues and is a structural component of fungi and insects but is not found in higher plants (Gooday, 1994). Endochitinase , the enzyme that randomly hydrolyses internal β-1,4- linkages of chitin, is constitutively expressed in many crop plants, including beans (Boller et al., 1983). The exact physiological function of chitinases in higher plants is not known, but their existence has been considered a measure of defense to pathogens and insects rather than a normal metabolite essential for plant metabolism (Huang, 2001). Chitinases may be involved directly in defense by breaking down fungal cell walls and indirectly involved by producing elicitors that induce other plant defenses. Chitinase activity in plants significantly increases in numerous incompatible host-parasite interactions. Increases in chitinase activity were detected in bean leaves inoculated with alfalfa mosaic virus (de Tapia et al., 1986). Many fungal pathogens contain β-1,3-glucan, chitin, and chitosan as cell wall components. It is conceivable that enzymes hydrolyzing these fungal cell wall components are able to inhibit fungal growth.

Peroxidases catalyse a number of reactions that contribute to cell wall strengthening (Hammerschmidt et al., 1982). These reactions include the incorporation of phenolics into cell walls and lignification and suberization of plant cell walls. Anionic peroxidase is located in the cell wall and catalyzes the polymerization of cinnamyl alcohols into lignin (Peng and Kuc, 1992). Peroxidases may also have a role in defense by producing toxic levels of hydrogen peroxide and phenolic free radicals that restrict pathogen growth (Nicholson and Hammerschmidt, 1992).

The objectives of this work were to: 1. Introgress genetic variability from landrace and wild bean genotypes, selected as putative sources of resistance to *S. sclerotiorum,* into adapted genetic background through inbred backcross method;. 2. Test the reaction of the plant introductions, adapted parents and inbred backcross lines to *S. sclerotiorum* inoculation; and 3. Evaluate the activity of endogenous and induced defense related proteins in a group of bean lines including wild, landrace and cultivated genotypes.

Materials and Methods

Development of inbred backcross lines

Plant Material

Seven populations were developed through the inbred backcross method by crossing four plant introduction (PI) accessions, including wild genotypes and landraces, with three adapted dry bean cultivars (Table 1). The plant accessions PI 318695, PI 313850, PI 325685, and PI 313609 were originally selected as potential sources for resistance to *S. sclerotiorum* based on positive greenhouse tests conducted at Michigan State University, North Dakota State University and University of Nebraska-Lincoln (Kolkman, 2000). The greenhouse tests included the straw test (Petzoldt and Dickson, 1996), the leaf-agar plug assay (Steadman et al., 1997) and the oxalic acid assay (Kolkman and Kelly, 2000). The accessions PI 325685, and PI 318695 are wild type accessions and are considered potential sources of novel genetic diversity for resistance to *S. sclerotiorum.* Accession PI 313850 was also identified by Miklas et al. (1999), as having putative physiological resistance to *S. sclerotiorum*, by screening a sub sample of the core collection of *P. vulgaris* accessions using straw test. The core sub sample represents the active USDA National Plant Germplasm System Collection of 1,698 bean accessions from Central America and South America.

Due to photoperiod sensitivity, the crosses between the accessions and adapted navy (Bunsi and Huron) and black (Tacana) bean cultivars were made under short days (10 hours light /14 hours dark) in a growth chamber during summer of 2000. Field screening for resistance to *S. sclerotiorum* of unadapted germplasm is not practical, since morphological and phenological traits, such as photoperiod sensitivity and climbing growth habit prevent the evaluation for field resistance. The inbred backcross (IBC) method (Bliss, 1993) was conducted to develop inbred backcross lines to transfer potential valuable genes from unadapted genotypes in a commercial cultivar background where they can be effectively evaluated. The IBC method differs from the recurrent backcross in that no selection for donor traits is practiced during the backcrossing. To retain most of the genetic variability from the exotic germplasm represented in the IBL, different BC_1F_1 individuals need to be used as parents in the backcrossing. To overcome the problem of lack of adaptation within the wild accessions, two backcrosses to the recurrent parent were made in order to introgress quantitative traits from the wild genotypes into the adapted cultivated background, and one backcross was made to introgress quantitative traits from cultivated landraces PI 313850 and PI 313609 into local cultivars. Therefore, the average percentage of the landraces and wild genotypes in the resulting inbred backcross lines (IBL) were 25 and 12.5%, respectively. During the development of IBL from crosses involving wild genotypes, the objective was to use all BC_1F_1 plants available to obtain the BC_2F_1 generation to have represented the maximum genetic variability derived from the wild parent. For example, more than 35 different BC_1F_1 plants were utilized in crosses to generate the BC_2F_1 population. Lines were advanced to BC_2F_3 in the greenhouse through single seed descent and $BC_2F_{3:4}$ IBL were tested in the field. For those populations derived from crosses with landraces, involving only one backcross, five seeds from each individual BC_1F_1 plant were bu!l

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bulked and the BC_1F_2 generation was advanced in the field, during summer 2001 and individual plants were harvested. Lines were advanced to BC_1F_4 in the greenhouse by single seed descent and $BC_1F_{4:5}$ IBL were tested in the field in 2002.

Field evaluations

The $BC_2F_{3:4}$ and $BC_1F_{4:5}$ populations were planted in the field as single rows in Saginaw, Michigan, in 2002. The goals of the experiment in Saginaw were to evaluate the adaptation, agronomic traits of the 638 IBL derived from seven different crosses (Table 3.1), and also increase seed of the selected populations for future studies. Plot rows were 6m in length, with 0.5 m row spacing. The plots were spaced planted with 20 seeds per 6m row. The experiment consisted of single row plots per IBL without replication due to limited seed availability. Standard agronomic practices for tillage, fertilization, and insect and weed control were applied to ensure adequate plant growth and development. No attempt was made to create favorable conditions for white mold development and no disease was observed during the growing season.

The IBLs were visually rated for agronomic and phenological traits, including: architecture and lodging, maturity and overall desirability. At physiological maturity, plots were evaluated for architecture, using a 1 to 5 scale, where 1 = fully upright, 3 = bush, and 5 = prostrate. Lodging was determined at the same stage, based on a 1 to 5 scale, where 1 = no lodging, 3 = moderate lodging, and 5 = excessive lodging. Maturity was rated comparing the IBL to the adapted parents and was classified in early (E), normal (N) and late (L). Desirability was rated using a scale from 1 to 9 where 1=undesirable plant type and 9= highly desirable plant type. Both populations of Tacana, with PI 313850 and PI 318695 were selected along with the cross of Huron and PI 318695 for further studies because they were agronomically adapted. Selected populations were harvested at maturity, and then plots were individually pulled and threshed. The 100 seed weight of individual IBL was determined after adjusting to 18 % seed moisture content.

Greenhouse Straw Tests

Two Tacana derived IBL populations developed with PI 313850 and PI 318695 were screened in two different experiments for physiological resistance to *S. sclerotiorum* mold using the straw test (Petzoldt and Dickson, 1996). The straw test was carried out with four replications for the Tacana/PI 318695 IBL population and three replications for the Tacana/PI 313850 population. The experiment was conducted in greenhouse maintained at 20-24°C. The temperature control is crucial for a consistent an effective screening, since higher temperatures affect the ability of the pathogen in developing the disease. A 15 cm diameter clay pot containing Bacto potting planting mix substrate (Michigan Peat Co.), with 4 plants, represented a replicate that were randomized in complete blocks. Plants were inoculated 28 days after planting. The growing tip of the main stem was removed and a plastic straw containing a potato dextrose agar (PDA) plug of three-day-old growing *S. sclerotiorum* mycelium was placed over the intact cut stem. Only the edges of the growing fungus on the petri dish plates were used to inoculate the plants. Eight days after inoculation, the white mold reaction was scored from 1 to 9, suggested by Petzoldt and Dickson (1996) and modified by Miklas et al., (1999).

Analysis of Plant Defense Related Proteins

Plant material and inoculation with *S. sclerotiorum*

Common bean cultivars Tacana, Raven, Bunsi, and PI 318695 and PI 313850 accessions were grown in 15 cm diameter pots containing Bacto potting planting mix substrate (Michigan Peat Co.) in the greenhouse. The second fully expanded trifoliate leaves of 28 day-old plants were excised and brought to the laboratory. Leaves were harvested from three pots per cultivar, and each pot contained two plants, thus providing six replicate samples per trifoliate per cultivar. Half of the leaves (three trifoliates per cultivar) were inoculated with a 5 mm plug containing a 3-day-old *S. sclerotiorum* culture grown in PDA. The inoculated trifoliates were kept in a petri dish containing wet filter paper (Whatman # 1; 125 mm diameter). The other half of the trifoliate leaves were kept in a petri dish with wet filter paper and a 1 cm PDA plug without the fungus was placed into its surface (control). The size of the lesions was measured using a digital caliper (Mitutoyo Corp.) at 48 hours after inoculation and the leaf tissue surrounding the lesions was collected, weighed, frozen in liquid nitrogen and stored at -80^{0}C until required for use in enzymatic assays of peroxidase, β-1,3-glucanase and chitinase. Twenty-day old plants of bean cultivars Tacana, Raven, Bunsi, and accessions PI 318695 and PI 313850 were also tested against *S. sclerotiorum* by performing a straw test. The plants were grown under the same time and conditions of those plants used to assay the defense related proteins. Clay pots were used with three plants represented the experimental unit and ten replications in a completely randomized design.

Sample preparation and protein extraction

The frozen leaf material was homogenized on ice in 0.08M potassium acetate buffer, pH 5.0, containing 1 mM EDTA (3 $ml.g^{-1}$ fresh weight). Reduced glutathione was added to the buffer immediately prior to homogenization to achieve a final concentration of 5 mM. The extracts were centrifuged at 10,000 *g* for 15 minutes and the supernatants were collected. Protein content in crude extracts was determined using the Bio-Rad Protein Assay, with bovine serum albumin (BSA) as standard. Samples of these supernatants with volumes that varied depending on initial mass of fresh weight were frozen at -80^0C for subsequent use in the enzymes assays.

Spectrophotometric assays of peroxidase and β-1,3-glucanase

Soluble peroxidase activity was determined at room temperature by measuring the appearance of pink/brown color resulting from guaiacol oxidation in the presence of hydrogen peroxide, based on the method described by Hammerschmidt et al (1982). The reaction mixture consisted of 50 μl guaiacol 0.02 M (Sigma), 0.5 ml 0.38 M H_2O_2 and 2.0 ml 0.2 M sodium phosphate buffer pH 6.0 that were added to a 3.0 ml glass cuvette and the spectrophotometer was set to zero at 470 nm. Suitable diluted plant extract (50 µl) was added and the cuvette inverted to mix ingredients prior to measuring optical density at 470 nm every 0.25 minute for 4 minutes. The slope of the optical density versus time was used as a relative measure of peroxidase activity. Results were expressed as ΔOD_{470}.g fresh weight^{-1} min^{-1}. Three replicates were used for each sample and results averaged.

The β-1,3-glucanase assay was performed according to Dann and Deverall (2000) which relies on the release rate of a measurable blue dye when the dye-bound substrate is hydrolyzed by endo-1,3-β-glucanase. The substrate, azurine-crosslinked pachyman (AZCL-Pachyman), was obtained from Megazyme Pty. Ltd. (Wicklow, Republic of Ireland). The substrate hydrates in water but is water insoluble. Hydrolysis by endo-1,3-β-glucanase releases water-soluble dyed fragments and the rate of release can be directly related to the enzyme activity. Samples of the crude extract preparation (0.1 ml) were added to eppendorf tubes and 0.4 ml potassium acetate buffer 10 mM, pH 5.0 was added. A separate tube containing 0.5 ml of the buffer was included as a substrate blank. After pre-equilibration for 5 minutes at 30^{0}C, the reaction was initiated by adding 0.1 ml of AZCL-pachyman substrate (prepared at the rate of 0.1 g substrate per 3.0 ml of 10 mM potassium acetate buffer, pH 5.0) to each tube. The reaction was stopped at 10 minutes by adding 0.7 ml of a 20% w/v Tris (Sigma Chemical Co, USA) solution. The tubes were vortexed, left at room temperature for 5 minutes and then centrifuged at 9,000 G for 2 minutes. The optical density of the supernatants was measured at 595 nm in the spectrophotometer (Dann et al., 1996). β-1,3-Glucanase activity was determined by using a standard curve prepared with laminarinase (EC 3.2.1.6, Sigma) with amounts that varied from 1.25 to 20 units.ml^{-1}. The enzyme activity was expressed as units.g fresh weight^{-1}. Three replicates were used for each sample and results averaged.

