QUANTITATIVE TRAIT LOCI ANALYSIS OF RESISTANCE TO WHITE MOLD (*SCLEROTINIA SCLEROTIORUM*) IN COMMON BEAN (*PHASEOLUS VULGARIS*)

By

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ABSTRACT

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White mold, caused by *Sclerotinia sclerotiorum* (Lib.) De Bary, is a serious yield reducing fungal pathogen of common bean (*Phaseolus vulgaris* L.). The objective of this study was to: identify QTL for resistance to white mold and associated agronomic traits in two inbred backcross line (IBL) populations derived from the recurrent black bean parent Tacana; two recombinant inbred line (RIL) pinto bean populations derived from a common parent AN-37 and to explore the role of defense genes during infection of bean genotypes with contrasting responses to white mold.

In the IBL populations selective phenotyping failed to detect QTL for field disease resistance but other agronomic traits less sensitive to environmental conditions were detected. Four novel QTLs for white mold resistance were identified in the greenhouse straw test on bean chromosomes Pv03, Pv07, Pv09 and Pv11, respectively and two previously mapped QTL were also validated on Pv02 and Pv04.

The two pinto bean RIL populations were genotyped with SSR, InDel and SNP markers resulting in two linkage maps of 1183 and 953 cM and a total of fifty QTL were identified in different years for eight traits (white mold disease incidence, seed yield, days to flowering, days to maturity, 100 seed weight, canopy height, lodging and the straw test). These QTL were located across all 11 chromosomes with LOD scores ranged from the threshold of 2.5 to 10.5. Six new QTL for yield were identified on Pv01, Pv02, Pv03, Pv05 and Pv09. Seven new QTL for white mold were identified on Pv01, Pv04, Pv07 and Pv08 and two were validated on Pv02 and Pv03.

Analysis of defense genes in response to white mold infection showed variable temporal transcription suggesting that the resistance reported in different bean genotypes to white mold could be due to different defense pathways. The induction of defense genes at different time points in the contrasting genotypes confirm the quantitative nature of the observed resistance to white mold in common bean.

DEDICATION

To the memory of Victor Baraza Msowoya; my inspiration, my hero.

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CHAPTER 1

LITERATURE REVIEW

Common bean

Common bean (*Phaseolus vulgaris* L.) is the most important legume for direct human consumption worldwide (Broughton et al., 2003). Dry beans provide a major source of quality protein which is high in lysine and therefore complements most cereals. In addition beans are high in carbohydrates, fiber, and minerals (calcium, potassium, phosphorus iron, zinc and magnesium) (Azarpazhooh and Boye, 2012). Bean consumption is high in Latin America and East Africa with a per-capita consumption of over 60kg per year in countries like Rwanda (Miklas and Singh, 2007). Consumption is also increasing in the developed countries as beans are promoted to combat such chronic illnesses like diabetes, kidney disease, hypertension and certain cancers (Bennink, 2002; Thompson et al., 2009). Apart from their health benefits, beans play an important role in environmental sustainability through nitrogen fixation and their ability to be intercropped (Bliss, 1993; Vance, 2001).

Global bean production worldwide was estimated to be at 23.3 million metric tons in 2011 representing an increase of over 70% from 1980. Most of the production is concentrated in the Americas with Brazil being the largest producer while the U.S. ranks second at 1.3MMT (FAOSTAT, 2011). The area under dry bean production in the U.S. has increased by a factor of 7% while Michigan which produces 13% of all US beans has decreased by 40% since 1980 (Kelly and Cichy, 2012). Significant production increases have also been observed in Africa (145%), Asia (36%) and Europe (35%) during the same period (FAOSTAT, 2011).

Taxonomy and Diversity

Common bean is diploid species that belongs to the family *Fabaceae* and genus *Phaseolus* (2n=22). It is a predominantly self-pollinating crop. The genus *Phaseolus* is made up of five domesticated species namely *P. vulgaris* (common bean), *P. coccineus* (scarlet runner bean), *P. dumosus* or *P. polyanthus* (year long bean), *P. acutifolius* (tepary bean) and *P. lunatus* (lima bean). These species belong to the four *Phaseolus* gene pools namely the primary, secondary, tertiary and quaternary respectively (Freytag and Debouck, 2002; Gepts, 2000). Crosses made in and between the primary and secondary gene pools result in viable hybrids especially when common bean is used as the female parent while those crosses with the tertiary gene pool require embryo rescue. However no successful hybrids have been produced between lima bean and common bean (as summarized by Singh, 2007).

Domestication

Common bean was domesticated around 10,000-8,000 years BP and early evidence indicated two centers of origin namely Mesoamerica and Andean America (Gepts et al., 1986; Gepts, 1998). This was supported by archeological findings that showed that wild forms are present in both regions and complementary varieties of the species also exist independently in both geographical areas (Kaplan, 1981). Molecular evidence also suggested that there are two centers of domestication. These are in the forms of variation observed in phaseolin protein markers (Gepts and Bliss, 1986), allozymes (Koenig and Gepts, 1989), RFLP patterns on nuclear DNA (Becerra and Gepts, 1994) and mitochondrial DNA (Khairallah et al., 1990). Reproductive isolation as observed in weak hybrids and segregation distortion between the two groups also lends more support to this theory (Gepts, 1985; Koinange and Gepts 1992). Recent genetic

studies however suggest that domestication of all common beans occurred in the Mesoamerican region. Bitocchi et al. (2012) analyzed nucleotides at five different legume anchor marker loci in both wild and domesticated *P. vulgaris* collected from the two regions and the intermediate region and their results indicated that both gene pools originated in central Mexico and moved to Northern Mexico and south to Argentina.

Races

Common bean (*P. vulgaris*) is divided into two major gene pools of Middle America and Andean based on their phylogenetic distances (Gepts and Bliss, 1986). The Andean gene pool is composed of large seeded kidney and cranberry bean races namely Chile, Nueva Granada and Peru while the Middle American gene pool is made up of four races namely Mesoamerica, Durango, Jalisco and Guatemala which consist mainly of small and medium sized beans (Singh et al., 1991; Beebe et al., 2000). In general the Middle American gene pool has more diversity and genetic variability than the Andean gene pool possibly due genetic bottlenecks occurring during domestication and limited migration from the center of origin (Bitocchi et al., 2013; Porch et al., 2013)

Pinto beans, which belong to the medium seeded Durango race, are the most commonly grown dry bean class in the US (Teran et al., 2009). Most Durango race beans have predominantly indeterminate prostrate growth habit. The Mesoamerican race consists of the small seed sized bean market classes which include black and navy beans. Black beans represent the largest commercial bean class in Michigan (41,000 ha harvested in 2009 representing 51% of MI and 58% of U.S. production (USDA NASS, 2012).

In addition to obvious seed trait differences, other major differences exist among classes in growth habit, disease reaction, and adaptation. The diversity in seed size, shape, and color permits the targeting of commercial classes to meet specific regional and cultural market needs. In addition the variability in the *Phaseolus* genus enables breeders to explore diverse germplasm as a source of favorable traits in the improvement of the crop. Most breeding programs aim at improving yield, pest and disease resistance and nutritional quality and abiotic stresses (Kelly and Cichy, 2012). There are many diseases that affect common bean including anthracnose, common bacterial blight, viral mosaics and white mold (Singh and Schwartz, 2010). In the US Midwest white mold is ranked by farmers as the most important disease limiting production (Lamey et al., 2000; Webster and Kelly, 2000).

White mold

White mold caused by the ascomycete fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is an important disease of many economically important crops worldwide although it is a more common pathogen in temperate regions (Purdy, 1979). The fungus is a necrotroph and attacks a wide host range that includes more than 400 species, primarily dicotyledonous species (Boland and Hall, 1994). The fungus produces compact resting bodies called sclerotia on the surface of diseased plants as well as inside infected stems and pods. These melanized resting structures survive in a dormant state in the soil when conditions are not favorable (winter/dry) for germination and may persist in the soil for 4 to 5 years (Steadman, 1983). Sclerotia can germinate carpogenically or through the growth of mycelia depending on environmental conditions. The sclerotia on the soil germinate carpogenically due to prolonged periods of continuous soil moisture and produce slender stalks with a disk or cup shaped apothecium in

which asci and ascospores are produced. When discharged the spores are blown by the wind. Direct infection of intact healthy tissues from germinating ascospores is not common in beans however initial infection occurs when ascospores germinate on senescing flower petals allowing the fungus to form infection hyphae and colonizes leaf, stem and pod tissues (Lumsden, 1976). Stems can get invaded near the soil line while pods or branches may be directly infected if they are in contact with the soil. Typical symptoms appear as water soaked lesions on stems and pods which expand rapidly under moist conditions resulting in a watery rotten mass covered by white fungal growth. Infected tissue will appear white due to bleaching by oxalic acid produced by the fungus (Schwartz et al., 1989). Oxalic acid was thought to be a necessary pathogenicity factor as shown by Godoy et al. (1990) who demonstrated that oxalic acid deficient mutants of *S. sclerotiorum* were non-pathogenic. Recent research however indicates that oxalic acid is a virulence factor and that different isolates of *S. sclerotiorum* produce varying levels of oxalates (Rollins and Dickman, 2001). The acid has a direct toxic effect through lowering of the pH of the environment within the middle lamellae, and binding calcium to form insoluble oxalate crystals and promoting the activity of cell wall degrading enzymes (Hegedus and Rimmer, 2005).

Disease Management

Yield losses resulting from white mold infection of beans in irrigated regions of the U.S. have been estimated from 20 to 100% (Kerr et al., 1978; Purdy, 1979) suggesting an integrated approach to control white mold. Agronomic practices that help control white mold in the field involve reducing the efficiency of sclerotia and ascospore germination by controlling humidity. High moisture within the plant canopy creates a favorable microclimate for ascospore

germination such that increasing plant and row spacing helps in managing the disease. This however may not always be economically feasible. Timing irrigations to allow drying of plant canopy and avoiding excessive irrigation during flowering and after petal drop also reduces disease severity (Schwartz and Steadman, 1980). Other cultural practices such as avoiding excessive nitrogen fertilizer that stimulates excessive vegetative growth; deep plowing and crop rotation contribute toward disease management. The use of fungicides such as benomyl (Schwartz, 1980) and more recently biocontrol agents like *Coniothyrium minitans* (a mycoparasite to *Sclerotinia sclerotiorum)* (Budge and Whipps, 2001) have been effective in controlling the disease in the field. Transgenic approaches have been demonstrated in other crops like soybean (*Glycine max*) and sunflower (*Helianthus sp*) where the overexpression of the wheat germin gene OXO which encodes for oxalate oxidase has resulted in the reduction of disease symptoms. Oxalate oxidase works by breaking down oxalic acid into carbon dioxide and hydrogen peroxide (Donaldson et al., 2001; Hu et al., 2003; Lane, 2000). Transformation of common bean using the OXO gene has not been reported. Breeding for resistant varieties of beans therefore is the most economical and effective way of controlling the disease in common bean.

Breeding for Resistance

Resistance to white mold is known to be complexly inherited in common bean (Fuller et al., 1984). When crosses are made between resistant and susceptible cultivars the progeny exhibit continuous variation indicating that trait is controlled by many genes and is affected by environmental conditions. Bean lines with complete resistance have not been identified but moderate levels of resistance to white mold have been identified in diverse common bean

genotypes (Miklas and Grafton, 1992; Kolkman and Kelly, 2002; Miklas et al., 2006a; Maxwell et al., 2007; Mkwaila et al., 2011). The moderate levels of resistance are usually enhanced by plant morphological avoidance mechanisms (Miklas et al., 2001; Kolkman and Kelly, 2002; Ando et al., 2007). There are several growth habits or plant architectural mechanisms that contribute to disease escape in common beans. Type I growth habit which is an erect determinate bush in contrast to the prostrate Type III indeterminate (Singh, 1982) reduces the severity of the disease by minimizing plant contact with the moist ground (Fuller et al., 1984). The density of the canopy also plays an important role in the development of the disease in the field by influencing the penetration of light and the circulation of air. The porosity of the canopy is a product of the plant growth habit, structure and branching patterns (Ando et al., 2007). A dense closed canopy creates a favorable microclimate for the germination of sclerotia, growth of the hyphae and development of infection structures on the plant surfaces (Schwartz et al., 1987). The ability of the plant to resist lodging will also determine whether the plant escapes white mold infection. Genotypes with stiffer stems have better standing ability that promotes air circulation and therefore avoid the disease (Ender and Kelly, 2005). These traits are moderately heritable and can be visually scored in the field (Miklas et al., 2004), however they do not always predict how a cultivar will respond to white mold infection in the field. For example the indeterminate type II cultivar Bunsi has an open porous canopy and partially prostrate growth habit that exhibits moderate levels of field resistance, whereas erect determinate cultivars like Newport and Midland exhibit high infection levels in the field (Kolkman and Kelly, 2002). The environment also has an impact on the severity of the disease in the field as some determinate open canopy cultivars behave as resistant phenotypes when grown in semiarid regions (Park et al., 2001). Another factor that contributes to disease avoidance is early maturity which enables the plant to

escape infection due to less vegetative growth (Park et al., 1993; Kolkman and Kelly, 2003). More recently Miklas et al. (2013) reviewed the role of plant architecture related traits that contribute toward the white mold resistance. Canopy porosity plant height and lodging were correlated with disease severity in the field however under high disease pressure these traits were not effective in reducing white mold infection levels. While direct selection for white mold resistance may be conducted based on disease scores in the field and greenhouse, many resistance lines exhibit poor agronomic performance and are not useful for release as cultivars. Most resistant sources are associated with linkage drag of unfavorable characteristics such as low yield or late maturity. The most commonly used resistant check and parent in white mold studies, G122 (cranberry bean) (Steadman et al., 2010) is low yielding and does not dry down uniformly to enable direct harvesting. Other sources of resistance like VCW54 obtained from introgression from *P. coccineus* (Singh et al., 2009) are equally unadapted and low yielding. The inbred backcross lines (IBL) introgressed from exotic accessions (Mkwaila et al., 2011) exhibit characteristics such as late maturity, small seed size associated with negative linkage drag from unadapted parental germplasm. Combining selection for white mold resistance with desirable agronomic traits is essential for the recovery of useful phenotypes with resistance suitable for variety release (Kelly et al., 2012).

Agronomic traits and heritability

Estimates of heritability have been reported for agronomic traits in a range of different populations being evaluated for white mold resistance in the field. Results vary depending on population and traits. Kolkman and Kelly (2002) and Ender and Kelly (2005) reported moderate estimates (0.24-0.25) for lodging and yield in navy and black bean populations respectively. In

inbred backcross line populations broad sense heritabilities were low in 'Tacana' x wild (TW) accessions cross while in the 'Tacana' x landrace (TL) population the estimates were higher (>0.30) for the number of days to flowering and maturity while low (0.14) for lodging (Mkwaila et al., 2011). In two RIL populations Soule et al. (2011) found higher heritability estimates (>0.48) for white mold resistance canopy height, days to maturity and lodging. More recently high broad-sense heritability estimates were obtained in a population of climbing beans for such traits as seed weight and days to maturity while yield and yield components had relatively low heritabilities (Checa and Blair, 2012). Trait correlations in these populations also exhibit varying levels and direction of relationship indicating possibility of generating a selection index. Miklas (2013) recently reported high positive correlations between canopy height and field disease scores (r = 0.20 to 0.82) and negative correlations between plant canopy porosity and disease incidence (r = -0.33 to -0.62).

Disease Screening

The interaction of avoidance mechanisms and environmental conditions makes it impossible to separate physiological resistance from disease avoidance in the field. Physiological resistance is controlled by genetic factors that may inhibit infection or spread of the pathogen in the host tissue. There are several methods of detecting physiological resistance to white mold in crops. Artificial inoculation techniques employ substrates colonized with fungal mycelia like agar plug, carrot, celery pieces. Ascospores are usually not used because they require long periods (4-6 weeks) to produce in vitro (Boosalis et al., 2000; Cline and Jacobsen, 1983) and will not always result in infection under controlled environments. Some methods include limited term inoculation where a mycelial-infested celery stem, is inoculated onto the plant and then removed

from the plant after a specified time (Hunter et al., 1981). In the detached leaf assay an agar plug is placed in the center of a detached leaf or trifoliate and disease is measured by size of necrotic lesion and in the cotyledon inoculation test (Steadman, 1997); a mycelial infested agar is placed on a germinating plant cotyledon prior to abscission (Kull et al., 2003). An indirect method for screening was developed by Kolkman and Kelly (2000) which employs oxalic acid solution (40mM) to screen for resistance. In the oxalate assay, seedlings are placed in oxalic acid solution and resistance is rated based on the extent of wilting. More recently a novel inoculation method called the drop mycelium test was developed in soybean (Chen and Wang, 2005). The method involves spraying a suspension of mycelia cultured in nutrient broth. This method has not been tested on beans however. The most commonly used method for screening bean germplasm for white mold resistance in the greenhouse is the straw test (Petzoldt and Dickson, 1996). In this test, mycelia from the expanding edge of a white mold colony on agar are applied with a soda straw or 10ul pipette tip to the severed end of a bean seedling plant and disease development is rated based on the progression of the fungus down the stem from the point of inoculation. The current broad categories are as follows: 1-3 (resistant) is where the lesion does not reach the first node; 4-6 (moderate) is where the lesion passes the first node but does not reach the second node; 7-9 (susceptible) is where the lesion extends to the third internode and may lead to plant death.

Marker assisted selection

To expedite germplasm enhancement and cultivar development marker assisted selection (MAS) is being incorporated in efforts to breed for white mold resistance. Markers are scored in place of the desired phenotypic with the aim of correlating the pattern to the genes or loci associated with

the trait in question. The sequence of polymorphism or banding pattern of the molecular marker is indicative of the presence or absence of a specific gene or chromosomal segment that is known to carry a desired allele. Since phenotypic disease scoring for white mold is confounded by plant architecture and environment in the field, MAS offers an efficient and reliable way to separate avoidance from genetic resistance through tagging genes or genomic regions that are associated with physiological resistance.

To realize the full potential of MAS, markers linked to disease resistance loci must be identified and possibly separated from those associated with more visible architectural avoidance traits. This is achieved through the construction of linkage maps which indicate the position and relative distances between markers along chromosomes. The basis of mapping is that genes and markers segregate through chromosome recombination such that genes or markers that are close together will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart. Several types of markers have been employed in mapping and tagging disease resistance in common beans. The first linkage map was created using a combination of biochemical markers and restriction fragment length polymorphisms (RFLP) markers (Vallejos et al., 1992). RFLPs are based on the differential hybridization of cloned DNA to DNA fragments in a sample of restriction enzyme digested DNAs; and are specific to a single clone/restriction enzyme combination. Later Random Amplified Polymorphic DNA (RAPD) markers were anchored to the map (Skroch at al., 1996). RAPD markers are based on the differential PCR amplification of a sample of DNAs from short oligonucleotide sequences. Locus specific co dominant markers been developed by amplifying, sequencing and cloning RAPD markers to obtain sequence characterized amplified region (SCAR) markers (Melotto et al., 1996). Amplified Fragment Length Polymorphisms (AFLP) have also been used to study

genetic diversity in beans (Tohme et al., 1996). These loci are generated using a procedure that combines restriction digestion and polymerase chain reaction (PCR) amplification. AFLPs are particularly useful in mapping because they generate a large number of polymorphic loci with a single amplification such that a genome can be saturated quickly. Targeted Region Amplified Polymorphism (TRAP) markers are also PCR-based and they take advantage of the available EST database sequence information to generate polymorphic markers targeting candidate genes. Essentially an 18-mer primer is derived from the EST sequence and pairs it with an arbitrary primer that targets the intronic and/or exonic region (AT- or GC- rich core) (Hu and Vick, 2003). Since TRAP markers are anchored based on arbitrary primers to amplify coding regions in the genome, the resulting polymorphism should be reflective of diversity within functional genes. Miklas et al. (2006b) showed that TRAP markers were linked to new QTL for disease resistance. Another kind of markers is the sequence related amplified polymorphisms (SRAP). SRAP markers have a great affinity to amplify gene-rich regions (Li and Quiros, 2001). They have potential for candidate gene analysis of QTL, and have been used to measure genetic diversity in pumpkin (*Cucurbita pepo* L.) germplasm (Ferriol et al., 2003). Since the development of simple sequence repeats (SSR) for common bean (Yu et al., 2000) more studies are increasingly relying on these markers to tag for important traits. These markers, also known as microsatellites consist of tandemly repeated nucleotide motifs that are a common feature of most eukaryotic genomes (Morgante et al., 2002). Since they have a number of repeats and due to the high allelic diversity, microsatellites are valuable as molecular markers particularly for closely related individuals. In addition they are codominant which eliminates the need for progeny testing of heterozygotes. The first SSR based map was constructed by Blair et al (2006) and since then subsequent maps have been integrated into the bean consensus map (Freyre et al., 1998; Vallejos et al., 1992).

A densely populated genome map enables the dissection of quantitative traits. With advances in genomics, the *P. vulgaris* genome has been sequenced using the Andean accession G19833 as the reference genotype. Single nucleotide polymorphisms (SNPs) were identified by aligning the whole genome sequence with paired end sequences of 19 other bean genotypes using the Illumina genome analyzer. SNPs are the most abundant polymorphisms in the genome (estimated at 1.8 million in common bean). Out of these a subset of about 9,000 loci were reliably scored on the genotypes of the BeanCAP collection and were also segregating in Jalo/BAT93 and Stampede/Red Hawk mapping populations and these were developed into a Illumina GeneChip consisting of 6,000 SNPs (Cregan, 2012). Due to their abundance, maps constructed using SNP cover most of the genome at a higher resolution.

Quantitative Trait Locus Mapping

Quantitative trait loci (QTL) analysis relies on detecting genotypic differences responsible for variation in trait means at a marker locus, by associating the phenotype and genotype of an organism. Several QTL studies have been conducted using different marker systems to identify white mold resistance in beans derived from different genetic backgrounds. The RIL populations developed for QTL identification in dry beans have been derived from several sources of white mold resistance PC-50 (pompadour bean), NY6020-4 (snap bean), Bunsi (navy bean) and G122 (cranberry bean). In a PC-50/XAN-159 RIL population, Park et al. (2001) identified three QTL explaining 26% of the variance for reaction to white mold infection on linkage groups Pv04, Pv07, and Pv08, and one QTL explaining 24% of the variance for reaction in the straw test. Three QTL were also identified on Pv04, Pv07, and Pv08, explaining 27% of field reaction to white mold in the same study. Miklas et al. (2001) detected a QTL on Pv07 near the *Phaseolin*

(*Phs*) locus explaining 38% and 26% of the variance for straw test and field response, respectively in an A 55/G 122 RIL population. A QTL on Pv01 conditioning 18% of the variance for field response was associated with the *fin* locus and a QTL explaining 34% of the variance for canopy porosity, suggesting a role of this locus in disease avoidance. In Bunsi/Newport and Huron/Newport RIL populations, (Kolkman and Kelly, 2003) found QTL on Pv02 and Pv07 that account for 12% and 17% of the variance in disease severity, respectively. A QTL conditioning 16% of the variance in oxalate resistance was also detected on Pv07. In a Benton/NY 6020-4 RIL population, Miklas et al. (2003) identified QTL on Pv06 and Pv08 that account for 12% and 38% of the variance in straw test disease reaction and 13% and 26% of the variance in field disease reaction, respectively. Ender and Kelly (2005) detected QTL on Pv02, Pv05, Pv07, and Pv08 that accounted for 10.1%, 10.7%, 14.7%, 9.2 % of the variance in field disease reaction, respectively, in a Bunsi/Raven RIL population. Miklas et al. (2007) also reported significant QTL on Pv02 and on Pv03 explaining from 8.7-22.7% and 5.3-15.7% of the variance white mold response in the field respectively, in a pinto x navy cross. Some of the markers associated with the QTL were recently validated in an independent study in snap beans (Chung et al., 2008). Other possible sources of white mold resistance in beans are scarlet runner bean (*P. coccineus)* (Miklas et al., 1998) and a wild Mexican accession (Terpstra and Kelly, 2007; Mkwaila et al., 2011). Based on these QTL studies marker assisted breeding has been implemented for QTL on Pv07, Pv08 (Miklas, 2007) and Pv02 (Ender et al., 2008) associated with resistance and plant architectural avoidance traits. More recently Soule et al. (2011) showed that resistance in the kidney bean line VA19 colocalized with QTL on Pv02 and Pv08. Interestingly the partial resistance in VA19 is not derived from previously recognized resistance sources. The colocalization of QTL to genomic regions where prior QTL were detected adds support to

validity of these QTL and more importantly provides breeders with tools to enhance resistance to white mold.

Objectives

The objectives of this research were to identify in both greenhouse and field experiments QTL associated with white mold resistance and agronomic traits including yield under white mold pressure in two inbred backcross populations developed from crosses between wild and landrace bean accessions exhibiting partial resistance to white mold in greenhouse evaluations with the recurrent parent 'Tacana', a black bean cultivar possessing field resistance and/or architectural avoidance to white mold and in two recombinant inbred line populations of type II pinto beans developed from a common resistance source AN-37 and to explore the pattern of expression of three defense genes in response to white mold infection in different common bean cultivars.

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CHAPTER 2

IDENTIFICATION OF QTL FOR AGRONOMIC TRAITS AND RESISTANCE TO WHITE MOLD IN WILD AND LANDRACE GERMPLASM OF COMMON BEAN

Introduction

In humid temperate regions, white mold is rated by bean producers as the most serious disease threat to the common bean crop (Webster and Kelly 2000). Fewer seeds per pod, decreased seed size, and, in severe cases, plant death contribute to severe yield losses associated with white mold infection (Kerr et al. 1978, Steadman 1983). Resistance to white mold is considered a complexly inherited trait with both physiological and architectural avoidance components but no source of complete resistance is currently available in cultivated bean germplasm (Soule et al. 2011). A wide array of both Andean and Middle American genotypes have been evaluated for reaction to white mold (Terán and Singh 2010) but it is probable that resistance to *S. sclerotiorum* exists in wild and landrace bean germplasm. Prior screening of wild and landrace plant accessions of *P. vulgaris* from the core germplasm collections have identified accessions possessing partial resistance to white mold in greenhouse evaluations (Grafton et al. 2002, Kolkman 2000, Miklas et al. 1999). In order to exploit potential physiological resistance present in undomesticated sources, the resistance must first be transferred into locally adapted backgrounds to facilitate field testing. One method that has been widely used to introgress exotic germplasm into cultivated background is the inbred backcross line (IBL) method (Tanksley and Nelson 1996). Quantitative trait loci (QTL) analysis can be conducted on the IBL populations to detect significant QTL from the undomesticated donor parent. The IBL breeding method has been used successfully to introduce quantitative variation for enhanced biological nitrogen fixation into cultivated beans (Bliss 1993) and root rot resistance from landrace cultivars

(Román-Avilés and Kelly 2005). The objective of this study was to identify in both greenhouse and field experiments QTL associated with white mold resistance and agronomic traits including yield under white mold pressure in two IBL populations developed from crosses between wild and landrace bean accessions exhibiting partial resistance to white mold in greenhouse evaluations with the recurrent parent 'Tacana', a black bean cultivar possessing field resistance and/or architectural avoidance to white mold.

Materials and Methods

Plant Material

This study was conducted on two IBL populations developed by Ender (2003). The first population, referred to herein as the TW population, consisted of 89 $BC_2F_{3:4}$ IBLs derived from a cross between 'Tacana', recurrent parent, and PI 318695. Tacana is a Middle American black bean cultivar from Mexico (López Salinas et al. 1997) and was selected for this study because of its upright architecture and partial resistance to white mold observed in the field in Michigan (Ender 2003). PI 318695 is a tiny seeded (3.6 g/100 seeds) photoperiod sensitive wild bean accession from Mexico that exhibited partial resistance to white mold in greenhouse screening evaluations (Grafton et al. 2002, Kolkman 2000). Two backcrosses to 'Tacana' were required in order to generate IBLs exhibiting sufficient agronomic adaptation to temperate climate in Michigan. The second population, referred to herein as the TL population, consisted of 75 $BC_1F_{4:5}$ IBLs derived from a cross between Tacana, recurrent parent and PI 313850 accession. PI 313850 is a large-seeded (64.4g/100 seeds) unadapted Andean landrace from Peru that was identified as having partial resistance to white mold based upon straw test data (Kolkman 2000,

Miklas et al. 1999). In the case of the TL population one backcross to Tacana was sufficient to develop agronomically adapted IBLs for field testing in Michigan.

Greenhouse Evaluations

The greenhouse straw test, as described by Petzoldt and Dickson (1996) was used to evaluate both the TW and TL populations for physiological resistance to white mold. The straw test was conducted on all IBLs and parents each consisting of three replications for four separate years (2003-2006). The greenhouse was maintained between 20 to 24^0C and plants were misted daily to promote disease development. Eight days after inoculation plants were rated for disease progression on a scale of 1 – 9 as described by Petzoldt and Dickson (1996) and modified by Miklas et al. (1999). The straw test has been widely used to measure physiological resistance to white mold in common bean (Pérez-Vega et al. 2011, Terán and Singh 2010).

Field Trials

Selective phenotyping was used to screen the IBL populations for resistance to white mold under field conditions, due to space limitations. The method of selective phenotyping implies the selection of the extremely high and low scoring individuals from the continuous distribution of a quantitative trait, and has been shown to be a powerful method for mapping (Jannink 2005). The IBL lines displaying the extreme ends of the distribution for resistance/susceptibility based on initial greenhouse straw test studies were further characterized for reaction to white mold in the field from 2003 to 2006. A subset of 30 IBLs from both the TW and TL populations was identified based on reaction to white mold using the straw test in 2003 (data not shown). Twenty of the most resistant and ten most susceptible genotypes of each population were selected for

field evaluation. Due to problems associated with evaluating excessively late maturing IBLs the subset of IBLs chosen for planting in 2004, 2005, and 2006 were further selected after 2003 field season based upon earlier maturity (<100d) while maintaining the same range in reaction to white mold in the straw test. Only the 2004-2006 field data was utilized for QTL analysis of the TW and TL populations for disease incidence and agronomic traits in field environments.

Plots were planted in naturally infested soil at the Montcalm Research Farm in Montcalm County, MI. The experimental design each year was an 8 x 8 lattice with three replications. Entries included 30 IBLs from each population, the recurrent parent, Tacana and three checks 'Bunsi', 'T-39' and 115M black bean (Wright and Kelly 2011). Four row plots measuring 6 m in length with 0.5 m row spacing were planted. The interior two rows of each plot were planted with the IBLs and the exterior two rows were planted with a susceptible cultivar 'Matterhorn'. To enhance white mold disease development in the plots, rainfall was supplemented with overhead irrigation as needed and vigorous plant growth and development was ensured using standard tillage, fertilization, and weed and insect control practices. All plots were rated for white mold and agronomic characteristics immediately prior to harvest when plants had reached physiological maturity. White mold disease was visually rated as a combination of incidence and severity on a scale of 1-9, where 1 = no diseased plants and 9 = 80-100% diseased plants and/or 60-100% infected tissue (Miklas et al. 2001). Agronomic traits that may impact white mold incidence and severity were also measured. Such traits included canopy height, days to flower and maturity, and lodging from 1 to 5, where 1 = no lodging and 5 = excessive lodging. Harvest occurred at maturity, when the center two rows of each plot were individually pulled and

threshed. Seed yield and the weight of 100 seeds were calculated after harvest and adjusted to 18% moisture content.

Genotyping of Mapping Populations

Plant tissue for DNA extraction was collected from three week old greenhouse plants from four to six individuals of each IBL and the parental genotypes from both populations according to method of Haley et al. (1994). SSR, SRAP and TRAP markers were screened on the parents of both IBL populations following protocols for SSR markers (Blair et al. 2003) and for SRAP and TRAP markers (Terpstra et al. 2006, Miklas et al. 2006). Only markers polymorphic between parents of either or both populations were genotyped on the entire population and marker genotyping was conducted on either 6% polyacrylamide gels or precast 4% agarose gels depending on the size of the polymorphism of interest.

