MAPPING QTL FOR AGRONOMIC AND CANNING QUALITY TRAITS IN BLACK BEAN (PHASEOLUS VULGARIS L.)

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A THESIS

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

MASTER OF SCIENCE

Crop and Soil Sciences

2008

ABSTRACT

MAPPING QTL FOR AGRONOMIC AND CANNINGQUALITY TRAITS IN BLACK BEAN (PHASEOLUS VULGARIS L.)

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Quantitative trait loci (QTL) analysis was used to identify QTL for agronomic performance and canning quality in a recombinant inbred line (RIL) population from the cross of the black bean cultivar 'Jaguar' and the breeding line 115M. A total of 96 RILs were evaluated for seven agronomic and four canning quality traits in replicated trials at one location over four years (2004-2007) in Michigan. SSR, TRAP, SRAP, and phenotypic markers were used to create a genetic map of the population consisting of 119 loci including a locus associated with resistance to a new race of bean rust isolated in Michigan. The map consisted of 15 linkage groups spanning 460cM (38%) of the bean genome. Composite interval mapping analysis identified a total of 20 QTL for 10 traits averaged across environments, while an additional 18 QTL were identified in one or more individual environments. QTL were identified on 10 linkage groups (LG). A major QTL for seed yield was identified on LG B10. A total of 7 QTL for yield, seed size, plant height, and canned bean texture showed positive alleles from 115M. Several QTL co-localized with regions identified in previous studies while others, particularly for canning quality, were unique. Rust resistance associated with 115M was mapped to LG B4 and flanked by two TRAP markers, both at an approximate distance of 3 cM. These results support the utility of TRAP markers to tag disease resistance loci and QTL and provide a valuable source of rust resistance for future black bean cultivars adapted to Michigan.

ACKNOWLEDGEMENTS

I would like to thank Dr. James Kelly, for serving as my major professor and advising this research. I appreciate his patience and understanding over the years. His enthusiasm for plant breeding and common bean has been inspiring. I feel fortunate to have had such an outstanding mentor in all aspects of practical scientific research and professional life.

I also appreciate the members of my guidance committee, Dr. James Hancock, Dr. Dechun Wang, Dr. Ryan Warner, and Mr. Greg Varner, who applied their varied expertise to improving this research and thesis. Their advice and helpful conversations have enhanced this work.

To the members of the bean lab, your friendship and discussions over the years have been great. Your help with many aspects of my research has also been crucial and truly appreciated. Thanks for making the field and lab such an enjoyable place to work and conduct research.

I also thank my wife, April, for her faith and support throughout this season of my life. I am also thankful to my family for inspiring me to learn more about the scientific aspects of agriculture, and for their interest and encouragement.

Finally, I would like to thank the many friends I made while at Michigan State University for creating such an enjoyable community in which to work and learn.

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Chapter 1: Literature Review

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important legume for direct human consumption worldwide. Worldwide production is nearly twice that of chickpea, the second most important legume (Broughton et al., 2003). Dry beans provide a major source of protein which accounts for 20-25% of seed weight (Ma and Bliss, 1978). This major storage protein known as phaseolin has an amino acid profile that complements the deficiencies in cereal proteins. Beans are superior to cereals in terms of micronutrient content and contain many important minerals (Ca, Cu, Fe, Mg, Mn, P, Zn) and vitamins (folate) (Welch et al., 2000). These nutritional properties are a critical component of the diet for over half a billion people worldwide (Gepts, 2001).

In areas of Latin America and Africa, beans are the primary protein source in the diet of many people with a per-capita consumption of over 60kg per year in parts of eastern Africa (Miklas and Singh, 2007). Developed countries concerned with healthy diets are steadily increasing consumption (Acosta-Gallegos et al., 2007), although consumer preference for bean size, shape, and color vary widely. Sustained bean consumption has been linked with reduced cholesterol levels and a lower risk of heart disease (Anderson et al., 1984; Winham et al., 2007) and certain cancers (Hangen and Bennink, 2003).

Beans are grown on more than 14Mha worldwide. Collectively, the Americas produce 6.7MMT. Brazil is the largest producer (2.5MMT) and consumer (>10kg/yr per-capita), followed by the US (1.3MMT) and Mexico (1.0MMT) (Singh, 1999). Production practices vary based on social and ecological factors. In Latin America, with the exception of Argentina, more than half the production takes place on farms <20ha, often on small parcels of marginal land. Similar production constraints exist in South and East Africa,

where diverse assortments of beans are often intercropped on marginal soil, and significant losses due to biotic and abiotic stress are common (Broughton et al., 2003).

The farm value of the U.S. dry bean crop for 2007 was approximately $677 million with an estimated 1.15 MMT harvested from 598,429 ha for an average yield of 1736kg/ha. These figures represented an increase in overall yield and a 22% increase in value of the crop, but a decrease in area harvested compared with the previous season. In Michigan the 2007 crop was valued at $88 million, with 141,500 metric tons produced on 78,917 hectares for an average of 1800kg/ha, representing a decrease in area harvested and yield, but an increase in crop value over previous years (USDA, 2008).

Domestication

Beans (*Phaseolus* spp. L.) are among the oldest crops in the New World. Along with maize and cassava, they have been a staple for generations of people in the Americas and around the world. Beans are extremely diverse in terms of morphological variation and environmental adaptation, resulting in crops which are suited for a range of agronomic niches and consumer preferences (Broughton et al., 2003).

Common bean was domesticated from a wild legume distributed from northern Mexico to northeastern Argentina (Gepts et al., 1986). *P. vulgaris* is one of five cultivated *Phaseolus* species native to the Americas. Domestication occurred over a period of 7000-8000 years (Gepts and Debouck, 1991), and radiocarbon dating of ancient beans confirmed this process was occurring 4000 years ago (Kaplan and Lynch, 1999). This process took place independently in South America and Middle America from morphologically and biochemically distinct populations (Chacon et al., 2005).

From domestication centers in South America, Mexico, and Central America, common bean production has expanded into a range of environments. In the Americas, it occurs from 35°S to >50°N latitude and from sea level to >3000masl (Gepts et al., 1988;

Beebe et al., 1997). Beans were introduced to Africa, Asia, Europe, and Oceana during early explorations of the Americas by European traders (Gepts and Bliss, 1988). Although common bean has diverged into diverse environments, hybrids between wild and domesticated beans are fully fertile. This presents opportunity for barrier-free introgression of favorable allelic diversity into cultivated bean (Koinange et al., 1996).

During domestication, common bean was transformed from a climbing, indeterminate vine to less vigorous indeterminate vines and determinate bush habits. Sensitivity to long day photoperiod was lost; leaves, pods, and seeds increased in size; and seed colors evolved from speckled gray, brown, beige, and cream colors to brighter solid, striped, or spotted colors. Pod structure was altered from highly fibrous to less fibrous, resulting in a loss of shattering at maturity (Gepts and Debouck, 1991). Several major genes and quantitative trait loci (QTLs) that influenced these domestication traits have been identified and mapped (Koinange et al., 1996; Freyre et al., 1998; Gepts, 1999).

Gene Pools and Races

Wild populations, as well as modern cultivars, can be grouped into two major gene pools, one located in Middle America and the other in the Central and Southern Andes. Based on recent DNA sequence information, it appears that these two gene pools originated from an ancestral group in Ecuador and northern Peru that spread both north and south, resulting in the evolution of the two geographically distinct groups (Debouck et al., 1993; Kami et al., 1995). Each gene pool can be recognized by differences in seed size, seed proteins (phaseolin), allozymes, morphological traits, and molecular markers (Gepts, 1988; Beebe et al., 2000; Blair et al., 2006b). Strong evidence exists for multiple, independent domestications of some races of Middle American beans, based upon multiple chloroplast haplotypes (Chacon et al., 2005). However, all Andean beans examined share a common

haplotype, supporting the hypothesis of a single domestication before diverging into their present domesticated races.

Each gene pool has been subdivided into races based on environmental adaptation as well as plant and seed morphology. These races are recognized by their specific physiological, agronomic, biochemical, and molecular characteristics. The Middle American gene pool consists of races Mesoamerica, Durango, Jalisco, and Guatemala, while the Andean group consists of races Chile, Nueva Granada, and Peru (Singh et al., 1991a; 1991b; Beebe et al., 2000). The fourth Mesoamerican race, Guatemala, was not initially identified by Singh et al. (1991a), but later proposed by Beebe et al. (2000) as a less well defined group encompassing accessions from Guatemala and areas of southern Mexico. Members of this race possess an indeterminate climbing growth habit and small seed size most similar to race Mesoamerica but nonetheless unique.

During domestication, the genetic base of many crop species has become increasingly narrow (Papa et al., 2005). At least 16-18% of the common bean genome was influenced by selection during domestication (Papa et al., 2007). While this process eliminated many genes for undesirable traits such as dispersal of seeds or excess vegetative growth, useful genes located in close proximity to these loci were excluded from the modern cultivated gene pool. Analysis of genomic regions surrounding these major domestication genes suggests they harbor much more genetic diversity in wild beans and represent an untapped source of genetic variability waiting to be introgressed into cultivated germplasm (Papa et al., 2005, 2007). Traits present in wild populations that are absent or underrepresented among domesticated germplasm include insect resistance and increased nutritional value (summarized in Table 2, Acosta-Gallegos et al., 2007).