Assay for chitinase activity

Chitinase activity was determined for diluted concentrated extracts as described by Trudel and Asselin (1989), modified by Velasquez and Hammerschmidt (personal communication). A gel containing 40 ml sodium phosphate buffer 0.01 M, pH 6.0 and 0.4 g agarose and glycol chitin (Sigma, 10 mg.ml^{-1}) was poured in a petri dish plate (125 mm). Glycol chitin was used as substrate and was prepared by acetylation of glycol chitosan (Molano et al., 1979). After the gel polymerized, small wells (2 mm diameter) were opened into the gel and 50 μg of the samples protein extract were poured into the openings. The gel was incubated for 4 hours at 37^{0}C in water bath. After incubation, 50 ml of Tris-HCl 0.5 M, pH 8.9 containing 0.01% w/v calcofluor white m2R (Sigma) was added into the gel. This staining solution was kept for 10 minutes, and then the gel was rinsed with distilled water. The petri dish plate was filled with distilled water for overnight period of time. The gel was placed under UV light, where dark lytic zones of chitinase activity could be visualized in a clear background. A standard curve of chitinase activity was prepared using a serial dilution from 1 to 20 units.ml^{-1} of commercially available chitinase prepared from *Streptomyces* *griseus* (Sigma). Pictures of the gels were taken under UV light, and the diameters of the lytic zones with chitinase activity were measured using image analysis software (Sigma Scan). Activity could be compared by the size of the lytic zone. The enzyme activity was expressed as units.g fresh weight^{-1}. Three replicates were used for each sample and the results were averaged.

Native polyacrylamide gel electrophoresis (PAGE) assays for peroxidase, β-1,3-glucanase and chitinase activity

Electrophoresis was carried out under non-denaturing conditions. Polyacrylamide resolving gels (7.5%, 1.5 mm) were prepared by mixing 4.9 ml deionized water, 2.5 ml 1.5 M Tris-HCl (pH 8.8), 2.5 ml 30% acrylamide/bis (29:1), 50 µl 10% (w/v) ammonium persulfate. The solution was degassed for 15 minutes, 5 µl of TEMED was added, and the gel was poured. The stacking gel (4.0%) was prepared by mixing 6.1 ml deionized water, 2.5 ml 0.5 M Tris-HCl (pH 6.8), 1.3 ml 30% acrylamide/bis, and 50 µl of 10% (w/v) ammonium persulfate. The solution was degassed for 15 minutes, then 10 µl of TEMED was added and the gel was poured. The upper tank buffer was prepared by mixing 5.2 g Tris base, 3.5 g glycine and 1 liter of distilled water. The lower tank buffer was prepared by mixing 14.5 g Tris base, 60 ml of 1 N HCl, and diluting to 1 liter with distilled water (Laemli, 1970). For chitinase detection gels, 0.01% (w/v) glycol chitin was added prior to polymerization of the gel. Equal amounts of protein (30 µg) were loaded onto the gels, and a constant current of 60 mA.gel^{-1}

was applied for 5 hours and the temperature set to 4^0C. A Mini-Protean III electrophoresis system from Bio-Rad was used to run the gels.

After electrophoresis, acidic peroxidase isoenzymes were visualized by soaking the gels in a staining solution that contained 200 ml of 0.1 M sodium acetate buffer, pH 5.0; 2 ml of 3-amino-9-ethyl-carbazole (prepared as 25 mg/ml of N,N-dimethylformamide), and 200 μl of 30% H_2O_2. After development of red-brown bands, the gels were refrigerated in 7% acetic acid until scanned or photographed (Graham et al., 1965).

Endo-1,3-β-glucanase isoenzymes were visualized by washing the electrophorized gels with distilled water three times and preincubated in 0.05 M potassium acetate buffer, pH 5.0, for 5 minutes with slow shaking. Then the gels were incubated at 40^0C for 30 minutes in a solution containing 75 ml 0.05 M potassium acetate buffer, pH 5.0 and laminarin (Sigma) predissolved in distilled water (1g/75 ml). The gels were washed three times with distilled water and placed onto a glass tray containing 200 ml 1.0 M NaOH and 0.3 g 2,3,5-triphenyltetrazolium chloride. The gels were kept in a boiling water bath until red bands appeared. The gels were transferred to a solution containing 3% glycerol, 40% methanol, 10% acetic acid, and 47% water (v/v) until photographed (Pan et al., 1989).

Chitinase isoenzymes were detected after incubating the gels for 2 hours at 37°C with reciprocal shaking in 0.1 M sodium acetate buffer, pH 5.0 containing 1% (v/v) Triton X-100. After the incubation, the gels were stained with a solution of 0.01% (w/v) calcofluor white m2R (Sigma) dissolved in 0.5 M Tris-HCl, pH 8.9

for 5 minutes. The dye solution was removed and the gels were kept in distilled water for about one hour at room temperature. Lytic zones were visualized by placing the gels under a UV light source, and the gels were photographed (Trudel and Asselin, 1989).

Data from the five genotypes including PI 313850, PI 318695, Tacana, Bunsi and Raven were analyzed for significance by one-way analysis of variance using the SigmaStat software. The straw test conducted in the two IBL populations was analyzed using PROC GLM (SAS, 1995) in a randomized complete block design with three replications for Tacana/PI 313850 and four replications for Tacana/PI 313695 IBL populations.

Results and Discussion

Development and evaluation of inbred backcross lines

Six hundred and thirty-eight inbred backcross progenies ($BC_2F_{3:4}$ and $BC_1F_{4:5}$) were developed from seven different crosses (Table 3.1). The inbred backcross lines may provide a unique opportunity for the evaluation of novel sources for resistance to *S. sclerotiorum* in an adapted cultivated genetic background. Field evaluation of landrace and wild types is not possible due to problems of adaptation of wild bean germplasm lacking the domestication syndrome traits (Koinange et al., 1996). Inbred lines can only be tested for field resistance against *S. sclerotiorum* if they are fully adapted to the local environmental conditions. Field screening for white mold resistance of unadapted germplasm is ineffective, since morphological and phenological traits, such as

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photoperiod sensitivity and climbing growth habit have undesired avoidance mechanisms and are could be falsely confounded with physiological resistance. The preliminary field test carried out in 2002, based on spaced plants and single plots, was the first opportunity to evaluate the IBL in terms of agronomic traits and adaptation to local conditions. The main objective was to identify the best populations in terms of adaptation and agronomic traits for further studies to detect enhanced levels of resistance to *S. sclerotiorum* among IBLs. Tacana/PI313850 and Tacana/PI 318695 IBL populations were selected from seven populations based on visually rated agronomic traits. Tacana was the adapted parent in both selected populations. Tacana (Lopez-Salinas et al., 1997) is an upright black bean that has more erect plant architecture compared to the other cultivated parents, Bunsi and Huron (Kelly et al., 1994) were used as parents to develop the other five backcross inbred populations. Lines within the two selected populations that showed extreme late flowering and maturity were discarded. Out of 97 and 116 IBL from Tacana/PI313850 and Tacana/PI 318695 populations, respectively, 75 and 89 IBL were chosen to be tested with the straw test to detect resistance to *S. sclerotiorum*.

Table 3.1. Number of inbred backcross lines developed in crosses between unadapted PI accessions with putative physiological resistance to *S. sclerotiorum* and three commercial dry bean cultivars, Tacana, Bunsi and Huron.

Accession PI	Improvement status	Origin	100 seed weight(g)	Generation	Tacana crosses	Bunsi crosses	Huron crosses
318695	Wild	Mexico	3.5	$BC_2F_{3:4}$	116	93	37
313850	Cultivated	Peru	61.0	$BC_1F_{4:5}$	97	115	-
325685	Wild	Mexico	3.6	$BC_2F_{3:4}$	-	100	-
313609	Cultivated	Colombia	69.2	$BC_1F_{4:5}$	-	-	80

Seed size was an important agronomic trait that was expected to be recovered with two backcrosses in the population derived from the cross between Tacana and the wild genotype PI 318695. Seed size ranged from 13.4 to 26.1g per 100 seeds in 89 IBL compared to 3.5 and 21.5g from the parents PI 318695 and Tacana, respectively (Table 3.2). Lodging and desirability scores indicate that lines with acceptable commercial architecture were developed and can be effectively tested in the field for resistance to *S. sclerotiorum*. On average the IBLs resulting from two backcrosses have 12.5% of contribution from the wild genotype. A third backcross was not considered necessary because it would reduce the average contribution of the exotic germplasm to 6.25% among the resulting IBLs. The goal was to retain as much genetic variability as possible from the wild source within IBL lines while maintaining adaptation, agronomic and seed size traits of the recurrent parent. The population derived from Tacana and the landrace PI 313850 was developed with one backcross, resulting in inbred backcross lines with 25% of genetic contribution from the landrace genotype. A landrace is already a cultivated genotype and therefore one backcross should be sufficient to recover adapted lines with acceptable combination of agronomic and seed size traits. Despite the gene pool differences between the Tacana (Middle American) and PI 313850 (Andean) parents, the population was well adapted and did not show the typical genetic incompatibility associated with gene pool crosses. The desirability scores of IBL indicated they possessed adequate adaptation and architecture (Table 3.3).

Table 3.2. Lodging, desirability coefficient, maturity, seed size and reaction to straw test in BC^2 IBL derived from the cross between Tacana and PI 318695, grown in Saginaw, MI, in 2002.

IBL code	Lodging[1]	DS[2]	Days to Maturity[3]	Seed size (g.100seed^{-1})	Straw Test[4]
8571	3	4	M	18.1	2.3
8540	4	4	E	19.0	2.5
8563	2	6	E	23.4	2.6
8553	3	5	M	20.2	2.7
8600	3	3	L	19.1	2.7
118-2	3	4	M	18.7	5.9
8501	4	3	E	16.2	6.3
8507	3	3	M	17.7	6.8
8599	3	4	M	18.4	7.1
8508	3	4	E	17.3	7.2
Average(89)	2.9	4.3	-	18.7	4.3
Range	2 – 4	3 – 7	-	13.4 – 26.1	2.3 – 7.2
Tacana	2.0	5.0	M	21.5	3.4
PI-318695	-	-	-	3.5[5]	4.5
Raven[6]	2.9	3.9	107	19.0	8.5
Bunsi[6]	3.5	4.0	109	22.8	6.6

[1] Lodging is rated in a 1 to 5 scale, where 1= no lodging, 3 = moderate lodging, and 5 = excessive lodging.

[2] Desirability score, the higher the score (from 1 to 9) the more desirable is the line.

[3] E=early, M= mid season and L=late, relative to days to maturity in Tacana.

[4] Straw test is scored in a scale from 1 to 9 where 1=no symptoms and 9= complete collapse of the plant.

[5] Seeds from plants grown in the greenhouse.

[6] Grown in Montcalm, MI, in 2002.

PI 313850 could be an important source of resistance to white mold for future breeding of Andean Kidney and Cranberry bean classes.

Greenhouse straw tests

Based on the reaction to *S. sclerotiorum* measured by the straw test, PI 313850 was the most resistant genotype, followed by PI 318695 and Tacana (Figure 3.1).The two populations derived from Tacana crossed with PI 318695 and PI 313850 were tested in relation to their reaction to *S. sclerotiorum* in the greenhouse using the straw test. In both populations significant differences ($P<0.001$) for reaction to *S. sclerotiorum* were detected among the IBL within each population. Many lines with low ratings for straw test were identified (Table 3.2, Figure 3.2 and Table 3.3 and Figure 3.3), suggesting that based on the reaction to *S. sclerotiorum*, putative resistance to white mold existed within both populations. The heritability estimative for the reaction to *S. sclerotiorum* in both populations were 0.65 and 0.59, suggesting that selection for reaction to straw test can be performed effectively in a breeding program.

Evaluation of defense related proteins activity in detached leaf assays

The response of bean genotypes PI 318695, PI 313850, Tacana, Bunsi and Raven to *S. sclerotiorum* and activity of defense-related enzymes was determined. The plant introductions and Tacana were used as parents to develop two inbred backcross population selected in Saginaw in 2002.

Table 3.3. Lodging, desirability coefficient, maturity, seed size and reaction to straw test in BC^2 IBL derived from the cross between Tacana and PI313850, grown in Saginaw, MI, in 2002.

IBL code	Lodging[1]	DS[2]	Days to Maturity[3]	Seed size (g.100seed^{-1})	Straw Test[4]
8607	4	3	M	25.8	1.3
8673	3	3	L	24.4	1.4
8601	2	3	M	27.1	1.8
8636	3	4	M	23.4	2.0
8604	2	4	M	23.2	2.0
8639	3	4	M	27.5	4.2
8690	2	5	M	23.9	4.3
8638	3	5	E	21.1	4.4
8695	3	3	L	28.6	5.1
8652	3	3	M	24.6	5.7
Average(76)	2.7	4.2	-	24.0	3.0
Range	2 - 4	2 – 6	-	18.3 – 31.9	1.3 – 5.7
Tacana	2	5	M	21.5	2.8
PI 313850	-	-	-	61.0[5]	3.3
Raven[6]	2.9	3.9	107	19.0	8.5
Bunsi[6]	3.5	4.0	109	22.8	6.6

[1] Scale from 1 to 5, where 1= no lodging and 5 = excessive lodging.

[2] Desirability score, the higher the score (from 1 to 9) the more desirable is the line.

[3] E=early, M=mid season and L=late, relative to Tacana.

[4] Scale from 1 to 9, where 1=no symptoms and 9= collapse of the plant.

[5] Seeds from plants grown in the greenhouse.

[6] Grown in Montcalm, MI, in 2002.

Bunsi and Raven were included as checks and they were chosen because they are the parents of the mapping population discussed in chapter 1.