Statistical Analysis

Data generated from all greenhouse and field experiments were analyzed as randomized complete block designs using PROC MIXED both individually and across field and greenhouse tests (SAS 1995). Phenotypic correlation coefficients for all measured field and greenhouse traits were calculated using PROC CORR (SAS, 1995). Narrow sense heritability h^2 was estimated on a progeny mean basis for the traits (Hallauer and Miranda 1981): as

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gy}^2 / y + \sigma_e^2 / ry}$$

where σ_g^2 = the variance due to genotypes, σ_y^2 = the variance due to years, σ_{gy}^2 = variance due to the interaction between genotype and year and σ_e^2 = experimental error, r= replications and y = years. Exact 90% confidence intervals were calculated for h^2 estimates were calculated as described by Knapp et al. (1985). All marker data for each population was entered into the JoinMap 4.0 software program to develop genetic linkage groups for each population (Van Ooiijen 2006). Linkage maps for both populations were anchored to the bean integrated map based on the presence of previously mapped SSR markers on each respective linkage group (Blair et al. 2003). Markers associated with resistance to white mold in either field or greenhouse evaluations and markers associated with select agronomic traits associated with white mold avoidance were detected. Initially single marker analysis was performed with QTL Cartographer (Basten et al. 2001) followed by interval mapping using MapQTL (Van Ooiijen 2004). Subsequently, multiple-QTL model (MQM) analysis, also known as composite interval mapping, was performed, using markers near LOD peaks >2.0 as covariates to increase the power and precision of QTL mapping. If new peaks appeared, these were also employed as a cofactor until no further cofactor effect was found. Cofactors without an effect were not included. The set of cofactors that was in use when the LOD profile stabilized was subsequently used in restricted MQM mapping. This method uses all selected cofactor markers (2-18 in this study), except the ones on the linkage group where the QTL is located. In QTL analysis, the mapping step size was 1 cM and additive effects were fitted.

Results

Disease incidence (DI) ranged from a low of 30% in 2004 to a high of 57% in 2006 in the TW population over the three years of field testing (Table 2.1). A very similar trend was observed in the TL population across years (DI ranged from 29% to 56%, Table 2.2). White mold ratings were highest in both populations in 2006 when above average rainfall was recorded in July, and scores were lowest in 2004 when the weather was exceptionally hot and dry (146mm below 30 year average). These data support the major role that weather plays in the incidence and development of white mold. Despite the higher white mold incidence in 2006 individual IBLs with low scores (DI~30%) were observed in both populations indicating that genetic resistance was expressed despite high white mold pressure. Seed yield under disease pressure followed very similar trends in both populations. Yields were highest in 2004 since supplemental irrigation favored plant growth and development as high temperatures prevented development of white mold (Tables 2.1 and 2.2). The lowest yields were recorded in 2006 when the incidence of white mold was the highest and reflects the negative impact high white mold pressure has on the yield of common beans. Other factors contributed to yield as white mold incidence was similar in 2003 and 2005 yet the yields were substantially different in both populations. The lower yields in 2005 were the result of dry conditions in August (50mm below 30-year average) during critical pod filling period, whereas some of the top yields of individual IBLs were recorded in 2004. Seed weight followed very similar trends as yield in both populations in 2004 as the lowest seed weights were observed in 2005 when overall yields were low due to drought stress. Since white mold contributed to the low yields in 2006, seed weight was not negatively affected to the same extent. Seed weight was generally lower than commercial standards, particularly in the TW population, due in part to the undomesticated wild parent and in part to the effects of disease

pressure. Overall seed weights were smaller in the TW population as compared to values in the TL population derived from the larger sized landrace accession. Canopy height, lodging, days to flower and maturity were consistent across populations and years with the lowest lodging scores recorded in the highest yielding year (2004, Tables 2.1 and 2.2). All phenotypic traits except disease incidence were significantly different among IBLs over three years in both populations except days to maturity and height in TL population.

Heritability estimates for the traits in the TW population were low to moderate except for the straw test (Table 2.1). Heritability of field disease incidence was noticeably low indicating that only 14% of the observed variation in the field was due to genotypic differences. Plant height and lodging also exhibited low heritability estimates. In the TL population heritability estimates were higher for each of the traits however the trend was the same. The straw test had a high heritability estimate (93%) whereas field disease incidence was substantially lower at 21% (Table 2.2).

Disease incidence was negatively correlated with yield, and seed weight but was positively correlated with height and lodging in the TW population (Table 2.3). Yield was strongly positively correlated with seed weight, and days to flowering, while being negatively correlated with height and lodging. Lodging was positively associated with seed weight and days to maturity. In the TL population yield was negatively correlated with seed weight and lodging but was positively correlated with canopy height (Table 2.3). The number of days to flowering was also positively correlated with plant height while lodging was negatively correlated with plant height. Days to maturity was positively correlated with disease incidence in the field.

A total of 106 polymorphic markers were used to genotype the TW population. These markers which included 39 SSR, 29 SRAP, and 38 TRAP markers and 72 markers; were placed on a total

of 17 linkage groups covering 251.2 cM (not shown). Common SSR markers between the TW map and the bean core maps BAT93/Jalo EEP558 (BJ, Freyre et al. 1998) and DOR364/G19833 (DG, Soule et al. 2011) were used to anchor linkage groups B2, B3, B4, B7, B9, and B11to the TW map. The DG map contains 246 markers of which 78 are microsatellites anchored by Blair et al. (2006b) and these were used to place previously unmapped markers especially TRAP's and SRAP's based on relative positions and orientation. Significant QTL identified by CIM from the TW population are presented in Table 2.4. A QTL for white mold resistance based on the greenhouse test was found on B2 (Fig. 2.1). This QTL explained about 14% of the phenotypic variation and was associated with straw test conducted in 2004, STRAW04 (Table 2.4). Another QTL was identified on B3 in association with the same straw test; STRAW04. This QTL had R^2 value of 13%. Based on straw test, STRAW06, a QTL was found on linkage group B9 which accounted for 16% of the phenotypic variation.

Seven agronomic traits were also analyzed through interval mapping and these included disease incidence, seed yield, 100 seed weight, days to flower and maturity, plant height and lodging given the diversity between the parents. There were no significant QTL for disease incidence in the field, but two QTLs for seed weight were found on B3 and on B9 based on 2006 field data. The QTL on B3 accounted for 21% of the phenotypic variation while the other QTL on B9 accounted for 49% of the variation in seed weight (Table 2.4). The same QTL on B4 were associated with plant height in 2006 and lodging in 2004. These two traits were also linked on B7 accounting for 48% of the phenotypic variation in each case. The alleles for the QTL on B4 came from Tacana parent while the QTL on B7 originated from the wild parent (Table 2.4). Fine mapping of this interval would further separate the different trait QTL.

A total of 81 polymorphic markers were genotyped in the TL population, comprising 20 SSR, and 61 SRAP markers. Five linkage groups were anchored to B2, B3, B4, and B7 and B11 based on SSR markers located on the two core maps (Fig. 2.1). Significant deviations from expected Mendelian segregation (1:1) segregation rates were observed. Markers showed significant distortion throughout the linkage groups in favor of Tacana alleles. However, less than half (27 of 55), of the distorted markers were placed on linkage groups in the TL population. The distortion however was not expected to affect QTL detection because the association between marker class and phenotype remains the same. Different straw tests were conducted on the TL population but significant QTL were only detected in 2006 on B4, B7 and B11 of the TL genetic linkage map (Table 2.5, Fig. 2.1). These QTLs accounted for 22 %, 33% and 15% phenotypic variation on the three linkage groups respectively.

Analysis of field disease incidence scores revealed no significant QTL in any of the three seasons. However, significant QTL for yield under disease pressure were detected on B2 across all three years on a region spanning about 10cM (Fig. 1). These QTL accounted for 38%, 31%, and 19% of the phenotypic variation from 2004-2006. QTL $SY4.5^{TL}$ on B4 and QTL $SY7.2^{TL}$ on B7 explained 30% and 36%; and from 31% to 16% of the phenotypic variation for yield in 2005 and 2006, respectively. There was also a coincidence with a QTL for seed weight (in 2006) on B2 (14%), a second QTL on B7 based on 2005 and 2006 data (R^2 = 21% and 27%, respectively) and a third on B11 in 2006 (R^2 =25%). These QTL are adjacent to the yield QTL at a distance of about 5 cM. Three QTLs for lodging were identified on B3, B4 and B7 based only on 2005 drought season. These QTL explained 16%, 38%, and 28% of the variation, respectively (Table 2.5).

Table 2.1: Means, ranges and heritability estimates for white mold resistance and agronomic traits in the 'Tacana' x PI 318695 (TW) IBL population in three greenhouse screening and field environments at the Montcalm Research Farm, Michigan from 2004 to 2006

Trait	2004[1] mean (range)	2005[1] mean (range)	2006[1] mean (range)	Genotypes IBLs	Years	Heritability[2] h^2 (90% CI)
Straw Test[3]	5.2 (3.2-7.1)	7.6 (3.6-9.0)	7.1 (4.9-9.0)	***	****	0.69 (0.59-0.76)
Disease Incidence (%)[4]	30 (22-41)	43 (23-67)	57 (27-83)	Ns	****	0.14 (0.10-0.41)
Seed Yield[5]	3450 (2789-4144)	1714 (1064-2654)	1378 (560-2117)	***	****	0.31 (0.27-0.59)
Seed weight[6]	19.5 (15.1-23.5)	14.8 (10.5-17.4)	17.8 (15.2-20.2)	**	****	0.34 (0.20-0.61)
Days to Flower	44.2 (39.0-48.0)	41.2 (37.5-43.0)	42.6 (41.0-44.0)	*	****	0.33 (0.20-0.61)
Days to Maturity	97.2 (94.6-101.3)	98.4 (96.0-100.0)	96.2 (92.6-99.1)	**	****	0.38 (0.12-0.64)
Lodging[7]	1.3 (0.9-2.4)	2.8 (2.0-4.5)	1.8 (0.9-3.0)	*	****	0.14 (0.10-0.50)
Height (cm)	47.9 (45.7-51.5)	50.7 (43.0-55.0)	52.6 (48.0-54.5)	*	****	0.10 (0.09-0.39)

*, **, ***, **** significant at $P < 0.05$, 0.01and 0.001and 0.0001 levels, respectively; ns= not significant based on ANOVA over years

[1]These experiments were conducted at the Montcalm Research Farm each year on 30 IBLs previously selected for reaction to white mold in straw test in 2003.

[2]Narrow sense heritability calculated on a progeny mean basis, (90% Confidence Limits).

[3]Greenhouse straw tests on 75 IBLs were rated on a scale of 1-9, where 1 = no infection and 9 = total plant collapse.

[4]Disease incidence is resistance to white mold measured on a 1-9 scale, where 1 = no diseased plants and 9 = 80-100% disease in the plot.

[5]Yield is expressed in kilograms per hectare adjusted to 18% moisture.

[6]Seed weight is expressed as the weight in grams of 100 seeds.

[7]Lodging is measured on a scale of 1 – 5, where 1 = erect and 5 = prostrate or 100% lodged.

Table 2.2: Means, ranges and heritability estimates for white mold resistance and agronomic traits in the 'Tacana' x PI 313850 (TL) IBL population in three greenhouse screening and field environments at the Montcalm Research Farm, Michigan from 2004 to 2006

Trait	2004[1] mean (range)	2005[1] mean (range)	2006[1] mean (range)	Genotypes IBLs	Years	Heritability[2] h^2 (90% CI)
Straw Test[3]	3.2 (2.4-5.3)	4.1 (2.9-8.3)	5.9 (3.0-9.0)	***	****	0.93 (0.90-0.94)
Disease Incidence(%)[4]	29 (22-44)	36 (23-57)	56 (30-83)	Ns	****	0.21 (0.13-0.27)
Seed Yield[5]	3483 (2296-4211)	1882 (795-2498)	1534 (560-2173)	***	****	0.89 (0.80-0.93)
Seed weight[6]	22.1 (19.7-26.3)	17.0 (14.8-20.6)	19.5 (17.2-26.1)	**	****	0.90 (0.83-0.94)
Days to Flower	44.4 (39.0-48.0)	41.6 (37.5-44.5)	42.5 (41.0-44.1)	*	****	0.75 (0.56-0.85)
Days to Maturity	96.3 (94.6-99.5)	98.5 (97.0-100.0)	95.7 (94.1-98.1)	Ns	****	0.21 (0.13-0.27)
Lodging[7]	1.1 (0.9-1.6)	2.3 (1.4-3.9)	1.3 (0.9-3.0)	*	****	0.13 (0.10-0.48)
Height (cm)	47.7 (43.9-51.3)	52.7 (45.5-55.0)	53.2 (50.0-54.0)	Ns	****	0.36 (0.12-0.62)

* ** *** **** , , , significant at P ‹ 0.05, 0.01and 0.001and 0.0001 levels, respectively; ns= not significant based on ANOVA over years

[1]These experiments were conducted at the Montcalm Research Farm each year on 30 IBLs previously selected for reaction to white mold in straw test in 2003.

[2]Narrow sense heritability calculated on a progeny mean basis, (90% Confidence Limits).

[3]Greenhouse straw tests on 75 IBLs were rated on a scale of 1-9, where 1 = no infection and 9 = total plant collapse.

[4]Disease incidence is resistance to white mold measured on a 1-9 scale, where 1 = no diseased plants and 9 = 80-100% disease in the plot.

[5]Yield is expressed in kilograms per hectare adjusted to 18% moisture.

[6]Seed weight is expressed as the weight in grams of 100 seeds.

[7]Lodging is measured on a scale of 1 – 5, where 1 = erect and 5 = prostrate or 100% lodged.

Table 2.3: Persons phenotypic correlation coefficients (r) between disease incidence, yield and agronomic traits across three field environments and greenhouse straw test evaluations in the TW (above the diagonal) and TL (below the diagonal) IBL populations

	Seed Yield	Seed Weight	Days to Flower	Canopy Height	Lodging	Days to Maturity	Disease Incidence	Straw Test
Seed Yield	--	0.57****	0.38****	-0.45****	-0.38****	0.05	-0.55****	-0.01
Seed Weight	-0.36*	--	0.37****	-0.10	0.53****	-0.25***	-0.26****	-0.10
Days to Flower	0.32	-0.54**	--	-0.09	-0.38****	-0.01	-0.17*	-0.16*
Canopy Height	0.83****	-0.53***	0.44**	--	0.10	0.11	0.48****	-0.10
Lodging	-0.59****	0.33	-0.10	-0.47**	--	0.30****	0.18*	0.15*
Days to Maturity	0.22	0.26	-0.01	0.24	0.14	--	-0.11	0.03
Disease Incidence	0.06	-0.26	0.16	0.25	-0.24	0.53**	--	0.02
Straw Test	0.18	-0.17	0.11	0.12	0.10	0.02	-0.10	--

*, **, ***, **** significant at P ‹ 0.05, 0.01, 0.001, and 0.0001 levels, respectively.

Table 2.4: Putative QTL for white mold resistance and agronomic traits identified in field and greenhouse environments in 'Tacana' x PI 318695 (TW) IBL population evaluated in Michigan during 2004-2006.

Trait	Year	Assigned QTL	Linkage Group	Indicative marker[1]	LOD score[2]	Adjusted R^2 (%)[3]	Additivity[4]
Straw Test							
	2004	WM2.3[BR,GC,TW]	B2	Me2Em4.350	2.88	14.3	-0.65
	2004	WM3.3[TW]	B3	BM189	2.52	13.2	-0.49
	2006	WM9.2[TW]	B9	F6R8.600	2.78	15.7	-0.52
Seed Weight							
	2006		B3	BM189	2.93	21.0	1.51
	2006		B9	F1R8.300	3.07	49.1	0.50
Canopy height							
	2006		B4	F5R5.180	3.20	29.0	0.71
	2006		B7	BM210	5.67	48.0	-0.42
Lodging							
	2004		B4	BMd-15	2.60	10.8	0.04
	2004		B7	F1R1.275	5.67	48.0	-0.42

[1] Primer information for SSR markers online at: http://www.css.msu.edu/bic/PDF/Bean SSR Primers 2007.pdf.

[2] LOD: Log of odds

[3] Proportion of the phenotypic variance explained by the QTL at peak LOD using MQM mapping with cofactor selection.

[4] Effect of substituting a single allele from one parent to another. Positive values indicate allele from Tacana and negative from Wild.

Table 2.5a: Putative QTL for white mold resistance and agronomic traits identified in field and greenhouse environments in 'Tacana' x PI 313850 (TL) IBL population evaluated in Michigan during 2004-2006.

Trait	Year	Assigned QTL	Linkage Group	Indicative marker[1]	LOD score[2]	Adjusted R^2 (%)[3]	Additivity[4]
Straw Test							
	2006	WM4.2^{R31,TL}	B4	F1R2.550	2.78	22.0	-0.94
	2006	WM7.5^{TL}	B7	F11R3.200	3.90	32.8	-1.09
	2006	WM11.1^{TL}	B11	BMd-33	2.15	15.4	-0.74
Seed Yield							
	2004	SY2.1^{TL}	B2	BMd-47	3.56	37.8	3.99
	2005		B2	BMd-47	3.13	30.9	3.72
	2006		B2	BMd-47	2.86	19.3	2.61
	2005	SY4.5^{TL}	B4	F16R8.100	4.38	30.4	3.76
	2006		B4	BMd-16	4.15	35.6	3.04
	2005	SY7.2^{TL}	B7	F5R5.225	3.81	30.5	3.43
	2006		B7	F5R5.225	2.63	15.5	2.16

[1] Primer information for SSR markers online at: http://www.css.msu.edu/bic/PDF/Bean SSR Primers 2007.pdf.

[2] LOD: Log of odds.

[3] Proportion of the phenotypic variance explained by the QTL at peak LOD using MQM mapping with cofactor selection.

[4] Effect of substituting a single allele from one parent to another. Positive values indicate allele from Tacana and negative from landrace.

Table 2.5b: Putative QTL for white mold resistance and agronomic traits identified in field and greenhouse environments in 'Tacana' x PI 313850 (TL) IBL population evaluated in Michigan during 2004-2006.

Trait	Year	Assigned QTL	Linkage Group	Indicative marker[1]	LOD score[2]	Adjusted R^2 (%)[3]	Additivity[4]
Seed Weight							
	2005		B7	F15R5.200	2.99	20.5	-1.86
	2006		B2	PV-gccacc001	2.50	13.7	-1.46
	2006		B7	F1R8.300	3.07	27.0	0.50
	2006		B11	F15R5.700	6.10	24.6	-3.38
Lodging							
	2005		B3	BM189	2.68	15.5	-0.80
	2005		B4	F16R8.100	3.00	38.2	-0.41
	2005		B7	F5R5.225	3.62	28.1	-0.41
Days to flower							
	2005		B11	F11R3.100	2.71	19.5	1.28
	2005		B11	BMd-33	2.27	18.0	1.26

[1] Primer information for SSR markers online at: http://www.css.msu.edu/bic/PDF/Bean SSR Primers 2007.pdf.

[2] LOD: Log of odds.

[3] Proportion of the phenotypic variance explained by the QTL at peak LOD using MQM mapping with cofactor selection.

[4] Effect of substituting a single allele from one parent to another. Positive values indicate allele from Tacana and negative from landrace

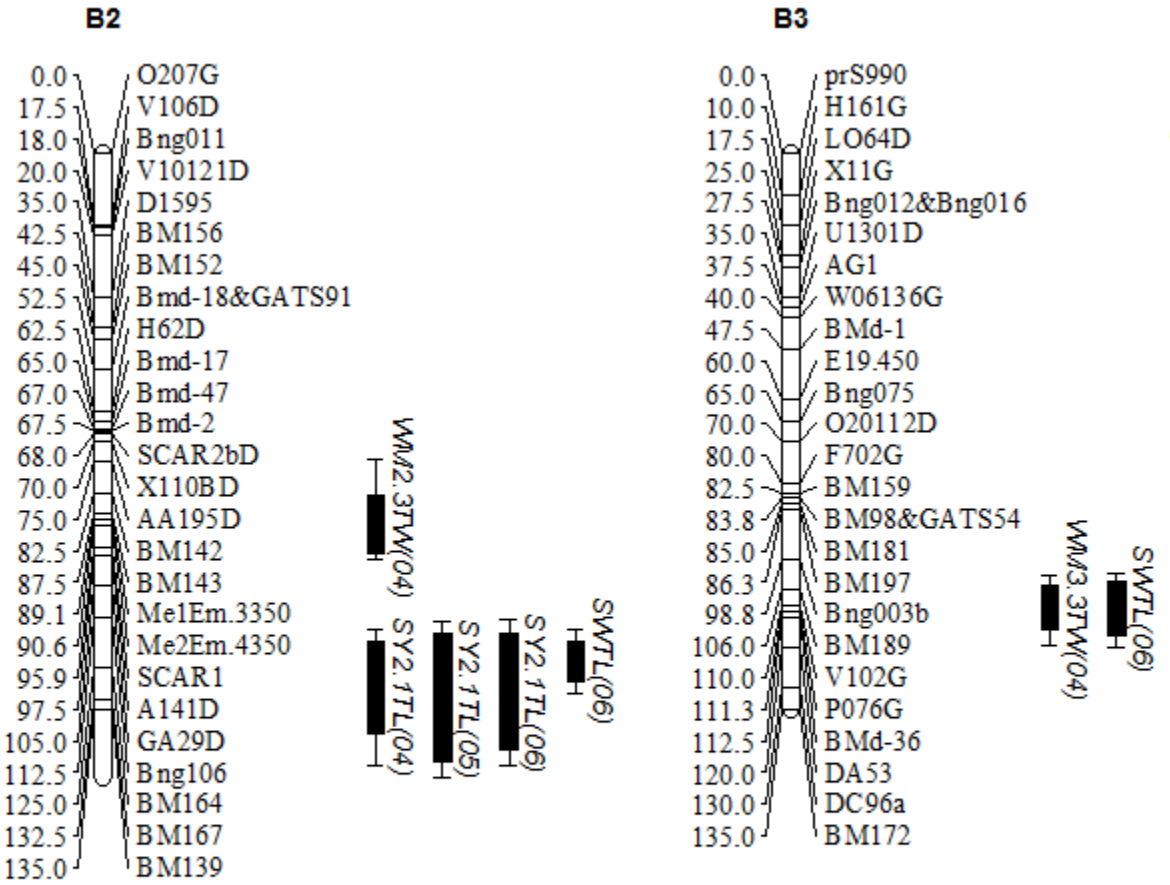

Fig. 2.1: Integrated bean linkage map showing relative positions of QTL for white mold resistance and agronomic traits evaluated over three years (2004-2006) in two inbred backcross line populations TW and TL at Montcalm Research Farm, Michigan, USA.
B2-B11=bean linkage groups, WM= greenhouse straw test, DF= number of days to flowering, HT= plant height, LDG= lodging, SW= 100 seed weight and SY=seed yield. QTL identified by population and year. Bar sizes indicate LOD support ranges for the significant QTL.

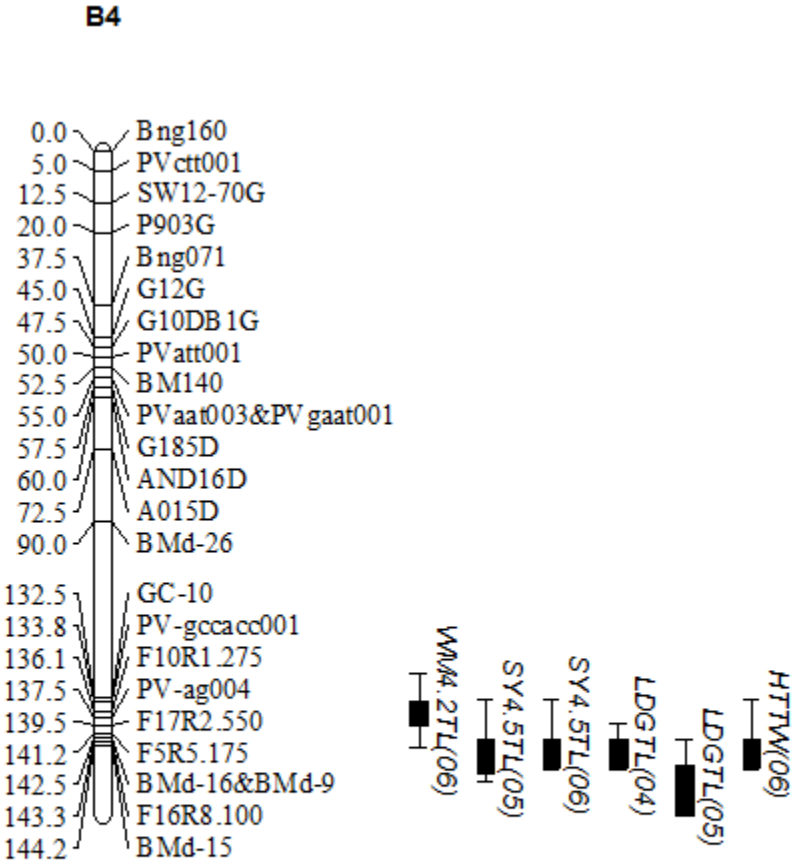
B4
0.0 Bng160
5.0 PVctt001
12.5 SW12-70G
20.0 P903G
37.5 Bng071
45.0 G12G
47.5 G10DB1G
50.0 PVatt001
52.5 BM140
55.0 PVaat003&PVgaat001
57.5 G185D
60.0 AND16D
72.5 A015D
90.0 BMd-26
132.5 GC-10
133.8 PV-gccacc001
136.1 F10R1.275
137.5 PV-ag004
139.5 F17R2.550
141.2 F5R5.175
142.5 BMd-16&BMd-9
143.3 F16R8.100
144.2 BMd-15
WM4.2TL(06)
SY4.5TL(05)
SY4.5TL(06)
LDGTL(04)
LDGTL(05)
HTTW(06)

Fig. 2.1 (cont'd).

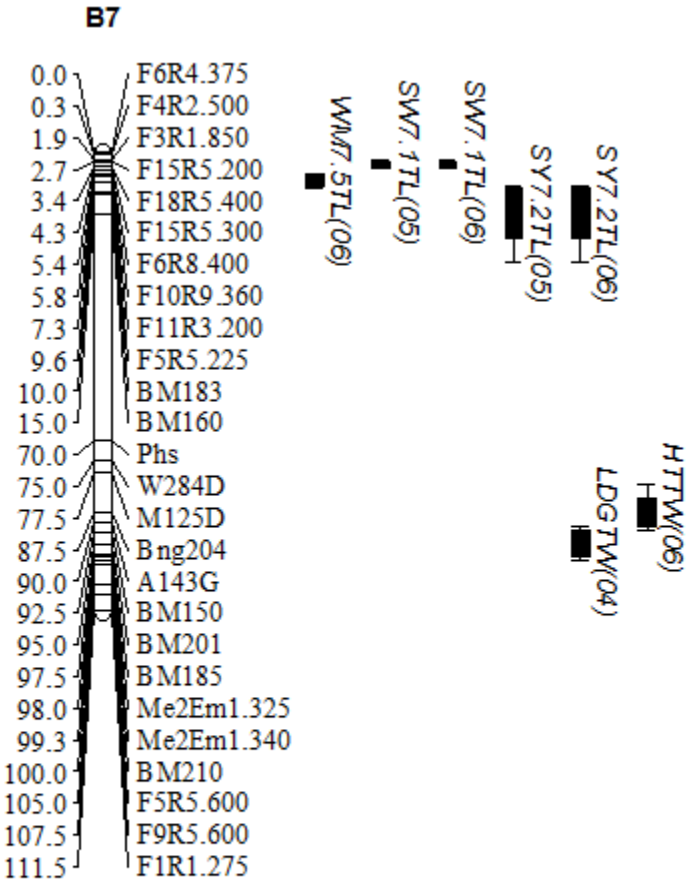
B7
0.0 F6R4.375
0.3 F4R2.500
1.9 F3R1.850
2.7 F15R5.200
3.4 F18R5.400
4.3 F15R5.300
5.4 F6R8.400
5.8 F10R9.360
7.3 F11R3.200
9.6 F5R5.225
10.0 BM183
15.0 BM160
70.0 Phs
75.0 W284D
77.5 M125D
87.5 Bng204
90.0 A143G
92.5 BM150
95.0 BM201
97.5 BM185
98.0 Me2Em1.325
99.3 Me2Em1.340
100.0 BM210
105.0 F5R5.600
107.5 F9R5.600
111.5 F1R1.275
WM7.5TL(06)
SW7.1TL(05)
SW7.1TL(06)
SY7.2TL(05)
SY7.2TL(06)
LDGTW(04)
HTTW(06)

Fig. 2.1 (cont'd).

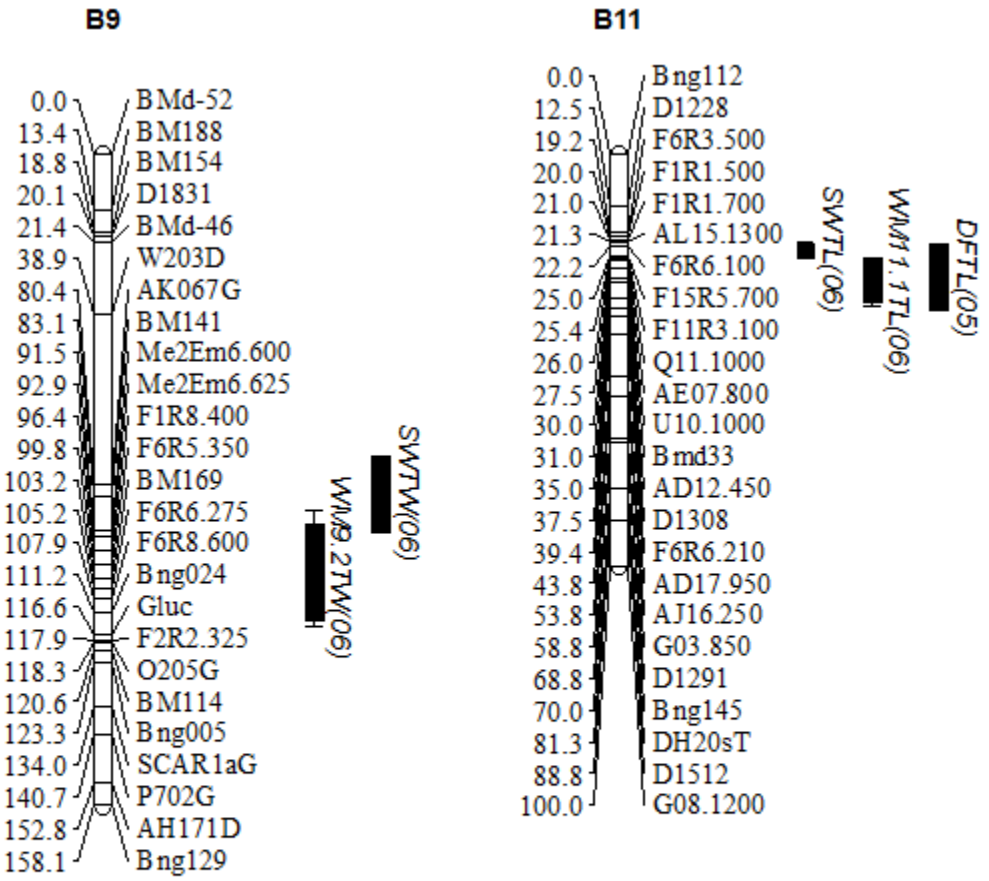

Fig. 2.1 (cont'd).

Discussion

The low heritability estimates for field disease incidence underscores the difficulty of evaluating white mold in the field and the importance of using separate greenhouse tests to assess physiological resistance to white mold in common bean. The high heritability estimates for the straw test indicates that it is a valuable screen to identify underlying mechanisms of resistance independent of confounding plant avoidance traits expressed in the field. Similar modest

heritability estimates for field white mold incidence and agronomic traits in common bean have been reported previously (Miklas et al. 2004, 2007). It is noteworthy that the economic trait of importance to growers, yield under disease pressure, had higher heritability estimates. The higher estimates for most traits in the TL population could be attributed to better combing ability between the two cultivated parents as opposed to the absence of desired agronomic traits in the wild parent in the TW population.

The association between disease incidence, plant height and lodging emphasizes the interplay between architectural traits and physiological resistance mechanisms. Lodging is also associated with disease development, specifically by reducing canopy porosity and assisting in development of a microclimate favorable for disease development (Coyne et al. 1974). The correlation between height and disease incidence may result from taller IBLs being more prone to lodging, although lodging and height were not correlated in the TW population. The straw test ratings were not significantly associated with disease incidence in either population, underscoring the complexity of white mold resistance and the interplay between plant avoidance mechanisms observed in the field and the partial physiological resistance of the original accessions assayed in the straw test. The correlation between days to maturity and lodging could be the consequence of the associations between disease incidence these two traits, as early flowering IBLs showed more disease and lodging is a consequence of disease development. The strong correlation between lodging and days to maturity may indicate that late maturing IBLs are either more prone to lodging as a result of increased vegetative growth or that linkage between these domestication-related traits was not broken in this population. Days to maturity was used as a selection criterion when selecting the IBLs tested in the field, and the narrow range of maturities evaluated may be responsible for the lack of correlations observed between days to maturity and other traits. As

expected disease incidence was negatively correlated with yield, and seed weight, as disease development has been shown to reduce yield and seed size in other studies (Steadman 1983, Kerr et al. 1978). In the TL population the negative correlation between seed weight and yield was expected as these are compensatory yield component traits in crosses between large and small seeded genotypes from the different gene pools (Blair et al. 2006a). The positive correlation between disease incidence and the number of days to maturity confirms that in some genotypes earliness serves as an escape mechanism from severe white mold infection (Kolkman and Kelly 2002).