Although significant progress has been made in improving yield and disease resistance, breeders have increasingly relied upon crosses between genetically related elite

germplasm to develop new cultivars (Sonnante et al., 1994). This practice has increased the probability of producing improved progeny but further narrowed the genetic base of the crop. Voysest et al. (1994) reported that among a subset of 130 bean cultivars each from both a Mesoamerican race and an Andean race, over 75% of the genes present originated in that same race, and in most cases could be traced back to about 12 parental lines based on analysis of pedigrees. However, they also noted a tendency toward inter-racial crosses in more recently developed bean cultivars, suggesting breeders are making an effort to maintain or increase the genetic diversity of future cultivars.

Preserving Genetic Diversity

Vavilov (1940) described the value of collecting and preserving wild crop relatives as sources for genetic improvement for modern agriculture. This potential motivated the creation of seed banks to preserve the wild, weedy, and landrace ancestors of crops so that they would be available to future generations of plant breeders. At least 700 such collections worldwide are estimated to contain over 2.5 million accessions of wild and domesticated germplasm (Tanksley and McCouch, 1997). Recently an underground vault in Norway was built to store duplicate samples of these accessions to further protect plant genetic resources on a long term basis (Charles, 2006).

A number of core collections, relatively small subgroups representing the diversity contained in these large collections, have been assembled to facilitate the use of these resources (Logozzo et al., 2007). Other genetic resources have been conserved *in situ*, either in the wild (Debouck et al., 2008) or in some cases on farms as cultivated landraces or farmer-maintained varieties (Lioi et al., 2005; Tiranti and Negri, 2007). Gomez (2004) found differences between some Nicaraguan bean landraces conserved *in situ*, and samples of those landraces conserved *ex situ,* suggesting that the natural environment may be the preferred location to maintain these resources. Due to the highly heterogeneous nature of landraces,

diversity can be lost during successive cycles of seed renewal in environments that differ from the area where this germplasm was collected.

Utilizing Genetic Diversity

Although germplasm collections have been established, many have been underutilized (Singh, 2001). Many breeders believe that there are useful genes available that could be utilized to further improve modern cultivars, but attempts at identifying and extracting these resources have been limited. One promising example was reported by Lippman and Tanksley (2001), where six QTL (collectively accounting for 67% of the variation for fruit weight) were detected in a population derived from a single inter-specific cross between a wild tomato and the largest cultivated tomato. Although the QTL had all been previously reported, this was the first time they had been detected together in the progeny from a single cross.

In order to move useful diversity for genetically complex, quantitative traits such as yield from unadapted backgrounds into elite cultivars, there is a need for prebreeding efforts. Through prebreeding, beneficial traits can be moved into an intermediate, adapted background, which facilitates the transfer into elite material without bringing along many undesirable characteristics associated with the original unadapted germplasm (Gepts, 2005).

Kelly et al. (1998) proposed a pyramid scheme for organizing pre-breeding in bean. The top tier consists of elite by elite crosses within market class, growth habit, and maturity groupings, which ensures short term progress in yield potential. The intermediate level would involve genetically distant but adapted crosses between market classes, growth habits, maturity groups, and races, requiring more time to obtain commercially accepted cultivars. At base level, few restrictions would govern breeding activities, presenting opportunity to introgress wild or unadapted genetics requiring several crosses to achieve an adapted phenotype.

Wild Beans to Improve Yield

Wild beans have been largely untouched as a genetic resource for increasing yield (Singh et al., 1995). Wild germplasm contains alleles, especially near loci associated with the domestication syndrome, which are not represented in domesticated cultivars (Papa et al., 2007). Some of these alleles have a positive effect on key traits such as yield, and should be transferred to a cultivated background and exploited to increase yield. When working in populations derived from crosses between domesticated and wild parents, the diversity of progeny can become problematic. Due to the large variation in traits related to domestication, alleles with relatively small effect are difficult or impossible to detect (Acosta-Gallegos et al., 2007). The inbred backcross line (IBL) (Bliss, 1993) combined with the advanced backcross-QTL (Tanksley and Nelson, 1996) method offers an opportunity to overcome this barrier and identify minor loci that would otherwise go undetected. The IBL method has been used extensively at the Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia) to introgress diversity from wild beans into domesticated germplasm (Beebe et al., 2004). Progeny from these crosses have been widely tested in Mexico and Michigan where some lines have surpassed all local checks and exceeded the yield of the domesticated recurrent parent by up to 27% (Beaver et al., 2003; Kelly, 2004). These results have stimulated further research to dissect the genetic architecture underlying the increased performance of progeny derived from wild by domesticated crosses.

Quantitative Genetic Variation

Through phenotypic selection, bean breeders have made significant progress in developing cultivars with superior performance across a range of environments by selecting for agronomic traits such as yield, disease resistance, and improved plant architecture (Kelly, 2001; Beaver et al., 2003; Miklas et al., 2006). To ensure continued progress in understanding these complexly inherited traits, phenotypic selection can be complemented by

the use of molecular markers to pinpoint specific locations within the genome that condition individual genetic components of complex traits, along with their relative individual effects (Beaver et al., 2003). Information from molecular analyses, combined with phenotypic evaluation, will contribute to continued development of agronomically superior cultivars.

In crop plants, quantitative variation affects many important traits, including yield, resistance to pathogens, and seed quality (Kelly et al., 1998; Miklas et al., 2006; Posa-Macalincag et al. 2002). Therefore breeding requires a means to analyze and discern the genetic basis of these complexly inherited traits. In the early twentieth century, Sax (1923) developed concepts to study genes affecting quantitative traits. He demonstrated that quantitative variation resulted from the combined effects of multiple genes with environmental effects by investigating the co-segregation of bean seed size with Mendelian markers for seed color and pattern (Gepts, 2001). Thoday (1961; as cited by Young, 1996) suggested if the segregation of simply inherited genes could be used to detect linked QTLs, eventually all QTLs involved in complexly inherited traits could be characterized.

However, until about 1980, quantitative traits analysis remained a statistical exercise rather than a detailed analysis of individual gene effects. The assumption remained that although multiple genes contributed to the expression of a quantitative trait, their individual effects were relatively equal and allelic differences were minimal compared to the effect of the environment. The varying effects of individual loci on the complex trait were largely overlooked under this system. Using this minimal framework, substantial progress was made in better understanding the genetics controlling many complex traits and making appropriate selections based on this knowledge (Asins, 2002).

Dissecting the genetic control of complex traits requires a joint analysis of genotypic and phenotypic data. Molecular linkage mapping and subsequent analysis of genetic regions affecting quantitative traits, or QTL, has been a useful approach to determine the minimum

number of genes involved, their relative phenotypic effects, as well as linkages that may exist between traits (Koinange et al., 1996).

Modern QTL analysis entails hybridizing parents that differ significantly in one or more quantitatively inherited traits, followed by phenotyping and genotyping of the progeny. Genotypic data are used to construct a linkage map of the population, which is subsequently used as a framework to locate regions of the genome significantly associated with phenotypic variation. Adjacent markers on the linkage map delineate the approximate location of the QTL; consequently, shorter intervals between markers will result in a more precise estimate of QTL size and location while indicating a tighter linkage between the markers and the genetic locus (Collard et al., 2005).

Once the location of the QTL and tightly linked markers is determined, plant breeders can use this information to make indirect selections based on the genotypes of the markers linked to the QTL. A selection process where marker data contributes to the selection decision is called marker-assisted selection (MAS), and has contributed to more rapid introgression of favorable alleles for disease resistance and agronomic traits into improved cultivars from various genetic backgrounds (Asins, 2002; Kelly et al., 2003; Kelly and Vallejo, 2006). These selections can be made despite environmental conditions that hinder the expression and selection of the linked phenotype. The process may also be more efficient and economical than phenotypic selection (Ender et al., 2007; Yu et al., 2000b). However, genotypic selections must be verified for phenotype to eliminate false positive or negative selections due to recombination between markers and their associated QTL (Collard et al., 2005).

Application of QTL Analysis and Marker Assisted Selection

QTL analysis, coupled with MAS, is a tool increasingly used by breeders to locate genes associated with quantitative traits in plant genomes and select individuals containing desirable combinations of those genes. This approach has the ability to overcome some of the limitations encountered when selecting for quantitative traits by conventional phenotypic selection (Blair et al., 2007). Using phenotypic selection alone, it can be difficult to identify and select individuals that carry a series of different beneficial alleles influencing a quantitative trait (Schneider et al., 1997; Tar'an et al., 2003). The situation is further complicated if the desirable phenotype is masked by the presence of undesirable alleles, which often occurs when genetic materials from the wild are introgressed. Tanksley and Nelson (1996) proposed the advanced backcross QTL (ABC-QTL) analysis to introgress and identify favorable alleles from wild relatives that are masked by unfavorable genotypes. This technique was utilized by Gur and Zamir (2004) to improve yield in tomato by up 50% using a wild species as a donor of favorable alleles. Similar improvements were obtained in rice (Tanksley and McCouch, 1997) and in bean (Blair et al., 2006), demonstrating that phenotypic selection can be enhanced by MAS.