The straw test results observed in the PI accessions and adapted bean varieties (Figure 3.1) were supported by the lesion size measured in detached leaves 48 hours post inoculation (Figure 3.4). Leaves of PI 313850 inoculated with *S. sclerotiorum* developed smaller lesions compared to all the other genotypes. Bunsi was considered a susceptible genotype based on the reaction to *S. sclerotiorum* with the detached leaf (Steadman et al., 1997) and straw (Pedzoldt and Dickson, 1996) tests. Bunsi has field resistance and is known as a source of resistance to *S. sclerotiorum*. Moderate correlation between field resistance to *S. sclerotiorum* and straw test rating has been reported (Hall et al., 1999; Myers et al., 1999; Miklas et al., 2001). However, the resistant genotypes Bunsi and G 122, normally scored greater than five in greenhouse straw tests. It is likely that the straw test measures only a single component (physiological resistance of stem internode and nodal tissue to pathogen invasion) of a complex of factors involved in physiological resistance (Miklas et al., 1999).

The activity of defense related enzymes peroxidase, β-1,3-glucanase and chitinase was evaluated 48 hours post inoculation. PI 313850 showed the highest peroxidase activity after infection, compared to the other four genotypes tested (Figure 3.5). Peroxidase activity was also higher in PI 313850 without the presence of the pathogen, suggesting that peroxidase was constitutively expressed in higher levels in this genotype. Tacana showed the highest β-1,3-glucanase activity with and without inoculation (Figure 3.6).

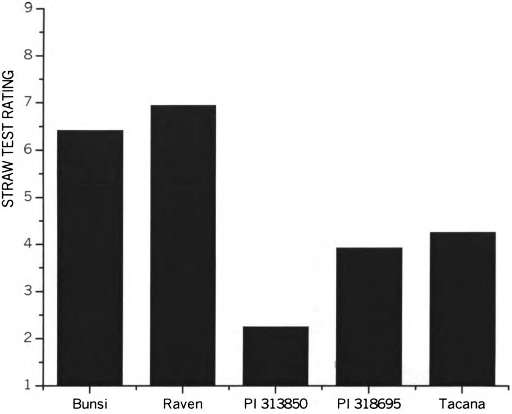

Figure 3.1. Reaction of adapted and exotic bean genotypes to inoculation with *S. sclerotiorum* using the straw test. Data represent mean of 10 replicates and the bars indicate standard deviation for each mean. $P<0.001$ for bean genotypes. The straw test was rated in a scale from 1 to 9 where 1= no symptoms and 9= total plant collapse.

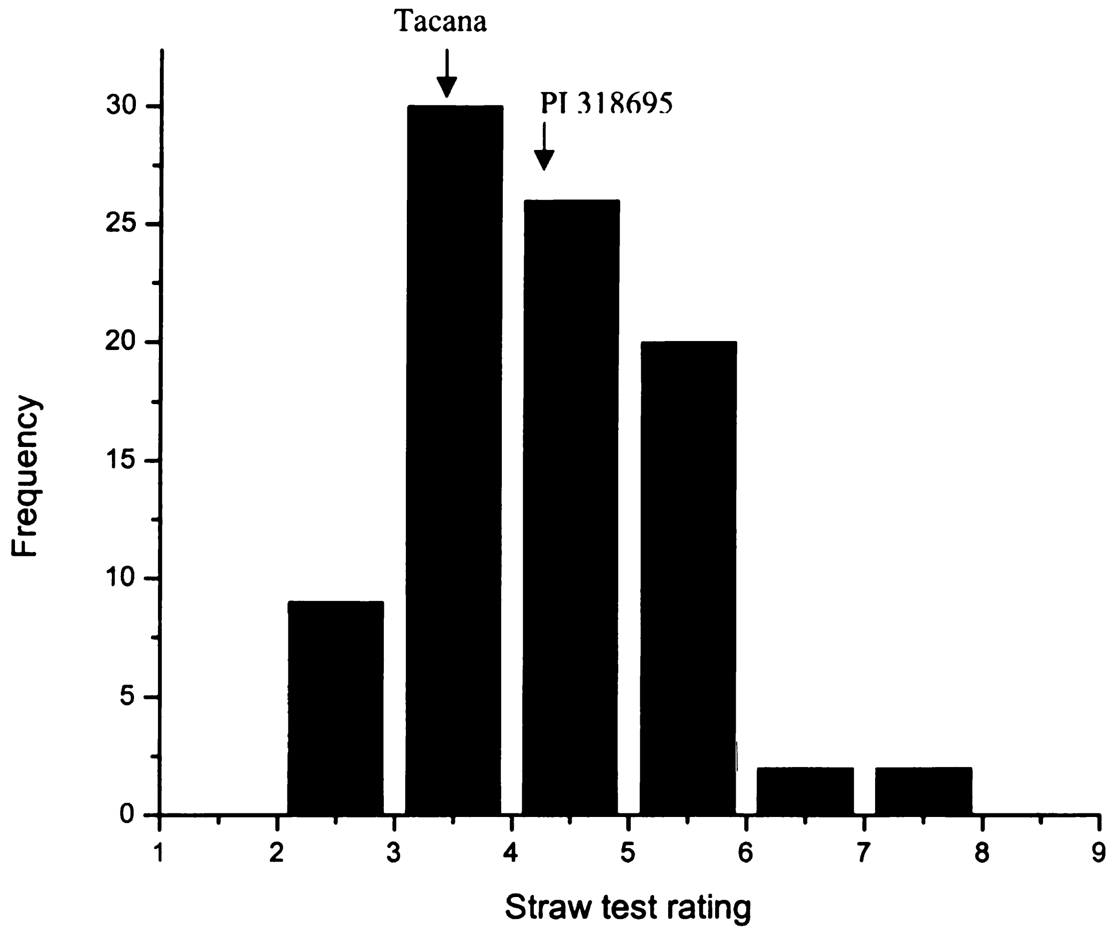

Figure 3.2. Reaction of 89 Tacana/PI 318695 inbred backcross lines (IBL) to inoculation with *S. sclerotiorum* using the straw test. $P < 0.001$ for IBL. The straw test was rated in a scale from 1 to 9 where 1= no symptoms and 9= total plant collapse.

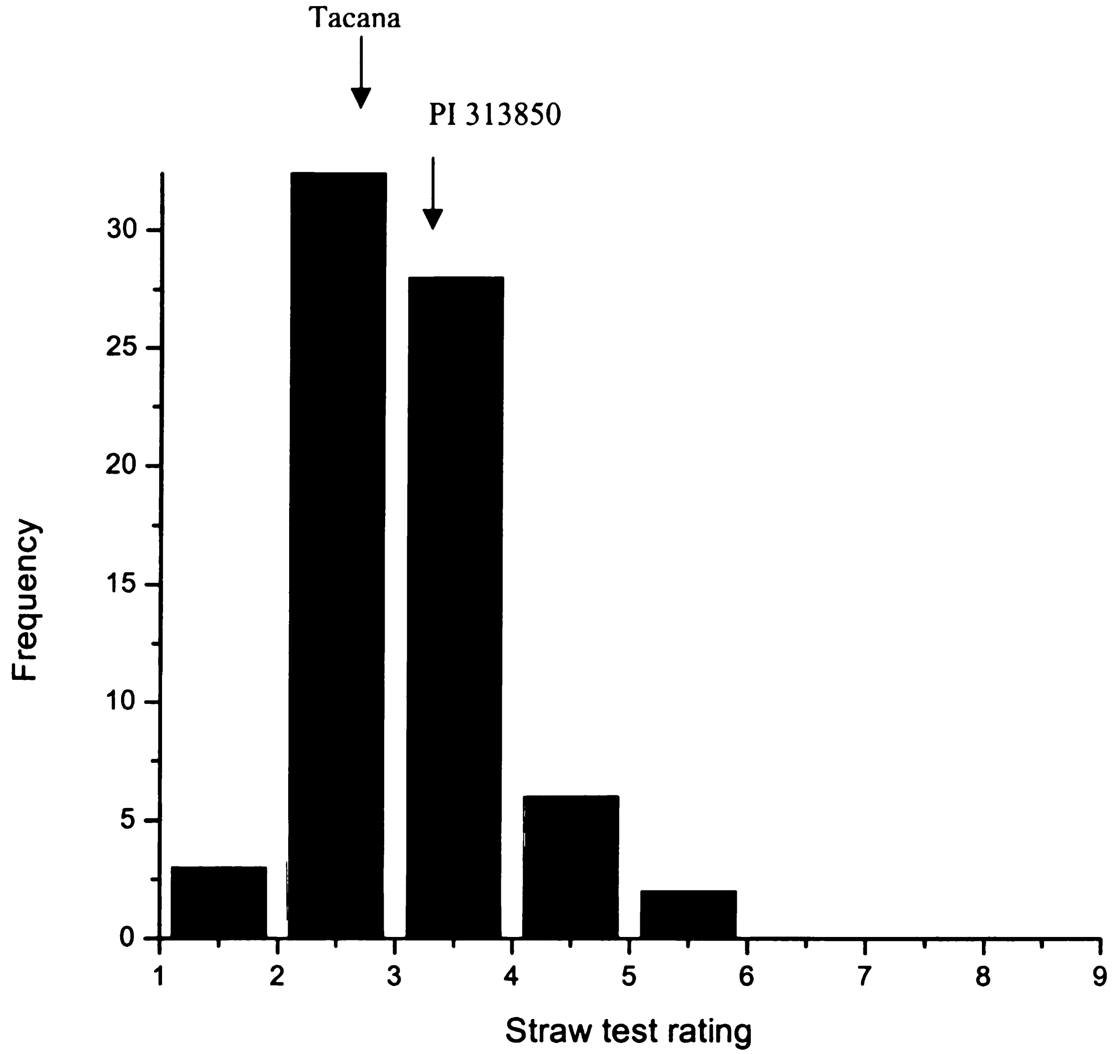

Figure 3.3. Reaction of 76 Tacana/PI 313850 inbred backcross lines (IBL) to inoculation with *S. sclerotiorum* using the straw test. $P < 0.001$ for IBL. The straw test was rated in a scale from 1 to 9 where 1= no symptoms and 9= total plant collapse.

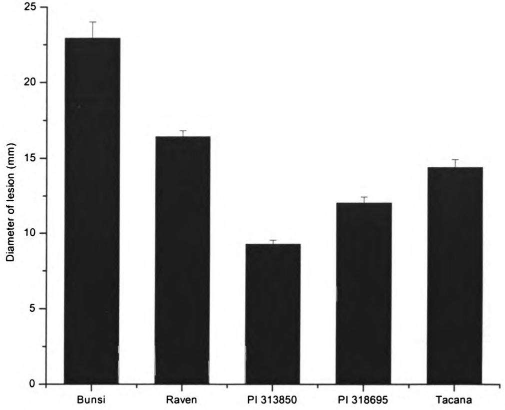

Figure 3.4. Diameter of lesion in detached leaves of five common bean accessions at 48 hours after inoculation with *S. sclerotiorum*. Data represent mean of 9 replicates, and the bars indicate standard deviation. P=0.003 for bean genotypes.

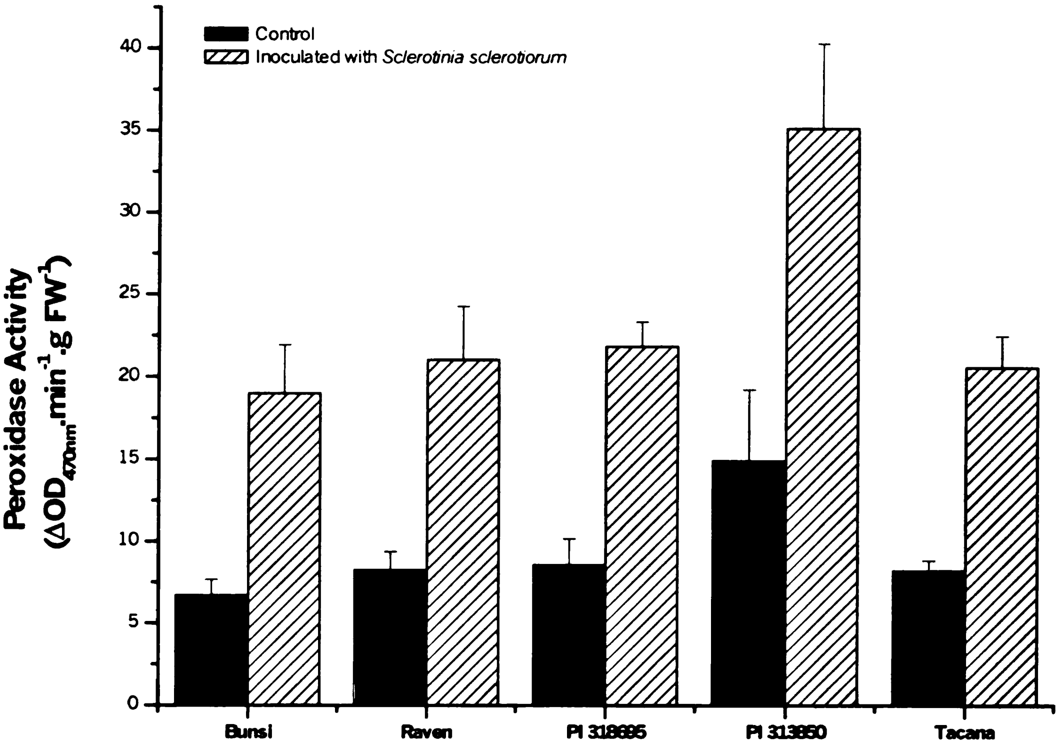

Figure 3.5. Peroxidase activity in detached leaves of five common bean accessions at 48 hours after inoculation with PDA plug (control), and PDA plug with *S. sclerotiorum* mycelia. Data represent mean of 9 replicates, and the bars indicate standard deviation. P=0.02 for genotypes when inoculated and non significant for genotypes without inoculation (control).