The location of the QTL for resistance on B2 based on the straw test in TW population suggests that it is the same QTL as were previously detected in Bunsi/Raven (Ender and Kelly 2005) and G122/CO72548 (Maxwell et al. 2007) populations. The nearest SSR markers on the DG map were BM142 and BM143. This is the tenth QTL identified in this region (Soule et al. 2011) which also contains plant defense genes (PGIP and CHS). This QTL identified in the TW population is designated WM2.3^{BR,GC,TW} according to the new QTL nomenclature guidelines (Miklas and Porch 2010). Based on the numerous QTL found in this region it is likely that allelic variations in PGIP or CHS genes may contribute to white mold resistance on B2. The QTL on B3 appears to be a new locus as the previous QTL reported in this region is WM3.1^{AN} near marker BMd-1 in the Aztec/ND88-106-04 population (Miklas et al. 2007). This new QTL is located near BMd-36 which is over 40cM away from previously described QTL (Soule et al. 2011, Pérez-Vega et al. 2011) and therefore is designated as WM3.3^{TW}, the third QTL for white mold resistance on B3. A third QTL was detected on the proximal end of B9 suggesting that it is a new locus as previously reported QTL on B9 in the G122/CO72548 population (Maxwell et al.

2007) was located on the distal end of the DG map. This QTL is located near F6R8.600 marker on B9 is designated WM9.2^{TW}. The nearest previously anchored marker to this QTL is BM114 which is found in the region of *gluc* gene in the BJ core map. Plant glucanases are suggested to play several physiological roles including plant protection. When fungal pathogens like *S. sclerotiorum* attack plants, glucanases aid in the release of polysaccharides from cell wall degradation and these act as elicitors which induce defense response in plants (Stintzi et al. 1993).

There was significant deviation from the expected genetic ratio in the TL population. Segregation distortion is a common phenomenon in plant mapping studies. In a previous QTL mapping study in bean using IBLs, segregation distortion was also noted (Blair et al. 2006a). The published study was not based on an inter-gene pool cross per se, but rather a cross between an Andean cultivar and a wild bean that is genotypically distinct from both major gene pools of common bean (Blair et al. 2006a) and it is speculated that the distorted ratios observed may be the direct result of inadvertent selection for pod, or seed domestication traits during population development. It is possible that inadvertent selection also contributed to the segregation distortion and absence of specific linkage groups including B1, which contains several domestication genes, in the TL population, as some IBLs were discarded during population development due to poor adaption to Michigan field conditions (Ender 2003). The deviant markers however did not significantly affect the accuracy of the genetic map or marker-trait associations. Three QTL for white mold resistance based on the straw test were identified. The QTL on B4 in the TL population is probably the same as WM4.2^{R31,TL} identified in R31 population as it is in the same vicinity as SSR marker BMd-15 in the DG map (Soule et al. 2011). The QTL on B7 is a novel QTL because it maps near marker BM160 which is on the

distal end of B7 away from where the previously reported QTL were located on the DG map. The new QTL differs from the WM7.1^{AG,PX,XC} QTL in the A55/G122 (Miklas et al. 2001), PC-50/XAN-159 (Park et al. 2001), and Xana/Cornell 49242 (Pérez-Vega et al. 2011) populations; from the WM7.2^{BN,BR} QTL in the Bunsi/Newport (Kolkman and Kelly 2003) and Bunsi/Raven (Ender and Kelly 2005) populations; from the WM7.3^{R31} QTL in the R31 population (Soule et al. 2011); and from the WM7.4^{XC} QTL in the Xana/Cornell 49242 population (Pérez-Vega et al. 2011). Approximately 12 cM was added to the integrated B7 linkage group since B7 is mostly composed of SRAP markers in this study. The new QTL is designated WM7.5^{TL}. No QTL have been reported for white mold resistance on B11. The QTL on this linkage group accounted for 15.4% of the variation in the TL population and is designated WM11.1^{TL}.

A better understanding of the associations between disease resistance and agronomic traits contributes to the development of more efficient strategies to select for resistance to white mold in common bean. Yield under disease pressure is very important since it is the primary trait of interest in most breeding programs. A major QTL, SY2.1^{TL} that ranged from 19 to 37% of the variation for yield under white mold pressure over three years was detected on B2 in the TL population. The consistency of yield QTL on B2, B4 and B7 underpins the accuracy of mapping for the trait. Kolkman and Kelly (2003) previously found the SY7.1^{BN} QTL for yield under white mold pressure on B7 that explained 36% of the phenotypic variation. Blair et al. (2006a) also mapped four QTL for yield on B4. The two QTL SY4.5^{TL} and SY7.2^{TL} from this study are

independent of those previously described. Seed weight and disease resistance colocalized on B3 (TW population) and on B7 and B11 (TL population, Fig. 1). QTL for seed weight, and white mold resistance were previously mapped to B2 (Kolkman and Kelly 2003). Seed weight is a component of yield which is a complex trait like white mold. To make genetic gains in yield the individual components like seed weight have to be improved individually. The colocalization of white mold resistance and seed weight offers the opportunity for simultaneous selection for yield and white mold resistance. Other traits like plant height and lodging are an important part of the architectural escape mechanisms of common bean from white mold. Beattie et al. (2003) also found QTL for plant height on B4 but it only accounted for only 10% of the phenotypic variation. The occurrence of these two traits in the same region on B4 and B7 in both populations confirms that these two traits may affect one another and in turn influence the reaction of bean plants to white mold. The QTL for lodging and plant height were in the same locations on B4 as the yield QTL SY4.5^{TL}. QTL for lodging have previously been identified on B3 (Ta'ran et al. 2002) on B4 and B7 (Beattie et al. 2003, Wright and Kelly 2011) with variation ranging from 13 to 18%.

The lack of significant QTL for disease incidence could be attributed to the small sample size and the extreme variation in environmental conditions for the development of white mold in the field. Selective genotyping and DNA pooling has been previously used to map QTL for white mold (Kolkman and Kelly 2003, Miklas et al. 2007) but it is only recommended when the reference populations are genetically narrow. We report here the application of selective phenotyping on a whole genome scale for QTL mapping when field space for extensive phenotyping is limited (Jannink 2005). The high R^2 values for yield, plant height and lodging are

indicative of how relatively consistent these traits are from year to year in comparison to disease incidence. The consistency of yield QTL across the years in the TL population suggests that yield under disease pressure can be used as a selection index for improving white mold resistance in common bean. In summary, selective phenotyping did not permit the detection of QTL for white mold resistance based on field disease incidence. Four new QTLs for white mold were identified and two previous QTL were confirmed based only on the straw test. QTL for yield under white mold pressure were identified on B2, B4 and B7 in the TL population. Deviation of markers from expected ratio in the inter gene pool TL population was observed but did not affect the QTL analysis..

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CHAPTER 3

QTL ANALYSIS OF WHITE MOLD RESISTANCE AND AGRONOMIC TRAITS IN TWO PINTO BEAN (*PHASEOLUS VULGARIS)* RIL POPULATIONS.

Introduction

White mold caused by the ascomycete fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is an important disease of common bean (*Phaseolus vulgaris*) where average yield losses are estimated to range from 20 to 80% under levels of severe infection (Kerr et al., 1978; Purdy, 1979). Controlling white mold requires an integrated approach. Cultural practices such as increasing plant and row spacing and reducing excessive irrigation at flowering help in managing the disease. However breeding resistant bean varieties is the most economical and effective way of controlling the disease. The earliest attempts at dissecting the nature of resistance to white mold involved the screening of a wide array of genetic materials to identify sources of resistance. Adams et al. (1973) identified black turtle soup (black bean) and some inter specific crosses between *Phaseolus vulgaris* and *P. coccineus* as having some moderate levels of resistance to white mold. In greenhouse screening Anderson et al. (1978) identified cultivars like Sanilac and Aurora that possess moderate levels of resistance to the disease. A single dominant gene responsible for resistance was identified in inheritance studies of interspecific crosses between *P. coccineus* and *P. vulgaris* (Abawi et al., 1978). Schwartz et al. (2006) also reported that resistance from *P. coccineus* is qualitative and under the control of a single gene. Similarly Genchev and Kiryakov (2002) screened progeny from a bayo breeding line A195 which exhibited disease inheritance patterns consistent with a single recessive gene. However none of the genes identified in these early studies have been mapped or verified in independent studies. Consequently they have not been deployed in breeding programs. Many inheritance studies show

that resistance to white mold is a complex trait that is under the control of multiple genes (Fuller et al., 1984). Park et al. (1999, 2001) developed a recombinant inbred line (RIL) mapping population between PC-50 and XAN-159 to study the inheritance of white mold resistance following infection with different *Sclerotinia* isolates. Three quantitative trait loci (QTL) were identified on bean chromosomes Pv01 and Pv04 accounting for 33% of the variation observed in reaction to isolate 152 and four QTL that explained 54% of the variance for reaction to isolate 279 were mapped to Pv01, Pv03, and Pv04. Miklas et al. (2001) identified a QTL on Pv07 near the seed protein *Phaseolin* (*Phs*) locus that explained 38% of the variance for straw test and 26% field response, respectively in a RIL population from G122 (a landrace from India with partial resistance to white mold) and A55 a Type II Middle American black bean. In the same population a QTL on Pv01 conditioning 18% of the variance for field response was associated with the determinate *fin* locus and another QTL explaining 34% of the variance for canopy porosity, suggesting that this locus contributes resistance through plant avoidance mechanisms (Miklas et al., 2001). Kolkman and Kelly (2003) found QTL on Pv02 and Pv07 that accounted for 12% and 17% of the variance in disease severity in Bunsi/Newport and Huron/Newport RIL populations, respectively. A QTL conditioning 16% of the variance for oxalate resistance was also detected on Pv07. Miklas and Delorme (2003) identified QTL on Pv06 and Pv08 that accounted for 12% and 38% of the variance in straw test disease reaction and 13% and 26% of the variance in field disease reaction, respectively in a Benton/NY 6020-4 RIL population. Ender and Kelly (2005) mapped QTL on Pv02, Pv05, Pv07, and Pv08 that accounted for 9 to 15% of the variance in field disease reaction, respectively, in a navy x black (Bunsi/Raven) RIL population. Miklas et al. (2007) reported significant QTL on Pv02 and on Pv03 explaining from up to 23% of the variance to white mold in the field in Aztec pinto /ND88-106-04 navy bean RIL

population. Maxwell et al. (2007) identified QTL for white mold resistance on Pv01, Pv02 and Pv08 in both field and greenhouse tests in a G122/C072548 population. Soule et al. (2011) mapped QTL in two populations Benton/VA19 and Raven/I9365-31 on Pv02, Pv04, Pv05, Pv06, Pv07 and Pv08 that accounted for 9 to 33% variation in field, greenhouse straw test and non-wounding tests. Perez-Vega et al. (2012) also mapped QTL associated with five different isolates of *S. sclerotiorum* on Pv01, Pv03, Pv06, and Pv07 in an Andean Xana/Cornell 49242 (white x black bean) RIL population. Mkwaila et al. (2011) identified QTL associated with white mold resistance in two inbred backcross line (IBL) populations from Tacana and a Mexican wild and Peruvian landrace bean accession on Pv02, Pv03, Pv04, Pv07, Pv09, and Pv11 that accounting for up to 32% observed variation in both straw test and field disease incidence. All these studies emphasize that the utility of QTL analysis for MAS depends on the population from which the QTL was identified. While some of the loci colocalize between studies, the markers associated with the trait may not segregate in different populations so breeders need to develop specific market class populations to identify markers for selection for resistance to white mold.

Pinto beans which belong to the Middle American Durango race are the most common market class grown in North America (Teran et al., 2009). They have medium size seed with a light tan color and an irregular mottled dark brown pattern (Freytag and Debouck, 2002). Early landraces and some traditional cultivars include the Common pinto (Teran et al., 2009), Othello (Burke et al., 1995) and Maverick (Grafton et al., 1997). These cultivars have a typical indeterminate, prostrate, type III growth habit that favors disease development. Plants develop a dense leaf canopy and easily lodge causing bean pods to come in contact with the ground (Grafton et al., 1988; Miklas et al., 2006). These decumbent types are therefore unsuited for direct harvest and are also readily attacked by pathogens especially white mold. In recent years, new type II

indeterminate upright cultivars have been bred to avoid white mold (Kelly, 2000). More recent releases include Santa Fe (Kelly et al., 2010), Lariat and Stampede (Osorno et al., 2010) and a private cultivar La Paz. The main objective of this study was to identify and validate QTL associated with white mold resistance and agronomic traits in two pinto bean RIL populations derived from the same resistant parent.

Materials and methods

Plant material

Two half-sib RIL populations of 94 $F_{4:7}$ lines derived from a common parent were developed through single seed descent (SSD). The common parent AN- 37 registered as USPT–WM-1 was released as a white mold tolerant germplasm (Miklas et al., 2006). AN-37 has Type IIb growth habit, open canopy, stay-green trait, but has an unacceptable small pinto seed. AN-37 was developed from Aztec pinto/ND88-106-04 navy cross where white mold resistance was derived from the navy parent (Miklas et al., 2007). The other parents P02630 and P02647 used in the development of the AP630 and AP647 RIL populations were MSU breeding lines that possessed high yield potential, superior seed quality, and upright plant type, but were susceptible to white mold. F_1 plants were grown and selfed in the greenhouse at East Lansing, MI during the spring of 2005. The F_2 populations were grown at Saginaw, MI during summer 2005 and over 100 single plants were randomly harvested in each population. Two cycles of SSD were conducted in the greenhouse at East Lansing, MI. No selection was practiced as the RILs were advanced through the $F_{2:3}$ and $F_{3:4}$ generations during 2005/06 winter. Single plants were harvested and

$F_{4:5}$ single rows were grown in 2006 and harvested in bulk to produce sufficient seed for future yield evaluations. In 2007, 109 RILs of AP630 population and 99 RILs of the AP647 population and parents were grown at the Montcalm Research Farm (MRF) near Entrican, MI in a field naturally infested with white mold. 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). The experimental design each year was a 96 entry randomized complete block design (RCBD) with three replications except in 2007 when only two replications were planted per population. The extra RILs were evaluated in small RCBD experiment in 2007. In later years, both populations were reduced to 94 RILs by eliminating lines that were not adapted to local growing conditions and the population size better fitted the field layout for irrigation purposes. The interior two rows of each 4 row plot were planted with the RILs and the exterior two rows were planted with a susceptible cultivar. The great northern cultivar 'Matterhorn' was used as the susceptible genotype in all years except 2007, when the susceptible pinto cultivar 'Othello' was used. In order to enhance white mold disease development in the plots, rainfall was supplemented with overhead irrigation as needed and vigorous plant growth and development was ensured using standard tillage, fertilization, and weed and insect control practices. All plots were rated for white mold incidence and agronomic characteristics immediately prior to harvest when plants had reached physiological maturity. White mold disease was visually rated as a combination of incidence and severity on a scale of 1-9, where 1 = no diseased plants and 9 = 80-100% diseased plants and/or 60-100% infected tissue (Miklas et al., 2001). Agronomic traits that impacted white mold incidence and severity were also measured. These traits included canopy height, days to maturity, measured as the number of days from planting when 90% of the pods where physiologically mature, and lodging from 1 to

5, where 1 = no lodging and 5 = excessive lodging. Harvest occurred at maturity, when the center two rows of each plot were individually pulled and threshed. Seed yield and the weight of 100 seeds were calculated after harvest and adjusted to 18% moisture content. Data on height, lodging, days to maturity and white mold incidence was collected at physiological maturity for four years (2007-2010) for AP630 and three years (2007, 2010 and 2011) for AP647 populations. Greenhouse straw tests were conducted from 2008-2010 for AP630 and 2010-2011 for AP647 to detect physiological resistance as described by Petzoldt and Dickson (1996). The straw test was carried out on four separate occasions, each consisting of three replications. On each occasion, clay pots with a 15 centimeter diameter were filled with Bacto potting mix (Michigan Peat Co., Michigan, USA) and four seeds were planted per pot. Each pot served as a replicate in a randomized complete block design. After germination and emergence, pots were thinned to three plants per pot. Plants were inoculated 28 days after planting as described by Petzoldt and Dickson (1996), with the following modifications. The growing tip of the plant was excised with a razor blade and discarded. Petri dishes of two to three day old cultures of *S. sclerotiorum* (collected from Montcalm Research Farm) on PDA were used as the inoculum source. The PDA plugs were placed in the top end of 10 uL pipette tips, which were then applied over the cut stem. A PDA plug without fungus was first placed in the pipette tip followed by a PDA plug with fungus in order to prevent the fungal plug from drying out prior to infection of the plant. The greenhouse was maintained between 20 and 24^{o} C and plants were misted daily to promote disease development. Eight days after inoculation plants were rated for disease progression on a scale of 1 – 9 as described by Petzoldt and Dickson (1996) and modified by Miklas et al. (1999). The rating scale is as follows: 1 = no symptoms, 2 = invasion of the stem past the site of inoculation but not to the first node, 3 = invasion of the stem to the first node, 4 = invasion of the

internode slightly past the first node, 5 = invasion to the middle of the internode, 6 = invasion to the second node, 7 = invasion slightly past the 2nd node, 8 = invasion to the middle of the second internode and beyond, 9 = total plant collapse.

SSR and InDel Marker Genotyping

Plant tissue was obtained from emerging leaves of 3 four week old plants pooled into one sample for each genotype from each population and DNA extraction was conducted using a mini-prep chloroform extraction protocol (Haley et al., 1994). SSR markers were screened on the parents of AP630 population. Markers that were polymorphic between the parents of the population were genotyped on the entire population. The polymorphic markers from the first population were screened on the second population. Bands were amplified using the following polymerase chain reaction (PCR) specifications, as modified from Blair et al. (2003): 20 uL reactions containing 0.1 mM forward SSR primer, 0.1 mM reverse SSR primer, 1X PCR Buffer (Invitrogen, Carlsbad, CA), 1.5 mM magnesium chloride, 0.2 mM dNTP mixture, 1 unit Taq polymerase (Invitrogen, Carlsbad, CA, USA), 2 uL DNA template, and ultra-pure water to volume amplified in a 96-well PTC-100 Programmable Thermal Controller (MJ Research, Inc.) with the following profile [94°C for 5 minutes; 30 cycles of 94°C for 1 minute, 47°C for 1 minute, and 72°C for 1 minute; 72°C for 5 minutes]. All SSR markers were screened on 6% polyacrylamide gels. Depending on the specific size polymorphism for an individual marker, SSR marker genotyping was conducted on 6% polyacrylamide gels. Insertion Deletion markers (InDels) (Moghaddam et al., 2013) were also screened on the parents of both RIL populations and polymorphic markers were genotyped on the entire population. The PCR reaction conditions for the InDel markers were as follows, 20 uL reactions containing 2uL of 5 uM forward primer, 2uL of 5 uM reverse

primer, 10 uL GoTaq Master mix (Invitrogen, Carlsbad, CA), 2 uL DNA template, and 5uL ultra-pure nuclease free water to volume amplified in a 96-well PTC-100 Programmable Thermal Controller (MJ Research, Inc.) with the following profile 3 min at 95°C for one cycle, 20 sec at 95°C -30 sec at 55°C-1 min at 72°C for 45 cycles, 10min at 72°C for one cycle. Polymorphic markers were then genotyped on either 6% polyacrylamide gels or 3% agarose gels, depending on the size of the polymorphism of interest.

All polyacrylamide gels were run on 38 x 50 cm Sequi-Gen GT sequencing cells (BioRad Laboratories Inc., Hercules, CA, USA) or 6% non-denaturing gels on vertical rigs (CBS Scientific) as described by Wang et al. (2003) to separate marker fragments. The denatured PCR product was mixed with 8 uL of formamide loading buffer comprised of 98% formamide; 10mM EDTA, pH 8.0; 1.0 mg/ml bromophenol blue; and 1.0 mg/ml xylene cyanol. Upon loading, samples were electrophoresed at 50-80 watts for between 1.5 and 3 hours. Visualization of fragments was accomplished using a silver staining kit procedure (Promega # Q4132). Depending on the marker, gels were scored either for presence or absence of the desired band or scored for the size of the band. 10 and 100 base pair ladders were included on each gel to facilitate the size estimation of bands. All agarose gels used were cast at 3% agarose in TAE buffer containing ethidium bromide (EmbiTec, San Diego, CA, USA). All agarose gels were run between 80 – 100 Volts for 0.5-1.5 hours in TAE buffer. Bands were visualized with ultraviolet light and scored for either presence or absence of the desired band or for the size of the desired band.

SNP Genotyping

SNP genotyping for both populations was conducted through the BeanCAP (www.beancap.org) project at the Soybean Genomics and Improvement USDA Laboratory (Beltsville Agricultural Research Center) in Maryland. The assays were performed on the Illumina HD Infinium Assay Platform as recommended by the manufacturer (Illumina). Genomic DNA was denatured and amplified isothermally overnight and the amplified product was fragmented, precipitated and resuspended in hybridization buffer. The DNA was then loaded on the BARCBEAN6K_3 Genechip for hybridization while the unhybridized fragments were washed away. The captured DNA served as the template for single base extension and determining the genotype call for the sample. The Genechip was then imaged using laser to record high resolution images of the genotype call. The SNP call data were then analyzed in Illumina Genome Studio software via auto call. The data was also manually adjusted for allele calls (the author acknowledges receipt of SNP marker data developed by the BeanCAP project at the Soybean Genomics and Improvement USDA Laboratory (Beltsville Agricultural Research Center) in Maryland by C. Quigley and P. Cregan (www.beancap.org).

Phenotypic Data Analysis

The trait data collected in each year were analyzed as separate variables using linear mixed models. Normality and homogeneity of variance assumptions were checked using box plots and Levene's test for unequal variances. When the Levene's test results were statistically significant at $p<0.01$, the analysis with unequal variances was performed by comparing performance of statistical model with homogeneous and heterogeneous variances. The model that generated the lowest Akaike Information Criterion (AIC) value was selected for further analyses. After checking for assumptions of zero mean and genetic variance the following model was adopted

$$y = \mu + \text{Re}\, p_i + RIL_j + e_{ij,}$$

where μ is the grand mean overall RILs, *Repi* is the random effect of block, *RILj* is the random effect of the RIL and *eij* is the residual (non-genetic effects) associated with a replication *k* from RIL *j* in block *i*. Since RCB design was used, block was assumed random so that differences between blocks could be recovered and RILs were fixed and for variance components year was considered a replication. The variance components of the random effects were estimated using the maximum likelihood method. All analysis of variance was performed by PROC MIXED in SAS software version 9.3 of the SAS System for Windows (2002). Heritability estimates were computed using mean squares of RILs and mean squares of error (residual) to obtain broad sense heritability. Transgressive segregation was determined for those RILs whose phenotypic values were outside the range of the parental values.

Genetic Linkage Map

Preliminary maps were constructed from the segregation data using JoinMap 4.0 (Van Ooiijen, 2006) and QTL IciMapping (Li et al., 2007) software was employed for the final maps. Pairwise recombination was estimated using Kosambi function and markers were ordered within a linkage group with a minimum LOD score of 3.0 using a Recombination Counting and Ordering (REC) algorithm. Linkage groups were assigned chromosome names based on marker position on integrated linkage maps. Linkage groups with less than three markers of unknown positions were considered artifacts. Segregation distortion was examined by testing the goodness of fit to the

expected Mendelian ratio 1:1. Map integration was conducted using relative positions of markers within each population.

QTL Estimation

The underlying assumption for all the traits under study was that they were controlled by multiple genes. The multiple QTL model was employed for each trait per year. The MQM analysis was conducted in QTL IciMapping (Li et al., 2007) using the function ICM-ADD which takes into account additive effects of the genotypes. Markers with more than 50% missing genotypes were eliminated. The first step was to scan the map for QTL single marker analysis with a step window of 1 cM and stepwise regression probability set at p = 0.001. Consequently a permutation test (1000) was conducted to determine the threshold level for significant QTL in simple interval mapping and the logarithm of odds (LOD) score was determined at 2.5. The composite interval mapping model was fitted to remove the non- significant QTLs and to generate the final QTL model. The direction and size of QTL model was determined and the proportion of variance due to the QTL was also estimated from the analysis output with support ranges. Since there were a lot of markers in the model the R^2 was adjusted to limit the overestimation of QTL effects according to the formula below.

$R^2_{adj} = 1- [(1-R^2) (n-1)] / n-k-1,$

where k is the number of markers and n is the population size.

The map and estimated positions of the QTL were converted from QTL IciMapping (which presents QTL as a single point on the map) to MapChart (Voorrips, 2002) format to indicate LOD support ranges.

Map Integration

The two maps were integrated in QTL IciMapping by adding the SSR data set to the SNP data. Map order and genetic distances were determined using default parameters of 2.0 LOD and Kosambi function. Where marker order and recombination distances were significantly discordant with known positions the markers were combined based on cM position with the SNP map as the reference**.** Single marker analysis followed by composite interval mapping were conducted on the half sib population AP647 based on SNP and SSR markers segregating between the two parents AN-37 and P02647 using the same parameters described in mapping the AP630 population.

Results

Frequency distributions and variation across the years

Significant variation was observed as shown by the data ranges among all the traits in the RIL populations which suggest that initial random selection in the segregating F2 population was unbiased. Across the years all the trait means exhibited mostly normal distribution (Fig. 3.1 and 3.2). There were no bimodal distributions indicating all the traits are continuous under quantitative inheritance.

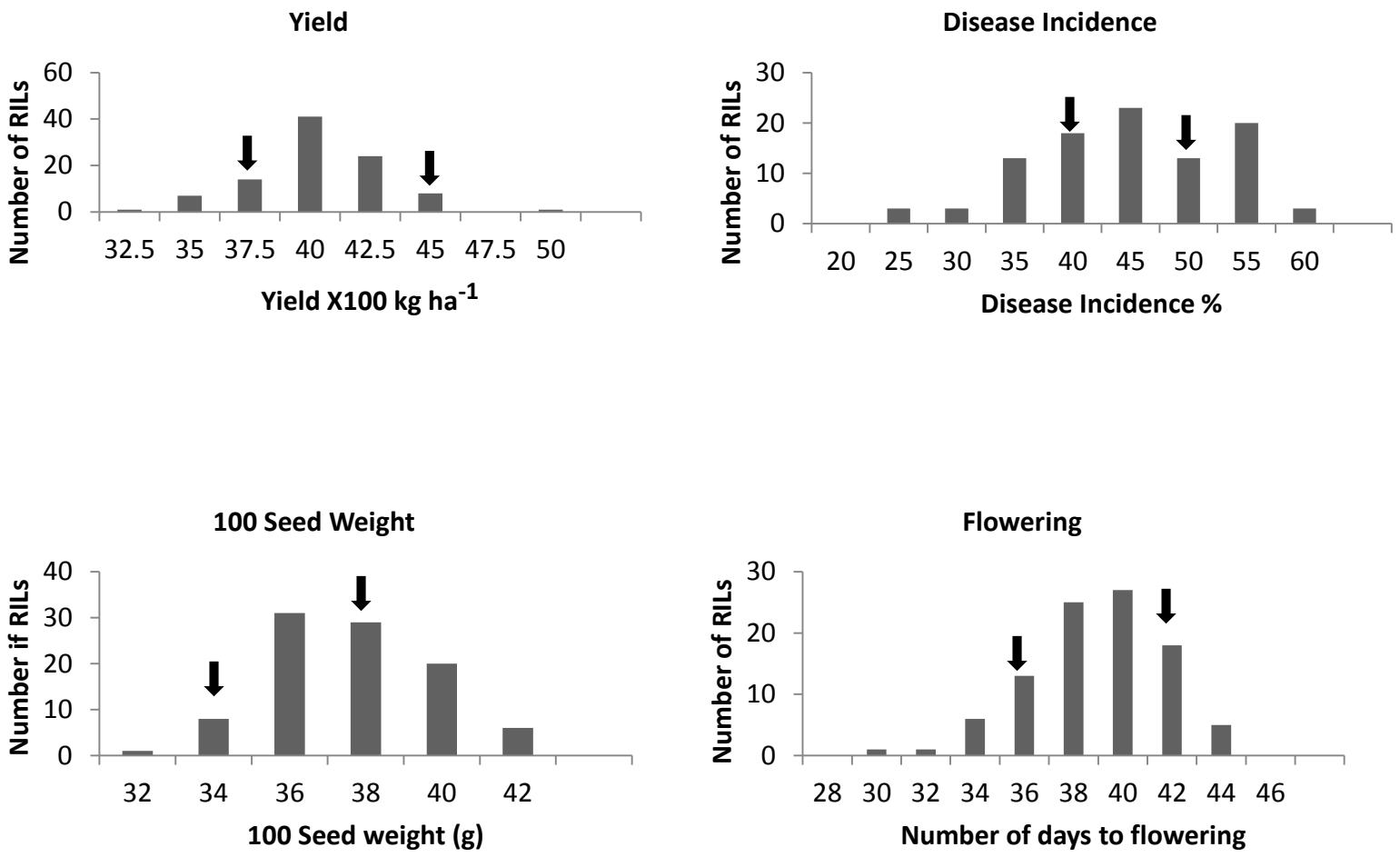


Figure 3.1 Population distributions for white mold incidence, straw test and agronomic traits in the AP630 population. Parents are indicated by arrows.

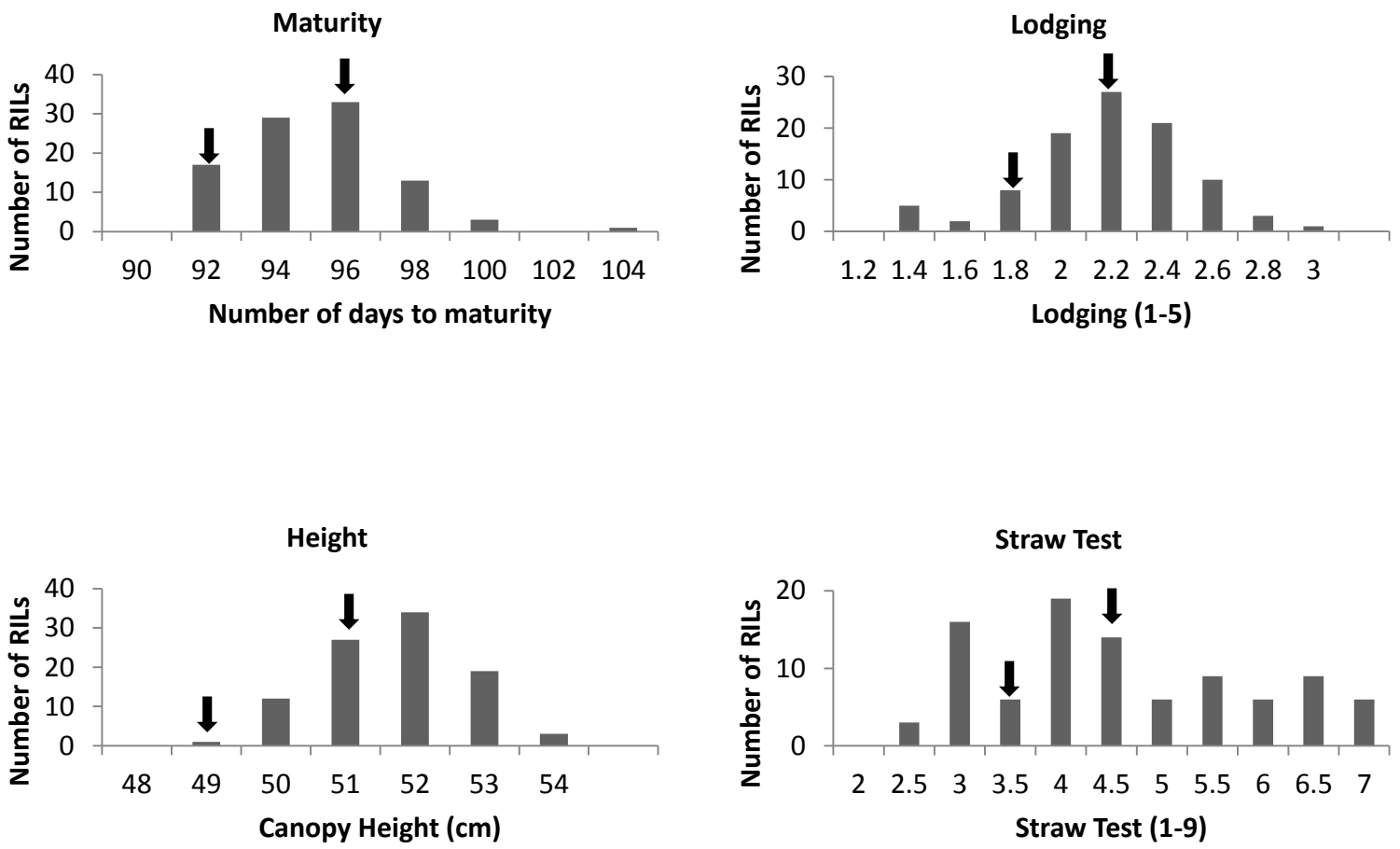


Figure 3.1 (cont'd).