Markers & Mapping

P. *vulgaris* is a diploid species that has 2n = 2x = 22 chromosomes. The 11 chromosomes are relatively small, and have all been identified (Cheng and Bassett, 1981). Arumuganathan and Earle (1991) determined the genome size was 0.65pg/haploid genome or 635mbp, one of the smallest in the legume family. Pedrosa et al. (2003) assigned all 11 chromosomes to their respective linkage groups (LGs) using fluorescence in situ hybridization.

To determine where genes are located in the genome, molecular linkage maps based on molecular markers have been developed. These maps provide approximate locations of

individual loci relative to each other. A number of different marker types have been used for different purposes and as new marker systems became available, they have often replaced older systems that had inherent limitations. Gepts et al. (2008) recently reviewed advances in marker technology for bean.

Biochemical Markers

Weeden (1984) first described allozymes in bean as a method to differentiate cultivars based on genotype. These early biochemical markers were used to confirm the geographic distribution of the wild common bean gene pools. Singh et al. (1991a) used allozymes to definitively divide the two major gene pools of P. *vulgaris* into three races each. Debouck et el. (1993) showed that in addition to the Middle American and Andean gene pools, an ancestral gene pool exists in Ecuador and northern Peru that is distinct from the other two gene pools. Seed protein markers (phaseolin) have also been used to characterize diversity among beans and provide evidence for multiple bean domestications based on differences in electrophoretic patterns (Gepts and Bliss, 1986). These early biochemical markers were useful, although their limited genome coverage and level of polymorphism imposed limitations on their application for characterizing genetic diversity in closely related groups of beans.

Molecular Markers and Bean Linkage Maps

Molecular markers based on random variation in genomic sequences later became available and expanded the application of genetic markers in bean breeding. Randomly amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams et al., 1990) have been widely used to tag and map disease resistance genes (Kelly, 1995) and in linkage map construction. Amplified fragment length polymorphism (AFLP) markers (Vos et al., 1995) are also based on arbitrary primer sequences and have been used widely for mapping and to assess genetic diversity. A number of sequences linked to resistance genes

and amplified by RAPD or some by AFLP markers have been converted to sequence characterized amplified region (SCAR) markers for use in resistance gene pyramiding (Kelly et al., 2003; Miklas et al., 2006).

Restriction fragment length polymorphism (RFLP) markers were used as framework markers in early linkage maps, which also integrated the information from the earlier biochemical markers. Vallejos et al. (1992) constructed a linkage map based primarily on RFLP markers and estimated the size of the bean genome at 1200 cM. Freyre et al. (1998) published a consensus map of the 11 LGs of bean that integrated several previous maps (Vallejos et al., 1992; Nodari et al., 1993; Adam-Blondon et al., 1994; Jung et al., 1996, 1997; Skroch et al., 1996) based on shared RFLP and RAPD markers. This map consisted of 550 RAPD, RFLP, SCAR, isozyme, and phenotypic markers, in addition to another 500 markers in common with the other bean maps, resulting in an average distance of 1-2cM between adjacent markers (Kelly et al., 2003). Linkage maps also delineate the locations of genes for phenotypic traits such as disease and insect resistance, seed size, color, storage proteins, and pod color.

Yu et al. (2000a) developed the first 37 common bean simple sequence repeat (SSR) markers, successfully assigned 15 of them to the Freyre et al. (1998) consensus map, and determined that SSR sequences were abundant in common bean. SSR markers have an advantage of being co-dominant, PCR based which allows automation, usually multi-allelic and hyper-variable, randomly and uniformly distributed throughout the genome, and accessible to multiple researchers as published primer sequences (Yu et al., 1999).

Since the introduction of SSRs for bean in 1999, additional markers utilizing a number of different sources of sequence information have been developed (Gaitan-Solis et al., 2002; Blair et al., 2003; Yaish and Vaiga, 2003; Guerra-Sanz, 2004; Caixeta et al., 2005; Frei et al., 2005; Buso et al., 2006; Benchimol et al., 2007; Hanai et al., 2007; de Campos et

al., 2007; Grisi et al., 2007). A small portion of these markers have been mapped to the consensus map, but most have not been widely utilized for mapping. Expanding the consensus map, Blair et al. (2003a) constructed the first map of bean based solely on simple sequence repeat (SSR) markers and then integrated those markers into the Freyre et al. (1998) and Vallejos et al. (1992) maps.

Despite recent interest in development of SSR markers for common bean, the bean genome has not been saturated so other marker systems must be used to construct an efficient linkage map from genetically related mapping populations. Sequence-related amplified polymorphism (SRAP) markers were originally developed as a simple, reliable, moderate throughput, reproducible, dominant marker system for *Brassica oleracea* (Li and Quiros, 2001). These markers have been utilized in diverse crops such as potato, rice, lettuce, Chinese cabbage, rapeseed, garlic, apple, citrus, and celery. The markers are based on pair-wise combinations of 17 or 18 nucleotide long primer sequences that target genomic sequences in open reading frames and have shown equivalent genome coverage as AFLP markers in *Brassica* spp. (Li and Quiros, 2001).

Hu and Vick (2003) developed the target region amplification polymorphism (TRAP) technique for use in *Helianthus annuus.* Miklas et al. (2006b) suggested tagging and mapping common bean genes involved in disease resistance using TRAP markers. TRAP markers use two primers of 18 nucleotides, one designed from expressed sequence tag (EST) sequence information, and the other of arbitrary sequence with either an AT- or GC-rich core targeted to an intron or exon, respectively (Hu and Vick, 2003). These markers have been used successfully for a number of crops and purposes, including fingerprinting of lettuce cultivars (Hu et al., 2005), gene tagging in sunflower (Rojas-Barros et al., 2005), and QTL mapping in a RIL population of wheat (Liu et al., 2005). In wheat, this marker system was

an efficient and robust technique to rapidly generate markers distributed across the genome and proved as useful as SSR markers for assigning linkage groups to chromosomes.

Mapping and Tagging Genes and QTL

The development of a bean consensus map has facilitated comparison between individual mapping and gene tagging studies. For example, clusters of resistance genes for bean rust, anthracnose, common bacterial blight, and white mold have been detected on LGs B1, B4, B7, and B11 (summarized by Miklas et al., 2006a). Tagging these genes with markers has allowed for indirect selection for disease resistance in both domestic and overseas breeding programs. In addition to tagging major resistance genes, marker assisted techniques have increased understanding of complexly inherited traits such as stress tolerance (Schneider et al., 1997), root architecture (Beebe et al., 2006), and quantitative disease resistance (Park et al., 2001; Miklas et al., 2007) through QTL analysis. Another group of genes associated with the domestication syndrome of common bean that influences photoperiod insensitivity, lack of seed dormancy, seed color patterns, and increased seed size, were identified and mapped to LG B1 (Koinange et al., 1996). A summary of the populations used for tagging and mapping a variety of genes and QTL between 1992 and 2004 was recently compiled by Miklas and Singh (2007). Mapping studies will continue to be useful to identify additional genetic diversity from wild or exotic germplasm introgressed into domesticated beans.

Introgression of wild bean germplasm into cultivated backgrounds has received considerable attention from scientists at CIAT, located in Cali, Colombia (Beebe et al., 2003). Based on information available from molecular diversity analyses of diverse bean germplasm (Tohme et al., 1996), a core collection was established and unique wild accessions were incorporated into a breeding program in order to transfer genetic diversity into a cultivated background for further analysis of desirable variation. Blair et al. (2006a)

identified 13 QTL associated with a wild Colombian bean that had a positive effect on plant height, yield and yield components in a population derived from a cultivated by wild cross. Guzman-Maldonado et al. (2003) identified 14 QTL and determined that a Mexican wild bean contributed alleles that increased seed mass, content of Ca, Fe, Zn, and tannins in the seed when crossed with a cultivated bean.

Studies using diverse genetic backgrounds from both gene pools of bean have identified QTL for varied agronomic traits. Park et al. (2000) identified QTL for seed size and shape. Tar'an et al. (2002) identified 14 QTL for yield and other agronomic traits. Beattie et al. (2003) used QTL analysis to identify 21 genomic regions associated with agronomic and architecture traits of a bean ideotype. Checa and Blair (2008) examined climbing ability and identified 23 QTL for growth habit components. Tsai et al. (1998) identified 6 QTL for nodule number involved in N-fixation.

Although QTL for disease resistance have shown practical application for MAS (Miklas et al. 2006a), application of QTL studies for other polygenic traits has been limited (Blair et al., 2007.). Complex agronomic traits targeted by QTL studies are often controlled by many minor loci, rather than a few regions with major effect, which increases the investment required to implement routine MAS.

The limited genome coverage of molecular maps in narrow intra-gene pool crosses in bean typically used to generate elite germplasm further restricts the ability to detect QTL related to major economic traits. However, markers can be useful in breeding programs even if the application is associating a particular phenotype with a trait that can then be targeted for phenotypic selection. This approach has been used to study nutrient uptake characteristics of different root structures and determine which root features should be selected to increase the efficiency of nutrient uptake in phosphorous deficient soils (Beebe et al., 2006; Tsai et al., 1998; Yan et al., 2005).