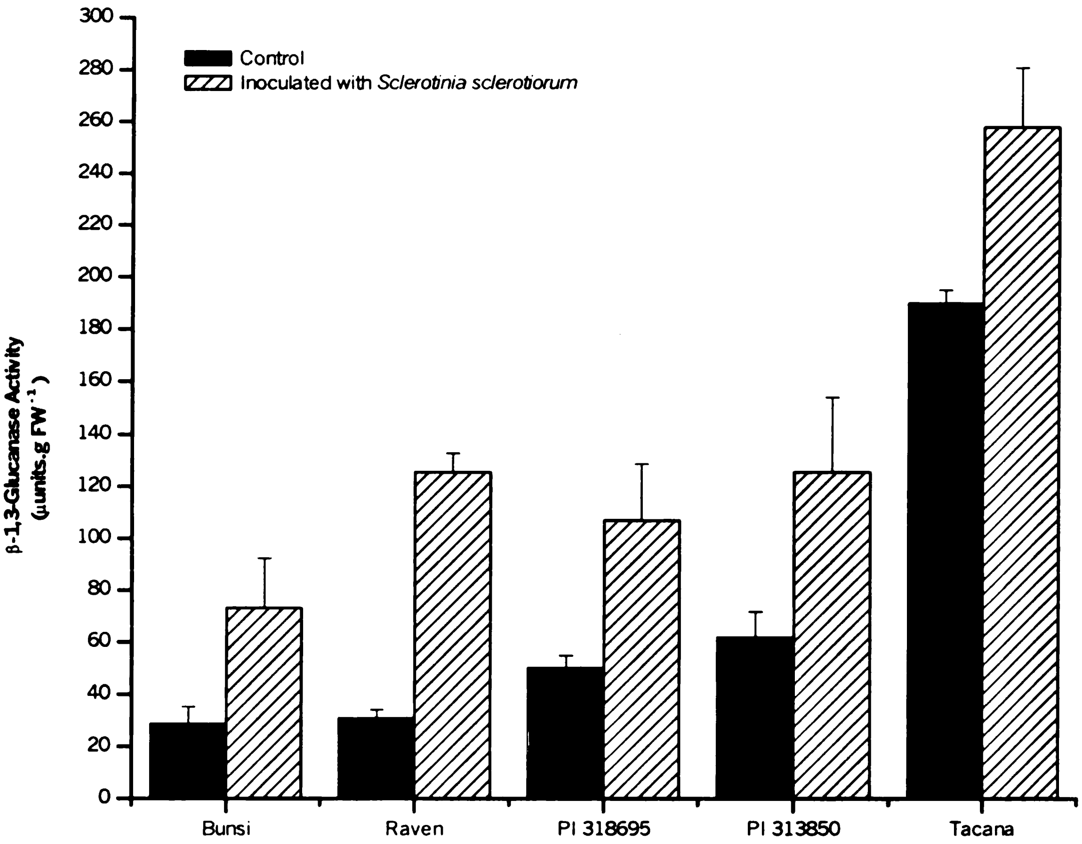

Figure 3.6. β-1,3-Glucanase activity in detached leaves of five common bean accessions 48 hours after inoculation with PDA plug (control), and PDA plug with *S. sclerotiorum* mycelia. Data represent mean of 9 replicates, and the bars indicate standard deviation. P=0.01 for genotypes when inoculated and P=0.03 for genotypes without inoculation (control).

The activity of the defense related enzyme chitinase was not increased when the leaves were inoculated with *S. sclerotiorum* and the five genotypes showed no significant differences in its activity (Figure 3.7).

Polyacrylamide gel electrophoresis (PAGE) for peroxidase and β-1,3-glucanase activity supported the previous data from spectrophotometric assay of enzymatic activity. PI 313850 showed the high peroxidase activity, but similar to Bunsi, after being inoculated with *S. sclerotiorum* (Figure 3.8). The peroxidase activity observed through PAGE is only associated with the acid isoforms and represent only part of the total peroxidase activity measured in the spectrophotometric assay. Tacana had the highest β-1,3-glucanase activity (Figure 3.9) and PAGE gel for chitinase activity confirmed that the five genotypes tested were not significantly different (Figure 3.10).

The different levels of enzymatic activity of defense related proteins detected in this study indicated that genetic variability for plant defense mechanisms exist among the five bean genotypes. Combining high activity of different defense related enzymes could result in higher levels of non-specific plant defenses. This hypothesis could be tested by producing recombinants from crosses between parents such as Tacana and PI 313850 and combining high activity of different defense related enzymes from different sources. The inbred backcross lines with putative resistance to *S. sclerotiorum* mold might represent a unique opportunity to introduce novel sources of resistance into different bean commercial classes.

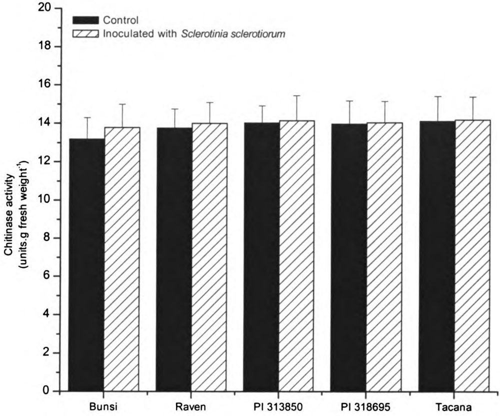

Figure 3.7. Chitinase activity in leaves of five common bean accessions 48 hours after inoculation with PDA plug (control), and PDA plug with *S. sclerotiorum* mycelia. Data represent mean of 9 replicates, and the bars indicate standard deviation. Differences were not significant among genotypes at P=0.05, with or without inoculation.

C I C I C I C I C I

C = Control
I = inoculated with
S. sclerotiorum

PI318695 PI313850 Bunsi Tacana Raven

Figure 3.8. Enzymatic activity of acidic peroxidase isoforms in detached leaves of five common bean accessions 48 hours after inoculation with PDA plug (control), and PDA plug with *S. sclerotiorum* mycelia.

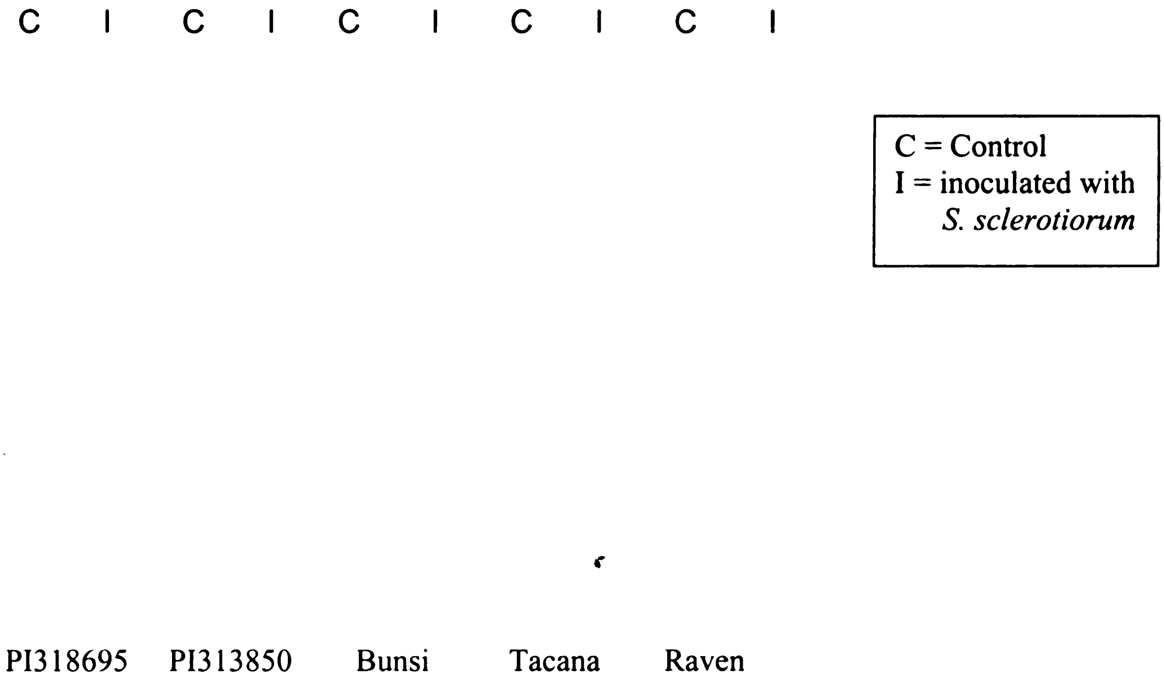

Figure 3.9. Glucanase activity in detached leaves of five common bean accessions 48 hours after inoculation with PDA plug (control), and PDA plug with *S. sclerotiorum* mycelia.

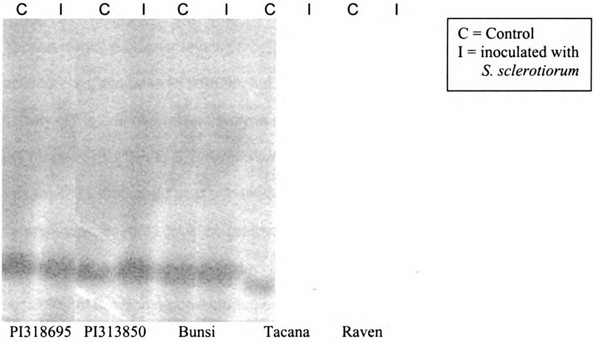

Figure 3.10. Chitinase activity in detached leaves of bean 48 hours after inoculation with PDA plug (control), and PDA plug with *S. sclerotiorum* mycelia.

Considering that Bunsi does not display high levels of resistance when inoculated using the straw test, the inbred lines identified using the straw test may have different mechanisms of defense other than those present in Bunsi. Tacana displayed higher levels of resistance when inoculated with *S. sclerotiorum*, similar to PI 318695, but lower than PI 313850. The differences in activity of the defense related enzymes in Tacana, PI 313850 and PI318695, and the range of reaction to *S. sclerotiorum* observed in the populations Tacana/PI313850 and Tacana/318695, suggest that different mechanisms of defense to white mold could be present in those genotypes. To identify and understand the defense mechanisms involved in resistance would open the possibility of developing new strategies to select new sources of resistance to *S. sclerotiorum*. The study of other plant defense-related genes and the putative association with physiological resistance to white mold should be considered. Polygalacturonase-inhibiting protein (*Pgip*) is particularly noteworthy, since polygalacturonase is generated by the pathogen during *S. sclerotiorum* infection (Favaron et al., 1994) and QTL for resistance to white mold were detected (see Chapter 1) in the same region on linkage B2 where *Pgip* was mapped (Kolkman and Kelly, 2003). The new sources of resistance could be combined with those currently available and result in enhanced levels of resistance to *S. sclerotiorum* in common bean.

References

Acosta-Gallegos, J., C. Quintero, J.Vargas, O. Toro, J.Tohme, and C. Cardona. 1998. A new variant of arcelin in wild common bean, *Phaseolus vulgaris* L., from southern Mexico. Genet. Resources and Crop Evol. 45:235-242.

Bhandal, I.S, and J.D. Paxton. 1991. Phytoalexins biosynthesis induced by the fungal glucan polytran I in soybean, pea, and sweet pepper tissue. Journal of Agriculture and Food Chemistry 39:2156-2157.

Bliss, F.A. 1993. Breeding common bean for improved biological nitrogen fixation. Plant and Soil 152:71-79.

Boller, T., A. Gehri, F. Mauch., and U. Vogeli. 1983. Chitinase in bean leaves: induction by ethylene, purification, and possible function. Planta 157:22-31.

Brown, A.H.D. 1989. Core collection: a practical approach to genetic resources management. Genome 31:818-824.

Cardona, C., J. Kornegay, C.E. Posso, F. Morales, and H. Ramirez. 1990. Comparative value of four arcelin variants in the development of dry bean lines resistant to the Mexican bean weevil. Entomol. Expt. Appl. 56:197-206.

Cardona, C., and J. Kornegay. 1999. Bean germplasm resource for insect resistance . pp. 85-99. In: S.L. Clement and S.S. Quisenberry (ed.) Global plant genetic resources for insect-resistance. CRC Press, Boca Raton, FL.

Concibido, C., B. La Vallee, P. Mclaird, N. Pineda, J. Meyer, L. Hummel, J. Yang, K. Wu, and X. Delannay. 2003. Introgression of a quantitative trait locus for yield from *Glycine soja* into commercial soybean cultivars. Theor. Appl. Genet. 106:575-582.

Dann, E.K., P. Mewly, J.P. Métraux, and B.J. Deverall.1996. The effect of pathogen inoculation or chemical treatment on activities of chitinase and β-1,3-glucanase and accumulation of salicylic acid in leaves of green bean, *Phaseolus vulgaris* L. Physiological and molecular Plant Pathology 49:307-319.