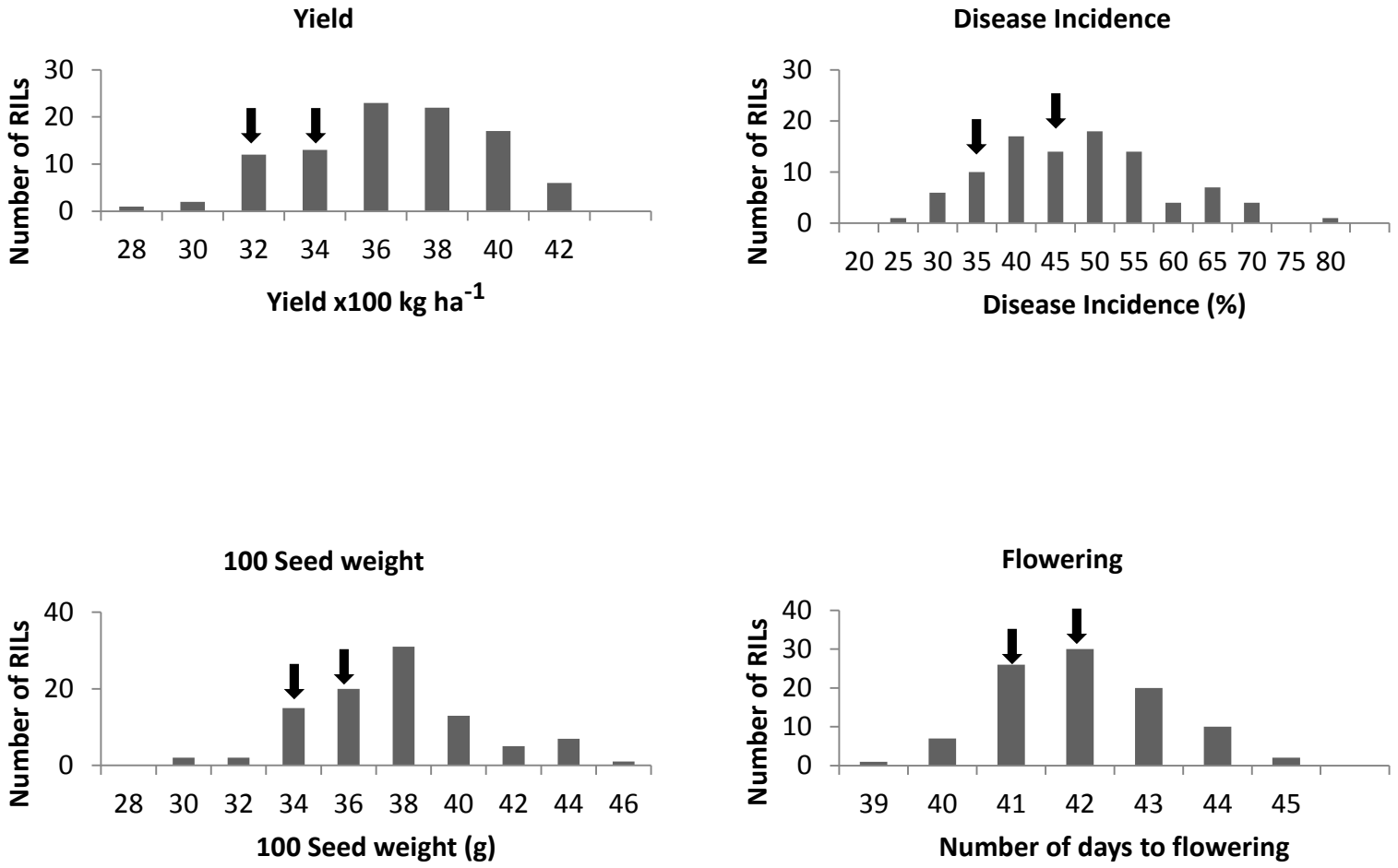


Figure 3.2 Population distributions for white mold incidence, straw test and agronomic traits in the AP647 population. Parents are indicated by arrows.

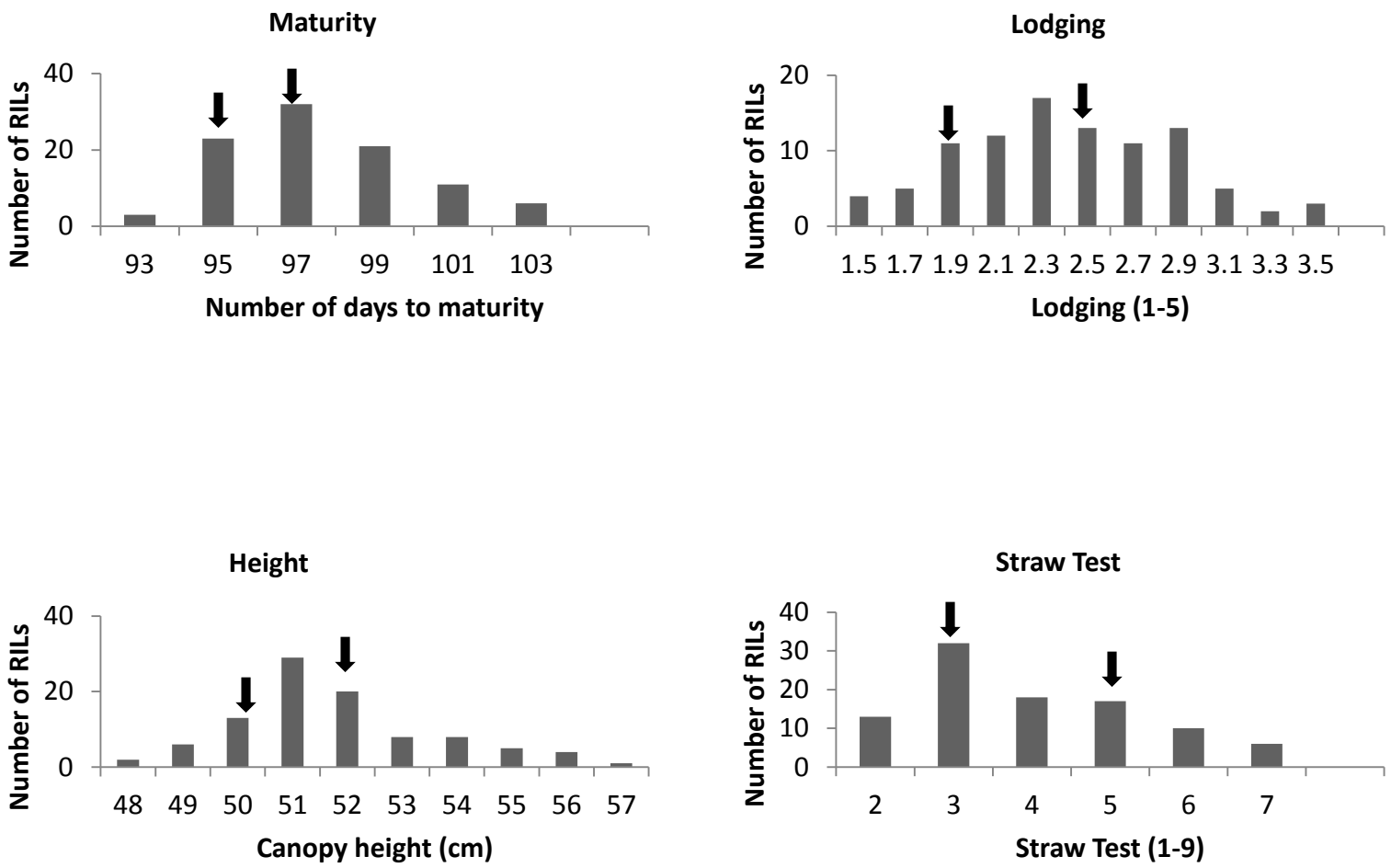


Figure 3.2 (cont'd).

In 2007 disease incidence ranged from 11 to 89% in the AP630 population and from 11 to 94% in the AP647 population (CV=41.5% in AP630 and CV=33.0% in AP647). The AP630 population ranged in yield from 3203 to 5152 kg ha^{-1} with a mean yield of 4177 kg ha^{-1} while AP647 had yield ranging from 2867 to 4648 kg ha^{-1} with a mean yield of 3763 kg ha^{-1}. Since only two replications of each population were evaluated in 2007 variability for yield was low; CV=11.5% in AP630 and CV=10.4% in AP647. Seed weight ranged from 29.4g -44.9g (CV= 5.2%) with a mean of 35.9g in AP630 and 26.3g - 42.7g in AP647 with a mean of 35.5g (CV=6.7%). Days to flowering ranged from 41.1-47.5 days with an average of 43.0 days (CV= 2.0%) in the AP630 population while AP647 had similar range and mean and CV=2.2%. Days to maturity ranged from 92-110 days with a mean of 97 days (CV=2.1%) in AP630 and 92 -104 days and an average of 98.1 days (CV=2.3%) in AP647. Lodging ranged from 1-3 with a mean of 2 (CV=23%) in AP630 and a similar range and an average of 1.8 (CV=26%) in AP647. Canopy height ranged from 42.0-52.5 cm with a mean of 47.5 cm (CV=3.0%) in AP630 and 41.5-54.0 cm and a mean of 48.4 (CV=3.7%) in AP647 (Tables 3.1; 3.2).

In 2008 disease incidence in the AP630 population ranged from 12 to 36% with a mean of 20% and high variability (CV=48%). Disease pressure was low despite the addition of 13 irrigations to promote disease development. Maximum temperatures during the growing season (June to September) were as high as 33^{o} C and may have suppressed disease development. Yields ranged from 3360 to 5824 kg ha^{-1} with a mean yield of 4480 kg ha^{-1} and variability was low (CV=9.7%). Seed weight ranged from 30.8-42.2g with a mean of 36.8g (CV=4.3%). Lodging ranged from 1-3 with a mean of 1.5 (CV=37.4%) while days to flowering ranged from 34-45 days with an average of 40 days (CV=2.1%). Days to maturity ranged from 92.5-99.5 days with

a mean of 94.3 (CV= 1.7%). Canopy height ranged from 48.5-53.3 cm with a mean of 50.4 cm (CV=3.7%). The straw test ranged from 1-6 with a mean of 5.3 (CV= 16.1%) (Table 3.1).

In 2009 disease incidence in the AP630 population ranged from 16.4 to 75.0% with a mean of 43.5% but variability was high (CV=40.4%). Yields ranged from 1982 to 3729 kg ha^{-1} with a mean yield of 3068 kg ha^{-1} with low variability (CV=15.2%). Seed weight ranged from 26.5 to 44.7g with a mean of 37.6g (CV=5.4%). Lodging ranged from 1-3 with a mean of 1.9 (CV=37.1%) while days to flowering ranged from 41-52.0 days with an average of 47.6 days (CV=1.8%). Days to maturity ranged from 97.3 to 106.5 days with a mean of 101.5 (CV= 4.0%). Canopy height ranged from 51.5 to 59.0 cm with a mean of 55.7 cm (CV=5.6%). The straw test ranged from 1-8 with a mean of 4.96 and moderate variability (CV= 14.2%) (Table 3.1).

In 2010 disease incidence ranged from 18.9 to 92% with a mean of 52.2% (CV=41.4 %) in the AP630 population and from 14 to 92 % in AP647 with a mean of 47.7% but variability was high (CV=47.2%). AP630 ranged in yield from 1814 to 3595 kg ha^{-1} with a mean yield of 2878 kg ha^{-1} and moderate variability (CV=16.2%) whereas the yield ranged from 2564 kg ha^{-1} to 4132 kg ha^{-1} with a mean of 3259 kg ha^{-1} in the AP647 population (CV=15.6%). Seed weight ranged from 26.4g to 40.0g with a mean of 33.6g (CV=6.6%) in AP630 and from 27.1g to 40.8g with a mean of 33.1g in the AP647 population (CV=6.5%). Days to flowering ranged from 37.3-41.3 days with an average of 38.6 days (CV= 2.8%) in the AP630 population while AP647 had a range of 37.3-41.3 days and mean of 39.1 and CV=3.3%. Days to maturity ranged from 90 days to 97 days with a mean of 94 days (CV=3.3%) in AP630 and 92 days to 101 days and an average of 95 days (CV=2.7%) in AP647. Lodging ranged from 1 to 4 with a mean of 2.9 (CV=39%) in AP630 and a similar range and an average lodging score of 2.6 (CV=48.6%) in AP647. Canopy

height ranged from 47.0 cm to 55.3 cm with a mean of 51.3 cm (CV=5.8%) in AP630 and 45.0 cm to 57.0 cm and a mean of 51.2 (CV=5.8%) in AP647. The straw test ranged from 3.0-8.0 with a mean of 3.37 in AP630 (CV=11.0%) and 1.00-9.00 with a mean of 3.6 (CV= 12%) in AP647 (Tables 3.1 and 3.2).

In 2011 disease incidence in the AP647 ranged from 16 to 72% with a mean of 48% and was associated with high variability (CV=47%). Yields ranged from 2620 to 5107 kg ha^{-1} with a mean yield of 3606 kg ha^{-1} and moderate variability (CV=13.3%). Seed weight ranged from 33.2 to 50.6g with a mean of 41.7g (CV=5.0%). Lodging ranged from 1.0 to 4.5 with a mean of 2.6 but variability was high (CV=40.3%) suggesting the scores were not very reliable. Days to flowering ranged from 37-47.0 days with an average of 42.2 days (CV=2.8%). Days to maturity ranged from 93 to 102 days with a mean of 97 (CV= 2.4%). Canopy height ranged from 50 to -60 cm with a mean of 54.5 cm (CV=5.4%). The straw test ranged from 1.0-9.0 with a mean of 3.66 and moderate variability (CV=10.0%) (Table 3.2).

Table 3.1: Means ranges and heritability estimates for white mold resistance and agronomic traits in the AN-37 x P02630 (AP630) pinto bean RIL population in greenhouse screening and field environments at the Montcalm Research Farm, Michigan from 2007 to 2010

Trait	2007 mean (range)	2008 mean (range)	2009 mean (range)	2010 mean (range)	Grand Mean	Heritability[1] h^2 (90% CI)
Disease Incidence (%)[2]	53.2 (11.1-88.9)	20.2 (11.9-36.3)	43.5 (16.4-79.4)	52.2 (18.9-92.1)	42.3	0.23 (0.03-0.41)
Straw Test (1-9)[3]	----	5.0 (1.0-6.0)	5.0 (1.0-8.0)	3.4 (3.0-8.0)	4.5	0.46 (0.29-0.58)
Yield (kg ha^{-1})[4]	4177 (3203-5152)	4480 (3360-5824)	3069 (1982-3729)	2878 (1814-3595)	3650	0.53 (0.37-0.64)
Seed weight (g)[5]	35.9 (29.4-44.9)	36.8 (30.8-42.8)	37.6 (26.5-44.7)	33.6 (26.4-40.0)	36.0	0.74 (0.65-0.80)
Days to Flower	43.0 (41.1-47.5)	40.0 (34.5-45.5)	47.6 (41.5-53.0)	38.6 (36.7-41.3)	42.3	0.62 (0.49-0.70)
Days to Maturity	97.7 (92.0-110.0)	94.3 (92.0-99.5)	101.5 (94.0-108)	94.3 (90.0-102.7)	97.0	0.34 (0.11-0.49)
Lodging (1-5)[6]	2.0 (1.0-3.0)	1.50 (1.0-2.5)	1.9 (1.0-3.0)	2.9 (1.0-4.7)	2.1	0.23 (0.03-0.41)
Canopy Height (cm)	47.5 (42.5-52.5)	50.4 (48.5-53.5)	55.7 (51.5-59.0)	51.3 (44.7-57.0)	51.23	0.25 (0.01-0.43)

[1]Broad sense heritability calculated on a progeny mean basis, (90% Confidence Limits).

[2]Disease incidence is resistance to white mold measured on a 1- 9 scale, where 1 = no diseased plants and 9 = 80-100% disease in the plot.

[3]Greenhouse straw tests on the RILS were rated on a scale of 1- 9, where 1 = no infection and 9 = total plant collapse.

[4]Yield is expressed in kilograms per hectare adjusted to 18% moisture.

[5]Seed weight is expressed as the weight in grams of 100 seeds.

[6]Lodging is measured on a scale of 1 – 5, where 1 = erect and 5 = prostrate or 100% lodged.

Table 3.2: Means ranges and heritability estimates for white mold resistance and agronomic traits in the AN-37 x P02647 (AP647) pinto bean RIL population in greenhouse screening and field environments at the Montcalm Research Farm, Michigan in 2007, 2010 and 2011.

Trait	2007 mean (range)	2010 mean (range)	2011 mean (range)	Grand Mean	Heritability h^2 (90% CI)[1]
Disease Incidence (%)[2]	40.4(11.1-94.4)	47.7 (14.4-92.1)	48.0 (16.0-7.02)	45.4	0.49(0.61-0.31)
Straw Test (1-9)[3]	-	3.6 (1.00-9.00)	3.7 (1.00-9.00)	3.6	0.67 (0.75-0.55)
Yield ($kgha^{-1}$)[4]	3763 (2867-4648)	2941 (2290-3690)	3606 (2620-5107)	3437	0.58 (0.68-0.43)
Seed weight (g)[5]	35.5 (26.3-42.7)	33.1(27.1-40.8)	41.7 (33.2-50.6)	36.8	0.88 (0.91-0.84)
Days to Flowering	43.4 (41.0-47.5)	38.9 (37.3-41.3)	42.2 (37.0-47.0)	41.5	0.74 (0.80-0.65)
Days to Maturity	98.1 (92.5-104.0)	95.1 (92.0-101.0)	97.2 (93.0-102.0)	96.8	0.72 (0.79-0.62)
Lodging (1-5)[6]	1.8 (1.0-3.5)	2.6 (1.0-4.3)	2.6 (1.0-4.5)	2.3	0.46 (0.59-0.26)
Canopy Height (cm)	48.4 (41.5-54.0)	51.2(45.0-57.0)	54.5 (50-60.0)	51.4	0.59 (0.69-0.44)

[1]Broad sense heritability calculated on a progeny mean basis, (90% Confidence Limits).

[2]Disease incidence is resistance to white mold measured on a 1- 9 scale, where 1 = no diseased plants and 9 = 80-100% disease in the plot.

[3]Greenhouse straw tests on the RILS were rated on a scale of 1- 9, where 1 = no infection and 9 = total plant collapse.

[4]Yield is expressed in kilograms per hectare adjusted to 18% moisture.

[5]Seed weight is expressed as the weight in grams of 100 seeds.

[6]Lodging is measured on a scale of 1 – 5, where 1 = erect and 5 = prostrate or 100% lodged.

Heritability Estimates

Broad sense heritability estimates (h^2 at p=0.001) for the traits in AP630 population ranged from low to high except for the straw test (h^2 =0.46), yield (h^2 =0.53), seed weight (h^2 =0.74) and days to flowering (h^2 =0.62) (Table 3.1). Field disease incidence (h^2 =0.23) was noticeably low in comparison to the greenhouse straw test (h^2=0.46) indicating that only 23% of the observed variation in the field was due to high levels of variability of this trait. Plant height and lodging also exhibited low heritability estimates (h^2=0.25 and h^2=0.23 at p=0.05 respectively). Seed weight and yield exhibited relatively higher heritability estimates (h^2=0.74 and 0.53 respectively). Higher heritability estimates for seed weight (h^2=0.88), days to flowering (h^2=0.74) and maturity (h^2=0.72) were observed in the AP647 population suggesting relatively more genetic control of these traits than yield (h^2 = 0.58), disease incidence (h^2 = 0.49) or lodging (h^2=0.46); respectively (Table3.2).

Trait Correlations

In the AP630 population all agronomic traits were negatively correlated with disease incidence in the field except for lodging (Table 3.3). There was small negative correlation (r = -0.14 ns) between the straw test and disease incidence in the field. Yield was positively correlated with seed weight (r = 0.57 p=0.001), days to flowering (r = 0.38 p=0.001) but negatively correlated with lodging (r = -0.38), and canopy height (r = -0.45 p=0.001). Seed weight was negatively correlated with the number of days to maturity (r = -0.25 p=0.05) but was positively correlated days to flowering (r = 0.37 p=0.001) and lodging (r = 0.53 p=0.001). Plant canopy height was negatively correlated with all traits in the AP 630 population.

In the AP647 population yield was positively and significantly correlated with all traits except lodging (r = -0.04 ns) and white mold incidence (r = -0.36 p=0.001). Similarly white mold affected incidence was negatively correlated to all traits except the number of days to maturity (r=0.46) and lodging (r = -0.41). The highest correlations were among plant height and other traits and white mold and other traits. Plant height was positively correlated with seed yield (r = 0.45 p=0.001), seed weight (r =0.38 p=0.001), days to flowering (r= 30 p=0.001) and negatively correlated with disease incidence (r = -0.41 p=0.001) and lodging (r = -0.21 p=0.05). In both populations there was no significant correlation either positively or negatively between the straw test and any of the traits measured in the field (Table 3.3).

Table 3.3: Persons phenotypic correlation coefficients (r) between disease incidence, yield and agronomic traits across three field environments and greenhouse straw test evaluations in the AP647 (above the diagonal) and AP630 (below the diagonal) pinto bean RIL populations

	Seed Yield	Disease Incidence	100 Seed weight	Days to Flowering	Days to maturity	Lodging	Canopy Height	Straw Test
Seed Yield	--	-0.36**	0.52***	0.38**	0.27**	-0.04	0.45***	0.00
Disease Incidence	-0.30**	--	-0.26*	-0.17	0.46***	0.41***	-0.41***	0.02
100 Seed weight	0.57**	0.06	--	0.37**	0.00	0.02	0.38**	-0.05
Days to Flowering	0.38**	-0.17	0.37**	--	0.60***	0.05	0.30**	0.03
Days to maturity	0.05	-0.47**	-0.25*	0.98***	--	0.18	0.35**	0.07
Lodging	-0.38	0.44**	0.53**	-0.38**	0.20*	--	-0.21*	-0.12
Canopy Height	-0.45**	-0.45**	-0.10	-0.09	-0.30**	-0.12	--	0.08
Straw Test	-0.01	-0.14	-0.10	-0.16	-0.15	-0.03	0.03	--

Significant at *p= 0.05, **p=0.01***, p=0.001 and ****p= 0.0001

Genotyping and Map Construction

A total of 440 SSR and 154 InDel markers were screened on the parents AN-37 and P02630 of the AP630 population. SSRs showed 54% polymorphism while InDels showed 20% polymorphism between parents. Only 107 markers polymorphic between parents were genotyped on the entire AP630 population. The same 107 markers were also screened on the AP647 population. Of these only 34 consisting of 6 InDels and 28 SSRs were polymorphic between parents of the second population and were genotyped in population AP647.

An initial map of AP630 was constructed using JoinMap version 3.0. All markers significantly deviating from the expected ratio of 1:1 were eliminated at this stage and an initial map of 325cM was generated (not shown). However the map distances and marker order were not concordant with existing linkage maps because most markers were clustered together within short (<5) cM distances and there were many unanchored small groups. QTL IciMapping was then used to order markers and chromosomes resulting in markers located across eight linkage groups of variable length. Since most of the SSR have been previously mapped and InDels have known positions on the *P. vulgaris* map, these were used to identify the bean chromosomes as anchor markers. A final map of 727 cM was obtained with 107 markers located approximately every 8 cM and was then used for QTL mapping of the AP630 (Appendix A1 SSR Map).

The two populations were also screened with the BARCBEAN6K_3 Genechip consisting of a total of 5,398 SNP markers. The two populations were genotyped resulting in an average hybridization rate of 45% for the AP630 and 44% for AP647 population. A total of 1024 markers were polymorphic in AP630 and 1016 markers were polymorphic in the AP647 population. Marker distortion was observed only in 5% of the SNPs in AP630 and these markers were excluded in final map if there was a duplicate locus. The final map assembly of the AP630

population resulted in 11 linkage groups corresponding to the 11 chromosomes of *P. vulgaris*. The average marker distance was estimated at 1.2 cM however there were regions of both high and low recombination on some linkage groups. The maximum distance between adjacent markers was approximately 22 cM on Pv04. The integrated map of AP630 covered a total of 1183 cM (Figure 3.3). Marker distortion was observed in 10% of the SNPs in AP647 but these were included in the analysis. The total map distance for AP647 was 953 cM represented on 11 linkage groups corresponding to 11 bean chromosomes with an average marker distance of 1.0 cM. Both high and low recombination regions were observed in AP647 and the maximum distance between two loci was 45 cM on Pv01 (Figure 3.4).

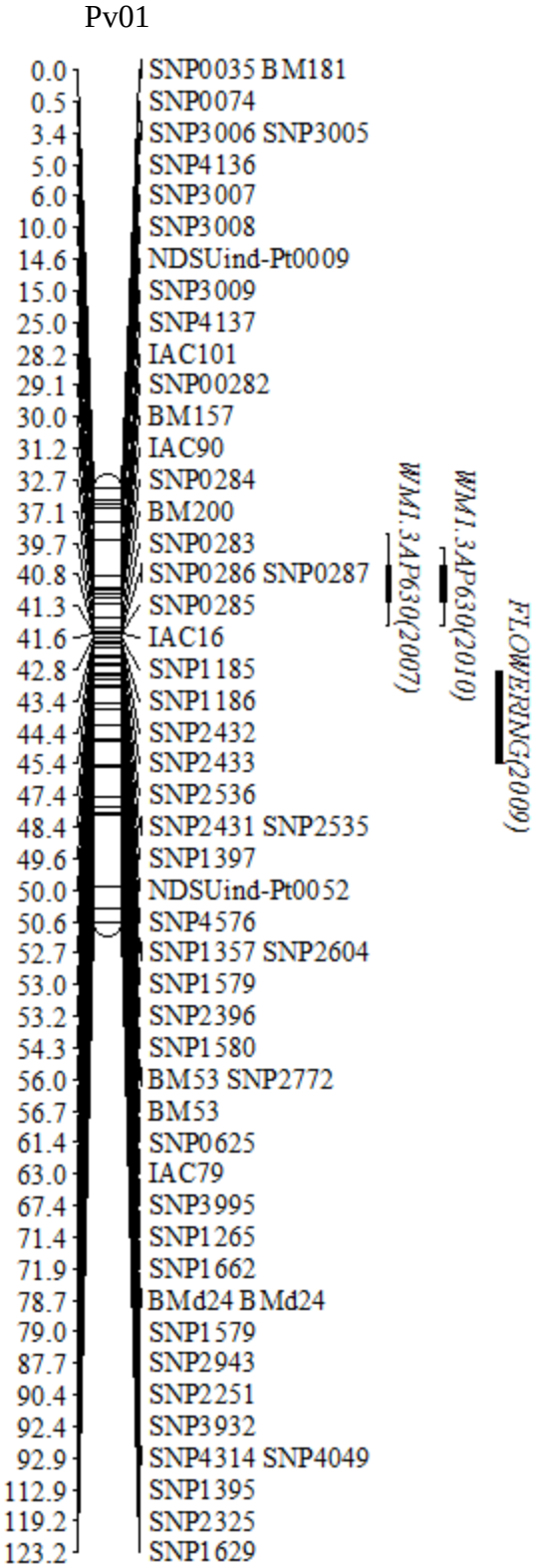
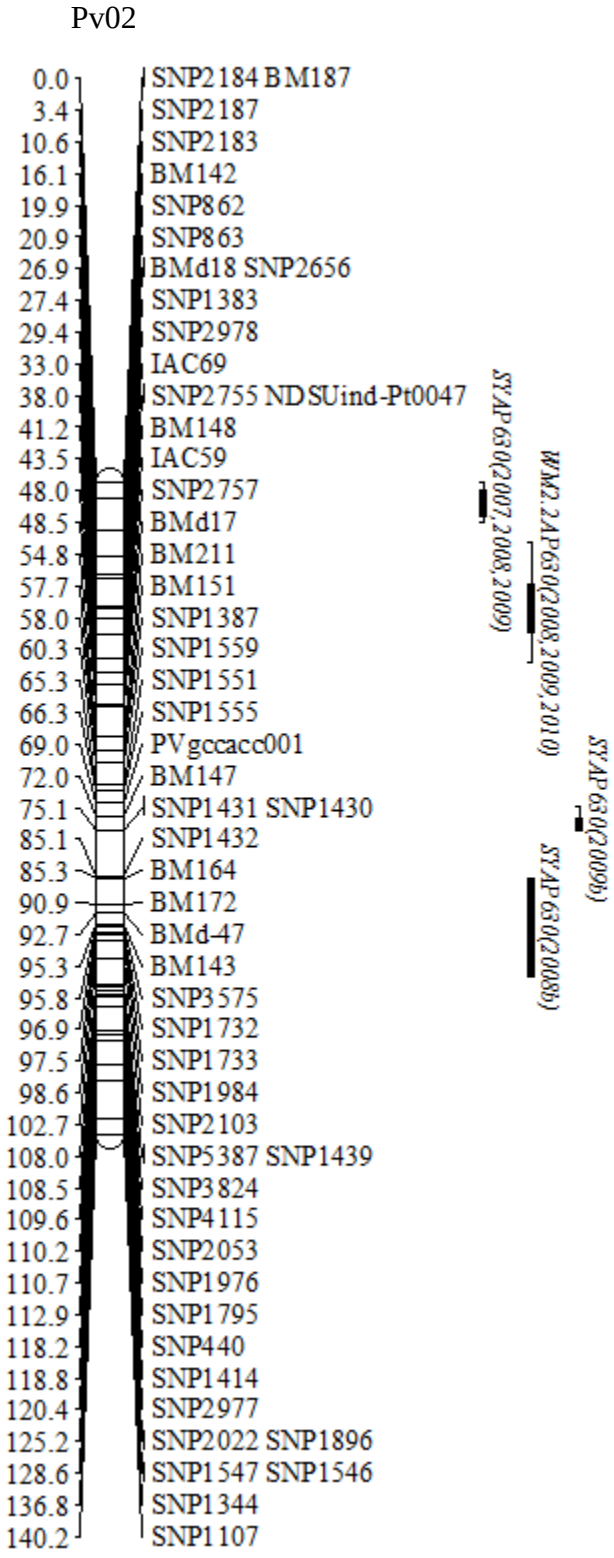


Fig. 3.3: Genetic linkage map showing relative positions of QTL for white mold resistance and agronomic traits evaluated in a pinto bean recombinant inbred line population AP630 grown over four years (2007-2010) at the Montcalm Research Farm, Michigan, USA. SY= Seed Yield, WM= White mold.