Disease Resistance

Anthracnose

A number of diseases reduce the productivity of common bean, so an important part of any breeding program involves resistance breeding. Among the diseases affecting bean production, anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cav., is considered the most serious disease of common bean worldwide (Kelly and Vallejo, 2004). This status is largely related to the seed-borne nature of the disease, and highly variable pathogenicity. Common bean and *C. lindemuthianum* co-evolved, leading to both Andean and Middle American pathogen groups (reviewed by Pastor-Corrales, 2004). This variability is classified by race with an internationally recognized binary code based on the disease reaction of 12 differential host cultivars that each carry a binary code number from 1 to 2048 (Pastor-Corrales, 1991). Under this system, a number is assigned to each virulent race based on the summation of the binary numbers of those differential cultivars that are susceptible to the race.

Genetic resistance is the most effective management strategy for dealing with bean anthracnose. Resistance to anthracnose is conferred by twelve major independent genes, denoted *Co-1* to *Co-13* (*Co-3/Co-9* are allelic), and follows the gene-for-gene theory. All but *co-8* behave as dominant genes, and various authors have demonstrated *Co-1, Co-3*, and *Co-4* to be part of an allelic series at three different loci (Table 2, Kelly and Vallejo, 2004; Goncalves-Vidigal et al., 2009). *Co-1*and *Co-12* are the only resistance genes of Andean origin, while the others originated in Middle American germplasm (Kelly and Vallejo, 2004; Goncalves-Vidigal et al., 2008). Historically, various letters have been used to denote these different resistance genes, but those designations have since been replaced with the *Co* symbol followed by a number, as proposed by Kelly and Young (1996).

Common Bean Rust

Another significant disease affecting bean production is common bean rust, caused by one of the most pathogenically variable rust fungi, *Uromyces appendiculatus* (Pers:Pers) Unger (Stavely et al., 1994). Pathogenic races of *U. appendiculatus* in common bean were first reported in 1935 (Harter et al., 1935; as cited by Stavely et al., 1994). Due to the variability of this pathogen, breeding for genetic resistance to rust has been complicated by the rapid breakdown of major resistance genes deployed in new cultivars. Efforts to prolong the life of currently known resistance genes include pyramiding of multiple genes and incorporation of different resistance characteristics (specific, slow rusting, reduced pustule size, age-dependent resistance, and pubescence) (Miklas et al., 2005).

The effectiveness of this strategy was confirmed in Honduras where a cultivar with single gene resistance succumbed to rust infection but another cultivar with additional resistance genes did not become infected with a newly emerging rust pathotype (Mmbaga et al., 1996). Specific races of rust exhibit patterns of virulence that reflect the division between the Andean and Middle American bean gene pools, suggesting a history of co-evolution between the rust pathogen and its host (Pastor-Corrales, 2004; Acevedo et al., 2008). Molecular analysis of the pathogen also confirms this pattern (Araya et al., 2004). Therefore the strategy to manage resistance to a wide range of rust races has been to pyramid major Ur-genes with overlapping resistance spectrums from both gene pools to provide durable rust resistance across a range of environments (Miklas et al., 2005).

To classify the variability of the rust pathogen, Steadman et al. (2002) proposed a new differential series of twelve bean cultivars, six each from the two gene pools. Each cultivar in this series was assigned a binary value, with the two gene pools considered separately. The sums of the binary values of the susceptible cultivars, determined for each of the two gene pools, are used to assign a race number to an unknown isolate of the pathogen.

This classification system better reflects the gene pool differences of rust isolates and resistance genes compared with the previously implemented differential series (Stavely et al., 1983).

Nine named resistance genes (*Ur-3, Ur-4, Ur-5, Ur-6, Ur-7, Ur-9, Ur-11, Ur-12,* and *Ur-13*) and four unnamed genes (one each in BAC6 (Ur-BAC6) and 'Ouro Negro' (Ur-ON), two in 'Dorado' (Ur-Dorado-53, Ur-Dorado-108) have been characterized, tagged with RAPD or SCAR markers, and mapped to five linkage groups (reviewed by Miklas et al., 2006a). Recently, Pastor-Corrales et al. (2008) tagged and mapped an additional unnamed gene from PI260418 to LG B4 that confers resistance to all but one known race of *U. appendiculatus*.

Based on these results and additional inheritance studies, it also seems that rust resistance genes are more clustered within the genome than anthracnose resistance genes (Miklas et al., 2005; 2006). Some rust resistance genes have been characterized as clusters of tightly linked loci as with *Ur-5* which is inherited as a complex of single dominant genes linked tightly in coupling (Stavely, 1984). *Ur-3,* which conditions slightly different reactions to the rust pathogen depending on the resistant cultivar used as a source, may also consist of a similar complex block of tightly linked genes (Miklas et al., 2006a).

Furthermore, *Ur-5* appears to be in close proximity, if not linked to Ur-Dorado-108 resistance (Miklas et al., 2000). Ur-ON is independent of these genes, but also resides on the same region of B4 (Alzate-Marin et al., 2004). A similar cluster resides on B11, where *Ur-3* and *Ur-11* are linked (Stavely, 1998), and Ur-Dorado-53 was mapped to the same region (Miklas et al., 2000). *Ur-6, Ur-7,* and Ur-BAC6 reside nearby on B11, but independent of the *Ur-3/Ur-11* cluster (Miklas et al., 2006a).

In addition to clustering of Ur-genes, resistance genes for anthracnose and rust co-localize by gene pool of origin at several genomic locations. The Andean genes *Co-1* and

Ur-9 co-localize on B1, while Mesoamerican genes *Co-3/Co-9*, *Ur-5*, *Co-10*, and Ur-Dorado-108 co-localize on B4. Additional Mesoamerican genes *Co-2*, *Ur-3*, Ur-11, and Ur-Dorado-53 co-localize on B11 (Miklas et al., 2006a). These associations suggest a mechanism such as duplication of ancestral gene sequences may have lead to these resistance gene clusters. Geffroy et al. (1999) examined the molecular basis of the genome at the B4 cluster and found the region was characterized by leucine-rich-repeats (LRRs) and possessed 11 resistance gene analogs, supporting the hypothesis of ancestral gene duplication and divergence at complex resistance clusters.

To date, all rust resistance genes characterized are dominantly inherited. Genes identified from the Mesoamerican gene pool include *Ur-3, Ur-5, Ur-7, Ur-11*, Ur-Dorado-53, Ur-Dorado-108, Ur-ON, and Ur-BAC6. These genes have conferred broader resistance to different rust races than those from the Andean gene pool, which include *Ur-4, Ur-6, Ur-9, Ur-12*, and *Ur-13*. (Kelly et al., 1996; Miklas et al., 2006a).

In temperate production areas of North America, *Ur-3* has shown impressive durability against the highly variable pathogen *U. appendiculatus* (Singh, 2005). However, this resistance could inevitably break down in the future, so additional information about relationships among resistance genes and more precise map locations will be needed to breed future rust resistant cultivars (Kelly et al., 2003).

Processing Quality

In addition to the agronomic traits, seed quality traits are also scrutinized by bean breeders. New cultivars must possess acceptable color, texture, and visual appearance when canned. A favorable combination of these traits is critical in determining whether a cultivar will be accepted by consumers (Hosfield and Uebersax, 1991). Commercial bean canners are constrained by these expectations, and additionally require beans with rapid, uniform hydration and a high water holding capacity. These characteristics ensure an efficient

canning process and result in increased washed-drained weight, therefore increasing processor yield (Hosfield, 1991). Cultivars that consistently fail to maintain a desirable color, texture, and visual appearance after the canning process may be discarded despite their agronomic merits.

Color

Consumers have specific preferences about the color and appearance of canned beans (Hosfield, 1991). Pigments in the seed coat of beans determine the absorption and reflectance of different wavelengths of light. During canning these pigments, especially anthocyanins, leach out of the bean and into the brine, which results in black beans that appear brown and unappealing (Bushey et al., 2000). Color of dry or canned beans can be measured on the Hunter L-scale, where 1=pure black and 100=pure white using the Hunter Lab Color and Color Difference meter (Hunter Laboratories, Reston, VA).

Texture

Texture is measured to quantify the consumer perception of chewing the cooked bean product (Ghaderi et al., 1984). This attribute is measured using a shear press in terms of kg force applied to a 100g sample of canned beans at a constant rate (Hosfield and Uebersax, 1980). An increased force required to shear a sample of beans corresponds to increased bean firmness (Bolles et al., 1990). A desirable texture for black beans is 45-75 kg force, and samples with texture measurements beyond this range may be perceived when chewed as being too soft or firm (BIC, 2008).

Visual Appearance and Washed-Drained Weight

Visual appearance is a subjective rating that considers the sum of individual quality components such as color, clumps, and splits, as well as the starchiness and consistency of the brine. Visual appearance provides a general index of a cultivar's suitability for commercial canning referenced to cultivars with demonstrated quality attributes (Hosfield

and Uebersax, 1984). Visual appearance correlates positively with texture, but negatively with washed-drained weight in navy beans (Walters et al., 1997). Although high washed-drained weight increases processor yield, the volume of canned beans produced from a given dry weight, the negative correlation indicates increasing this value excessively may decrease consumer acceptance.

Inheritance of Canning Quality Components

Each individual component of canning quality is moderately to highly heritable, but behaves in a complex, quantitative manner (Hosfield et al., 1984; Wassimi et al., 1990; Walters et al., 1997). In addition to this complexity, environmental effects such as location (Ghaderi et al., 1984; Shellie and Hosfield, 2001) or year (Hosfield et al., 1984) interact with individual components of canning quality in some studies while others have shown insignificant interactions (Wassimi et al., 1990). The effect of location or year may influence these components more than genotype in some seasons (Walters et al., 1997).