Dann, E.K. and B.J. Deverall. 2000. Activation of systemic disease resistance in pea by an avirulent bacterium or a benzothiadiazole, but not by a fungal leaf spot pathogen. Plant Pathology 49:324-332.

De Tapia, M., P. Bergmann, P. Awade, and G. Burkard. 1986. Analysis of acid extractable bean leaf proteins induced by mercuric chloride treatment and alfalfa mosaic virus infection. Partial purification and characterization. Plant Sci 45:167-177.

Favaron, F., R. D'Ovidio, E. Porceddu, and P. Alghisi. 1994. Purification and molecular characterization of soybean polygalacturonase-inhibiting protein. Planta 195:80-87.

Freyre, R., P.W. Skroch, V. Gelfroy, A.F. Adam-Blondon, A. Shirmohamadali, W.C. Johnson, V. Llaca, R.O Nodari, P.A Pereira, S.M. Tsai, J. Tohme, M. Dron, J. Nienhuis, C.E. Vallejos, and P. Gepts. 1998. Towards and integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theor. Appl. Genet. 97:847-856.

Gooday, G.W. 1994. Cell walls. In: N.A. R. Gow and G.M. Gadd (eds). The growing fungus. Chapman and Hall: London. P. 43-62.

Graham, R.C., U. Lundholm, and M.J. Karnovsky. 1965. Cytochemical determination of peroxidase activity with 3-amino-9-ethylcarbazole. Journal of Histochemistry and Cytochemistry 13:150-152.

Hall, R., L.G. Phillips, and D. Mooij. 1999. Field and greenhouse evaluation of the straw test for resistance of dry bean to white mold. Ann. Rep. Bean Improv. Coop. 42:55-56.

Hammerschmidt, R., E. M. Nuckles, and J. Kúc. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiological Plant Pathology 20:73-82.

Hartman, J.B., and D.A. St. Clair. 1998. Variation for insect resistance and horticultural traits in tomato inbred backcross populations derived from *Lycopersicon pennelli*. Crop Sci. 38:14501-1508.

Huang, J-S. 2001. Plant pathogenesis and resistance: biochemistry and physiology of plant-microbe interactions.

Hunter, J.E., M.H. Dickson, and A. Cigna. 1981. Limited-term inoculation: a method to screen bean plants for partial resistance to white mold. Plant Disease 65:414-417.

Ininda, J., W.R. Fehr, S.R Cianzio, and S.R. Schnebly. 1996. Genetic gain in soybean populations with different percentages of plant introduction parentage. Crop Sci. 36:1470-1472.

Kelly, J.D., G.L. Hosfield, G.V. Varner, M.A. Uebersax, M.E. Brothers, and J. Taylor. 1994. Registration of 'Huron' navy bean. Crop Sci. 34:1408.

Koinange, E. M.K., S.P. Singh, and P. Gepts. 1996. Genetic control of the domestication syndrome in common bean. Crop Sci. 36:1037-1045.

Kolkman J.M. 2000. Genetic dissection of resistance to white mold (*Sclerotinia sclerotiorum*) in common bean (*Phaseolus vulgaris*).Ph.D. Dissertation, pp. 124. Michigan State University. East Lansing, MI.

Kolkman J.M, and J.D. Kelly. 2000. An indirect test using oxalate to determine physiological resistance to white mold in common bean. Crop Sci. 40:281-285.

Kolkman, J.M. and Kelly, J.D. 2003. QTL conferring resistance and avoidance to white mold in common bean. Crop Sci. 43:539-548.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Leubner-Metzger, G., and F. Meins.. 1999. Functions and regulation of plant β-1,3-glucananes (PR-2). In: S. K. Datta and S. Muthukrishnan (eds). Pathogenesis-related proteins in plants. CRS Press: New York. p 49-76.

Lopez-Salinas, E., J.A. Acosta-Gallegos, E.N. Becerra-Leor, G. Frayre-Vazques, S.H.Orozco, and S.E. Beebe. Registration of 'Negro Tacana" common bean.

McClean, P.E., J.R. Myers, and J.J. Hammond. 1993. Coefficient of parentage and cluster-analysis of Nort-American dry bean cultivars. Crop Sci. 33:190-197.

Miklas, P.N., K.F. Grafton, and B.D. Nelson. 1992a. Screening for partial physiological resistance to white mold in dry bean using excised stems. J. Amer. Soc. Hort. Sci. 117:321-327.

Miklas, P.N., K.F. Grafton, G.A. Secor, and P.E. Mcclean. 1992b. Use of pathogen filtrate to differentiate physiological resistance of dry bean to white mold disease. Crop Sci. 32:310-312.

Miklas, P.N., K.F. Grafton, and P. E. McClean. 1993. Estimating phenylalanine ammonia-lyase activity in common beans inoculated with *Sclerotinia sclerotiorum*. HortScience 28:937-938.

Miklas, P.N., R. Delorme, R. Hannan, and M.H. Dickson. 1999. Using a subsample of the core collection to identify new sources of resistance to white mold in common bean. Crop Sci. 39:569-573.

Miklas PN, R Delorme, WC Johnson, and P Gepts. 2001. QTL conditioning physiological resistance and avoidance to white mold in dry bean. Crop Sci. 41:309-315.

Molano, J., I. Polacheck, A. Duran, and E. Cabib. 1979. An endochitinase from wheat germ. Activity on nascent and preformed chitin. The Journal of Biological Chemistry 254: 4901-4907.

Myers, J.R., B. Gilmore, and D. Kean. 1999. Correlation between the field and straw test for white mold resistance in common bean. Annu. Rep. Bean Improv. Coop. 42:57-58.

Nicholson, R.L., and R. Hammerschmidt. 1992. Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30:369-389.

Osborn, T.C., D.C. Alexander, S.S.M. Sun, C. Cardona, and F.A. Bliss. 1988. Insecticidal activity and lectin homology of arcelin seed protein. Science 240:207-210.

Pan, S.Q., X.S. Ye, and J. Kúc. 1989. Direct detection of β-1,3-glucanase isozymes on polyacrylamide electrophoresis and isoelectrofocusing gels. Analytical Biochemistry 182:136- 140.

Peng M., and J. Kúc. 1992. Peroxidase-generated hydrogen peroxide as a source of antifungal activity *in vitro* and on tobacco leaf disks. Phytopathology 82:696-699.

Petzoldt, R., and M.H. Dickson. 1996. Straw test for resistance to white mold. Annu. Rep. Bean Improv. Coop. 39:142-143.

SAS Institute. 1995. The SAS system for Windows. Release 6.12. SAS Inst., Cary, NC.

Schwartz, H.F., D.H. Casciano, J.A. Asenga, and D.R. Wood. 1987. Field measurement of white mold effects upon dry beans with genetic resistance or upright plant architecture. Crop Sci. 27:699-702.

Singh, P.S. 1999. Integrated Genetic Improvement. In:Common bean improvement in the twenty-first century. S.P. Singh (ed). Kluver Academic Publishers, Dordrecht, The Netherlands.

Skinner, D.Z., G.R. Bauchan, G. Auricht, and S. Hughes. 1999. A method for the efficient management and utilization of large germplasm collections. Crop Sci. 39:1237-1242.

Steadman. J.R. 1983. White mold- a serious yield-limiting disease of bean. Plant Disease 67:346-50.

Steadman, J.R., K. Powers, and B. Higgins. 1997. Screening common bean or white mold resistance using detached leaves. Annu. Rep.Bean Improv. Coop. 40:140-141.

Sutton, D.C., and B.J. Deverall. 1984. Phytoalexin accumulation during infection of bean and soybean by ascospores and mycelium of *Sclerotinia sclerotiorum*. Plant Pathology 33:377-383.

Tanksley, S.D., and J.C. Nelson. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTL form unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92:191-203.

Tanksley, S.D., and S.R. McCouch. 1997. Seed banks and molecular maps: Unlocking genetic potential from the wild. Science 277:1063-1066.

Thompson, J., and R.L. Nelson. 1998. Utilization of diverse germplasm for soybean yield improvement. Crop Sci. 38:1362-1368.

Thorne, J.C., and W.R. Fehr. 1970. Exotic germplasm for yield improvement in 2-way and 3-way soybean crosses. Crop Sci. 10:677-678.

Trudel, J., and A. Asselin. 1989. Detection of chitinase activity after polyacrylamide gel electrophoresis. Analytical Biochemistry 178: 362-366.

Van Loon, L.C., 1999. Occurrence and properties of plant pathogenesis-related proteins. In: S. K. Datta and S. Muthukrishnan (eds). Pathogenesis-related proteins in plants. CRS Press: New York. p 1-19.

Webster, D., and J.D. Kelly. 2000. Comentary on germplasm enhancement and cultivar development. pp. 55-59. In: S. Singh (ed). Bean research, production and utilization. Proc. of the Idaho Bean Workshop, August 3 and 4, 2000. Twin Falls, Idaho.

GENERAL DISCUSSION AND FUTURE RESEARCH

General Discussion

Complete field resistance to white mold does not exist in bean, but genotypes with partial resistance that includes physiological resistance and/or morphological avoidance mechanisms are available. The goal of the research reported here was to enhance the resistance of current bean varieties, develop methods to more efficiently transfer resistance to a wide range of seed types and identify new and novel sources of resistance to white mold in exotic bean germplasm.

The first objective was to identify QTL associated with resistance to white mold using a multi-trait bulked segregant analysis approach. The RIL population used in this study was derived from a cross between Bunsi and Raven. Bunsi is recognized as a source of resistance to white mold and Raven, despite its upright plant architecture that suggests avoidance to white mold, is highly susceptible and represents a low level of physiological resistance. A QTL for DSI was detected on linkage group B2, confirming the QTL detected previously by Kolkman and Kelly (2003) in a different RIL population. The data support the existence of a QTL for resistance to white mold on B2, in a region of the bean genome where several host response genes related to general defense mechanisms that may play an important role in disease resistance reside. PvPR-2, a low molecular weight acidic protein induced during fungal elicitation (Walter et al., 1990), a polygalacturonase-inhibiting protein (Toubart et al., 1992), and chalcone synthase, (Ryder et al., 1987), an isoflavonoid-derived phytoalexin involved in host defense are all located in this region on B2. Plant defense-related genes may be triggered as a general resistance response to *S. sclerotiorum* infection. QTL associated with resistance to common bacterial blight (Nodari et al., 1993), and root rot (Schneider et al., 2001) were also identified in this region of B2.

A novel QTL associated with resistance to white mold detected in this study was mapped to B5. A third QTL for DSI mapped to B7 in the same region where Kolkman and Kelly (2003) identified a major QTL for resistance to white mold and other associated traits, including days to flowering and maturity, architecture, lodging, seed size and yield. The genomic region on B7 where the QTL was detected corresponded to the linkage B7-b in this study. Miklas et al., (2001) and Park et al., (2001) also detected QTL for resistance to white mold on B7. However, those QTL were mapped close to the *Phaseolin* gene, a different region on B7, that corresponds to linkage B7-a in this study, suggesting that the QTL identified for DSI in Bunsi/Raven RIL population is different from the QTL detected on B7 in Andean genotypes G122 and PC-50 by Miklas et al., (2001) and Park et al., (2001).

The fact that different QTL were identified in 2001 and 2002 suggested that depending on the environmental conditions, different mechanisms were playing a role in resistance to white mold. The environmental conditions in 2001 were more favorable for white mold and plant structure was not detected as an important factor in disease development. Plant architecture played an important role in avoiding the disease in 2002 where high temperatures and low precipitation prevailed. The results suggested that under conditions of high disease pressure, avoidance mechanisms may not be sufficient to restrict disease development and physiological resistance would play an increasingly important role in field resistance. Bunsi and Raven are contrasting genotypes for architecture, and the effect of upright plant type on resistance to white mold was evaluated in different years with different environmental conditions. Associations between the different agronomic traits and DSI identified in this study, indicate that plant architecture and resistance to lodging are traits that need to be considered when selecting for resistance to white mold. An upright structure and medium plant width that ensures a porous canopy should represent ideal plant architecture with avoidance to white mold. However, avoidance mechanisms have to be combined with physiological resistance to obtain high levels of resistance to white mold.

Resistance to white mold is a quantitative trait, problematic to measure, significantly influenced by other traits and environment. Complex traits exhibiting low heritability make MAS an important approach to enhance resistance. The identification of different QTL is crucial because they may represent an opportunity to combine through MAS, different plant strategies for resistance to white mold that might result in an enhanced and stable resistance across environments. MAS provides an opportunity to enhance resistance by combining QTL regardless of function in resistance to white mold. However, a better understanding of the defense mechanisms directly related to resistance to white mold is needed. Since the contribution of each QTL to a quantitative trait is small, pyramiding different QTL is necessary to significantly improve the genetic gain for a complexly inherited trait. The potential of using MAS for resistance to white mold was confirmed in a F4 derived RIL population originated from a cross between Bunsi and Midland.