Pv02
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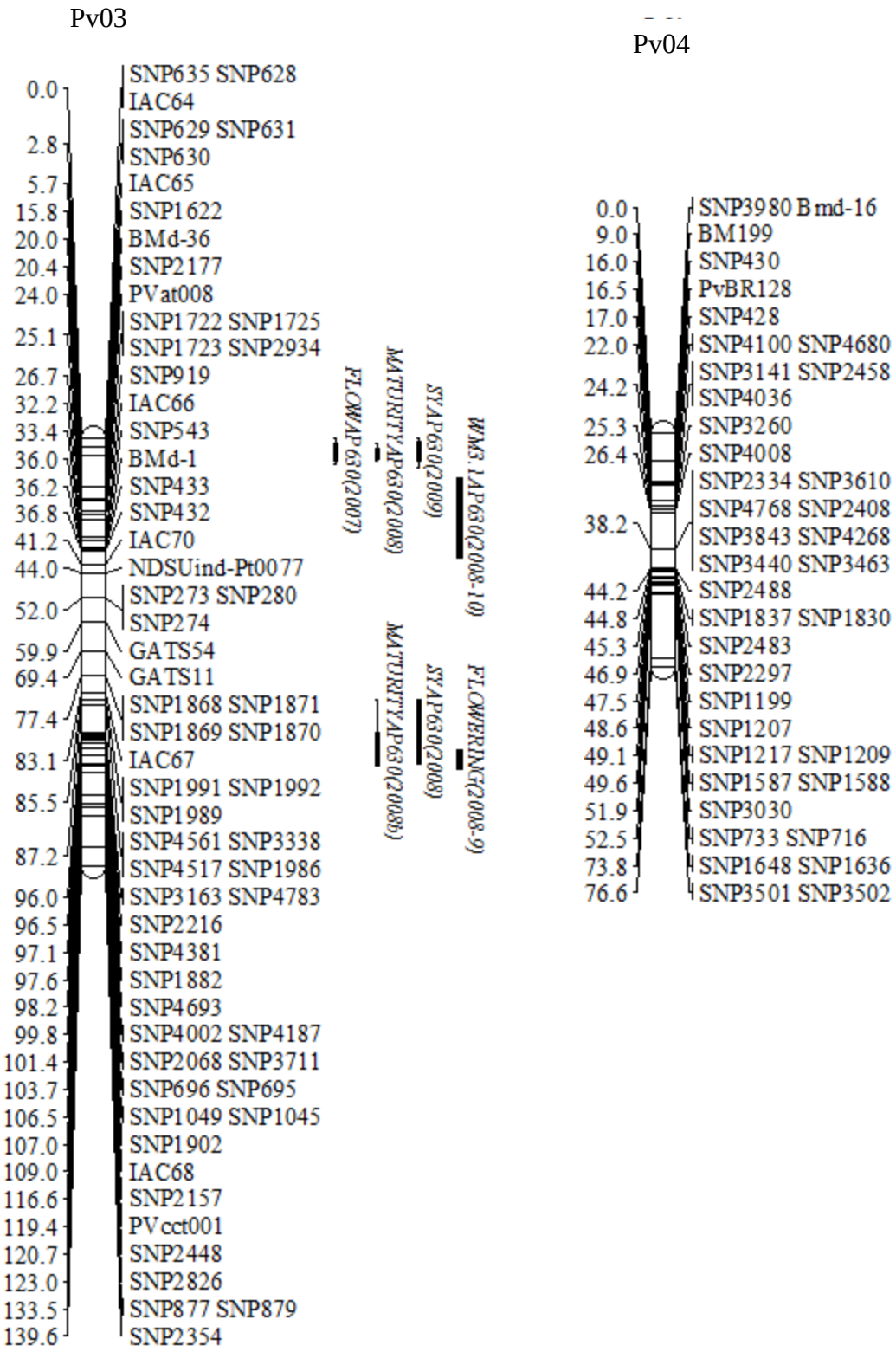
3.4 SNP2187
10.6 SNP2183
16.1 BM142
19.9 SNP862
20.9 SNP863
26.9 BMd18 SNP2656
27.4 SNP1383
29.4 SNP2978
33.0 IAC69
38.0 SNP2755 NDSUind-Pt0047
41.2 BM148
43.5 IAC59
48.0 SNP2757
48.5 BMd17
54.8 BM211
57.7 BM151
58.0 SNP1387
60.3 SNP1559
65.3 SNP1551
66.3 SNP1555
69.0 PVgccacc001
72.0 BM147
75.1 SNP1431 SNP1430
85.1 SNP1432
85.3 BM164
90.9 BM172
92.7 BMd-47
95.3 BM143
95.8 SNP3575
96.9 SNP1732
97.5 SNP1733
98.6 SNP1984
102.7 SNP2103
108.0 SNP5387 SNP1439
108.5 SNP3824
109.6 SNP4115
110.2 SNP2053
110.7 SNP1976
112.9 SNP1795
118.2 SNP440
118.8 SNP1414
120.4 SNP2977
125.2 SNP2022 SNP1896
128.6 SNP1547 SNP1546
136.8 SNP1344
140.2 SNP1107
SYAP630(2007,2008,2009)
WM2.2AP630(2008,2009,2010)
SYAP630(2009)
SYAP630(2008)

Fig. 3.3 (cont'd).

.


Pv03
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IAC64
2.8 SNP629 SNP631
SNP630
5.7 IAC65
15.8 SNP1622
20.0 BMd-36
20.4 SNP2177
24.0 PVat008
25.1 SNP1722 SNP1725
SNP1723 SNP2934
26.7 SNP919
32.2 IAC66
33.4 SNP543
36.0 BMd-1
36.2 SNP433
36.8 SNP432
41.2 IAC70
44.0 NDSUind-Pt0077
52.0 SNP273 SNP280
SNP274
59.9 GATS54
69.4 GATS11
77.4 SNP1868 SNP1871
SNP1869 SNP1870
83.1 IAC67
85.5 SNP1991 SNP1992
SNP1989
87.2 SNP4561 SNP3338
SNP4517 SNP1986
96.0 SNP3163 SNP4783
96.5 SNP2216
97.1 SNP4381
97.6 SNP1882
98.2 SNP4693
99.8 SNP4002 SNP4187
101.4 SNP2068 SNP3711
103.7 SNP696 SNP695
106.5 SNP1049 SNP1045
107.0 SNP1902
109.0 IAC68
116.6 SNP2157
119.4 PVcct001
120.7 SNP2448
123.0 SNP2826
133.5 SNP877 SNP879
139.6 SNP2354
FLOWAP630(2007)
MATURITYAP630(2008)
STAP630(2009)
WMS.1AP630(2008-10)
MATURITYAP630(2008)
STAP630(2008)
FLOWERING(2008-9)
Pv04
0.0 SNP3980 Bmd-16
9.0 BM199
16.0 SNP430
16.5 PvBR128
17.0 SNP428
22.0 SNP4100 SNP4680
SNP3141 SNP2458
24.2 SNP4036
25.3 SNP3260
26.4 SNP4008
SNP2334 SNP3610
SNP4768 SNP2408
38.2 SNP3843 SNP4268
SNP3440 SNP3463
44.2 SNP2488
44.8 SNP1837 SNP1830
45.3 SNP2483
46.9 SNP2297
47.5 SNP1199
48.6 SNP1207
49.1 SNP1217 SNP1209
49.6 SNP1587 SNP1588
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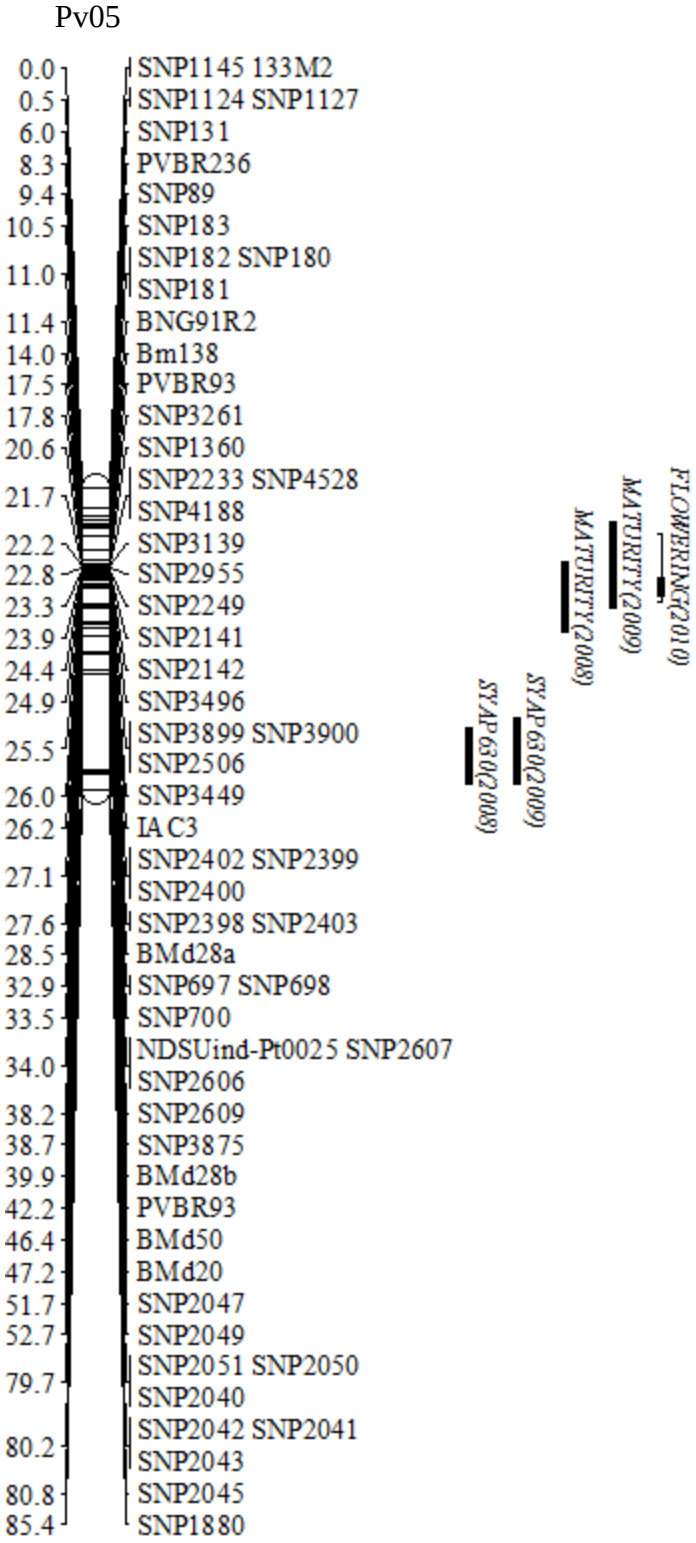
73.8 SNP1648 SNP1636
76.6 SNP3501 SNP3502

Fig. 3.3 (cont'd).


Pv05
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0.5 SNP1124 SNP1127
6.0 SNP131
8.3 PVBR236
9.4 SNP89
10.5 SNP183
11.0 SNP182 SNP180
SNP181
11.4 BNG91R2
14.0 Bm138
17.5 PVBR93
17.8 SNP3261
20.6 SNP1360
21.7 SNP2233 SNP4528
SNP4188
22.2 SNP3139
22.8 SNP2955
23.3 SNP2249
23.9 SNP2141
24.4 SNP2142
24.9 SNP3496
25.5 SNP3899 SNP3900
SNP2506
26.0 SNP3449
26.2 IAC3
27.1 SNP2402 SNP2399
SNP2400
27.6 SNP2398 SNP2403
28.5 BMd28a
32.9 SNP697 SNP698
33.5 SNP700
34.0 NDSUind-Pt0025 SNP2607
SNP2606
38.2 SNP2609
38.7 SNP3875
39.9 BMd28b
42.2 PVBR93
46.4 BMd50
47.2 BMd20
51.7 SNP2047
52.7 SNP2049
79.7 SNP2051 SNP2050
SNP2040
80.2 SNP2042 SNP2041
SNP2043
80.8 SNP2045
85.4 SNP1880
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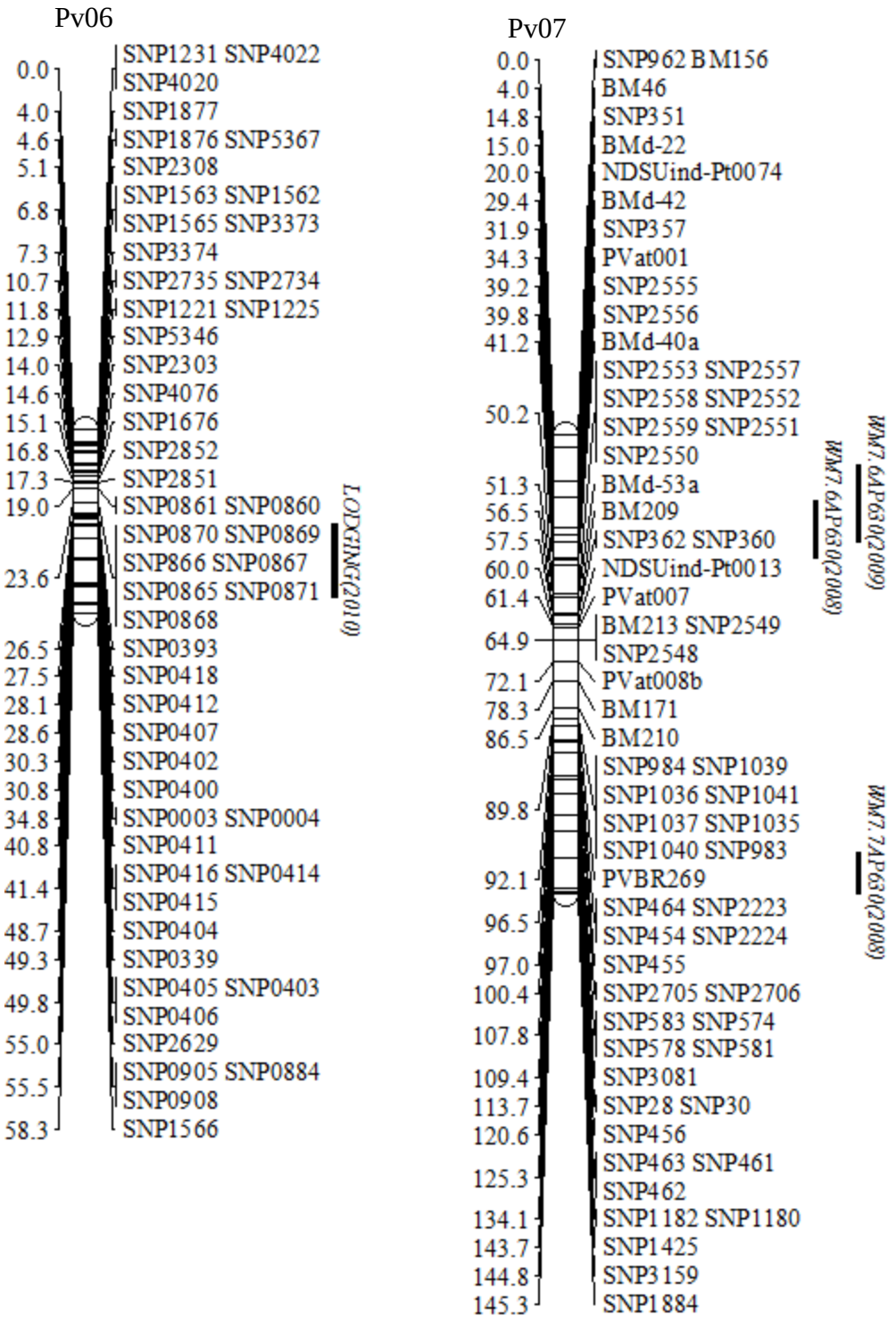
SYAP630(2009)
MATURITY(2008)
MATURITY(2009)
FLOWERING(2010)

Fig. 3.3 (cont'd).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SNP4020
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7.3 SNP3374
10.7 SNP2735 SNP2734
11.8 SNP1221 SNP1225
12.9 SNP5346
14.0 SNP2303
14.6 SNP4076
15.1 SNP1676
16.8 SNP2852
17.3 SNP2851
19.0 SNP0861 SNP0860
SNP0870 SNP0869
SNP866 SNP0867
23.6 SNP0865 SNP0871
SNP0868
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27.5 SNP0418
28.1 SNP0412
28.6 SNP0407
30.3 SNP0402
30.8 SNP0400
34.8 SNP0003 SNP0004
40.8 SNP0411
41.4 SNP0416 SNP0414
SNP0415
48.7 SNP0404
49.3 SNP0339
49.8 SNP0405 SNP0403
SNP0406
55.0 SNP2629
55.5 SNP0905 SNP0884
SNP0908
58.3 SNP1566
LODGING(2010)
Pv07
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4.0 BM46
14.8 SNP351
15.0 BMd-22
20.0 NDSUind-Pt0074
29.4 BMd-42
31.9 SNP357
34.3 PVat001
39.2 SNP2555
39.8 SNP2556
41.2 BMd-40a
SNP2553 SNP2557
SNP2558 SNP2552
50.2 SNP2559 SNP2551
SNP2550
51.3 BMd-53a
56.5 BM209
57.5 SNP362 SNP360
60.0 NDSUind-Pt0013
61.4 PVat007
BM213 SNP2549
64.9 SNP2548
72.1 PVat008b
78.3 BM171
86.5 BM210
SNP984 SNP1039
SNP1036 SNP1041
89.8 SNP1037 SNP1035
SNP1040 SNP983
92.1 PVBR269
SNP464 SNP2223
96.5 SNP454 SNP2224
97.0 SNP455
100.4 SNP2705 SNP2706
SNP583 SNP574
107.8 SNP578 SNP581
109.4 SNP3081
113.7 SNP28 SNP30
120.6 SNP456
SNP463 SNP461
125.3 SNP462
134.1 SNP1182 SNP1180
143.7 SNP1425
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WM7.6AP630(2008)
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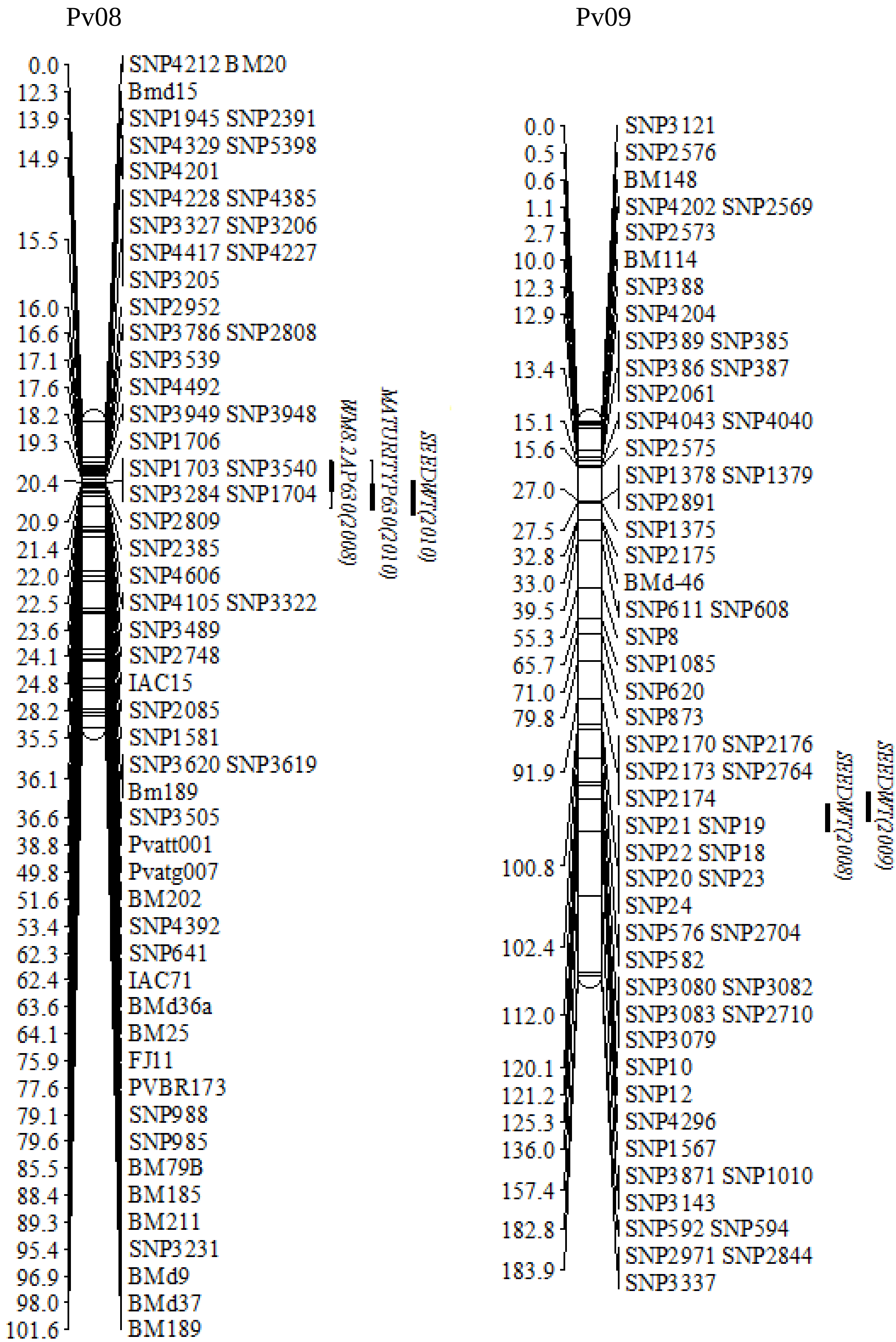
Fig. 3.3 (cont'd).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SNP4201
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SNP4417 SNP4227
SNP3205
16.0 SNP2952
16.6 SNP3786 SNP2808
17.1 SNP3539
17.6 SNP4492
18.2 SNP3949 SNP3948
19.3 SNP1706
20.4 SNP1703 SNP3540
SNP3284 SNP1704
20.9 SNP2809
21.4 SNP2385
22.0 SNP4606
22.5 SNP4105 SNP3322
23.6 SNP3489
24.1 SNP2748
24.8 IAC15
28.2 SNP2085
35.5 SNP1581
36.1 SNP3620 SNP3619
Bm189
36.6 SNP3505
38.8 Pvatt001
49.8 Pvatg007
51.6 BM202
53.4 SNP4392
62.3 SNP641
62.4 IAC71
63.6 BMd36a
64.1 BM25
75.9 FJ11
77.6 PVBR173
79.1 SNP988
79.6 SNP985
85.5 BM79B
88.4 BM185
89.3 BM211
95.4 SNP3231
96.9 BMd9
98.0 BMd37
101.6 BM189
WM8.2AP63(2008)
MATURITYP63(2010)
SEEDWT(2010)
Pv09
0.0 SNP3121
0.5 SNP2576
0.6 BM148
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2.7 SNP2573
10.0 BM114
12.3 SNP388
12.9 SNP4204
SNP389 SNP385
13.4 SNP386 SNP387
SNP2061
15.1 SNP4043 SNP4040
15.6 SNP2575
27.0 SNP1378 SNP1379
SNP2891
27.5 SNP1375
32.8 SNP2175
33.0 BMd46
39.5 SNP611 SNP608
55.3 SNP8
65.7 SNP1085
71.0 SNP620
79.8 SNP873
91.9 SNP2170 SNP2176
SNP2173 SNP2764
SNP2174
SNP21 SNP19
100.8 SNP22 SNP18
SNP20 SNP23
SNP24
102.4 SNP576 SNP2704
SNP582
SNP3080 SNP3082
112.0 SNP3083 SNP2710
SNP3079
120.1 SNP10
121.2 SNP12
125.3 SNP4296
136.0 SNP1567
157.4 SNP3871 SNP1010
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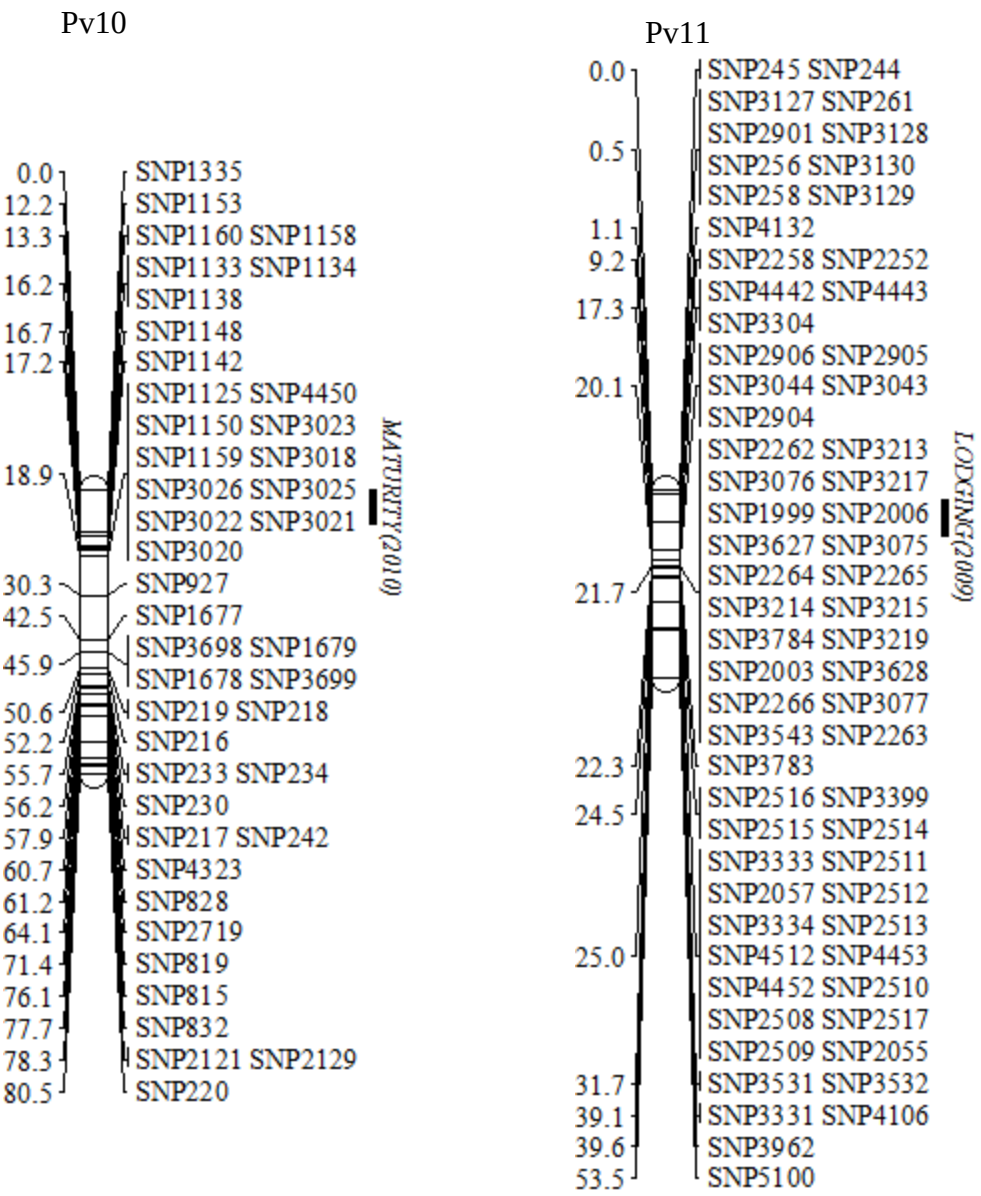
SNP3337
SEEDWT(2008)
SEEDWT(2009)

Fig. 3.3 (cont'd).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SNP1125 SNP4450
SNP1150 SNP3023
SNP1159 SNP3018
18.9 SNP3026 SNP3025
SNP3022 SNP3021
SNP3020
MATURITY2010
30.3 SNP927
42.5 SNP1677
SNP3698 SNP1679
45.9 SNP1678 SNP3699
50.6 SNP219 SNP218
52.2 SNP216
55.7 SNP233 SNP234
56.2 SNP230
57.9 SNP217 SNP242
60.7 SNP4323
61.2 SNP828
64.1 SNP2719
71.4 SNP819
76.1 SNP815
77.7 SNP832
78.3 SNP2121 SNP2129
80.5 SNP220
Pv11
0.0 SNP245 SNP244
SNP3127 SNP261
SNP2901 SNP3128
0.5 SNP256 SNP3130
SNP258 SNP3129
1.1 SNP4132
9.2 SNP2258 SNP2252
SNP4442 SNP4443
17.3 SNP3304
SNP2906 SNP2905
20.1 SNP3044 SNP3043
SNP2904
SNP2262 SNP3213
SNP3076 SNP3217
SNP1999 SNP2006
SNP3627 SNP3075
21.7 SNP2264 SNP2265
SNP3214 SNP3215
SNP3784 SNP3219
SNP2003 SNP3628
SNP2266 SNP3077
SNP3543 SNP2263
22.3 SNP3783
24.5 SNP2516 SNP3399
SNP2515 SNP2514
SNP3333 SNP2511
SNP2057 SNP2512
SNP3334 SNP2513
25.0 SNP4512 SNP4453
SNP4452 SNP2510
SNP2508 SNP2517
SNP2509 SNP2055
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53.5 SNP5100
LODGING2009

Fig. 3.3 (cont'd).