Due to the inherent environmental effects associated with evaluating canning quality traits, MAS for individual components has been proposed. This technique has been used successfully to improve disease resistance and abiotic stress tolerance (Kelly et al., 2003; Miklas et al., 2006). Previous attempts to identify QTL associated with canning quality traits have shown varied results. Walters et al. (1997) identified a group of RAPD markers associated with visual appearance, texture, and washed-drained weight in navy bean, but also found many of these associations were location and population specific, limiting their widespread use in breeding programs.

Posa-Macalincag et al. (2002) later screened two populations of red kidney beans with the same RAPD markers, but could not significantly associate them to any quality trait. Instead, two different QTL were detected, each associated with both visual appearance and splitting, and in different genomic regions than the markers identified by Walters et al.

(1997). These QTL were also population and environment specific. One QTL was located on B8 of the bean core map, which has been previously been associated with seed traits including the C locus for seedcoat pattern (McClean et al., 2002), the R gene for dominant red seedcoat pattern (Miklas et al., 2000; Bassett, 1998) and QTL for seed size and shape (Park et al., 2000). The other QTL was detected for the same traits in a different population, but on a different linkage group not aligned with the core map. These results suggest population, environment, and gene pool specificity of markers associated with seed quality traits, which underscores the difficulty in identifying reliable markers that are useful across a wide range of genetic backgrounds and seed types.

Indirect Screening Methods for Canning Quality

Black beans are especially prone to loss of seed color during the canning process and may appear brown rather than black, making them visually unappealing to consumers. Current canning methods require a minimum of three years to generate a sufficient quantity of seed before the first evaluation can be made for canning quality (Bushey and Hosfield, 2007a). Limited work has been undertaken to develop an informative early-generation screen to allow processing evaluation at an earlier generation in the breeding process. Ruengsakulrach et al. (1991) approached this problem both directly by canning a smaller sample (28.5g solids) and indirectly by correlating pasting torque values of whole bean flour (2g sample) to shear texture values. Those methods were both successful in predicting processing quality of later generations. Lu et al. (1996) examined the chemical composition of navy beans and found a significant correlation between soluble-pectin content measured in a small quantity of seed and visual score of a much larger canned sample of the same variety. Bushey and Hosfield (2007b) soaked a small quantity of black beans in a hot brine to simulate the blanching that occurs prior to canning, and then measured the color of the brine to predict color loss during canning. Although several of these methods showed good

correlation to canned bean quality, they still require a substantial investment of time and skill to complete, and have not been adopted by the bean community.

Conclusion

After review of previous work related to common bean agronomic and quality traits, the present study was undertaken to further dissect key economic traits by studying quantitative variation for yield and canning quality traits in a recombinant inbred line population of black beans. The goal of this work was to identify genomic regions associated with these traits that could be utilized by bean breeders working to enhance yield while maintaining canning quality of future black bean cultivars.

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Chapter 2: Identification of QTL for agronomic and canning quality traits in a black bean population

Abstract

Quantitative trait loci (QTL) analysis was used to identify QTL for agronomic performance and canning quality in a recombinant inbred line (RIL) population from the cross of the black bean cultivar 'Jaguar' and the breeding line 115M. A total of 96 RILs were evaluated for seven agronomic and four canning quality traits in replicated trials at one location over four years (2004-2007) in Michigan. SSR, TRAP, SRAP, and phenotypic markers were used to create a genetic map of the population consisting of 119 loci including a locus associated with resistance to a new race of bean rust isolated in Michigan. The map consisted of 15 linkage groups spanning 460cM (38%) of the bean genome. Composite interval mapping analysis identified a total of 20 QTL for 10 traits averaged across environments, while an additional 18 QTL were identified in one or more individual environments. QTL were identified on 10 linkage groups. A major QTL for seed yield was identified on B10 while B05 contained the greatest number of independent loci. A total of 7 QTL for yield, seed size, plant height, and canned bean texture showed positive alleles from 115M. Several QTL co-localized with regions identified in previous studies while others, particularly for canning quality, were unique.

Introduction

All breeding programs share a common goal to develop high yielding cultivars with desirable agronomic and quality traits (Brick and Grafton, 1999). However, genetic improvement of yield potential in bean cultivars has been lower than in other crops (Nienhuis and Singh, 1988; Kelly et al., 1998). Improving yield requires breeding for the interrelated effects of growth habit, seed size, maturity, and gene pool (Kornegay et al., 1992), while the end result, improved cultivars, must fit the constraints of a particular production system to be accepted by the marketplace (Kelly, 2001).

Bean breeders have used varied selection strategies to adapt basic breeding methods for increasing yield. Recurrent selection was successful in generating an upright type II pinto cultivar (Kelly and Adams, 1987) and generally increased yield potential in other studies (Ranalli et al., 1991; Ramalho et al., 2005). Selection based on an individual yield component was not successful (Nienhuis and Singh, 1988), likely restricted by yield component compensation (Adams, 1967). Early generation yield testing (EGT) can be effective (Singh et al., 1990), although the resources necessary for implementing EGT for yield may limit its application in most breeding programs (Kelly et al., 1998). Singh (1994) proposed gamete selection for the simultaneous selection of multiple traits, although the single seed descent (SSD) method resulted in more lines with more desirable combinations of traits (Singh, 1997).

Other approaches to breeding for yield in bean were conceptualized and implemented successfully. Adams (1973) proposed breeding for an ideotype. Wallace et al. (1993) proposed breeding for physiological efficiency by simultaneously selecting for the interrelated traits biomass, harvest index, and maturity. Singh (1992) suggested specific combining abilities should guide breeding decisions. Beebe et al. (2008) showed that

breeding for abiotic stress tolerance also improved harvest index and increased yield in favorable environments.

In addition to manipulating the diversity present in the cultivated gene pool, breeders have targeted wild germplasm as a source of additional favorable alleles. Effective utilization of wild germplasm requires the efficient introgression of genetic diversity into an adapted growth habit that can be grown and evaluated across a range of environments (Kelly, 2000). Historically, breeders have struggled to uncover useful alleles masked by undesirable traits such as climbing growth habit and photoperiod sensitivity in wild beans. Knowledge of the domestication syndrome, a relatively small group of loci controlling a large proportion of the differences in growth habit, seed/pod traits, and photoperiod sensitivity between wild and cultivated bean (Koinange et al., 1996), has led to renewed hope for capturing favorable variation from the wild. The proliferation of molecular markers linked to domestication traits has also made the process more attainable in recent years.

Breeding techniques such as the inbred-backcross line method (IBL) (Bliss, 1993) have been especially well suited for transferring genetic diversity from wild beans into an adapted background. The Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia) has extensively utilized IBL to introgress diversity from wild beans of Colombian origin into domesticated germplasm (Beebe et al., 2004). Molecular methods were used to identify wild beans that were unique from the Middle American, Andean, or northern Andean groups of origin (Tohme et al., 1996). One of these wild beans, G24423, was crossed with the Mexican cultivar 'Tacana' (Lopez-Salinas et al., 1997) to establish an inbred backcross BC_2 population that closely resembled the cultivated parent while lacking the undesirable characteristics of the wild parent. G24423 possesses a type IV, indeterminate-climbing growth habit and small seed size (9g per 100 seed) typical of wild beans (Beebe et al., 2001). The IBL population that resulted from these crosses at CIAT was first evaluated in Mexico.

One $BC_2F_{4:7}$ line yielded 5790 kg/ha in Michigan, which surpassed all local checks and exceeded the yield of the domesticated recurrent parent by 27% (Beaver et al., 2003; Kelly, 2004). The same high yielding line, 115M, continued to perform well in the 2004-06 national cooperative dry bean nursery trials, producing the top mean yield in the black bean market class across 12 locations (Hang, 2004; 2005; 2006). These results encouraged further research to dissect the genetic architecture underlying the increased performance of progeny derived from wild by domesticated crosses.

Quantitative trait loci (QTL) analysis is a useful tool for exploring the genetic control and variation of complex traits. QTL are defined as regions of the genome statistically associated with phenotypic variation of a quantitative trait (Doerge, 2002). Identification of QTL requires the construction of a genetic linkage map in a population segregating for traits of interest along with phenotypic data for the traits. Identification and mapping of QTL provides a starting point for marker assisted selection (MAS), which can be utilized to improve traits with low heritabilities or those that are difficult or expensive to measure using direct phenotypic selection (Collard et al., 2005). QTL analysis can also provide a method to locate genes of interest for future fine mapping, validation, or map-based cloning studies (Li et al., 2006; Liu et al., 2008).

Past QTL studies in bean have examined diseases, insects, and abiotic stresses that limit yield. To date, QTL analysis for disease resistance has been the focus of extensive research, and MAS based on these QTL studies has been widely implemented in breeding for resistance to bean golden mosaic virus and common bacterial blight (Miklas et al., 2006). Several studies have examined agronomic traits contributing directly to yield potential, either by measuring total yield or its individual components such as plant height, seeds per pod, pod number, or seed size (Beattie et al., 2003; Tar'an et al, 2002; Blair et al., 2006). Although

these studies have identified some similar QTL, many of the results have been unique to specific populations or environments. These results underscore the difficulty of defining the genetic elements contributing to complex traits such as yield and suggest that further studies are warranted.