A strategy proposed to improve resistance to white mold in common bean would combine phenotypic selection for agronomic traits with molecular markers linked to QTL associated with physiological resistance to *S. sclerotiorum*. Considering that most of the agronomic traits have a high heritability, selection could effectively be conducted in early generations and in preliminary yield trials. Environments without favorable conditions for white mold could be used to avoid problems regarding cause/effect that frequently occur with traits such as architecture and lodging that can significantly affect or be influenced by disease occurrence. Scoring agronomic traits in the absence of disease would allow a more efficient selection for these traits that could be combined with molecular markers for physiological resistance. Combining QTL for white mold resistance on different linkage groups with agronomic traits would require large populations in order to select lines that display the desired combination of markers and agronomic traits. Selection for yield under high white mold pressure might be an effective final step to improve resistance to white mold in agronomically acceptable genotypes. Selection only based on disease severity or incidence can result in lines with limited potential use in breeding due to combinations of undesirable agronomic traits. Pyramiding multiple QTL with individual minor effects is a challenging task, but the tools are available and MAS should play a more important role in plant breeding for quantitative traits in the future.

The third aspect of this study was to introgress putative sources of white mold resistance into cultivated bean varieties. The plant introductions were selected based on different greenhouse tests used to detect physiological resistance to *S. sclerotiorum*. Bean has a wide genetic diversity among landraces, wild types and species that is an invaluable source for important traits including resistance to white mold. Field screening for white mold resistance of unadapted germplasm is ineffective, since morphological and phenological traits, such as photoperiod sensitivity and climbing growth habit prevent field evaluation and could be confounded with physiological resistance. The putative sources of resistance to white mold have to be introduced into adapted genetic background for testing under field conditions. From seven populations developed using inbred backcross method, two populations, Tacana/PI 313850 and Tacana/PI 318695, were selected in the field for further studies based on acceptable adaptation and plant architecture.

In both selected populations significant differences for reaction to *S. sclerotiorum* were detected among the IBL within each population. Many lines with low ratings for the straw test were identified, suggesting that based on the reaction to *S. sclerotiorum*, both populations have putative resistance to white mold. Based on desirability score, architecture, lodging and seed size, the inbred backcross method was effective to recover adaptation and acceptable agronomic traits. The IBL can be tested in the field under white mold pressure to identify sources of resistance.

The different levels of enzymatic activity of defense related proteins detected in this study suggested that genetic variability for plant defense mechanisms may exist among the plant introductions and bean varieties tested. High activity of different defense related enzymes could result in higher levels of non specific plant defenses which could influence the reaction to *S. sclerotiorum*. This hypothesis could be tested by measuring the activity of defense related enzymes in the Tacana/PI 313850 IBL selected in the straw test, since Tacana and PI 313850 expressed high β-1,3-glucanase and peroxidase activity, respectively, when inoculated with *S. sclerotiorum*. The interactions of different defense related enzymes with white mold resistance remains to be elucidated.

The inbred backcross lines with putative resistance to white mold might represent an unique opportunity to introduce novel sources of resistance into different bean commercial classes. Considering that Bunsi does not display high levels of resistance when inoculated with *S. sclerotiorum* in the straw test, the resistant inbred lines identified using the straw test may have different mechanisms of defense other than those present in Bunsi. To identify and understand the defense mechanisms involved in resistance would provide the possibility of developing new strategies to select novel sources of resistance to *S. sclerotiorum*. The novel sources of resistance could be combined with those currently available sources and result in enhanced levels of resistance to white mold in common bean.

Future Research

White mold is a complex disease and a combination of approaches is needed to improve resistance to *S. sclerotiorum* in common bean. Suggestions of further studies that could follow the current research are discussed.

1. Select the recombinant inbred lines derived from Bunsi/Raven cross with the best marker combination associated with resistance to *S. sclerotiorum.* Test those lines in the field in different environments to confirm the efficiency of the QTL identified in this study. RIL lacking the markers linked to QTL for resistance could be used as controls.

2. Convert AFLP markers linked to QTL for resistance to white mold into SCAR markers to increase the throughput of MAS selection and help reduce screening costs. The regions where the QTL mapped were relatively saturated with markers. The selection of an adequate AFLP marker size (> 200bp) to be converted is therefore possible.

3. Test the IBL derived from Tacana/PI 313850 and Tacana/PI 318695, selected in the straw tests and based on agronomic traits, for the reaction to *S. sclerotiorum* under high disease pressure in field conditions. Indirect tests to detect physiological resistance including inoculation of detached leaves, straw test, and resistance to oxalate should also be conducted. Activity of defense

related enzymes should be assayed, especially β-1,3-glucanase and peroxidase activity in IBLs of Tacana/PI 313850 and correlations with resistance could be determined.

4. Select from the Tacana/PI 313850 and Tacana/PI 318695 populations, IBL that combine resistance to white mold and desired agronomic traits, for QTL detection studies. Developing mapping populations by crossing with a white mold susceptible genotype but with an acceptable upright plant structure, such as Raven, should be an effective strategy to detect QTL associated with physiological resistance, since both parents would be upright.

5. Identify QTL associated to resistance to *S. sclerotiorum* in genetic sources in both gene pools including landrace and wild accessions. Bunsi is the only reported Middle American bean which has QTL detected for white mold. Pyramiding different QTL from different genetic sources could be an effective strategy to improve resistance to white mold in bean. QTL identified in PI 313850 could be combined with QTL already detected in large seeded Andean genotypes G 122 and PC 50 and be introduced into Andean Cranberry and Kidney bean classes. Transfer QTL between gene pools has to be considered but may show limitation due the incompatibility in crosses involving genotypes from Andean and Middle American gene pools. The cross between Tacana and PI 313850 resulted in IBL with acceptable adaptation and agronomic traits suggesting the inbred backcross method may

be effective to transfer QTL for resistance to white mold between the Andean and Middle American gene pools. To identify adapted varieties, landraces or wild types with resistance to white mold is an important contribution to bean breeding programs but the incorporation of new sources of resistance to *S. Sclerotiorum* into new bean varieties is essential. However, plant introductions already identified as putative sources of resistance to white mold, such as those used in this study, should be first exploited. In a medium and long term, more screening of core collections should be conducted and maybe based on better understanding of physiological resistance. Searching for resistance in related species is an alternative, but genetic diversity in bean primary gene pool should be exploited first.

6. Study polygalacturonase-inhibiting protein (*Pgip*) and putative association with physiological resistance to white mold should be considered. *Pgip* is particularly noteworthy since polygalacturonase is generated by the pathogen during *S. sclerotiorum* infection and QTL for resistance to white mold were detection in the same region on B2 where *Pgip* was mapped. The limitation is to develop a high throughput protocol to measure *Pgip* in beans, since most of the methods currently available are based on measuring mRNA derived from *Pgip* gene. The detection of *Pgip* by use of polyclonal antibodies could be an alternative methodology that would allow screening of large populations.

7. Transform bean plants with *oxalate oxidase germin* gene from wheat might be an effective approach to increase resistance, since oxalic acid is the primary pathogenicity factor of *S. sclerotiorum*. Results obtained with soybean transformed with *oxalate oxidase* gene were promising (Donaldson et al., 2001). However technical limitations in bean transformation and marketing genetically modified organisms (GMO) are issues that have to be considered in this approach.

References

Donaldson, P.A., T. Anderson, B.G. Lane, A.L. Davidson, and D.H. Simonds. 2001. Soybean plants expressing an active oligomeric oxalate oxidase from the wheat *gf-2.8* (germin) gene are resistant to the oxalate-secreting pathogen *Sclerotinia sclerotiorum*. Physiological and Molecular Plant Pathology 59:297-307.

Kolkman, J.M. and J.D. Kelly. 2003. QTL conferring resistance and avoidance to white mold in common bean. Crop Sci. 43:539-548.

Miklas P.N., R. Delorme, W.C. Johnson, and P. Gepts. 2001. QTL conditioning physiological resistance and avoidance to white mold in dry bean. Crop Sci. 41:309-315.

Nodari, R.O., S.M. Tsai, P. Guzman, R.L. Gilbertson, and P. Gepts. 1993. Towards an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. Genetics 134:341-350.

Park S.O., D.P. Coyne, J.R. Steadman, and P.W. Shroch. 2001. Mapping of QTL for resistance to white mold disease in common bean. Crop Sci. 41:1253-1262.

Ryder, T.B., S.A. Hedrick, J.N. Bell, X. Liang, S.D. Clouse, and C.J. Lamb. 1987. Organization and differential activation of a gene family encoding the plant defense enzyme chalcone synthase in *Phaseolus vulgaris*. Mol. Gen. Genet. 210:219-233.

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

Toubart, P., A Desiderio, G. Salvi, F. Cervone, L. Daroda, and G. De Lorenzo. 1992. Cloning and characterization of the gene encoding the endopolygalacturonase-inhibiting protein PGIP) of *Phaseolus vulgaris* L. Plant J. 2:367-373.

Walter, M.H., J. Liu, C. Grand, C.J. Lamb, and D. Hess. 1990. Bean-pathogenesis-related proteins deduced from elicitor-induced transcripts are members of a ubiquitous new class of conserved PR proteins including pollen allergens. Mol. Gen. Genet. 222:353-360.

APPENDICES

APPENDIX A

Table A1. Disease severity index (DSI), disease incidence (DI), yield, seed size, days to flowering, days to maturity, lodging and architecture of Bunsi/Raven RILs grown under white mold pressure at Montcalm, MI, in 2001.

RIL (105-)	DSI (%)	DI (%)	Yield (kg/ha)	Seed size (g.100seed^{-1})	Days to Flowering	Days to maturity	Lodging[1]	Architecture[2]
1	28	52	4820	23.9	44	105	3.2	1.9
2	29	56	4719	23.5	44	107	2.6	3.0
3	41	76	4136	22.9	45	105	3.3	2.0
4	34	59	4181	22.1	43	105	3.0	3.0
5	38	69	4708	24.0	48	109	2.1	3.5
6	47	79	3912	24.8	48	110	3.0	3.0
7	53	88	4102	21.1	45	104	2.4	2.0
8	48	89	3721	21.6	39	103	3.8	2.5
9	32	50	5044	22.7	44	107	2.8	2.5
10	57	96	3183	22.2	48	112	2.8	2.5
11	48	79	4001	21.3	44	105	3.9	3.0
12	52	80	3710	24.3	40	104	3.3	3.5
13	41	72	4259	21.4	42	105	3.3	3.5
14	55	76	3766	25.4	44	104	3.3	2.0
15	29	52	4696	24.6	43	102	3.3	2.5
16	38	65	4114	20.9	45	107	3.9	4.0
17	33	59	5021	22.4	43	105	2.0	1.0
18	55	80	4091	24.0	46	107	3.6	3.0
19	38	63	4102	22.3	40	101	2.5	1.0
20	34	62	4797	23.3	46	107	2.8	2.5
21	42	67	3968	24.4	45	105	3.3	3.0
22	56	85	3351	21.3	46	105	4.7	3.0
23	69	96	2746	21.5	40	103	4.3	2.5
24	54	79	3564	20.0	45	110	3.7	3.0

Continuation of Table A1.

RIL (105-)	DSI (%)	DI (%)	Yield (kg/ha)	Seed size (g.100seed^{-1})	Days to Flowering	Days to maturity	Lodging[1]	Architecture[2]
25	41	72	4752	25.2	44	109	3.2	3.0
26	42	69	5156	22.5	43	107	2.6	3.0
27	56	82	4338	23.4	47	108	3.8	2.0
28	47	75	4528	22.4	46	108	3.3	4.0
29	60	88	3609	23.6	44	107	3.6	3.0
30	54	96	3508	21.2	40	101	3.9	2.5
31	32	57	5133	23.6	45	106	2.2	2.0
32	55	83	3452	21.1	38	104	4.4	3.0
33	46	73	3497	22.8	37	98	3.1	2.0
34	40	70	4349	21.8	41	99	4.2	2.5
35	64	92	3094	21.8	43	105	4.8	2.5
36	57	81	3620	21.9	46	106	3.0	2.5
37	36	63	4326	24.2	46	110	2.6	3.0
38	43	73	4069	19.7	38	98	4.4	3.0
39	42	66	4035	22.2	45	105	3.0	2.5
40	32	61	4584	23.5	47	111	3.5	3.5
41	42	76	3968	20.4	45	103	3.8	3.5
42	32	62	4775	18.9	45	107	3.0	2.5
43	52	78	4270	20.8	44	106	3.9	3.5
44	46	81	4136	21.7	44	110	3.8	3.0
45	42	73	3968	20.6	43	107	3.7	3.0
46	44	73	3800	22.1	37	99	4.8	2.5
47	59	99	4506	21.8	44	102	4.7	3.0
48	51	78	4282	21.6	45	99	2.7	2.0
49	50	81	4214	26.1	46	104	3.0	2.0

Continuation of Table A1.