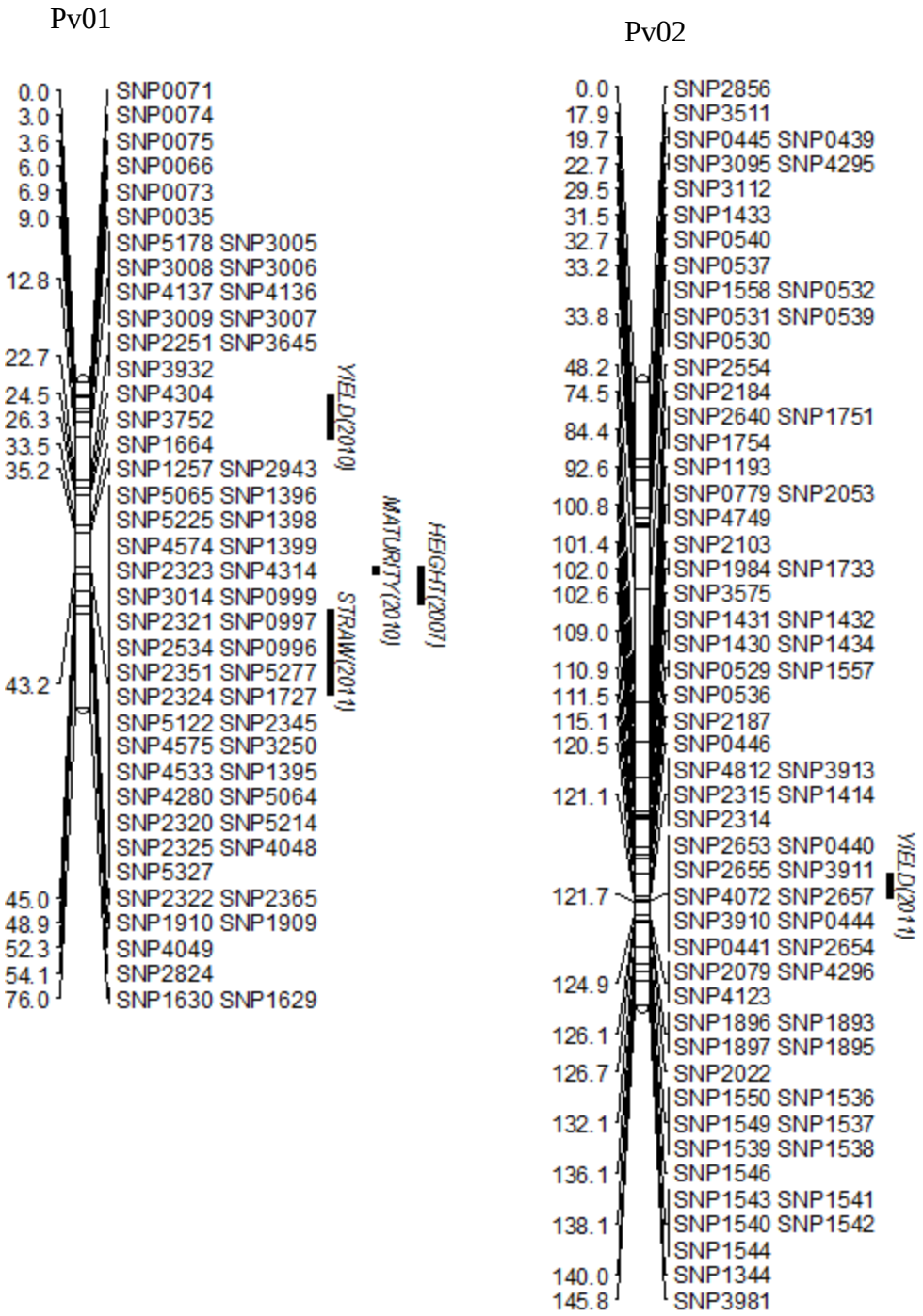
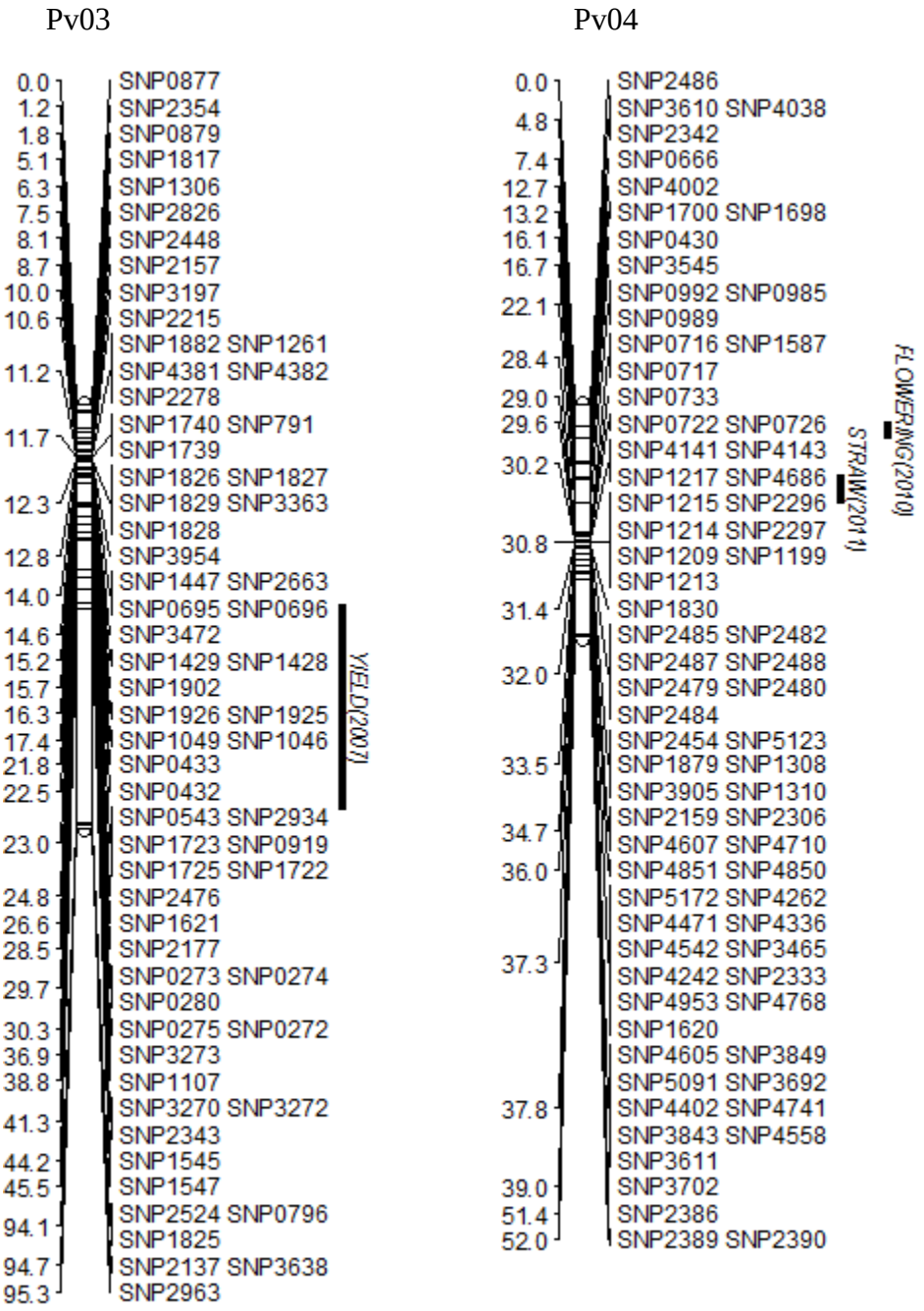


Fig. 3.4: Genetic linkage map showing relative positions of QTL for white mold resistance and agronomic traits evaluated in a pinto bean recombinant inbred line population AP647 grown over three years (2007, 2010, 2011) at the Montcalm Research Farm, Michigan, USA. SY= Seed yield, WM= White mold.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8.1 SNP2448
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10.6 SNP2215
SNP1882 SNP1261
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SNP2278
SNP1740 SNP791
11.7 SNP1739
SNP1826 SNP1827
12.3 SNP1829 SNP3363
SNP1828
12.8 SNP3954
SNP1447 SNP2663
14.0 SNP0695 SNP0696
14.6 SNP3472
15.2 SNP1429 SNP1428
15.7 SNP1902
16.3 SNP1926 SNP1925
17.4 SNP1049 SNP1046
21.8 SNP0433
22.5 SNP0432
SNP0543 SNP2934
23.0 SNP1723 SNP0919
SNP1725 SNP1722
24.8 SNP2476
26.6 SNP1621
28.5 SNP2177
SNP0273 SNP0274
29.7 SNP0280
30.3 SNP0275 SNP0272
36.9 SNP3273
38.8 SNP1107
SNP3270 SNP3272
41.3 SNP2343
44.2 SNP1545
45.5 SNP1547
SNP2524 SNP0796
94.1 SNP1825
94.7 SNP2137 SNP3638
95.3 SNP2963
YIELD(2007)
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SNP3610 SNP4038
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7.4 SNP0666
12.7 SNP4002
13.2 SNP1700 SNP1698
16.1 SNP0430
16.7 SNP3545
SNP0992 SNP0985
22.1 SNP0989
SNP0716 SNP1587
28.4 SNP0717
29.0 SNP0733
29.6 SNP0722 SNP0726
SNP4141 SNP4143
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SNP1215 SNP2296
SNP1214 SNP2297
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SNP1213
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SNP2485 SNP2482
SNP2487 SNP2488
32.0 SNP2479 SNP2480
SNP2484
SNP2454 SNP5123
33.5 SNP1879 SNP1308
SNP3905 SNP1310
SNP2159 SNP2306
34.7 SNP4607 SNP4710
36.0 SNP4851 SNP4850
SNP5172 SNP4262
SNP4471 SNP4336
SNP4542 SNP3465
37.3 SNP4242 SNP2333
SNP4953 SNP4768
SNP1620
SNP4605 SNP3849
SNP5091 SNP3692
37.8 SNP4402 SNP4741
SNP3843 SNP4558
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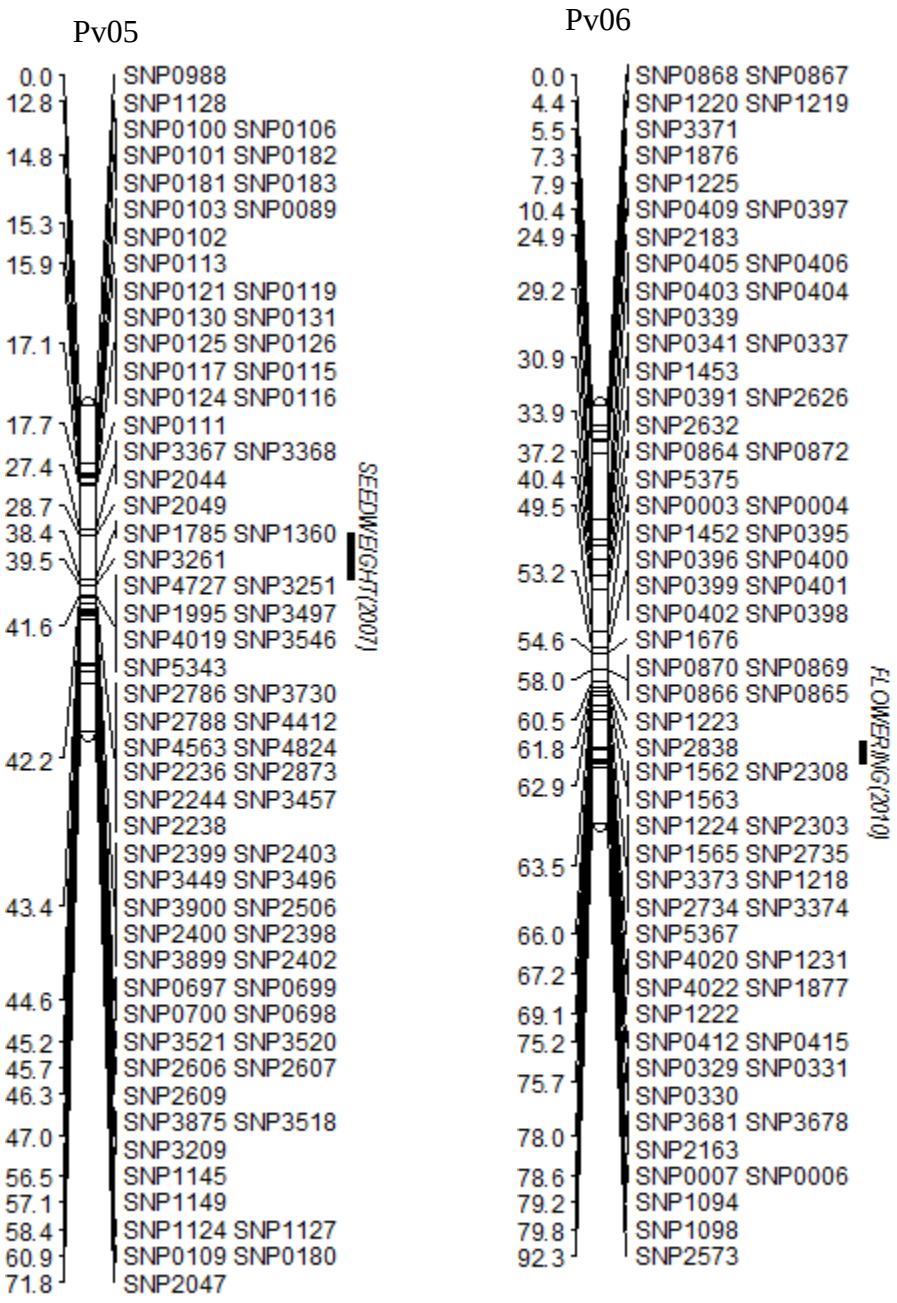
STRAW(2014)
FLOWER(2010)

Fig. 3.4 (cont'd)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SNP0121 SNP0119
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SNP0117 SNP0115
SNP0124 SNP0116
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SNP3367 SNP3368
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SNP5343
SNP2786 SNP3730
SNP2788 SNP4412
SNP4563 SNP4824
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SNP3449 SNP3496
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SNP2400 SNP2398
SNP3899 SNP2402
SNP0697 SNP0699
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SNP3875 SNP3518
47.0 SNP3209
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57.1 SNP1149
58.4 SNP1124 SNP1127
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7.3 SNP1876
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10.4 SNP0409 SNP0397
24.9 SNP2183
SNP0405 SNP0406
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SNP0339
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30.9 SNP1453
SNP0391 SNP2626
33.9 SNP2632
37.2 SNP0864 SNP0872
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SNP1452 SNP0395
SNP0396 SNP0400
53.2 SNP0399 SNP0401
SNP0402 SNP0398
54.6 SNP1676
58.0 SNP0870 SNP0869
SNP0866 SNP0865
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SNP1224 SNP2303
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SNP4022 SNP1877
69.1 SNP1222
75.2 SNP0412 SNP0415
SNP0329 SNP0331
75.7 SNP0330
SNP3681 SNP3678
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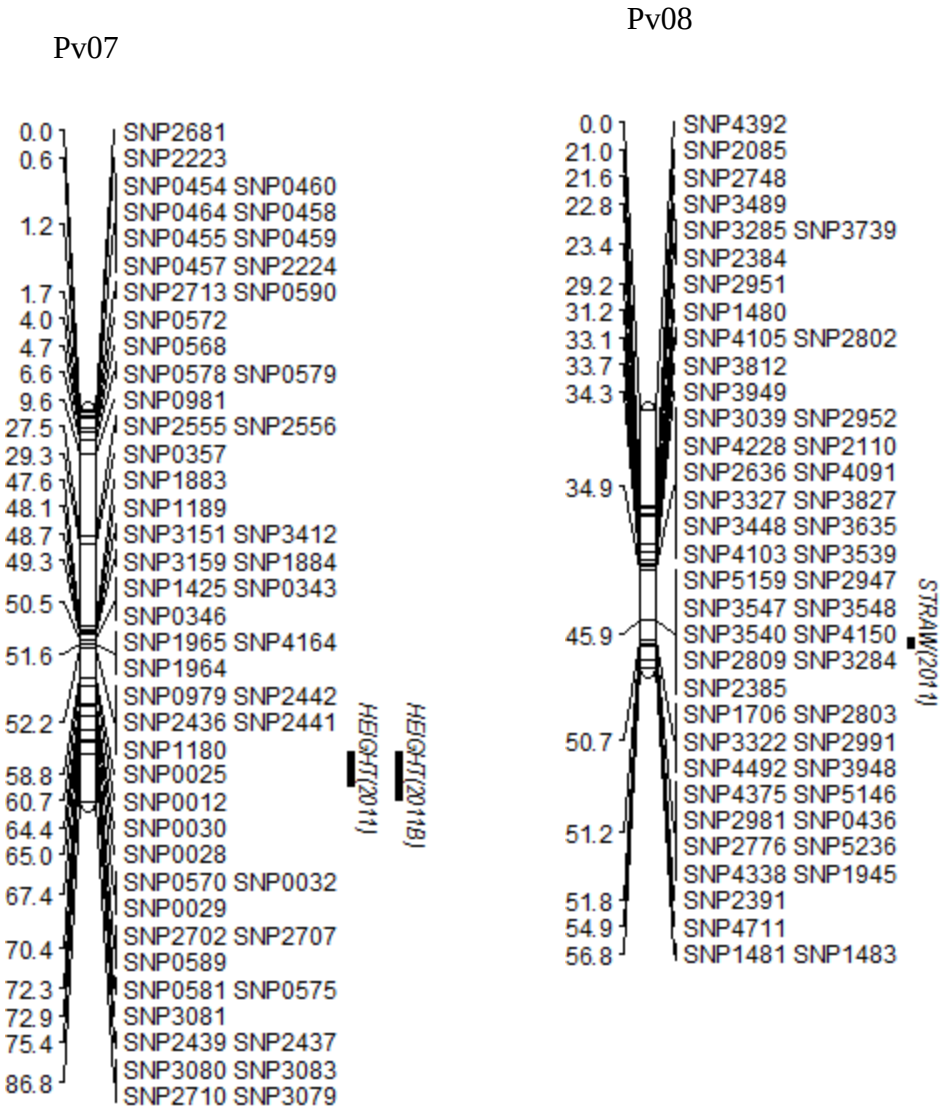
FLOWERWG(2010)

Fig. 3.4 (cont'd).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SNP0464 SNP0458
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4.0 SNP0572
4.7 SNP0568
6.6 SNP0578 SNP0579
9.6 SNP0981
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29.3 SNP0357
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48.7 SNP3151 SNP3412
49.3 SNP3159 SNP1884
SNP1425 SNP0343
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SNP1965 SNP4164
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SNP0979 SNP2442
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SNP1180
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SNP3080 SNP3083
86.8 SNP2710 SNP3079
HEIGHT(2011)
HEIGHT(2011B)
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SNP3285 SNP3739
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31.2 SNP1480
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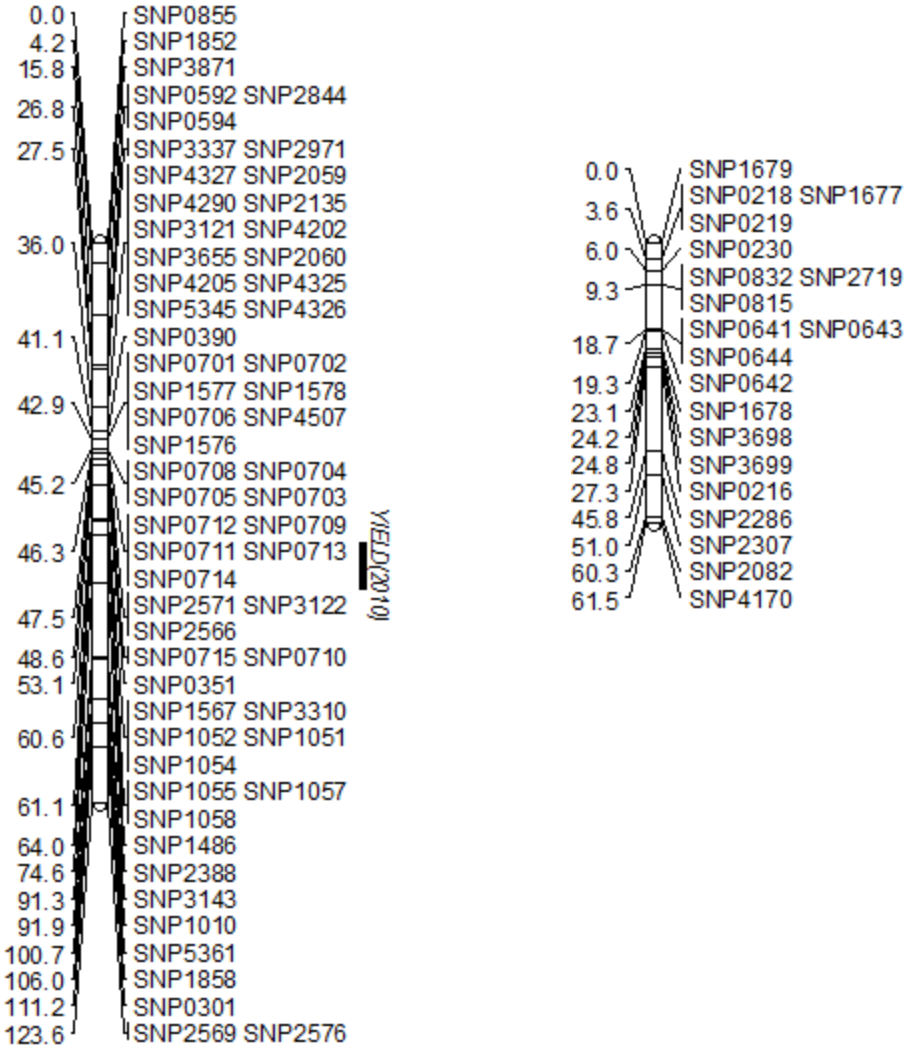
STRAW(2011)

Fig. 3.4 (cont'd).


Pv09
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SNP0594
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SNP4327 SNP2059
SNP4290 SNP2135
SNP3121 SNP4202
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SNP4205 SNP4325
SNP5345 SNP4326
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SNP1577 SNP1578
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SNP0708 SNP0704
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SNP2571 SNP3122
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SNP0219
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SNP0815
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SNP0644
19.3 SNP0642
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24.8 SNP3699
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61.5 SNP4170

Fig. 3.4 (cont'd).

Pv11

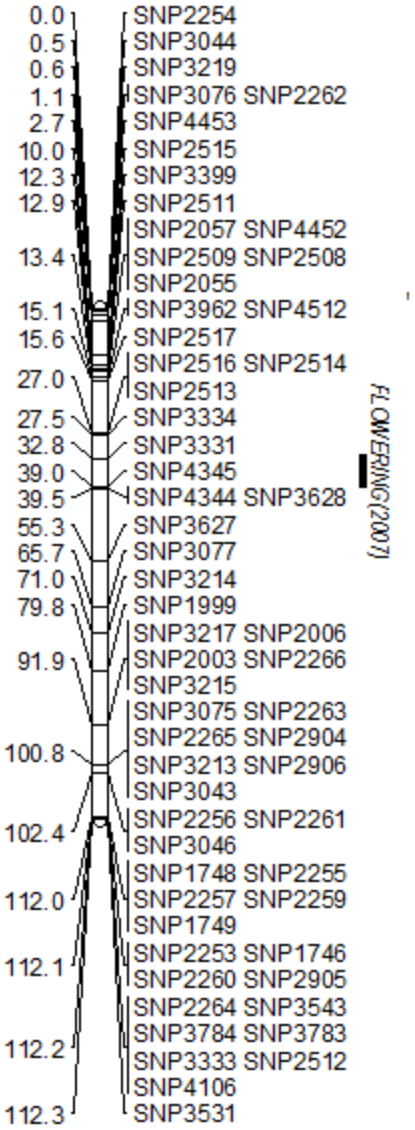

Fig. 3.4 (cont'd).

QTL Analysis in AP630 population

Eight traits namely yield, white mold disease incidence, 100 seed weight, days to flowering, days to maturity, plant height, lodging, and greenhouse disease severity (straw test) were analyzed through the multiple QTL model. Thirty six QTL were identified in different years for seven traits (white mold disease incidence, seed yield, days to flowering, days to maturity, 100 seed weight, lodging and the straw test) and these QTL were located on 10 linkage groups. LOD scores ranged from the threshold of 2.5 to 10.47 (Table 3.4, Fig. 3.3).

Table 3.4a: QTL for white mold resistance identified in field and greenhouse environments in pinto bean RIL population AP630. evaluated in Michigan during 2007-2011

Trait Name	**Year**	**QTL Name**	**Chromosome**	**Indicative Marker[1]**	**LOD[2]**	**R^2(%)[3]**	**Add[4]**
Disease Incidence	2007	WM1.3^{AP630}	Pv01	IAC101	3.31	33.0	14.83
	2010	WM1.3^{AP630}	Pv01	BM157	2.52	19.9	1.46
	2008	WM3.1^{AN,AP630}	Pv03	IAC66	3.42	22.8	-1.76
	2010	WM3.1^{AN,AP630}	Pv03	Pvat008	2.79	27.5	-1.48
	2008	WM8.5^{AP630}	Pv08	Bmd-15	3.47	25.1	-1.50
Straw Test	2008	WM2.2$^{BN..AP630*}$	Pv02	IAC69	4.03	22.9	-1.57
	2007	WM7.7^{AP630}	Pv07	BARCBEAN6K_3-SNP1180	3.56	22.3	-0.57
	2009	WM2.2$^{BN..AP630*}$	Pv02	IAC69	2.79	33.7	-1.71
	2010	WM2.2$^{BN..AP630*}$	Pv02	IAC69	8.18	41.5	-3.04
	2008	WM7.6^{AP630}	Pv07	BMd40	7.13	40.2	-3.00
	2009	WM7.6^{AP630}	Pv07	NDSU_IND_7_34.5855	4.41	41.9	3.02

Table 3.4a (cont'd).

Trait Name	Year	QTL Name	Chromosome	Indicative Marker[1]	LOD[2]	R^2(%)[3]	Add[4]
Seed yield	2007	$SY2.2^{AP630}$	Pv02	BM142	3.37	11.6	-1.70
	2008	$SY2.2^{AP630}$	Pv02	BM142	3.90	26.0	-3.81
	2009	$SY2.2^{AP630}$	Pv02	BM142	3.25	30.0	-4.30
	2008	$SY5.4^{AP630}$	Pv05	NDSU_IND_5_28.0255	2.77	32.3	-14.82
	2009	$SY5.4^{AP630}$	Pv05	Bmd-28b	3.24	30.4	14.53

* $WM2.2^{BN,HN,BR,AN,R31,BV,AP630}$

[1]Primer information for SSR markers online at: http://www.css.msu.edu/bic/PDF/Bean_SSR_Primers_2007.pdf. InDels available at www.beancap.org/Indel-Markers-bean-NDSU.xls and SNPs available online www.beancap.org.

[2]LOD: Log of odds.

[3]Proportion of the phenotypic variance explained by the QTL at peak LOD using MQM mapping with cofactor selection.

[4]Effect of substituting a single allele from one parent to another. Positive values indicate allele from AN-37 and negative from P02630.

Table 3.4b: QTL for agronomic and phenological traits identified in field and greenhouse environments in pinto bean RIL population AP630 evaluated in Michigan during 2007-2011.

Trait Name	Year	Chromosome	Indicative Marker[1]	LOD[2]	R^2 (%)[3]	Add[4]
Days to flowering	2007	Pv03	IAC64	2.58	19.7	-5.07
	2008	Pv03	BARCBEAN6K_3-SNP0695	3.99	9.4	1.01
	2009	Pv01	BARCBEAN6K_3-SNP2604	3.28	10.8	-0.62
	2009	Pv03	BARCBEAN6K_3-SNP0695	5.13	20.4	0.75
	2010	Pv05	BNG91R2	3.75	12.4	1.26
Days to maturity	2008	Pv03	BMd-36	5.1	23.5	-3.92
	2008	Pv05	BNG91R2	2.9	21.3	-3.96
	2009	Pv05	PVBR93	2.9	15.5	-3.99
	2010	Pv08	IAC15	5.6	31.7	-1.69
	2008	Pv03	BARCBEAN6K_3-SNP0695	10.5	40.0	0.96
	2008	Pv07	BARCBEAN6K_3-SNP0455	7.0	21.5	-0.72
	2008	Pv10	BARCBEAN6K_3-SNP1335	5.7	19.6	0.72
	2009	Pv05	BARCBEAN6K_3-SNP3261	2.8	15.0	1.12
	2010	Pv03	BARCBEAN6K_3-SNP2448	5.1	23.7	1.08
	2010	Pv10	BARCBEAN6K_3-SNP1153	5.7	19.6	0.72
Seed weight	2008	Pv09	BARCBEAN6K_3-SNP1701	6.5	27.6	-1.50
	2010	Pv08	BARCBEAN6K_3-SNP1706	7.5	25.3	-2.00
	2010	Pv09	BARCBEAN6K_3-SNP0012	3.7	15.1	-1.19
Lodging	2008	Pv06	BARCBEAN6K_3-SNP0860	2.7	14.0	-0.15
	2009	Pv11	BARCBEAN6K_3-SNP3962	3.6	67.6	-0.51

[1]Primer information for SSR markers online at: http://www.css.msu.edu/bic/PDF/Bean_SSR_Primers_2007.pdf. InDels available at www.beancap.org/Indel-Markers-bean-NDSU.xls and SNPs available online www.beancap.org

[2]LOD: Log of odds. [3]Proportion of the phenotypic variance explained by the QTL at peak LOD using MQM mapping with cofactor selection.

[4]Effect of substituting a single allele from one parent to another. Positive values indicate allele from AN-37 and negative from P02630

Yield (SY)

Seven QTL associated with yield were identified on chromosomes Pv02 and Pv05 though composite interval mapping. In 2007 a QTL in a 16 cM interval between BM142 and Bm187 on Pv02 that accounted for 11.6% of the observed variation associated with alleles from P02630 had a LOD score of 3.37. In 2008 a QTL in the same 16 cM interval between BM142 and Bm187 on Pv02 that accounted for 26% of the observed variation associated with alleles from P02630 had a of LOD score of 3.9. In 2009 a QTL in the same 16 cM interval between BM142 and Bm187 on Pv02 that accounted for 30% of the observed variation associated with alleles from P02630 had a LOD score of 3.25. In 2008 a QTL in a 12 cM interval between BARCBEAN6K_3-SNP1431 and BARCBEAN6K_3-SNP2054 towards the distal end of Pv02 accounted for 16% of the observed variation associated with alleles from AN-37 and had a LOD score of 3.2. In 2009 a QTL in the interval between BARCBEAN6K_3-SNP1893 and BARCBEAN6K_3-SNP1107 on Pv02 that accounted for 13.5% of the observed variation associated with alleles from P02630 had a LOD score of 2.9. In 2008 a QTL in a 10 cM interval between NDSU_IND_5_28.0255 and BMd28b on Pv05 that accounted for 32% of the observed variation associated with alleles from AN-37 had a LOD score of 2.77. In 2009 a QTL in the same 10 cM interval between NDSU_IND_5_28.0255 and BMd28b on Pv05 that accounted for 30% of the observed variation associated with alleles from P02630 had a LOD score of 3.24 (Table 3.4, Fig. 3.3).

In addition, QTL for yield were also identified on Pv03, Pv08 and Pv11 using single factor analysis. In 2009 markers associated with yield on Pv03 spanned from 87-106 cM (BARCBEAN6K_3-SNP1986-BARCBEAN6K_3-SNP1048) having LOD scores ranging from 2.5-3.4 and accounting for 12-18% of the phenotypic variation with alleles from P02630. In 2009 QTL associated with yield on Pv08 spanned from 14-28 cM (BARCBEAN6K_3-SNP1107-

BARCBEAN6K_3-SNP2085) having LOD score of 2.5 and accounting for 11.4% of the phenotypic variation with alleles from P02630. In 2008 QTL associated with yield on Pv08 at 62 cM (BARCBEAN6K_3-SNP0642-BARCBEAN6K_3-SNP0644 region) had a LOD score of 3.3 and accounted for 17% of the phenotypic variation with alleles from P02630. In 2007 a QTL associated with yield on Pv11 at 21 cM (BARCBEAN6K_3-SNP3543) had a LOD score of 2.5 and accounted for 13% of the phenotypic variation with alleles from AN-37 (Appendix Single Marker Analysis)

White Mold Disease Incidence (WM)

Five QTL on chromosomes Pv01, Pv03 and Pv08 were associated with disease incidence in the field. In 2007 a QTL in a 10 cM interval between NDSU_IND_1_16.1931 and IAC101 on Pv01 that accounted for 33% of the observed variation associated with alleles from AN-37 had a LOD of score 3.31. In 2010 a QTL in a 20 cM interval between NDSU_IND_3_50.0189 and IAC67 on Pv03 that accounted for 22% of the observed variation associated with alleles from AN-37 had a LOD score of 3.4. In 2009 a QTL in the same 20 cM interval between NDSU_IND_3_50.0189 and IAC67 on Pv03 that accounted for 28% of the observed variation associated with alleles from AN-37 had a LOD score of 2.8. In 2010 a QTL in the same 20 cM interval between NDSU_IND_3_50.0189 and IAC67 on Pv03 that accounted for 19% of the observed variation associated with alleles from AN-37 had a LOD score of 2.9. In 2010 a QTL in a 12 cM interval between BMd15 and IAC15 on Pv03 that accounted for 25% of the observed variation associated with alleles from P02630 had a LOD score of 3.5 (Table 3.4, Fig.3.3).

Using single marker analysis, QTL for white mold disease incidence were also identified on Pv02 and Pv03. QTL associated with disease incidence in 2008 spanned a 85-122 cM region

(BARCBEAN6K_3-SNP1431-BARCBEAN6K_3-SNP3575) on Pv02 with a LOD score of approximately 3.0 that accounted for 13% of the phenotypic variation from P02630 alleles. QTL at 36, 99-107 and 122 cM on Pv03 were associated with disease incidence in 2010 (LOD 2.5-3.3) that explained 12.6-17.9% of the observed variation and the alleles originated from P02630 (Appendix Single Marker Analysis).

Straw Test (WM)

Six QTL associated with greenhouse straw test were identified on chromosomes Pv02 and Pv07 though composite interval mapping. In 2008 a QTL in a 10 cM interval between BM14 and IAC69 on Pv02 that accounted for 23% of the observed variation associated with alleles from P02630 had a LOD score of 4.0. In 2009 a QTL in the same 10 cM interval between BM14 and IAC69 on Pv02 that accounted for 34% of the observed variation associated with alleles from P02630 had a LOD score of 2.8. In 2010 a QTL in the same 10 cM interval between BM14 and IAC69 on Pv02 that accounted for 42% of the observed variation associated with alleles from P02630 had a LOD score of 8.8. In 2007 a QTL in a 10 cM interval between BARCBEAN6K_3-SNP1180 and BARCBEAN6K_3-SNP3159 on Pv07 that accounted for 23% of the observed variation associated with alleles from P02630 had a LOD score of 4.0. In 2008 a QTL in a 15 cM interval between BMd40 and NDSU_IND_7_34.5855 on Pv07 that accounted for 42% of the observed variation associated with alleles from AN-37 had a LOD score of 4.0. In 2009 a QTL in a 13 cM interval between BM46 and NDSU_IND_7_34.5855 on Pv07 that accounted for 40% of the observed variation associated with alleles from P02630 had a LOD score of 7.0 (Table 3.4, Fig. 3.3).

Days to Flowering

Five QTL associated with the number of days to flowering were identified on chromosome Pv01 Pv03 and Pv05 though composite interval mapping. In 2009 a QTL in a 16 cM interval between BARCBEAN6K_3-SNP2604 and BARCBEAN6K_3-SNP1579 on Pv01 that accounted for 11% of the observed variation associated with alleles from P02630 had a LOD score of 3.28. In 2007 a QTL in a 5 cM interval between BARCBEAN6K_3-SNP0695 and BARCBEAN6K_3-SNP1049 on Pv03 that accounted for 9% of the observed variation associated with alleles from AN-37 had a LOD score of 4.0. In 2009 a QTL in a 15 cM interval between BARCBEAN6K_3-SNP0695 and BARCBEAN6K_3-SNP1049 on Pv03 that accounted for 20% of the observed variation associated with alleles from AN-37 had a LOD score of 5.0. In 2010 a QTL in a 15 cM interval between BNG91R2 and BARCBEAN6K_3-SNP2249 on Pv05 that accounted for 12% of the observed variation associated with alleles from AN-37 had a LOD score of 3.8 (Table 3.4 and Fig. 3.3).