In addition to high yield, acceptable canning quality in cultivars is a trait valued by consumers, bean processors and plant breeders (Posa-Macalincag et al., 2002). Consumers desire canned beans that are visually appealing, with a color and texture that are pleasing to the palate following processing. Processors seek to provide bean products that satisfy these requirements, but are also concerned with the logistics of efficiently processing beans. Therefore they desire beans with a durable seed coat that will hydrate efficiently and uniformly during blanching and have a high water holding capacity that increases processor yield (Walters et al., 1997). Plant breeders are faced with the challenge of providing bean cultivars that address quality standards from both of these perspectives.

Components of canning quality are quantitatively inherited and exhibit a continuous range of phenotypes (Hosfield et al., 1984; Walters et al., 1997; Posa-Macalincag et al., 2002). Consequently, developing cultivars that possess a balance of these components that collectively contribute to acceptable or superior canning quality requires constant evaluation at all stages of the breeding process. Typically, breeders invest 3 or more years in early generation line development before initial quality evaluations are made and inferior lines can be eliminated. This delay not only adds time and cost to developing new cultivars, but it also limits the number of lines that can be reasonably evaluated. Due to the difficulty and expense associated with selecting for canning quality, breeders would benefit from alternative selection methods that can be used confidently to select for superior canning quality (Walters et al., 1997).

A number of selection strategies have been suggested or implemented based on a limited amount of previous research in this area. Wassimi et al. (1990) suggested recurrent selection might be the most effective means of combining desirable characteristics, while Walters et al. (1997) and Posa-Macalincag et al. (2002) revealed potential for MAS of QTL in guiding selection of some components. While these studies illustrate the potential for MAS, they also suggested some limitations due to population or gene pool specificity of some markers. Indirect phenotypic selection methods have been considered for traits correlated to canning quality based on studies by Bushey and Hosfield (2007), Lu et al. (1996), Ruengsakulrach et al. (1991) and Shellie and Hosfield (1991). These methods evaluated various physical or chemical characteristics of a small sample of seed and correlated the results with those of traditional canning protocols. In practice, these early generation selection methods have not been widely utilized, suggesting the need for continued research in this economically important area of bean breeding.

The objectives of the current study were: 1) Develop a linkage map utilizing a population of 96 $F_{4:5}$ RIL individuals derived from the cross 'Jaguar' by 115M. 2) Collect phenotypic field data over four seasons to conduct QTL analysis of yield and other agronomic traits. 3) Measure color, texture, visual appearance, and washed-drained weight to conduct QTL analysis of canning quality traits in the same RIL population.

Materials and Methods

Plant Material

'Jaguar' (Kelly et al., 2001) and the breeding line 115M (CIAT) were used as parents to develop 96 $F_{4:5}$ recombinant inbred lines (RILs). The initial cross was made in 2001 and advanced to the F_2 generation in the Michigan State University greenhouse. The F_2 family was planted at the Saginaw Valley Bean and Beet Research Farm in 2002, where 96 plants were randomly chosen to establish the RIL population through single seed descent. 'Jaguar'

is a black bean cultivar adapted to Michigan growing conditions. 115M was selected for its high yield potential from an inbred backcross line (IBL) population developed at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia (Kelly, 2004; Acosta-Gallegos et al., 2008).

115M was developed by the inbred-backcross technique by crossing 'Tacana' (Lopez-Salinas et al., 1997) and the wild bean G24423, followed by two backcrosses to the recurrent parent 'Tacana'. This IBL population was phenotypically very similar to the recurrent parent, and 46 of the IBL were evaluated in Michigan in 2000. From this field trial, four lines, including 115M, were selected that significantly exceeded the yields of 'Tacana', as well as local checks in Michigan (Kelly, 2004). 'Tacana' is a black bean cultivar from Mexico. G24423 is a wild bean from Colombia originally selected for its unique molecular marker pattern (Tohme et al., 1996).

Field Trials

To investigate the agronomic potential of the 96 RILs, the population, along with 'Jaguar', 'Tacana', 115M, and the commercial check cultivar 'T-39', were evaluated at the Saginaw Valley Bean and Beet Research Farm from 2004 to 2007. Plots consisted of four rows 6.4M in length, with 0.5M row spacing. They were organized in a 10 x 10 lattice with three replications. Standard agronomic practices were followed to ensure adequate crop growth and development. Data were collected for days to flower, plant height, lodging, days to maturity, and overall agronomic desirability. Yield and 100-seed weight data, standardized to 18% moisture, were collected by direct harvesting 4.6m of the middle two rows of each plot.

Canned Bean Evaluation

The population was also evaluated for canned bean color, texture, visual appearance, and washed-drained weight. Color measurements were recorded as a luminosity (L) value on the Hunter LAB scale using a LabScanXE (Hunter Laboratory, Reston, VA) where 1=black and 100=white. Texture measurements were made with a Kramer Shear Press (Food Technology Corp., Sterling, VA). For each genotype, two 100g samples taken from a single can of thermally processed beans were tested using the bean processing methodology posted on the Bean Improvement Cooperative website (BIC, 2008). Visual appearance was subjectively rated on a 1=undesirable to 7=desirable scale by a group of panelists. Washed-drained weight was determined as the weight of the entire canned bean sample rinsed under cold water and allowed to drip dry for 2 minutes on a standard number 8 (2.36mm) sieve. Data was collected from beans that were grown and canned during the years 2005-2007.

DNA Isolation and Molecular Marker Analysis

The RIL population and parents were grown in the greenhouse and DNA was extracted from young trifoliate leaf tissue bulked from three to four individual plants per genotype using a modified CTAB method (Haley et al., 1994). DNA concentrations were determined with a fluorometer (Hoeffer DyNA Quant 200, San Francisco, CA) according to the manufacturer's procedure and adjusted to 40 ng μl^{-1} for use in PCR. Molecular markers screened for polymorphisms between the parents 115M and 'Jaguar' included 444 SSR, 64 SRAP, 220 TRAP, and 7 SCAR markers. Those that were polymorphic between parents were used to genotype the population.

SSR Markers

Amplification reactions were performed with 1 µl of DNA diluted to 40 ng μl^{-1}, 1.0µl of (2mM) primer, 0.2µl (1U) of Taq polymerase, 0.6µl (50mM) $MgCl^2$, 2.0µl (10x) PCR

buffer, 0.8µl of a 5mM mix of dNTPs, and 14.4µl sterile distilled water. PCR was conducted in a 96 well PTC-100 Programmable Thermal Controller (MJ Research, Inc., Waltham, MA) programmed for 1 cycle of 5 minutes at 94°C, followed by 30 cycles of 1 minute at 94°C, 1 minute at 47°C, and 1 minute at 72°C, and a final extension step at 72°C for 5 minutes. Prior to loading on gels, 8µl of formamide loading buffer was added to each sample, which was then denatured for 5 min. at 94° C. PCR products were separated on 6% denaturing polyacrylamide gels in 0.5x TBE buffer, electrophoresed on Sequi-Gen GT Sequencing Cells (Bio-Rad Laboratories, Hercules, CA) at a constant power of 1800W for approximately three hours, and silver stained with a Silver Sequence kit (Promega, Madison, WI) according to the manufacturer's procedure for viewing.

SRAP and TRAP Markers

Amplification and electrophoresis on agarose gels was performed as described by Terpstra et al. (2006) for most SRAP and all TRAP markers. The remainder of the SRAP markers were electrophoresed and viewed as described above using polyacrylamide gels.

Phenotypic Markers

Segregation for resistance to race 73 of *Colletotrichum lindemuthianum*, the causal agent of anthracnose, and race 3:22 of *Uromyces appendiculatus* were assayed in the RIL population following the methods of Kelly et al. (1994) and Stavely (1983).

Data Analysis, Linkage Map Construction, and QTL Analysis

Analysis of variance for all traits in a given year and a combined analysis as a randomized complete block design (RCBD) across years were performed with Proc GLM in the Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC). The mean values for each trait across years were used to calculate Pearson correlation coefficients among traits.

Linkage analysis was performed on genotypic data using JoinMap 3.0 (Van Ooijen and Voorrips, 2001). The Kosambi mapping function was used, which assumes the existence of interference that is negatively related to recombination frequency. A minimum logarithm of odds (LOD) threshold of 3.0 and recombination frequency smaller than 0.300 was used to divide the 181 markers into linkage groups, determine marker order, and calculate relative map positions. LOD scores are = log (L1/L0), where L1 is the likelihood for the alternative hypothesis and L0 is the likelihood of the null hypothesis. A LOD score of 3 means the alternative hypothesis is 1000 times more likely than the null hypothesis. Linkage groups were identified and named according to the core reference map (Freyre et al., 1998) based on microsatellite map locations previously assigned in Blair et al. (2003a) and Grisi et al. (2007). Remaining linkage groups were anchored by mapping one or more markers in a subset of the BAT93/JaloEEP558 RIL population.