RIL (105-)	DSI (%)	DI (%)	Yield (kg/ha)	Seed size (g.100seed^{-1})	Days to Flowering	Days to maturity	Lodging[1]	Architecture[2]
50	52	82	3497	20.6	46	105	3.9	2.5
51	51	89	3396	20.8	45	103	3.5	3.0
52	52	83	4102	20.9	44	104	3.7	3.0
53	42	76	4427	23.5	39	101	3.7	2.5
54	47	72	3531	24.6	44	106	4.4	2.5
55	55	89	2858	21.5	45	107	4.3	4.5
56	53	80	4024	21.2	47	107	3.0	2.9
57	35	59	4170	21.8	40	100	3.5	2.5
58	50	80	3049	19.0	44	105	4.4	3.0
59	42	67	4360	20.7	40	98	4.1	2.5
60	51	82	3934	21.0	45	108	4.2	4.0
61	31	59	4607	24.4	40	103	2.5	1.5
62	38	71	4708	20.9	43	104	3.0	1.5
63	40	71	5212	22.2	46	104	2.5	2.5
64	55	84	3654	20.8	47	106	3.0	3.0
65	55	91	4214	20.9	45	102	3.3	2.5
66	47	80	4091	22.4	44	104	2.7	2.0
67	45	70	4304	21.1	44	105	4.2	2.5
68	47	79	3688	20.8	44	103	2.5	1.5
69	34	61	5571	22.8	45	103	2.2	2.0
70	43	72	4125	21.8	45	104	2.3	1.5
71	55	84	3542	20.4	45	105	3.9	2.0
72	41	69	5268	21.9	45	109	3.4	3.0
73	31	69	4842	20.9	45	108	2.7	3.5
74	42	68	4405	22.6	45	103	2.9	3.5

Continuation of Table A1.

RIL (105-)	DSI (%)	DI (%)	Yield (kg/ha)	Seed size (g.100seed^{-1})	Days to Flowering	Days to maturity	Lodging[1]	Architecture[2]
75	23	42	4618	21.0	43	105	3.3	3.0
76	41	72	4562	22.5	43	99	2.0	1.5
77	36	64	4472	21.2	41	102	2.6	2.5
78	45	72	4270	23.1	44	104	3.4	2.5
79	32	60	5010	20.4	47	107	3.0	1.5
80	40	70	4528	22.7	44	107	3.6	3.0
81	44	73	4360	21.6	44	105	2.8	1.9
82	43	85	3744	20.6	45	108	3.8	4.0
83	51	86	4394	20.3	44	108	3.3	3.0
84	51	74	3699	23.9	45	109	3.7	3.0
85	56	83	3329	24.8	45	104	3.1	2.5
86	40	71	4181	20.4	37	104	3.6	2.9
87	45	76	4046	20.8	45	105	4.0	2.5
88	33	61	4864	24.7	45	109	2.0	3.0
89	45	80	4147	24.5	45	106	3.4	3.0
90	30	54	4977	24.8	46	106	2.1	3.0
91	49	79	4349	23.7	46	107	3.2	2.5
92	51	89	4259	21.1	45	102	3.7	3.0
93	47	77	3463	22.4	44	102	3.2	1.5
94	47	81	3564	22.2	45	103	4.5	3.0
95	56	84	3116	22.7	46	105	2.7	2.5
96	73	85	2130	19.1	42	104	4.9	3.5
97	37	63	4562	25.2	43	102	2.6	2.0
98	40	67	4326	21.4	40	102	3.0	3.0

[1] Scale 1 to 5 where 1=no lodging and 5=excessive lodging. [2] Scale 1 to 5 where 1=fully upright and 5=prostrate.

Table A2. Disease severity index (DSI), disease incidence (DI), yield, seed size, days to flowering, days to maturity, lodging and architecture of Bunsi/Raven RILs grown under white mold pressure at Montcalm, MI, in 2002.

RIL (105-)	DSI (%)	DI (%)	Yield (kg/ha)	Seed size (g.100seed^{-1})	Days to Flowering	Days to maturity	Lodging[1]	Architecture[2]
1	14	28	5436	24.2	44	101	2.3	2.4
2	24	55	4371	22.3	45	110	2.9	2.5
3	13	34	3833	22.2	46	105	2.9	2.6
4	32	61	4472	20.7	46	104	3.0	2.9
5	35	75	4752	21.8	53	112	3.1	2.8
6	39	76	4405	23.5	47	117	3.3	3.1
7	25	58	4069	19.0	46	106	3.0	2.4
8	33	66	4652	20.4	44	105	3.4	2.6
9	35	69	4797	20.9	45	111	3.1	2.6
10	34	66	3845	20.1	51	108	2.6	2.4
11	17	37	4943	21.8	44	107	3.4	3.6
12	22	40	4663	23.9	44	105	2.7	2.6
13	17	42	4562	22.6	43	112	2.9	3.1
14	24	53	4965	22.7	45	105	2.7	2.4
15	21	46	4652	22.8	44	102	3.3	2.9
16	15	39	4035	18.7	48	105	3.0	3.4
17	10	23	4977	21.3	44	103	1.9	1.9
18	35	69	4517	22.6	45	111	3.7	2.9
19	21	36	4338	21.4	44	97	2.6	2.5
20	31	71	4270	22.1	46	112	3.6	2.9
21	21	45	4629	22.2	45	107	2.8	2.6
22	36	61	3777	19.9	47	104	4.1	3.5
23	27	46	4450	20.7	45	98	2.7	2.5
24	45	85	4282	19.5	48	106	3.5	3.0

Continuation of Table A2.

RIL (105-)	DSI (%)	DI (%)	Yield (kg/ha)	Seed size (g.100seed^{-1})	Days to Flowering	Days to maturity	Lodging[1]	Architecture[2]
25	8	23	4551	26.2	45	109	2.1	2.6
26	15	33	5268	20.2	45	107	2.3	2.4
27	32	66	4573	22.5	49	110	3.1	3.1
28	51	83	4013	19.5	50	108	3.4	3.4
29	36	69	4752	23.3	46	111	3.3	2.7
30	22	44	3699	19.5	44	104	3.2	3.0
31	11	28	5627	23.0	45	108	2.2	2.2
32	14	32	4898	19.4	44	103	3.0	3.1
33	10	23	4326	24.1	43	97	2.6	2.1
34	41	68	4170	18.8	43	94	3.7	3.0
35	40	76	4573	21.9	45	100	4.0	3.1
36	34	61	4472	20.9	47	106	2.7	2.5
37	29	56	5425	23.0	44	113	2.4	2.7
38	12	24	4416	18.5	43	96	3.1	3.0
39	23	61	4562	21.9	46	111	3.2	2.5
40	37	71	4595	22.8	52	112	3.4	3.0
41	32	64	4607	19.2	47	106	2.9	3.1
42	27	62	4528	18.4	46	111	3.3	3.2
43	16	34	4640	20.6	45	105	2.9	2.6
44	36	66	4259	18.5	46	112	3.9	3.5
45	29	73	4405	20.4	44	112	4.0	3.7
46	26	45	4181	20.2	43	96	3.8	2.7
47	28	57	4640	19.7	46	103	3.5	3.1
48	27	45	4416	20.6	45	100	2.4	2.2
49	48	73	3878	23.8	44	110	3.2	2.5

Continuation of Table A2.

RIL (105-)	DSI (%)	DI (%)	Yield (kg/ha)	Seed size (g.100seed^{-1})	Days to Flowering	Days to maturity	Lodging[1]	Architecture[2]
50	34	63	4685	19.3	45	105	3.4	2.7
51	51	91	3127	18.5	49	101	3.9	3.1
52	45	75	4595	20.2	42	109	3.4	2.5
53	28	47	4551	21.7	44	98	2.9	2.8
54	25	44	4057	22.3	44	101	4.1	3.2
55	46	85	4259	20.2	44	113	4.1	3.9
56	23	44	4461	21.1	47	110	2.7	2.1
57	13	29	4259	19.9	45	97	3.0	2.5
58	34	66	3688	17.5	45	101	3.8	3.3
59	22	40	4663	19.0	42	94	3.3	3.0
60	30	61	4349	19.3	47	107	3.4	3.2
61	9	18	4708	23.4	44	104	1.9	1.8
62	21	50	4629	19.8	45	107	2.8	2.3
63	37	71	4752	20.2	49	108	2.8	2.6
64	39	67	3990	19.6	45	106	3.4	2.9
65	21	40	4831	20.9	46	101	2.6	2.3
66	20	41	4551	21.4	45	108	2.2	2.1
67	33	69	4248	21.0	45	106	3.9	2.6
68	26	50	4876	19.6	44	106	2.7	2.5
69	30	72	4629	21.0	47	106	3.3	2.9
70	16	36	4652	19.8	46	100	2.2	2.1
71	26	49	4405	20.8	46	108	3.1	2.9
72	34	70	4439	20.1	48	112	3.1	3.0
73	23	56	4797	18.2	47	116	3.1	3.7
74	27	48	4921	20.3	47	102	3.2	3.1

Continuation of Table A2.

RIL (105-)	DSI (%)	DI (%)	Yield (kg/ha)	Seed size (g.100seed^{-1})	Days to Flowering	Days to maturity	Lodging[1]	Architecture[2]
75	18	38	4831	21.0	44	106	2.6	2.4
76	14	34	5033	22.7	45	99	1.6	1.9
77	12	31	4383	19.3	44	100	2.5	2.7
78	27	56	3912	22.3	45	109	3.3	3.1
79	32	65	4360	19.2	48	110	3.5	3.0
80	18	47	5302	20.7	46	110	3.2	2.9
81	25	52	4539	20.5	45	107	2.6	2.2
82	37	73	4170	19.8	46	114	3.6	3.7
83	47	78	4270	16.7	48	110	4.0	3.6
84	22	49	5178	23.0	44	109	2.5	2.5
85	23	45	4719	24.3	44	104	2.3	2.3
86	11	35	4640	19.8	43	105	3.0	3.0
87	42	74	3150	20.1	48	112	3.8	3.5
88	13	33	4932	22.2	46	107	2.3	2.4
89	18	37	4562	24.1	44	108	2.8	2.6
90	32	57	4203	22.3	46	110	3.2	2.7
91	43	76	4439	23.5	50	112	3.0	2.5
92	22	45	4483	20.2	47	104	3.5	3.5
93	16	31	4730	20.9	46	106	1.9	2.0
94	42	73	4808	21.0	45	105	4.1	3.5
95	39	80	3475	20.2	48	105	2.8	2.0
96	33	64	3901	18.8	44	106	4.1	3.8
97	40	62	4764	21.5	44	92	3.4	2.5
98	25	47	5066	20.1	43	102	2.6	2.6

[1] Scale 1 to 5 where 1=no lodging and 5=excessive lodging. [2] Scale 1 to 5 where 1=fully upright and 5=prostrate.

Table A.3. Primer combination and fragment size of AFLP markers tested in Bunsi/Raven RIL population.

AFLP marker	Primer combination[1]	AFLP marker	Primer combination
1	$E_{AAC}M_{CTT}130$	27	$E_{AGC}M_{CAA}480$
2	$E_{AAC}M_{CTT}207$	28	$E_{AGC}M_{CAA}520$
3	$E_{AAC}M_{CTT}223$	29	$E_{AGA}M_{CTG}190$
4	$E_{AAC}M_{CAA}83$	30	$E_{AGA}M_{CTG}400$
5	$E_{AAC}M_{CAA}380$	31	$E_{AGA}M_{CTG}440$
6	$E_{AAC}M_{CAA}147$	32	$E_{AGA}M_{CAT}102$
7	$E_{AAC}M_{CAA}320$	33	$E_{AGA}M_{CAT}135$
8c[2]	$E_{AAC}M_{CAA}177/222$[3]	34	$E_{AGA}M_{CAT}148$
9	$E_{AGG}M_{CTG}170$	35	$E_{AGA}M_{CAT}165$
10	$E_{AGG}M_{CTG}340$	36	$E_{AGA}M_{CAT}180$
11	$E_{ACT}M_{CTT}103$	37	$E_{AGA}M_{CAT}200$
12	$E_{ACT}M_{CTT}170$	38c	$E_{AGA}M_{CAT}349/350$
13	$E_{ACT}M_{CTT}210$	39	$E_{ATA}M_{CAG}130$
14	$E_{ACT}M_{CTT}280$	40	$E_{ATA}M_{CAG}157$
15	$E_{ACT}M_{CTT}290$	41	$E_{ATA}M_{CAG}85$
16	$E_{ACT}M_{CTT}670$	42	$E_{ATA}M_{CAG}90$
17	$E_{ACT}M_{CTT}700$	43	$E_{AAT}M_{CAA}285$
18	$E_{ACT}M_{CTT}720$	44	$E_{AAT}M_{CAA}250$
19	$E_{ACC}M_{CTT}290$	45	$E_{AAT}M_{CAA}134$
20	$E_{ACC}M_{CTT}330$	46	$E_{ACT}M_{CCA}190$
21	$E_{ACC}M_{CTT}350$	47	$E_{ACT}M_{CCA}120$
22	$E_{ACC}M_{CTT}550$	48	$E_{ACT}M_{CCA}110$
23c	$E_{AGT}M_{CTT}219/220$	49	$E_{AAT}M_{CAA}400$
24	$E_{AGT}M_{CTT}300$	50	$E_{AAT}M_{CAA}115$
25	$E_{AGT}M_{CTT}500$	51	$E_{AAT}M_{CAA}105$
26	$E_{AGC}M_{CAA}400$	52	$E_{AGG}M_{CAA}170$

Continuation of Table A.3.