Days to maturity

Ten QTL associated with the number of days to maturity were identified on chromosomes Pv03, Pv05, Pv07, Pv08 and Pv10 using composite interval mapping. In 2008 a QTL in a 15 cM interval between BMd-36 and IAC65 on Pv03 that accounted for 23.5% of the observed variation associated with alleles from P02630 had a LOD score of 5.1. In 2008 a QTL in a 6 cM interval between BARCBEAN6K_3-SNP0695 and BARCBEAN6K_3-SNP1049 on Pv03 that accounted for 40% of the observed variation associated with alleles from AN-37 had a LOD score of 10.5. In 2010 a QTL in an 8 cM interval between BARCBEAN6K_3-SNP2448 and BARCBEAN6K_3-SNP2826 on Pv03 that accounted for 24% of the observed variation

associated with alleles from AN-37 had a LOD score of 5.1. In 2008, 2009 and 2010 markers BNG91R2, PVBR93 and BARCBEAN6K_3-SNP3261 on Pv05 associated with days to maturity explained 15-21% of total variation observed (LOD 2.75-2.93) and the contributing alleles originated from both parents. In 2008 a QTL in a 3 cM interval between BARCBEAN6K_3-SNP0455 and BARCBEAN6K_3-SNP2705 on Pv07 that accounted for 21.5% of the observed variation associated with alleles from P02630 had a LOD score of 6.9. In 2010 a QTL in a 12 cM interval between IAC15 and BMd-15 on Pv08 that accounted for 32% of the observed variation associated with alleles from P02630 and a LOD score of 5.6. A QTL explaining 19.6% of the total variation in 2008 spanned a 12cM region between BARCBEAN6K_3-SNP1153 and BARCBEAN6K_3-SNP1335 markers on Pv10 with a LOD score of 5.7 and associated with alleles from AN-37 (Table 3.4 and Fig. 3.3).

Seed weight

Three QTL associated with 100 seed weight were identified on chromosomes Pv08 and Pv09 through composite interval mapping. A QTL explaining 25% of the total variation in 2008 spanned a 20 cM region between BARCBEAN6K_3-SNP4296 and BARCBEAN6K_3-SNP1567 markers on Pv09 with a LOD score of 6.0 and associated with alleles from P02630. In 2010 a QTL in a 10 cM interval between BARCBEAN6K_3-SNP1701 and BARCBEAN6K_3-SNP1703 on Pv08 that accounted for 28% of the observed variation associated with alleles from P02630 had a LOD score of 6.5. In 2010 a QTL in a 9 cM interval between BARCBEAN6K_3-SNP0012 and BARCBEAN6K_3-SNP4296 on Pv09 that accounted for 15% of the observed variation associated with alleles from P02630 had a LOD score of 3.71 (Table 3.4 and Fig. 3.3).

Lodging

Two QTL were identified on chromosome Pv06 and Pv11. In 2008 a QTL in a 23 cM interval between BARCBEAN6K_3-SNP 860 and BARCBEAN6K_3-SNP870 on Pv06 that accounted for 14% of the observed variation associated with alleles from P02630 had a LOD score of 2.67. In 2009 a QTL in a 48 cM in the interval between BARCBEAN6K_3-SNP3962 and BARCBEAN6K_3-SNP5100 on Pv11 that accounted for 68% of the observed variation associated with alleles from P02630 and had a LOD score of 3.57 (Table 3.4 and Fig. 3.3).

QTL Analysis in AP647 half-sib population

A total of 1016 SNP and 34 SSR markers were segregating in the AP647 population. Eight traits namely yield, white mold disease incidence, 100 seed weight, days to flowering, days to maturity, plant canopy height, lodging and greenhouse straw test were analyzed through single marker analysis and interval mapping (IM). Fourteen QTL spread across 10 linkage groups were identified in different years associated with seed yield, the greenhouse straw test, 100 seed weight, and number of days to maturity, number of days to flowering and plant canopy height. LOD scores ranged from 2.5 to 6.9 for these traits (Table 3.5, Figure 3.5)

Table 3.5: QTL for white mold resistance and agronomic traits identified in field and greenhouse environments in pinto bean RIL population AP647 evaluated in Michigan during 2007, 2010 and 2011

Trait Name	Year	QTL Name	Chromosome	Indicative Marker[1]	LOD[2]	R^2(%)[3]	Add[4]
Seed yield	2007	$SY3.4^{AP647}$	Pv03	BARCBEAN6K_3-SNP1547	3.0	45.1	3.0
	2010	$SY1.1^{AP647}$	Pv01	BARCBEAN6K_3-SNP0035	3.2	50.9	4.6
	2010	$SY9.3^{AP647}$	Pv09	BARCBEAN6K_3-SNP1486	2.6	40.7	4.2
	2011	$SY2.4^{AP647}$	Pv02	BARCBEAN6K_3-SNP2187	2.6	18.4	-1.2
Straw test	2011	$WM1.4^{AP647}$	Pv01	BARCBEAN6K_3-SNP2824	4.0	35.8	2.3
	2011	$WM4.3^{AP647}$	Pv04	BARCBEAN6K_3-SNP3545	3.4	42.9	2.5
	2011	$WM8.6^{AP67}$	Pv08	BARCBEAN6K_3-SNP3948	2.6	15.0	0.8
Seed weight	2007		Pv05	BARCBEAN6K_3-SNP2049	2.9	21.7	1.4
Days to maturity	2010		Pv01	BARCBEAN6K_3-SNP5327	2.7	15.9	-0.6
Canopy Height	2007		Pv01	BARCBEAN6K_3-SNP1909	6.2	58.0	5.2
	2011		Pv07	BARCBEAN6K_3-SNP2437	5.3	48.8	-5.2
Days to flowering	2007		Pv11	BARCBEAN6K_3-SNP3331	7.0	42.2	-2.9
	2010		Pv04	BARCBEAN6K_3-SNP2342	3.6	24.2	-0.5
	2010		Pv06	BARCBEAN6K_3-SNP0006	3.6	19.3	-0.5

[1]Primer information for SSR markers online at: http://www.css.msu.edu/bic/PDF/Bean_SSR_Primers_2007.pdf. InDels available at www.beancap.org/Indel-Markers-bean-NDSU.xls and SNPs available online www.beancap.org

[2]LOD: Log of odds.

[3]Proportion of the phenotypic variance explained by the QTL at peak LOD using MQM mapping with cofactor selection.

[4]Effect of substituting a single allele from one parent to another. Positive values indicate allele from AN-37 and negative from P02647

Seed yield

Four QTL associated with seed yield were identified on chromosomes Pv01, Pv02 Pv03 and Pv09 through composite interval mapping. A QTL in marker interval BARCBEAN6K_3-SNP0035 and BARCBEAN6K_3-SNP5178 at 3 cM on Pv01 had a LOD score of 3.20 that accounted for 60.9% of the observed variation associated with alleles from AN-37in 2010. In 2011 a QTL in marker interval BARCBEAN6K_3-SNP2187 and BARCBEAN6K_3-SNP0446 at 116 cM on Pv02 had a LOD score of 2.64 that accounted for18.4% of the observed variation associated with alleles from P02647. In 2007 the QTL in marker interval BARCBEAN6K_3-SNP1547 and BARCBEAN6K_3-SNP2524 at position 74 cM on Pv03 had a LOD score of 2.99 that accounted for 75.1% of the observed variation associated with alleles from AN-37. A QTL in marker interval BARCBEAN6K_3-SNP1486 and BARCBEAN6K_3-SNP2388 at 66 cM on Pv09 had a LOD score of 2.57 that accounted for 40.7% of the observed variation associated with alleles from AN-37 in 2010 (Table 3.5, Figure 3.4)

Straw Test

Three QTL associated with greenhouse straw test were identified on chromosome Pv01 Pv04 and Pv08 though composite interval mapping in 2011. A QTL in marker interval BARCBEAN6K_3-SNP2824 and BARCBEAN6K_3-SNP1630 at position 19 cM on Pv01 had a LOD score of 4.01 that accounted for 35.8% of the observed variation associated with alleles from AN-37. A QTL in marker interval BARCBEAN6K_3-SNP3545 and BARCBEAN6K_3-SNP0992 at position 18 cM on Pv04 had a LOD score of 3.37 that accounted for 42.9% of the observed variation associated with alleles from AN-37. A QTL in marker interval BARCBEAN6K_3-SNP3948 and BARCBEAN6K_3-SNP4375 at position 51 cM on Pv08 had a

LOD score of 2.55 and accounted for 15% of the observed variation associated with alleles from AN-37 (Table 3.5, Figure 3.4).

Number of days to flowering

Three QTL associated with the number of days to flowering were identified on chromosomes Pv04 Pv06 and Pv11 through composite interval mapping. In 2010 a QTL spanning the interval between BARCBEAN6K_3-SNP2342 and BARCBEAN6K_3-SNP0666 at 7 cM on Pv04 had a LOD score of 3.64 that explained 25.2% of phenotypic variation with alleles from P02647. On Pv06 the QTL spanned the interval between BARCBEAN6K_3-SNP0006 and BARCBEAN6K_3-SNP1094 at 79 cM with a LOD score of 3.63 that explained 19.3% of phenotypic variation with alleles from P02647. In 2007 the QTL in marker interval BARCBEAN6K_3-SNP3331 and BARCBEAN6K_3-SNP4345 at position 68 cM on Pv11 had a LOD score of 6.97 that accounted for 82.2% of the observed variation associated with alleles from P02647 (Table 3.5, Figure 3.4).

Number of days to maturity

One QTL associated with the number of days to maturity was identified on chromosome Pv01 though composite interval mapping in 2010 in the marker interval BARCBEAN6K_3-SNP5327 and BARCBEAN6K_3-SNP2322 at position 15 cM. The QTL had a LOD score of 2.66 and accounted for 15.9% of the observed variation associated with alleles from P02647 (Table 3.5, Figure 3.4).

100 Seed weight

One QTL associated with seed weight was identified on chromosome Pv05 though composite interval mapping in 2007 in the marker interval BARCBEAN6K_3-SNP2049 and BARCBEAN6K_3-SNP1785 at position 38 cM. The QTL had a LOD score 2.94 and accounted for 21.7% of the observed variation associated with alleles for smaller seed size from AN-37 (Table 3.5, Figure 3.4).

Plant canopy height

Two QTL associated with plant canopy height were identified on chromosome Pv01 and Pv07 through composite interval mapping. In 2007 a QTL in marker interval BARCBEAN6K_3-SNP1909 and BARCBEAN6K_3-SNP4049 at position 17 cM on Pv01 with a LOD score 6.21 accounted for 58% of the observed variation associated with alleles from AN-37. In 2011 a QTL spanned the interval between BARCBEAN6K_3-SNP2437 and BARCBEAN6K_3-SNP0462 at 83 and 86 cM on Pv07 had LOD score of 5.33 that explained 49% associated with alleles from P02647 (Table 3.5, Figure 3.4).

Discussion

Frequency Distributions

The continuous distributions exhibited by all the traits confirm the quantitative nature of inheritance of the traits measured in this study. Desirable phenotypes beyond the range of either of the parents were detected in a few individuals and one of the RILs in the AP630 population that exhibited yield potential combined with high levels of resistance to white mold was released

as the new pinto bean cultivar Eldorado (Kelly et al., 2012). The variation in yield and disease scores across the years emphasizes the need for multi-year testing to map white mold resistance in bean genetic populations. Transgressive segregation was also observed in both directions for all the traits in both populations as RILs expressed trait mean values above or below parental means. These two populations represent an intra-gene pool and intraracial cross and transgressive segregation in these two populations was advantageous in that it enabled the phenotypic expression of underlying genetic variation in the parental lines. The higher yields and lower disease scores in AP630 in comparison to AP647 could be due to better general combining ability between AN-37 and P02630 parents as opposed to the P02647 parent. Better combining ability was exhibited as greater yield stability observed across the years with the some of the same RILs genotypes showing consistent yield in different years. These populations proved ideal for dissecting complex traits through genetic mapping with P02630 being a better genetic donor for yield traits while P02647 exhibited improved architectural traits.

Estimates of heritability were similar to those previously reported in other bean populations (Kolkman and Kelly, 2002; Miklas et al., 2004) underscoring the fact that all favorable agronomic traits need to be considered simultaneously to advance breeding objectives. Park et al. (2001) reported low heritability estimates (0.23-0.24) for white mold resistance in the PC-50/XAN-159 (PX) population however the population was only evaluated in the greenhouse. Miklas et al. (2004) also reported low to high heritability estimates (0.21-0.90) in a Bunsi-derived $F_{5:8}$ RIL population (Aztec/ND88-106-04 (AN)) evaluated in multiple locations for white mold resistance and agronomic traits. The range heritability estimates from different studies suggests that these estimates depend on the genetic background of the material under evaluation, the size and the level of homozygosity of the population, and the methods of

computing variance components. The PX population has introgression alleles from *P. acutifolius* introduced for common bacterial blight (CBB) resistance (Park et al., 2001) while AN population (Miklas et al., 2004) is an intra gene pool cross similar to the two populations under this study. The higher heritability estimates observed indicate that selection can be effectively implemented in advanced RIL populations. Alternatively it would be interesting to compute heritability in multisite experiments in order to make comparison with results obtained from other studies. While these populations had RILs with a good representation of medium sized pinto seed, seed size is rarely used as a selection criterion unless crosses are made among highly contrasting individuals. The high heritability of seed size observed in these populations could be due to the reproducibility when measuring the trait in comparison to other traits. Seed size is the most stable trait among yield components followed by the number of pods per plant (Adams, 1967; Nienhuis and Singh, 1986). While yield is more variable among environments, the negative correlation with disease incidence allows breeders to use yield as a basis for indirect selection to improve white mold resistance. This is likely achieved through disease tolerance mechanisms where plants are still able to produce high yields even under disease pressure. In comparison to other studies of more diverse crosses (Blair et al., 2006; Singh et al., 2009; Mkwaila et al., 2011; Checa and Blair, 2012) yields had moderately higher heritability estimates. White mold is a disease of highly productive environments where management and climatic factors combine to optimize yields but create local environmental conditions favorable for disease development. In order to breed for this situation, breeders need to include yield in their selection criterion as these are the environments where the disease will strike hardest as low yielding lines that escape disease do not offer the genetic potential needed in these high productivity environments.

Correlation analysis

The results from correlation analysis indicate that there is a complex relationship between agronomic traits and white mold resistance in the field. White mold incidence clearly reduces yields, and plant vigor and it might be expected that smaller earlier maturing plants would escape infection. However our data suggests that the longer plants take to reach physiological maturity the less likely they will get infected. It is probable that plants that mature later tend to remain physiologically active longer and are able to ward off late infection of the necrotrophic fungus. Miklas et al. (2004) demonstrated that the stay-green trait (where stems remain green when pods have reached physiological maturity and have dried down) in the Bunsi cultivar is the main mechanism regulating the plants ability to tolerate white mold pressure. This trait is not detectable in the greenhouse straw test. However variable maturity and dry down is a problem for farmers requiring the use of plant dessicants when direct harvesting bean as green stems at maturity do not thresh well and the stem sap can stain the bean seed. The negative correlation between plant height and lodging is not surprising as canopy height is taken at harvest after the weaker plants have lodged. This observation could be due to advances made in breeding for upright Type II pinto beans. The bean plant architecture has been modified from the predominantly shorter decumbent Type III plants to taller upright Type II plants that enable direct harvesting (Kelly, 2000). In order to maintain the upright architecture Type II plants must possess stiff stalks that support the full weight of the plant and resist lodging (Ender and Kelly, 2005). Negative correlations between plant height and lodging have also been demonstrated in a navy x pinto cross although there was also the environmental effect in different experimental locations (Miklas et al., 2004). The lack of correlation between the greenhouse straw test with all other traits is not surprising as lines such as VCW54 exhibit resistance in the greenhouse (Singh

et al., 2009) but do not perform well in field conditions. Many genotypes screened in the greenhouse that show high levels of white mold resistance in the straw test are among the lowest yielding in the field. The straw test is useful in studies that focus on the genetic and physiological basis of resistance. It is possible that the straw test is affected by such underlying morphological factors such as structural support through lignin (thick stems, larger cells), the internode length, and related traits which may have a negative effect on the number of branches and number of pods and consequently yield. Stem thickness and internode length could also be affecting lodging in the field which aggravates white mold infections. Alternatively genotypes that do not have high infection rates under natural conditions are usually more susceptible when challenged with the mycelia plug in the greenhouse (Kolkman and Kelly, 2001). Breeders would make more gains in selecting for resistance by focusing on other agronomic traits or developing other more informative greenhouse tests which closely mimic natural conditions.

Genetic Linkage Mapping

The map sizes (1183 cM and 953 cM for AP630 and AP647, respectively) obtained from this study are a reasonable representation of the bean linkage map which is estimated at 1200 cM (Freyre et al., 1998; Soule et al., 2011). The maps from this study have a higher marker density and are the first attempt to map QTL for white mold and agronomic traits using SNP markers. In previous white mold mapping studies, genome coverage has ranged from around 200 to 1000 cM (Ender and Kelly, 2005; Mkwaila et al., 2011; Perez-Vega et al., 2010). When results of JoinMap and QTL IciMapping are compared, maps of different sizes and orders were generated. JoinMap had uneven clustering, low map coverage and inconsistent marker order. Based on marker segregation these discrepancies could not have resulted from biological reasons such as

segregation distortion but rather from different computational algorithms. In contrast the recombination counting and ordering (REC) method of QTL IciMapping resulted in a more consistent map compared with other maps of white mold resistance in common bean (Soule et al., 2011). However it is possible that QTL IciMapping could have overestimated the effects of QTL as observed by the higher R^2 values. Similar results have been reported in mapping studies in other crops such as soybean where several algorithms have been employed to improve mapping and QTL analysis (Kim et al., 2012). Several QTLs were identified using different methods based on the same genotypic and phenotypic data sets and so the use of multiple algorithms enhances the reliability of maps and QTL detection.

Yield QTL Analysis

Chromosome Pv01

The only QTL detected on Pv01 for seed yield appears to be unique to the AP647 population and is named $SY1.1^{AP647}$. No prior QTL for yield have been reported on chromosome Pv01. This QTL is associated with alleles from AN-37 implying that this locus can be used for selection against low yielding individuals depending on the segregation of the flanking markers.

Chromosome Pv02

The seed yield QTL identified on Pv02 appears to be similar to the previously described QTL reported by Blair et al. (2006). The common marker between the two maps is the SSR BM142 but the QTL from this study is responsible for greater variation (26%) than that reported in the Andean x wild *P. vulgaris* cross used by Blair et al. (2006). The AP630 population exhibited

consistent yield performance across the years and so this QTL is more robust and is supported by the relatively higher R^2 values (11-26%). It is interesting to note that in the IBL population of Tacana x landrace (TL) a similar QTL was identified for yield under disease pressure $SY2.1^{TL}$ (Mkwaila et al., 2011). The QTL is flanked by two markers spaced 16cM apart at the distal end of Pv02 in the current map. However on the consensus map these markers are spaced further apart and are located near the middle of the linkage group (Soule et al., 2011). This observation could be due to the fixed order effect when fitting consensus map using different mapping populations. The other two QTL associated with SNP markers in AP630 are different from $SY2.1^{TL,AP630}$**.** In the absence of any overlap between loci these QTL are now named $SY2.2^{AP630}$ and $SY2.3^{AP630}$**,** respectively. The QTL for yield on Pv02 in AP647 is not close to any previously identified QTL and is designated $SY2.4^{AP647}$.

Chromosome Pv03

QTL for yield were also identified on Pv03 by Blair et al. (2006) near SSR markers BM172 and BM98 and by Beattie et al. (2004) based on RAPD markers grown in different environments. The QTL from this present study was from AN-37 parent in a single environment and is designated $SY3.4^{AP647}$**.**

Chromosomes Pv05 and Pv09

The first QTL reported for yield was identified in navy bean RIL population (Tar'an et al., 2002) and was associated with SSR marker BNG161. Beattie et al. (2004) also reported two QTL for

yield on the same chromosome in a different navy bean RIL mapping population. The yield QTL on Pv05 identified in this study is different as no QTL for yield have been reported in its proximity on chromosome Pv05 and therefore is designated $SY5.4^{AP630}$. Similarly, two QTL have been reported on Pv09 (Tar'an et al., 2002; Blair et al., 2006) and differ from the QTL in this study which is designated $SY9.3^{AP647}$.

Other researchers have also reported yield QTL on most linkage groups of the bean map. Blair et al. (2006) and Mkwaila et al. (2011) also identified seed yield QTL on a predominantly SSR map on Pv04 while Tar'an et al. (2006) found yield QTL on Pv11. Kolkman and Kelly (2002) identified QTL for yield on Pv07 based on RAPD markers while Wright and Kelly (2011) reported a major yield QTL $SY10.2^{J115}$ on Pv10 using TRAP markers.

All the QTL associated with yield could be further investigated by studying other yield related components like number of pods per plant. Although seed size is a yield component there was no co-localization observed with yield QTL. The large number of yield QTL on the bean genetic linkage map confirms the complex nature of the trait and indicates the pleiotropic effect these loci may have on each other. The large numbers, small effects and possible interaction between QTL presents a challenge for breeders if the QTL has only been detected in a single environment or year. However there is also opportunity to implement QTL pyramiding and introgression to improve the efficiency of MAS. Since SNP markers have specific known positions in the genome and map at a high density they can be employed as markers for candidate gene analysis.

White mold QTL Analysis

Chromosome Pv01

The QTL for white mold resistance on Pv01 does not share markers with either WM1.1^{AG,XC} or WM1.2 ^{GC} previously mapped by Miklas et al. (2001), Perez-Vega et al. (2012) and Maxwell et al. (2007). The two former AG and GC populations share a common parent G122 which has been associated with resistance in both the straw test and disease avoidance in the field due to a porous canopy. The open porosity of G122 is attributed to the determinate locus *fin* however canopy porosity is also influenced by low plant vigor (Miklas et al., 2001). The QTL from this study designated WM1.3^{AP630} is possibly associated with disease avoidance as Bunsi was the grandparent and used as the source of the resistance. This is demonstrated in greenhouse trials where Bunsi is ranked as susceptible in the straw test while in the field it exhibits disease avoidance through open canopy (Kolkman and Kelly, 2003; Ender and Kelly, 2005) and the stay green trait (Miklas et al., 2004). The QTL associated with disease resistance in the greenhouse observed in the AP647 population in the greenhouse had alleles from AN-37 parent and is named WM1.4^{AP647}.

Chromosome Pv02

The major QTL WM2.2 previously identified in many studies is likely to be the same as the one identified here as WM2.2^{BN,HN,BR,AN,R31,BV,AP630} . This QTL has been attributed to both field and greenhouse resistance detected by the straw test. In the BN (Bunsi/Newport) population the observed resistance was from Bunsi while in the HN (Huron/Newport) population the

resistance was conferred by C-20 grandparent (Kolkman and Kelly, 2003). Both Bunsi and C-20 exhibit disease avoidance through open canopy porosity and upright plant architecture. In the BR (Bunsi/Raven; Ender and Kelly, 2005), AN (Aztec/ND88-106-04; Miklas et al., 2007), R31 (Raven/I9365-31; Soule et al., 2011) populations, the QTL was also associated with reduced white mold incidence in the field. However, in the BV (Benton/VA19; Soule et al., 2011) population the resistance was only detected in the greenhouse straw test and non-wounding tests. The current study supports the finding that the loci has mechanisms associated with both types of resistance (Soule et al., 2011). This is important as our greenhouse phenotypic data did not exhibit any correlation with field disease scores. The region on Pv02 has defense genes such as chalcone synthase, pathogenesis related proteins and polygalacturonic inhibitor proteins which are induced following infection with many pathogens including *S. sclerotiorum.*

Chromosome Pv03

The QTL identified on Pv03 in AP630 is associated with similar markers as $WM3.1^{AN}$ identified in a navy pinto cross (Aztec/ND88-106-04, AN; Miklas et al., 2007). The AN-37 parent of this current study was a white mold germplasm line released from the AN mapping population (Miklas et al., 2006). This QTL is likely the same QTL and is therefore named $WM3.1^{AN,AP630}$.

Chromosome Pv04

The QTL identified on Pv04 in AP647 is a new locus and is in close proximity to a QTL for days to flowering. This chromosome has QTL associated with both the straw test and white mold incidence in the field. The other QTL identified on the same linkage group are $WM4.1^{PX}$ and

WM4.2^{R31,TL} (Park et al., 2001; Soule et al., 2011; Mkwaila et al., 2011). The new QTL from this study is designated WM4.3^{AP647}.

Chromosome Pv07

The QTL WM7.6^{AP630} on Pv07 was associated with markers BM209 and BARCBEAN6K_3-SNP1180 which are >30 cM away from other known QTL on this chromosome. The previous reported QTL were associated with resistance to specific isolates of *S. sclerotiorum* in the genotype Xana (Perez-Vega et al., 2012). The resistance associated the WM7.1^{AG,PX,XC} QTL that resides near the *Phs* seed protein locus on Pv07 is unknown. The resistance observed in these populations was derived from the Andean G122 genotype, the most commonly used resistance check in multisite trials (McCoy et al., 2012). Kolkman and Kelly (2003) and Ender and Kelly (2005) also identified WM7.2^{BN,BR} QTL based on field resistance and resistance to oxalic acid in the greenhouse. WM7.2^{BN,BR} share the Bunsi resistance while WM7.3^{R31} possesses resistance from I9365-31 derived from an interspecific cross between *P. vulgaris*/*P. coccineus* (Soule et al., 2011; Miklas et al., 1998). Both WM7.4^{XC} and WM7.5^{TL} were associated with the straw test in their respective populations (Miklas et al., 2013). Both the *Phs* locus identified by Miklas (2007) and the AFLP markers by Ender et al. (2008) have been used to introgress resistance into Middle American beans. Selection with WM7.6^{AP630}QTL could be improved by cloning an InDel marker NDSU_IND_7_34.5855 near the loci BM209. The QTL at the lower end of the chromosome is another new QTL designated WM7.7^{AP630}.

Chromosome Pv08

The QTL identified on Pv08 in AP630 was associated with white mold resistance in the field in 2008. With further validation this is the fifth QTL on this linkage group and is designated WM8.5^{AP630}**.** Two of previous QTL WM8.3^{B60,GC,BV} and WM8.4^{BR,GC,R31} share the common Bunsi source of resistance. In the B60 population (Bunsi/NY6020-4 snap bean; Miklas et al., 2003) resistance was based on both straw test and field disease resistance while in BR (Bunsi/Raven) (Ender and Kelly, 2005) resistance was based on field disease incidence only. The QTL detected in the AP647 population is at the distal end on the chromosome and is new locus designated WM8.6$^{AP647.}$ The colocalizing of QTL for resistance seem to suggest that the genes controlling resistance to white mold are part of a similar pathway whether the resistance is observed in the field or in the greenhouse.

Days to flowering and maturity

The QTL identified on Pv01 in AP630 associated with days to flowering was located on the same region as the QTL for seed weight. The QTL for days to flowering were also linked to the number of days to maturity and seed yield in two genomic regions of Pv03. Similarly days to flowering is also associated with the days to maturity on Pv05. All the alleles from P02630 and P02647 were associated with late flowering and possibly longer days to maturity. Other researchers have also identified several loci on the *P. vulgaris* map associated with flowering. Blair et al. (2006), and Perez-Vega et al. (2011) have mapped QTL on Pv01, Pv02, Pv06, Pv09, and Pv11. The number of days to flowering is an important phenological trait not only in relation to disease resistance and yield but also to other abiotic stresses. In both populations AN-37 flowered earlier and the QTL on Pv05 and the proximal end of Pv03 were influenced by alleles

from this parent. All the QTL on Pv04, Pv06 and Pv11 were associated with late flowering in AP647.

QTL for days to maturity have been mapped previously to Pv01, Pv02 and Pv06 (Perez-Vega et al., 2010) using mostly AFLP markers and those on Pv09 and Pv11 were based on a growth habit (GH) morphological marker (Tar'an et al., 2002). The QTL identified on Pv05 in AP630 population appears to be different from that of Blair et al. (2006) which explained 37% total variation in a single environment. The QTL on Pv01 (AP647) and on the proximal end of Pv03 and Pv05 (AP630) can be used in MAS in selecting for early maturity while QTL for maturity on the distal regions of Pv03, Pv05 and Pv10 (AP630) are associated with longer days to maturity coming from the white mold resistant AN-37 parent.

Plant canopy height and lodging

The QTL for plant canopy height on Pv01 in AP647 is a new locus as no QTL for plant height have been reported on this chromosome. The QTL colocalized with a QTL for shorter days to maturity. On Pv07 the QTL for plant height could be similar to the one identified in the Bunsi/Newport population (Kolkman and Kelly, 2002). Plant canopy height is important in disease avoidance in common bean. A tall plant canopy is associated with more air circulation and plants that are less likely to be in contact with the moist soil thereby avoiding infection. Lodging and plant height are closely related in that taller but weak plants tend to lodge more while upright types with stiff stems tend to remain upright. Canopy height measurements are taken at harvest time when weaker stemmed plants have already lodged and this explains why high canopies are correlated with low disease. Lodging helps to create a microclimate under the decumbent canopy which limits air flow and enhances white mold infections in the field.

Conclusion

This study suggests that moderate levels of white mold resistance have been transferred from navy bean into type II pinto beans. Agronomic traits exhibited moderate heritability estimates indicating that advances in breeding simultaneously for multiple traits can be made. However correlations between greenhouse and field disease scores were very weak similar to data observed in other mapping populations. The straw test is useful in screening diverse genotypes when trying to identify potential sources of resistance however this resistance has to be confirmed in the field. This is the first high density mapping study of white mold resistance with SNP markers in common bean. The markers associated with QTL for white mold resistance on Pv02, Pv03 and Pv07 can be used for selection or as indicators of regions that warrant future genomic analysis to identify biological factors involved in conferring the resistance to white mold in common bean.

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CHAPTER 4

PRELIMINARY ANALYSIS OF DEFENSE GENES IN RESPONSE TO WHITE MOLD INFECTION IN DIFFERENT BEAN (*PHASEOLUS VULGARIS)* CULTIVARS

Introduction

The development of resistant varieties for quantitatively inherited disease is a challenge to most breeders. White mold (*Sclerotinia sclerotiorum*) resistance in common bean (*Phaseolus vulgaris*) is known to be a complex trait (Fuller et al., 1984) such that dissection of resistance mechanisms requires quantitative trait loci analysis. QTL analysis relies on detecting genotypic differences in trait means at a marker locus, based on the principle of association between the phenotype and genotype of markers. Several QTL studies have been conducted on different genetic backgrounds; using different marker systems to identify white mold resistance in beans (Soule et al., 2011). The focus of QTL analysis however is shifting from simply discovery of QTL associated with quantitative disease resistance to determining the biological function underlying the QTL (Kelly and Vallejo, 2005). The interpretation of QTL results is usually limited due to lack of information on the genetics, biochemistry and physiology underlying trait expression. Understanding the function of quantitative resistance genes and their mode of expression is necessary to enable breeders to select which QTL to deploy in marker assisted selection. Some QTL explain a significant amount of variation but are associated with plant avoidance mechanisms which confound the discovery of physiological resistance (Kelly and Vallejo, 2005). QTL that have mapped to the same regions as major resistance genes or defense response in plants provide a bridge in the understanding the functional significance of QTL. Such QTL also

serve as a link in identifying candidate genes that influence disease resistance (Geffroy et al., 2000; Pflieger et al., 2001).

The QTL associated with white mold resistance on chromosome Pv02 has shown consistent expression in several different independent studies (Kolkman and Kelly, 2003; Ender and Kelly, 2005, Soule et al., 2011) including the current study. This QTL has not been highly correlated with disease avoidance traits indicating that these loci are associated with physiological disease resistance. The physiological disease resistance is expressed both in the field and greenhouse tests. The QTL in this region is in close proximity with three defense response genes namely chalcone synthase, the pathogenesis related gene PvPR-2 and polygalacturonase inhibiting protein (PGIP). PGIP are important in inhibitors of cell wall degrading enzymes produced by most pathogens. The cell wall is the primary barrier that pathogens must overcome in the infection process. Chalcone synthase catalyses the reactions in the biosynthesis of antimicrobial phytoalexins which are low molecular weight compounds locally produced in response to infection (Hahlbrock and Scheel, 1989). PR proteins are also induced in response to infection or wounding or abiotic stress. Endochitinase and glucanase (located on Pv09) are PR proteins that are induced by fungal infection (Lamb et al., 1989). Phenylalanine ammonia lyase (PAL) which catalyzes the conversion of L-phenylalanine into *trans*-cinnamate is also important in the defense pathway in response to pathogen attack (Cramer et al., 1989). It is a member of a multigene family that is located on Pv01 and Pv07 chromomsomes. The detection of QTL for disease resistance in similar genomic regions seems to suggest that these proteins are involved in host defense following *S. sclerotiorum* infection in beans.