QTL analysis was performed for the combined environment using the mean for each of the 11 traits across the four seasons for each line, and separately for each individual environment using the mean for each line in the respective year. Windows QTL cartographer version 2.5 (Wang et al., 2007) was used to identify QTL for days to flower, plant height, lodging, maturity, overall desirability, seed yield and 100-seed weight, in addition to canned bean color, texture, visual appearance, and washed-drained weight. The Composite Interval Mapping (CIM) function set to a window size of 10cM, 5 background markers, 2cM walk speed, and a forward and backward regression model was used to identify QTL. Significant QTL for individual traits were determined by the location of the peak LOD score at a genome wide empirical threshold of p=0.05 after 1000 permutation tests (Churchill and Doerge, 1994). Linkage maps and QTL were displayed using Mapchart v2.2 (Voorips, 2002).

Results

Field Trials

Mean squares for genotype and environmental differences were significant ($p<0.0001$) for all seven agronomic traits measured (Table 2.1). Significant genotype by environment (GxE) interaction was detected for all traits except days to flowering. With the exception of seed weight, all other agronomic traits were significantly correlated with three or more other traits (Table 2.2). Yield was positively correlated with days to flowering, lodging score, and plant height. Desirability score was inversely correlated with days to flowering, maturity, lodging, and height. Days to flowering, maturity, lodging score, and plant height were all positively correlated with each other.

Although significant differences were observed in the combined analysis across all four years on an entry mean basis for all seven traits across the population (Table 2.3), the means for the parents were significantly different only for 100-seed weight, maturity, lodging score, and desirability. All traits showed transgressive segregation and nearly normal distributions (Figure C.1). There were two significant differences between 115M and its recurrent parent 'Tacana', 115M yielded 435 kg/ha more and flowered one day later. 115M yielded significantly more, had increased plant height and desirability score, and decreased lodging score when compared to the check, 'T-39'. 'Jaguar' possessed significantly smaller seed size, lodging score, and increased plant height when compared to the check cultivar 'T-39'.

Nine lines yielded in the top 10% of the yield trial two or more years, and two lines appeared in this group all four years (Tables 2.4, 2.7). Similarly, five lines ranked among the bottom 5% of the trial for two or more years. In contrast to the consistently high yielding lines, the same line consistently yielded the least in all four years. The combined average yield for the 96 RILs was 3058 kg/ha, with a range of 2249 to 3654 kg/ha (Table 2.3).

Within individual years, yields ranged from a low of 1214 kg/ha in 2004 to a high of 4261 kg/ha in 2006, a range of 3047 kg/ha (Table 2.4). These extremes were observed in the driest and wettest years, respectively. In 2004, total precipitation from June 1 to September 30 measured 195mm, while 328mm was recorded during the same time period in 2006 (Figure 2.1).

Seed size, recorded as 100-seed-weight, varied significantly by as much as 20% (Table 2.4). With the exception of 2005, where mean seed size for the population increased significantly to 22.8g during the second driest season, average seed size varied between 19.0 to 20.4g. Both low and high yielding lines exhibited a range of seed sizes (Table 2.4), which agreed with the lack of correlation between yield and seed weight (Table 2.2).

Days to flower and maturity remained relatively constant from one year to the next (Table 2.8). However, flowering was delayed by 8-10 days in 2007, and maturity was similarly delayed. Lodging scores were the lowest in 2004, highest in 2006, and were equivalent between the high and low yielding groups. Similarly, plant height remained consistent from 2004-2007. Average desirability scores were similar between 2004 and 2005, but decreased in 2006 and 2007.

Canning Traits

Significant differences among lines in the population were observed for canned bean color, texture, and washed-drained weight. Mean squares for genotype, environment, and the genotype by environment interaction were significant at $p<0.0001$, both averaged over 2005-2007 and for each individual year, (Table 2.10). Significant differences were also observed among seasons for all population mean values of each seed quality trait (Table 2.11).

The population showed a range of variation for canned bean color, texture, visual appearance, and washed drained weight. The distributions for color, visual appearance, and washed-drained weight were normally distributed (Figure C.1) between 115M and 'Jaguar'.

The parents showed the greatest difference for texture (23.1 kg-force), and the population followed a bimodal distribution. Although a majority of the lines were normally distributed, there was a second group that more closely resembled the firmer texture of 115M. Washed-drained-weight was normally distributed.

Beans retained the most color in 2006 with a mean color (luminosity) of 14.2 and exhibited the greatest color loss in 2005 with a mean color of 17.6 (Table 2.11). The softest textures were measured in 2007 with a mean of 53.5kg-force needed to compress a 100g sample of beans to the point of catastrophic bean failure. Conversely, beans from 2005 had the firmest texture of the three seasons with a mean of 63.9kg-force. Washed-drained weight was negatively correlated to color and texture, and positively correlated with visual appearance, with a low value of 243g in 2005 and a high of 254g in 2007 (Tables 2.2, 2.11). Color was inversely related to visual appearance and washed-drained-weight.

Markers and Linkage Map

A total of 182 loci were included in the linkage analysis which resulted in 119 markers placed on the linkage map divided among 15 linkage groups for a total map distance of 460cM (Figure 2.2; Figure C.2). The number of markers per linkage group varied from 2 to 24. Markers clustered on B5, B6, and B10, while their distribution was more uniform for the remaining linkage groups. Three linkage groups consisting of a total of seven markers were not successfully anchored to one of the 11 bean linkage groups (Freyre et al., 1998), and B9 was the only linkage group not represented in the current map.

Polymorphism levels observed with the molecular markers used in this study ranged from low to moderate depending on marker type. A total of 444 SSR, 220 TRAP, 64 SRAP, and 7 SCAR markers were screened. Fifty six SSR markers (12.6%) amplified polymorphic fragments between the parent lines. Twenty one SRAP (32.8%) primer pairs amplified 42 clearly scorable fragments, while 55 TRAP (25%) produced 81 scorable fragments.

Two phenotypic markers for disease resistance were also placed on the linkage map. 'Jaguar' possesses the *Co-1* gene that resides on B1 and conditions resistance to race 73 of *Colletotrichum lindemuthianum* (Kelly et al., 2001; Vallejo and Kelly, 2008), the causal agent of anthracnose, but was susceptible to race 3:22 of *Uromyces appendiculatus*, which causes common bean rust. Conversely, 115M was susceptible to anthracnose, and possessed an unknown gene that conditioned resistance to rust race 3:22. Forty eight lines in the population were resistant to race 73 of anthracnose, while 27 were susceptible and 21 lines segregated for resistance reactions (Table C.7). These results did not fit the expected 1:1 segregation ratio for a single resistance gene in a RIL population ($p=0.0153$). Sixty three lines were resistant to race 3:22 of common bean rust, while 15 were susceptible and 18 lines segregated for both reactions. These results deviated significantly ($p<0.0001$) from a 1:1 ratio in favor of the resistant (115M) allele (Table 3.3).

The phenotypic marker for anthracnose resistance was used to anchor a linkage group to B1, which allowed the SSR IAC28 to be mapped to B1 for the first time (Figure 2.2). The phenotypic marker for rust resistance was mapped to B4, in the same region as the rust resistance genes Ur-Dorado-108 and *Ur-5* (Miklas et al., 2000). All markers on this linkage group exhibited a skewed segregation toward the 115M allele, which was consistent with the skewed phenotypic marker distribution. Mapping rust resistance is discussed further in Chapter 3.

QTL Analysis

Composite interval mapping identified 20 QTL associated with 10 traits in 13 marker intervals on 10 linkage groups when data was combined for all four environments (Table 2.12; Figure 2.2). QTL per linkage group ranged from 1 to 4, with clusters of 2 or more QTL occurring on 4 linkage groups. Individual QTL explained 7 to 22% of the phenotypic variation, and total phenotypic variation explained for a trait varied from 14% for plant

height and canned bean visual appearance to 46% for canned bean color (Table 2.12). The number of QTL per trait ranged from 1 to 4. Individual environments varied for both the total number of QTL and the number of traits for which those loci were detected (Table 2.13). In 2006, 16 QTL were detected for 10 traits, while in 2004, 11 QTL for 5 traits were identified. The total number of QTL for 2005 and 2007 were intermediate between these results, with 13 and 12 QTL identified for these environments, respectively. Eighteen additional QTL were detected in one or more single environment that were not present in the combined environment.

Yield

A single major QTL for yield that originated in 115M was identified on B10 with R^2=0.19 and an additive effect of 127 kg/ha (Table 2.12). No other significant QTL for yield were detected in the combined environment. Linkage groups B3, B5, B10, and B11 possessed significant QTL in one or more environments from 2004-2006, while none were detected in the 2007 (Table 2.13). The R^2 values ranged from 0.08 to 0.28, and additive effects varied from 41-192kg/ha. The only QTL detected from 'Jaguar' was located on B3, increased yield by 168kg/ha in the 2004 environment, and was located 20cM from a QTL detected in 2006 from 115M (Figure 2.2).

Seed Size

The alleles from 115M at loci on B6 and B11 each increased seed size by 0.3g per 100 seed and had R^2 values of 0.08 and 0.11, respectively, in the combined environment (Table 2.12). QTL identified in one or more environments were located on B5, B6, B8, and B11, controlled relatively small proportions of the variation in seed size (R^2 = 0.09-0.15), and had additive effects of 0.4-0.5g (Table 2.13). In contrast to seed yield where both parents contributed alleles with an additive effect in some environments, only alleles from 115M contributed to increased seed size.