AFLP marker	Primer combination[1]	AFLP marker	Primer combination
53	$E_{AGG}M_{CAA}85$	79	$E_{AAA}M_{CAA}550$
54	$E_{ACA}M_{CCG}100$	80	$E_{AAA}M_{CAA}600$
55	$E_{ACA}M_{CCG}120$	81	$E_{AAA}M_{CAA}650$
56	$E_{ACA}M_{CCG}200$	82	$E_{ATG}M_{CTC}110$
57	$E_{ACA}M_{CCG}460$	83	$E_{ATG}M_{CTC}220$
58	$E_{ACC}M_{CTG}255$	84	$E_{ATG}M_{CTC}330$
59	$E_{ACC}M_{CTG}350$	85	$E_{ATG}M_{CTC}82$
60	$E_{ATC}M_{CCT}330$	86	$E_{ATG}M_{CTG}120$
61	$E_{ATC}M_{CCT}90$	87	$E_{ATG}M_{CTG}400$
62	$E_{ACC}M_{CTC}450$	89	$E_{AGC}M_{CAA}105$
63	$E_{ACC}M_{CTC}86$	90	$E_{AGC}M_{CAA}128$
64	$E_{AGT}M_{CAT}220$	91	$E_{AGC}M_{CAA}190$
65	$E_{AGT}M_{CAT}280$	92	$E_{ACT}M_{CAC}120$
66	$E_{AGT}M_{CAT}360$	93	$E_{ACT}M_{CAC}205$
67	$E_{AGT}M_{CAT}480$	94	$E_{ACT}M_{CAC}400$
68	$E_{AGT}M_{CAT}75$	95	$E_{ACT}M_{CAT}220$
69	$E_{AGT}M_{CAT}90$	96	$E_{ACT}M_{CAT}260$
70	$E_{AAA}M_{CAA}104$	97	$E_{ACT}M_{CAT}550$
71	$E_{AAA}M_{CAA}170$	98	$E_{ACT}M_{CAT}85$
72	$E_{AAA}M_{CAA}190$	99	$E_{AAA}M_{CAG}100$
73	$E_{AAA}M_{CAA}195$	100	$E_{AAA}M_{CAG}103$
74	$E_{AAA}M_{CAA}223$	101c	$E_{AAA}M_{CAG}195/197$
75	$E_{AAA}M_{CAA}225$	102	$E_{AAA}M_{CAG}210$
76	$E_{AAA}M_{CAA}227$	103	$E_{AAA}M_{CAG}410$
77c	$E_{AAA}M_{CAA}269/270$	105	$E_{ACC}M_{CCT}310$
78	$E_{AAA}M_{CAA}500$	106	$E_{ACC}M_{CCT}82$

Continuation of Table A.3.

AFLP marker	Primer combination[1]	AFLP marker	Primer combination
107	$E_{ACC}M_{CCT}97$	114	$E_{ACT}M_{CAA}460$
108	$E_{ACC}M_{CGA}142$	116	$E_{ACT}M_{CAA}75$
109	$E_{ACC}M_{CGA}300$	117	$E_{ACT}M_{CAA}580$
110	$E_{ACC}M_{CGA}400$	118	$E_{ACT}M_{CAA}550$
111c	$E_{AGG}M_{CAC}145/147$	119	$E_{ACT}M_{CAA}360$
112	$E_{AGG}M_{CAC}256$	120	$E_{ACT}M_{CAA}90$
113	$E_{AGG}M_{CAC}78$	121c	$E_{ACT}M_{CAA}330/320$

[1] The letters E and M indicate the restriction enzymes EcoRI and MseI. The three letters following E indicate the EcoRI (ANN) selective nucleotides and the three letters following M indicate the MseI (CNN) selective nucleotides. The number represents the approximate size of the polymorphic band.

[2] c indicates codominant marker

[3] The two numbers indicate the size of both fragments of a codominant marker.

APPENDIX B

Table B.1. Lodging, desirability coefficient, maturity, seed size and reaction to straw test in 89 BC2 IBL derived from the cross between Tacana and PI 318695, grown in Saginaw, MI, in 2002.

IBL code (02T-)	Lodging[1]	DS[2]	Days to Maturity[3]	Seed size (g.100seed^{-1})	Straw Test[4]
8571	3	4	M	18.1	2.3
8540	4	4	E	19.0	2.5
8563	2	6	E	23.4	2.6
8553	3	5	M	20.2	2.7
8600	3	3	L	19.1	2.7
8580	2	5	M	22.8	2.7
8554	3	5	E	21.2	2.8
8533	2	4	M	16.9	2.8
8523	2	6	E	21.4	2.9
8537	3	4	M	22.6	3.0
8535	3	5	E	18.8	3.0
8509	3	4	M	18.5	3.0
139-1	3	4	E	20.0	3.1
8568	2	5	M	21.5	3.1
8595	3	4	L	17.7	3.2
8575	4	3	E	20.1	3.2
8596	2	5	M	20.7	3.3
8506	3	4	M	19.0	3.3
8577	3	4	L	26.1	3.3
8570	3	4	M	20.5	3.3
8574	2	7	E	21.4	3.3
8515	3	6	E	21.3	3.3
8527	3	4	E	19.6	3.3
8592	2	5	L	17.3	3.5
8551	2	4	M	16.7	3.6
8550	3	4	E	20.0	3.6
8561	3	5	E	15.2	3.6
8543	2	5	E	24.3	3.6
122-1	3	4	E	18.0	3.7
8526	3	3	L	15.5	3.7
8582	2	4	E	18.3	3.8
8503	3	5	E	19.4	3.8
8566	3	4	L	20.1	3.8
8558	3	6	E	18.3	3.8
97-3	3	6	E	17.6	3.8
95-3	3	4	E	18.0	3.9
8597	3	3	M	16.9	3.9
111-1	3	3	L	18.0	3.9

Continuation of Table B.1

IBL code (02T-)	Lodging[1]	DS[2]	Days to Maturity[3]	Seed size (g.100seed^{-1})	Straw Test[4]
8573	4	3	E	19.2	3.9
122-3	2	5	M	17.5	4.0
8588	3	4	L	19.4	4.1
8545	3	4	E	18.0	4.1
8586	2	4	M	17.8	4.1
127-2	3	4	M	18.8	4.1
8518	3	4	E	16.8	4.2
8536	3	4	E	13.4	4.2
8549	2	4	M	18.5	4.3
139.5-4	3	4	M	16.3	4.3
8524	3	5	E	20.5	4.4
8517	2	5	E	18.8	4.4
8581	2	5	E	22.0	4.4
8541	2	5	M	19.1	4.5
8531	3	4	E	17.7	4.5
8579	4	3	M	16.4	4.6
8547	3	4	L	20.9	4.8
8585	3	4	M	17.7	4.8
8532	4	3	M	16.8	4.8
8562	4	3	E	14.6	4.8
8556	3	7	E	16.1	4.8
8589	3	4	L	19.1	4.8
8598	4	3	M	18.1	4.8
8590	3	4	L	15.9	4.9
8576	3	5	M	22.2	4.9
8534	2	4	M	15.7	4.9
8587	3	3	M	18.8	4.9
8548	4	3	M	18.1	5.0
8530	2	6	E	20.7	5.1
97-2	4	3	M	16.4	5.1
114-4	3	4	M	16.4	5.2
8522	3	5	E	16.1	5.2
8564	3	4	E	20.7	5.3
8542	2	6	M	16.5	5.3
8544	2	4	E	14.4	5.3
8578	3	4	L	18.9	5.3
8520	3	4	M	17.4	5.4
8593	3	4	M	17.5	5.4
8529	3	5	E	19.2	5.4
8572	3	4	M	20.1	5.5
8512	3	4	M	19.9	5.6

Continuation of Table B.1

IBL code (02T-)	Lodging[1]	DS[2]	Days to Maturity[3]	Seed size (g.100seed^{-1})	Straw Test[4]
8538	4	3	E	15.3	5.6
8557	3	5	E	17.3	5.7
8504	2	4	M	18.8	5.8
8516	2	4	L	19.7	5.9
126-2	2	5	M	21.0	5.9
118-2	3	4	M	18.7	5.9
8501	4	3	E	16.2	6.3
8507	3	3	M	17.7	6.8
8599	3	4	M	18.4	7.1
8508	3	4	E	17.3	7.2
Average(89)	2.9	4.3	-	18.7	4.3
Range	2 – 4	3 - 7	-	13.4 – 26.1	2.3 – 7.2
Tacana	2.0	5.0	M	21.5	3.4
PI-318695	-	-	-	3.5[5]	4.5

[1] Lodging is rated in a 1 to 5 scale, where 1= no lodging, 3 = moderate lodging, and 5 = excessive lodging.

[2] Desirability score, the higher the score (from 1 to 9) the more desirable is the line.

[3] E=early, M= mid season and L=late, relative to days to maturity in Tacana.

[4] Straw test is scored in a scale from 1 to 9 where 1=no symptoms and 9= complete collapse of the plant.

[5] Seeds from plants grown in the greenhouse.

Table B.2. Lodging, desirability coefficient, maturity, seed size and reaction to straw test in 76 BC2 IBL derived from the cross between Tacana and PI 313850, grown in Saginaw, MI, in 2002.

IBL code (02T-)	Lodging[1]	DS[2]	Days to Maturity[3]	Seed size (g.100seed^{-1})	Straw Test[4]
8607	4	3	M	25.8	1.3
8673	3	3	L	24.4	1.4
8601	2	3	M	27.1	1.8
8636	3	4	M	23.4	2.0
8604	2	4	M	23.2	2.0
8668	2	3	E	23.9	2.1
8659	2	5	E	24.3	2.2
8683	2	5	E	23.6	2.2
8605	3	5	M	24.3	2.2
8621	2	4	L	24.0	2.3
8649	3	4	E	22.3	2.3
8679	2	4	M	27.5	2.3
8635	2	3	E	26.5	2.3
8655	4	3	L	25.5	2.3
8629	2	4	M	23.6	2.3
8640	4	4	L	22.6	2.3
8645	3	3	L	18.3	2.3
8611	3	3	E	23.8	2.4
8628	4	3	M	26.5	2.4
8633	2	3	E	22.7	2.5
8657	3	2	M	26.3	2.5
8627	2	4	E	21.3	2.6
8631	2	4	M	24.1	2.6
8684	3	4	M	25.7	2.6
8692	2	5	M	23.2	2.7
8606	4	4	M	23.8	2.7
8613	3	4	M	24.2	2.7
8615	2	4	M	23.4	2.7
8693	2	4	M	22.8	2.7
8642	3	5	E	23.8	2.7
8622	2	4	E	22.7	2.8
8641	2	5	E	24.4	2.8
8691	2	5	E	24.1	2.8
8632	3	4	L	31.9	2.8
8644	3	4	M	23.9	2.8

Continuation of Table B.2.

IBL code (02T-)	Lodging[1]	DS[2]	Days to Maturity[3]	Seed size (g.100seed^{-1})	Straw Test[4]
8678	3	5	E	23.7	2.9
8660	2	5	M	23.0	2.9
8680	2	5	E	23.0	2.9
8647	4	4	M	21.3	2.9
8616	2	4	M	23.3	3.0
8670	2	5	E	23.9	3.1
8675	3	5	M	23.3	3.1
8685	2	5	M	21.6	3.1
8688	4	3	M	24.7	3.1
8686	2	5	M	22.3	3.2
8612	2	4	M	24.5	3.2
8651	3	4	M	25.1	3.2
8662	3	4	M	24.3	3.2
8694	2	4	M	21.6	3.2
8650	2	5	E	25.1	3.3
8676	3	5	M	22.5	3.3
8681	3	5	E	23.5	3.3
8689	2	5	M	21.6	3.3
8656	3	4	E	27.5	3.3
8696	2	5	M	22.8	3.3
8624	3	4	M	24.9	3.3
8663	3	5	M	24.6	3.4
8630	3	4	M	23.4	3.5
8677	2	6	M	25.0	3.6
8666	3	5	E	24.6	3.6
8623	3	3	L	25.6	3.6
8625	2	4	E	23.6	3.7
8626	2	5	E	23.5	3.8
8637	3	2	L	25.1	3.8
8667	3	5	E	22.8	3.9
8671	3	5	M	24.1	3.9
8661	3	5	M	23.5	3.9
8653	3	5	M	21.9	4.0
8672	3	6	M	24.7	4.1
8608	3	4	M	23.0	4.2
8639	3	4	M	27.5	4.2
8690	2	5	M	23.9	4.3

Continuation of Table B.2.

IBL code (02T-)	Lodging[1]	DS[2]	Days to Maturity[3]	Seed size (g.100seed^{-1})	Straw Test[4]
8638	3	5	E	21.1	4.4
8695	3	3	L	28.6	5.1
8652	3	3	M	24.6	5.7
Average(75)	2.7	4.2	-	24.0	3.0
Range	2 – 4	2 – 6	-	18.3 – 31.9	1.3 – 5.7
Tacana	2	5	M	21.5	2.8
PI 313850	-	-	-	61.0[5]	3.3

[1] Scale from 1 to 5, where 1= no lodging and 5 = excessive lodging.

[2] Desirability score, the higher the score (from 1 to 9) the more desirable is the line.

[3] E=early, M=mid season and L=late, relative to Tacana.

[4] Scale from 1 to 9, where 1=no symptoms and 9= collapse of the plant.

[5] Seeds from plants grown in the greenhouse.