The expression of most defense genes varies in different tissues and in timing of expression. Most genes exhibit low constitutive expression levels. PGIP have been shown to be variably

expressed in bean plants (Salvi et al., 1990; Dovidio et al., 2004). In *Brassica napus* the PGIPs are differentially regulated in response to *Sclerotinia* infection and hormone treatment which demonstrates that these genes are indeed an important part of the plant defense response (Hegedus et al., 2008).

Bean lines with complete resistance to white mold have not been reported however partial resistance has been identified in specific cultivars (Kolkman and Kelly, 2002; Miklas and Grafton, 1992; Miklas et al., 2006). Since there are no specific resistance genes associated with white mold, it is likely that the physiological resistance detected in greenhouse tests is primarily dependent on defense response genes. Several sources of partial resistance that have been identified (Bunsi, G122, PC 50, AN 37, A195) and have been used as parents in developing mapping populations to study the inheritance of white mold. These genotypes show significant differences in disease reaction from susceptible genotypes such as Beryl and Matterhorn which exhibit high infection in the greenhouse screening straw test (Soule et al., 2011).

The objective of this research was to investigate the role of PGIP, Glucanase, and PAL genes in the defense response of different genotypes following infection with *Sclerotina sclerotiorum*.

Materials and Methods

Four cultivars of beans AN-37, G122, P02630 and Beryl were chosen based on their different reaction to white mold in the straw test. G122 is cranberry landrace from India (Miklas et al, 2001), AN-37 is pinto line derived from a navy line carrying resistance from Bunsi a source of resistance to white mold (Miklas et al., 2006). Both of these cultivars are moderately resistant, while Beryl (a great northern) and P02630 (MSU pinto breeding line) are susceptible. The plants were grown in the greenhouse under the same conditions as when conducting the straw test.

Briefly plants were inoculated 21 days after planting as described by Petzoldt and Dickson (1996), with the following modifications. The growing tip of the plant was excised with a razor blade and discarded. Petri dishes of two to three day old cultures of *S. sclerotiorum* on PDA were used as the inoculum source. The PDA plugs were placed in the top end of 10 uL pipette tips, which were then applied over the cut stem. A PDA plug without fungus was first placed in the pipette tip followed by a PDA plug with fungus in order to prevent the fungal plug from drying out prior to infection of the plant. The plug was affixed to the freshly cut edge of the stem with the pipette tip remained in contact with the stem for 8 days. Control plants were wounded without infection. All pots were kept at 25°C in the greenhouse. Tissue sampling was conducted from both the necrotic part and the chlorotic area of each lesion within 2 cm of the lesion edges at 0, 24, 48 hours post inoculation (hpi) and 7 days post-inoculation (dpi) and immediately frozen in liquid nitrogen prior to RNA extraction. All samples collected had three biological replicates.

RNA was extracted from plant tissues using both Qiagen RNeasy Plant Kit and the standard Trizol protocol (Invitrogen). To remove any genomic DNA contamination, RNA was treated with RNAse-free DNAse I (Promega) followed by enzyme inactivation (2.5 mM EDTA, 65°C/10 min) and ethanol precipitation.

The primers used in this experiment were as shown in Table 4.1. They were designed based on exons such that DNAse treatment was needed to avoid amplification of genomic DNA.

Table 4.1: Primers designed and used in semi quantitative reverse transcription-polymerase chain reaction (RT-PCR) for amplifying defense-associated genes of *Phaseolus vulgaris*

Gene	**Forward Primer 5'**	**Reverse Primer 5'**	**PCR product size**	**Annealing Temperature (^{0}C)**	**Accession Number**
Pvpgip 1	TCTTTGAGAAC TGCACT	CGTCGAATGTG ATTCCTC	240	55	AJ864506.1
Phenyl ammonia lyase	AAGCCATGTCC AAAGTGCTG	GAGTTCTCCGT TGCCACCT	240	64	M11939
Glucanase	GCTGTAAGGGC TCAAGGCCTC	CCAAGTACACA CGTGCGTTGTC	427	65	X53129
Actin	CACCGAGGCA CCGCTTAATC	CGGCCACTAGC GTAAAGGGAA	126	55	AB067722

First strand cDNA was synthesized using 2 µg DNAse-treated total RNA using superscript III Reverse Transcriptase (Invitrogen), following the supplier recommendations. 5 µL of first strand cDNA was amplified in a final reaction volume of 25 µL containing 1X Taq DNA polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM specific primers, and 5 U Taq DNA polymerase (Invitrogen). PCR was initially performed at 94°C for 4 min, and then 27 cycles as follows: 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The annealing temperatures were adjusted for each primer to obtain specific amplification of expected band size. PCR products were resolved by electrophoresis on a 3% w/v agarose gel. Two replicates of reverse transcription PCR experiments were performed for all genes analyzed. Gene expression levels were analysed via density analysis of Gel Doc EZ imager and Image Lab™ software. Amplified product intensity was expressed as relative absorbance units. The ratio between the relative absorbance units

determined for the amplified gene of interest and internal control (Actin) was calculated to normalize for initial variations in sample concentration and as a control for reaction efficiency (ie the same intensity in PCR reactions halted at the exponential phase).

Results

Three of the cultivars inoculated with the pathogen *S. sclerotiorum* showed significant differences in disease progression and in final disease scores taken at 8 days. There was no significant difference between AN-37 and P02630 genotypes (Table 4.2).

Table 4:2 Effect of inoculation of the cultivars with *Sclerotinia sclerotiorum*

Genotype	**Disease score**[1]
AN-37	3a
BERYL	7b
G122	1c
P02630	4a

[1]Greenhouse straw tests on the cultivars were rated on a scale of 1- 9, where 1 = no infection and 9 = total plant collapse.
Scores followed by different letters are significantly different from each other ($p < 0.05$).

Defense gene expression

All the results for the non-wounding experiments showed similar expression patterns to the 0 hpi time line in the challenged plants so they are not presented separately.

Glucanase

In general there was no glucanase expression (Figure 4.1 and 4.2) detected in the cultivars AN-37 and P02630 while some low levels of transcription took place at 0 hpi. Gene expression

peaked at 24 hpi for AN-37 and G122 and at 48 hpi for P02630. Beryl which had much lower expression at these time points had no expression at 48hpi. At 7 days all the cultivars had some level of glucanase present including Beryl which regained expression and P02630 had the strongest signal relative to the other lines.

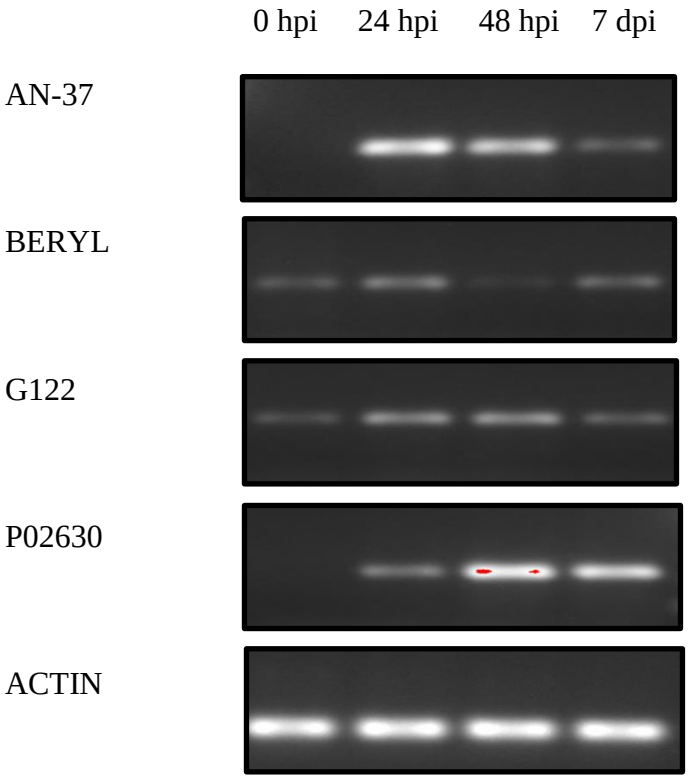

Figure 4.1 RT-PCR product analysis for the glucanase gene, indicating the differences in signal strength at four different time points in four bean cultivars challenged with *Sclerotinia sclerotiorum*. PCR products, amplified by the primer pairs as described under Materials and Methods, were separated on a 3 % agarose gel.

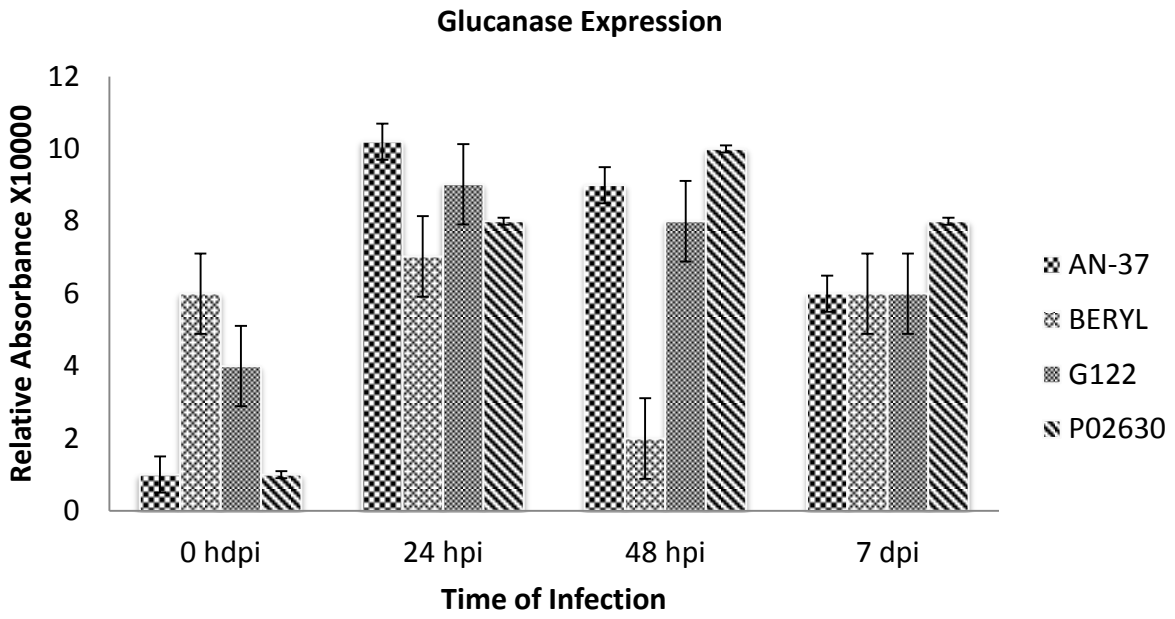

Figure 4.2: Cultivar-specific differential expression of glucanase gene in bean plants infected with *Sclerotinia sclerotiorum* at four time points. Error bars indicate the standard error of means of two biological replicates of the experiment

Phenylalanine Ammonia Lyase (PAL)

Overall phenylalanine ammonia lyase had low expression levels in response to *S. sclerotiorum* inoculation (Figure 4.3 and 4.4) There was no transcription in response to wounding at 0 hpi in all the cultivars however Beryl and G122 peaked at 24 hrs while the most abundant transcripts were at 48 hrs in the two pinto lines AN-37 and P02630. All four cultivars retained some enzyme expression at 7 days.

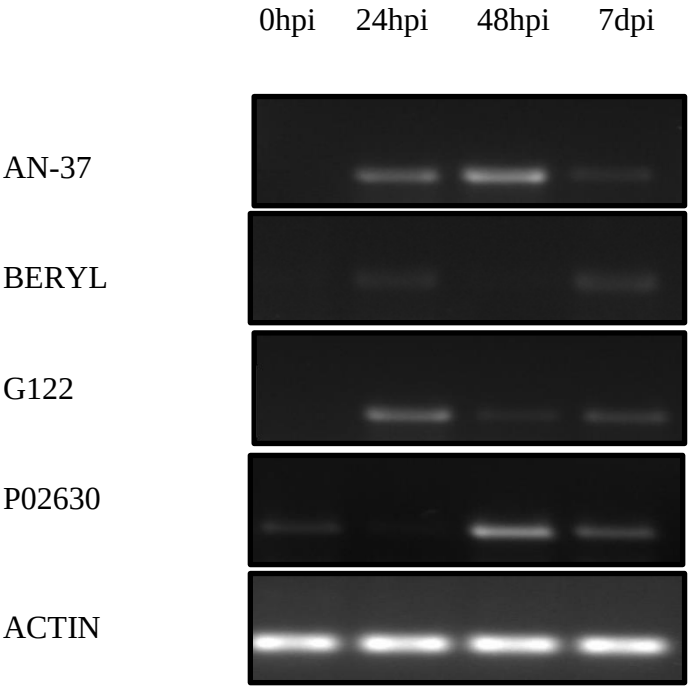

Figure 4.3: RT-PCR product analysis for the phenylalanine ammonia lyase gene, indicating the differences in signal strength at four different time points in four bean cultivars challenged with *Sclerotinia sclerotiorum*. PCR products, amplified by the primer pairs as described under Materials and Methods, were separated on a 3 % agarose gel.

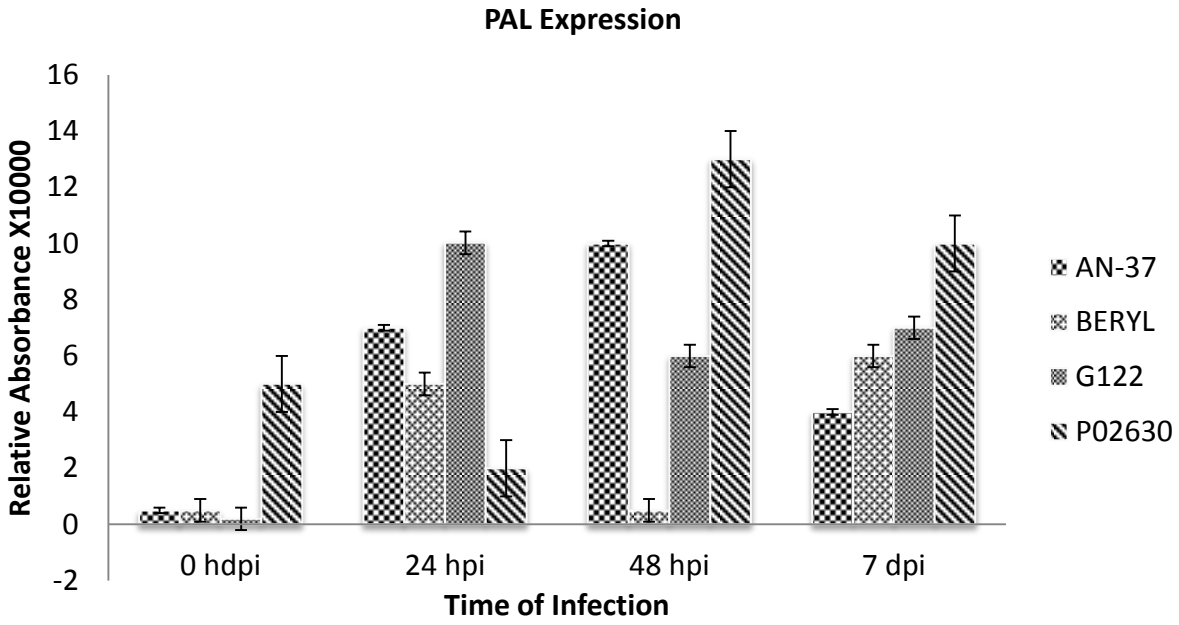

Figure 4.4 Cultivar -specific differential expression of phenylalanine ammonia lyase gene in bean plants infected with *Sclerotinia sclerotiorum* at four time points. Error bars indicate the standard error of means of two biological replicates of the experiment

Polygalacturonase Inhibitor Protein 1

There was also variable expression of PGIP1 in the different cultivars. In contrast to the other genes PGIP was induced with different signals in response to wounding at 0 hpi. The resistant line G122 showed the strongest signal at 0 and 48 hpi while AN-37 expressed the gene at 48 hpi only. At 7 dpi there was no signal except in P02630 (Figure 4.5 and4.6).

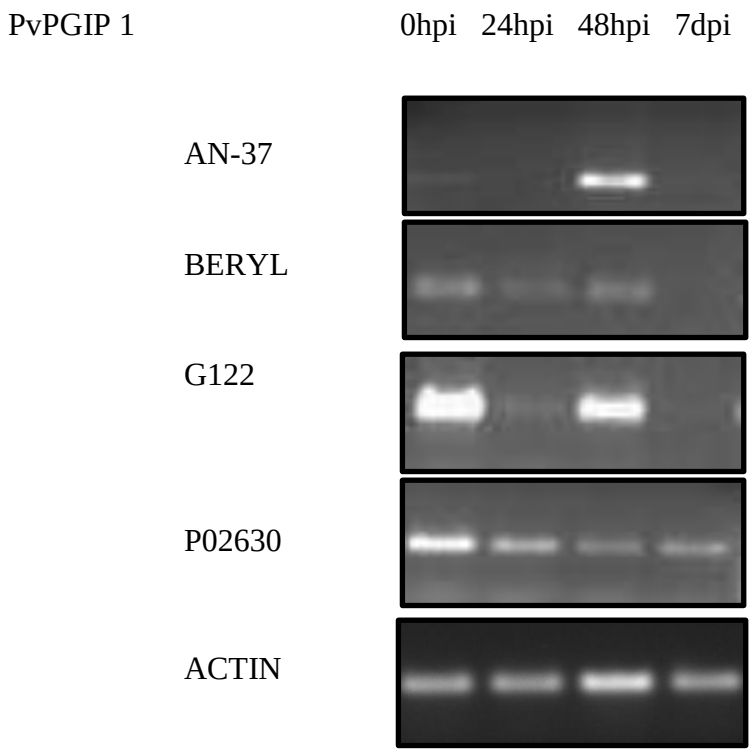

Figure 4.5: RT-PCR product analysis for the polygalacturonase inhibitor protein 1 PvPGIP1 gene, indicating the differences in signal strength at four different time points in four bean cultivars challenged with *Sclerotinia sclerotiorum*. PCR products, amplified by the primer pairs as described under Materials and Methods, were separated on a 3 % agarose gel.

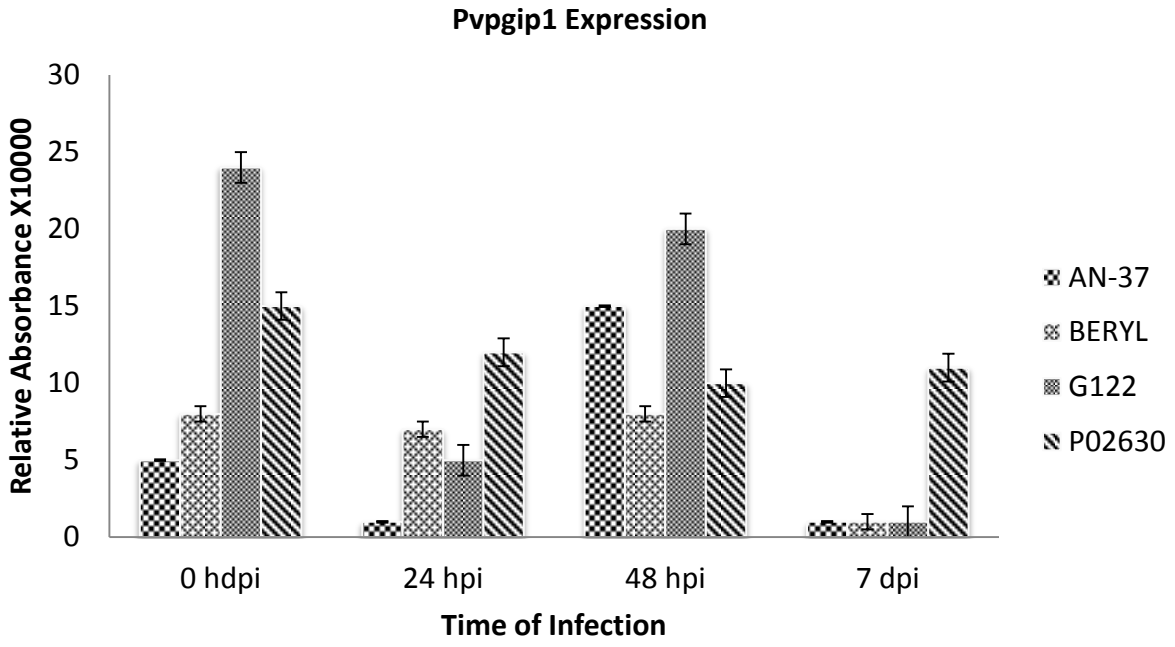

Figure 4.6 Cultivar -specific differential expression of polygalacturonase inhibitor protein 1 PvPGIP1 gene in bean plants infected with *Sclerotinia sclerotiorum* at four time points. Error bars indicate the standard error of means of two biological replicates of the experiment

Discussion

This study was mainly focused on the cultivar specific response of three defense genes following inoculation with *S. sclerotiorum*. The expression patterns exhibited by the three genes confirm that they have a role in defense response following infection with *S. sclerotiorum*. D'ovidio et al. (2006) showed that members of the bean PGIP family differ in their response to *Phytopthora megasperma*. PGIP 2 was the most abundant transcript in their experiment and PGIP1 was only induced in wounded plants. Oliveira et al. (2010) showed that PGIP1 reaches peak expression levels at 72 and 96hpi similar to our results. Our study shows the effect of genotype on the expression of PGIP 1. The other variation could be due to the differences in response to actual fungal infection in our study in contrast to using elicitor molecules like salicylic acid and glucans

employed by D'ovidio et al. (2006). The induction of PAL at 48 hrs suggests that some phytoalexins play a role in plant defense following *S. sclerotiorum* infection (Cramer et al., 1989). However based on relative amounts of transcripts in comparison to the other two genes this pathway may only offer generalized defense response.

Glucanase is an important pathogenesis related (PR) protein whose induction has been shown to be systemically spread to different plant parts following induction (Mauch and Staeheline, 1989). The most distinct temporal expression was in the pinto lines AN-37 and P02630 suggesting that it is a distinct defense response however the availability of transcripts in the other genotypes at 0 hpi indicates that the gene also responds to wounding.

On a whole these results confirm the quantitative nature of different levels of resistance to white mold infection observed in different bean cultivars. These genes could be deployed in overexpression experiments however due to cultivar difference the results may be variable.

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APPENDIX

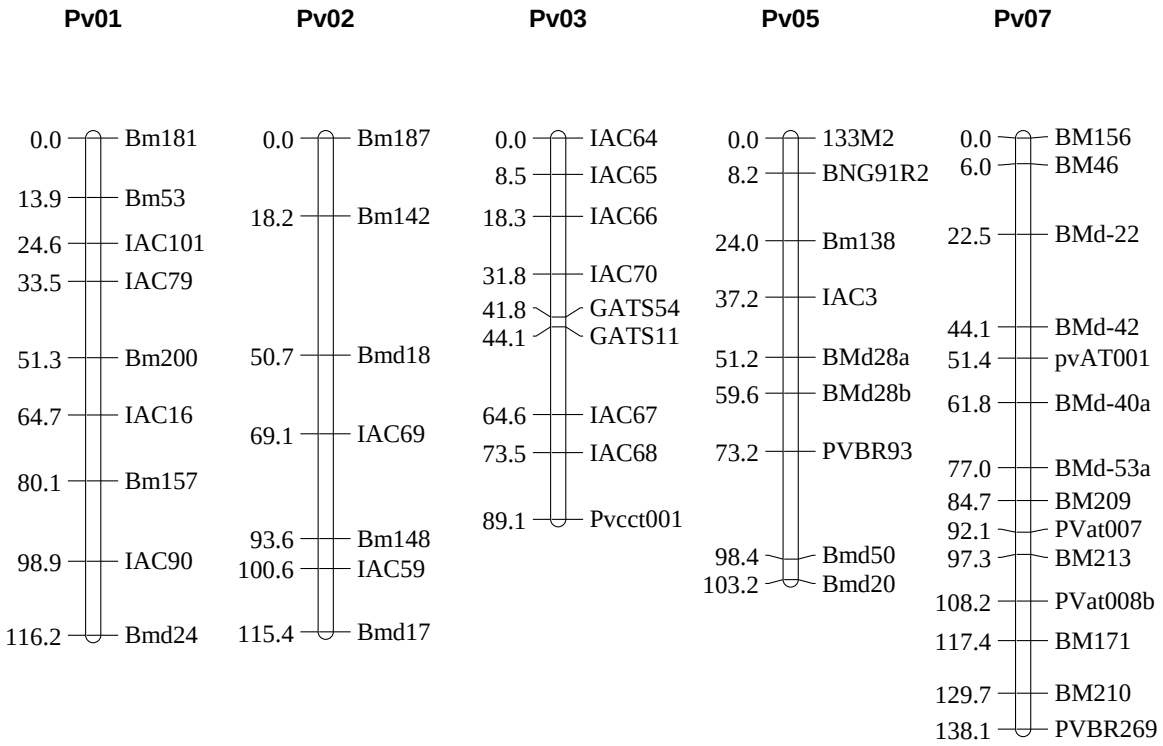

Figure A. 1 SSR MAP

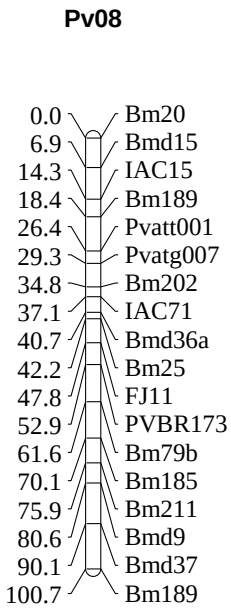
Pv08
0.0
6.9
14.3
18.4
26.4
29.3
34.8
37.1
40.7
42.2
47.8
52.9
61.6
70.1
75.9
80.6
90.1
100.7
Bm20
Bmd15
IAC15
Bm189
Pvatt001
Pvatg007
Bm202
IAC71
Bmd36a
Bm25
FJ11
PVBR173
Bm79b
Bm185
Bm211
Bmd9
Bmd37
Bm189

Fig A.1 (cont'd).

Table A.1. Single Marker Analysis for white mold and yield in AP630

Trait Name	Marker Name[1]	Chromosome	Position	LOD[2]	R^2 [3](%)	Add[4]
YIELD8	SNP1048	3	106.49	2.73	13.82	1.41
YIELD8	SNP1050	3	106.49	2.73	13.82	1.41
YIELD8	SNP1047	3	106.49	3.38	17.92	1.61
YIELD8	SNP1991	3	85.52	2.58	12.96	1.55
YIELD8	SNP1992	3	85.52	2.65	13.08	1.57
YIELD8	SNP1993	3	85.52	2.66	12.85	1.56
YIELD8	SNP1990	3	85.52	2.65	13.08	1.57
YIELD8	SNP5118	3	85.52	2.66	12.85	1.56
YIELD8	SNP1989	3	85.52	2.58	12.96	1.55
YIELD8	SNP1986	3	87.17	2.68	14.05	1.54
YIELD8	SNP4002	3	99.81	2.83	13.20	1.37
YIELD8	SNP4187	3	99.81	2.66	12.91	1.36
YIELD8	SNP3065	3	99.81	2.66	12.91	1.36
YIELD8	SNP3916	3	99.81	2.65	13.34	1.38
YIELD8	SNP1728	2	100.34	2.65	12.80	1.35
YIELD8	SNP1729	2	100.34	2.64	13.03	1.37
YIELD8	SNP2069	2	101.43	2.59	12.38	1.33
YIELD8	SNP2068	2	101.43	2.53	12.53	1.34
YIELD8	SNP695	2	103.68	2.50	13.75	1.42
YIELD8	SNP1049	2	106.49	3.24	16.50	1.54
YIELD8	SNP1045	3	106.49	3.27	16.63	1.55
YIELD8	SNP1046	3	106.49	3.27	16.63	1.55
YIELD8	SNP1926	3	106.49	2.78	13.89	1.41
YIELD8	SNP1925	3	106.49	2.73	13.82	1.41
YIELD8	SNP3987	3	106.49	2.73	13.82	1.41
WHITEMOLD8	SNP1991	3	85.52	2.92	13.56	-2.25
WHITEMOLD8	SNP1992	3	85.52	3.02	13.82	-2.28
WHITEMOLD8	SNP1993	3	85.52	2.93	13.26	-2.25
WHITEMOLD8	SNP1990	3	85.52	3.02	13.82	-2.28
WHITEMOLD8	SNP5118	3	85.52	2.93	13.26	-2.25
WHITEMOLD8	SNP1989	3	85.52	2.92	13.56	-2.25
WHITEMOLD10	SNP433	3	36.22	2.67	13.08	-6.50
WHITEMOLD10	SNP432	3	36.75	2.67	13.03	-6.46
WHITEMOLD10	SNP4002	3	99.81	2.89	13.43	-6.55
WHITEMOLD10	SNP4187	3	99.81	2.63	12.60	-6.34
WHITEMOLD10	SNP3065	3	99.81	2.63	12.60	-6.34
WHITEMOLD10	SNP3916	3	99.81	2.95	14.64	-6.84
WHITEMOLD10	SNP1728	3	100.34	3.37	16.11	-7.19
WHITEMOLD10	SNP1729	3	100.34	3.61	17.40	-7.48

Table A.1 (cont'd)

Trait Name	Marker Name[1]	Chromosome	Position	LOD[2]	R[2 3](%)	Add[4]
WHITEMOLD10	SNP2070	3	101.43	3.50	16.47	-7.26
WHITEMOLD10	SNP2069	3	101.43	3.28	15.03	-6.93
WHITEMOLD10	SNP2068	3	101.43	3.72	17.87	-7.58
WHITEMOLD10	SNP3711	3	101.43	3.19	15.50	-7.06
WHITEMOLD10	SNP696	3	103.68	2.87	14.16	-6.80
WHITEMOLD10	SNP695	3	103.68	3.05	15.00	-7.01
WHITEMOLD10	SNP1049	3	106.49	3.33	16.30	-7.23
WHITEMOLD10	SNP1045	3	106.49	3.02	15.29	-7.01
WHITEMOLD10	SNP1046	3	106.49	3.02	15.29	-7.01
WHITEMOLD10	SNP1926	3	106.49	3.20	14.94	-6.91
WHITEMOLD10	SNP1925	3	106.49	3.34	15.67	-7.08
WHITEMOLD10	SNP3987	3	106.49	3.34	15.67	-7.08
WHITEMOLD10	SNP1050	3	106.49	3.34	15.67	-7.08
WHITEMOLD10	SNP1047	3	106.49	2.69	14.00	-6.73
WHITEMOLD10	SNP1048	3	106.49	3.34	15.67	-7.08
WHITEMOLD10	SNP1902	3	107.03	2.79	13.13	-6.49
WHITEMOLD10	SNP2826	3	122.97	3.21	14.73	-6.87
STRAW1	SNP1425	7	143.72	3.05	17.23	-0.50
YIELD8	SNP644	8	62.25	3.32	16.61	-1.54
YIELD8	SNP643	8	62.25	3.32	16.61	-1.54
YIELD8	SNP642	8	62.25	3.32	16.61	-1.54
YIELD9	SNP4246	8	14.94	2.51	10.89	-1.08
YIELD9	SNP2952	8	16.02	2.80	12.17	-1.15
YIELD9	SNP3786	8	16.56	2.60	11.66	-1.09
YIELD9	SNP3588	8	16.56	2.69	11.45	-1.09
YIELD9	SNP1706	8	19.26	2.99	13.57	-1.19
YIELD9	SNP1702	8	19.26	2.80	12.17	-1.15
YIELD9	SNP2951	8	19.26	2.80	12.17	-1.15
YIELD9	SNP1701	8	19.26	2.80	12.17	-1.15
YIELD9	SNP1708	8	20.35	2.61	11.43	-1.10
YIELD9	SNP1707	8	20.35	2.61	11.43	-1.10
YIELD9	SNP1705	8	20.35	2.99	12.75	-1.15
YIELD9	SNP2085	8	28.16	2.66	11.57	-1.14
YIELD7	SNP3543	11	21.73	2.52	13.10	1.32

[1]Primer information for SNPs available online www.beancap.org

[2]LOD: Log of odds.

[3]Proportion of the phenotypic variance explained by the QTL at peak LOD using MQM mapping with cofactor selection.

[4]Effect of substituting a single allele from one parent to another. Positive values indicate allele from AN-37 and negative from P02647.