Days to Flowering

QTL from Jaguar on B11 and from 115M on LG2 delayed flowering by 0.3d each in the combined environment (Table 2.12). No additional loci influenced this trait in any individual environment but the two QTL consistently showed equivalent effects on days to flowering in 2004 and 2006 (Table 2.13).

Maturity

In the combined environment, two alleles from 115M delayed maturity by 0.5d each (Table 2.12). These QTL resided on B5 and LG2, and both had R^2 values of 0.19. In one or more individual environments, additional QTL from 'Jaguar' on B1, B3, and B7 also delayed maturity by less than one day (Table 2.13). In 2006 and 2007, three loci accounted for 50% and 36% of the total variation in maturity.

Lodging

Two loci increased lodging score in both the combined and individual environments (Tables 2.12, 2.13). These QTL with R^2 values of 0.13 and 0.15 were associated with the 115M allele on linkage groups B4b and B6 and increased lodging score minimally by 0.2 points each. Rust resistance in 115M also mapped in the same region as the lodging QTL on the lower end of linkage group B4b, and was the marker most tightly linked to the lodging QTL in 2007.

Height

A single QTL that slightly increased plant height was detected on B5 in the combined environment and was associated with the 115M allele (Table 2.12). Additional QTL on linkage groups B3, B6, and B11 were detected in one or more environments and associated with the 'Jaguar' allele (Table 2.13). An additional QTL from 115M was detected on B6 in 2004 at a distance of 18cM from the QTL detected in 'Jaguar' in 2006 (Figure 2.2).

Desirability

Two QTL were detected for desirability score on linkage group B5 and B6 (Table 2.12). Increased desirability was associated with the 'Jaguar' allele and each locus had an additive effect of 0.2. No additional QTL were detected in individual environments, but the locus on B6 accounted for 20% of the variation for this trait in 2004 (Table 2.13).

Canned Bean Color

QTL influencing canned bean color retention in the combined environment resided on linkage groups B3, B5, B8, and B11 and collectively accounted for 46% of the variation for color. Each locus on B3, B5, and B8 decreased black color by 0.4 (increased L-value) and originated in 115M while the locus on B11 decreased color by 0.3 and originated in 'Jaguar' (Table 2.12). An additional QTL was detected on B1 in 2007, for a total of four QTL from 115M that decreased color in one or more environments (Table 2.13).

Texture

Variation for canned bean texture was associated with two regions of linkage group B1 and one region of B6 in the combined environment (Table 2.12). Together, the three QTL accounted for 42% of the variation for texture and at each locus the 115M allele increased texture by 2.0-3.6kg force. An additional QTL was identified on B11 in 2005 that increased texture by 2.9kg force (Table 2.13).

Visual Appearance

For the combined environment, a single QTL associated with the 'Jaguar' allele on linkage group B8 slightly increased visual appearance by 0.1 (Table 2.12). However, in 2006, this region was associated with the 115M allele and increased visual appearance by 0.4, and an additional QTL for this trait was also detected on B5 and associated with the 'Jaguar' allele in 2005.

Washed-Drained Weight

Washed-drained weight represented the only trait for which no stable QTL across environments were identified (Table 2.12). In 2006, three QTL were detected on linkage groups B3 and B10 (Table 2.13). On B3 and the upper end of B10, the 'Jaguar' allele increased washed-drained weight by 1.65g while on the lower end of B10 the 115M allele resulted in a similar increase (Figure 2.2).

Co-localized QTL

QTL were detected at four locations that co-localized in the genome for the combined environment. On linkage group B6 QTL that co-localized for lodging and agronomic desirability were detected. QTL for canned bean color and visual appearance resided on B8. A region of the LG2 possessed QTL for both days to flowering and maturity. A complex cluster of 4 QTL was identified on B5 for maturity, plant height, overall agronomic desirability, and canned bean color. Within this cluster, QTL for maturity and plant height were detected adjacent to each other. Agronomic desirability and canned bean color QTL co-located with each other, as well as both maturity and plant height. This cluster represents the only location where a seed quality QTL co-located with QTL for agronomic traits. Only one QTL was detected on each of the remaining linkage groups (Figure 2.2), with the exception of three QTL distributed across B11.

QTL x Environment Interactions

Significant environmental interactions (QxE) were observed for one or more QTL detected for each trait except days to flowering (Table 2.13). The proportion of QTL for a particular trait that showed an environmental interaction varied. QxE was frequently detected for yield, seed size, desirability, and canned bean color, while fewer QTL for maturity, lodging, height, texture, visual appearance, and washed-drained weight showed an environmental interaction.

Discussion

Field Trials

The evaluation of the recombinant inbred line population over four years provided the opportunity to observe these genotypes in the field under a range of environmental conditions. The four growing seasons represented a range of conditions from dry and hot environments that limited yields to years with more moderate temperatures and adequate precipitation that maximized yield potential (Figure 2.1). In 2004 and 2005, adequate soil moisture was present at planting, but was followed by below normal precipitation throughout the growing season. In 2006, growing conditions were average early in the season; July rainfall was more than twice the 30-year average, which led to increased vegetative growth, while August had a 20-day period without rain. June and July were dryer than normal in 2007, followed by above average rainfall in August.

From a breeding standpoint, these conditions provided a challenge to identify stable lines that consistently performed despite different environmental conditions. Breeding line B04431 with a 4 year mean of 3654kg/ha, significantly exceeded the mean yield of the population, 'Jaguar', and the check cultivar 'T-39', but failed to yield significantly more than the high yielding parent 115M. Several other lines also consistently produced yields above the test mean and in the top 10% of the population (Table 2.4, 2.7). Similarly, there were lines that ranged in yield depending on the year (Table 2.7), and those that consistently produced poor yields (Table 2.4). B04442 with a mean of 2249 kg/ha was consistently the lowest yielding line in the population every year.

The normal distributions and transgressive segregation provided an opportunity to identify lines that exceeded the average yield of 115M by up to 298kg/ha (Table 2.9), although this difference was statistically insignificant (LSD=321kg/ha). Although modest in comparison to 115M, which has produced record high yields, the top yielding lines in the

population represent a much larger yield advantage compared to many of the other elite breeding lines trialed during the same seasons. Conversely, eleven lines yielded significantly less than 'Jaguar', up to 799kg/ha less. These results illustrate the difficulty of generating progeny with increased yield potential, and the relative ease of recovering lines with inferior performance compared to the agronomically desirable parents.

Although significant genotype by environment interactions were present in all years, the accumulated data on these lines provided adequate information to select the best lines for use in future crosses with other elite lines. Those lines with stable yield potential across diverse environments represent useful germplasm that will be utilized to improve the yield potential of future breeding lines.

Compared to the other black bean yield trials conducted at the same site during the same years, the mean yield of the population was higher in all seasons but 2005. This difference may reflect the extended dry conditions during most of the growing season (Figure 2.1), which appeared to limit the overall yield potential of this population more than the genetically diverse lines in the standard breeding trials. In other studies, 'Tacana', the recurrent parent of 115M, has shown less tolerance for drought stress compared with other black bean cultivars despite its superior yield potential under improved growing conditions (Beebe et al., 2008). 115M appears to exhibit the same characteristic that limited the yields of the population during the dry conditions of July and August 2005. Similarly, yields for the population were also substantially less in 2004, the overall driest of the four seasons (Figure 2.1).

Correlations among traits generally agreed with those of other studies. Seed size was the only trait not correlated to any other traits, which agreed with results in a navy bean population (Tar'an et al., 2002). The same population showed positive correlations among days to flower, maturity, and plant height, but no correlation between lodging and either days

to flower or maturity was detected. The inverse relationship of desirability with days to flower, maturity, lodging, and plant height was expected since later maturity and increased lodging decrease desirability rating.

Canning Traits

The variation observed among years for processed bean characteristics of the population agrees with previous studies that have shown seed quality traits vary widely based on environmental conditions among growing seasons (Posa-Macalincag et al., 2002; Walters et al., 1997). Differences in weather patterns such as air temperature or available moisture at critical times during the development of the bean crop have been implicated as contributing to the large range in bean quality traits from one season to the next. However, genetic differences still account for a significant portion of this variation in both the current population and previous studies (Shellie and Hosfield, 1991; Posa-Macalincag et al., 2002).

The inverse relationship observed between texture and washed drained weight agrees with studies in both navy and black beans (Wassimi et al., 1990), and in three navy bean populations (Walters et al., 1997). This relationship is logical, since higher washed-drained weight results from more water entrainment in the bean, which makes the bean easier to break apart, or exhibit a softer texture.

'Jaguar' and 115M differed substantially in canning quality traits, particularly for texture. These contrasts led to a distribution of progeny, and made this population especially suitable for detecting QTL for canning quality (Figure 2.2). Therefore more variation was explained by the analysis of canning quality traits than for that of the agronomic traits.

Despite the variation for these traits, most lines appeared brown and washed out with considerable loss of bean integrity after canning. Unfortunately, some of the highest yielding lines were among the least desirable based on canning traits. Due to the importance of canned bean visual appearance to commercial processors and consumers, these lines failed to

meet minimum quality standards when compared visually with check cultivars. The generally undesirable canned bean appearance of members of the population that closely resembled that of 115M was unexpected at the beginning of the study since 'Jaguar' possesses acceptable canning quality traits.

Although every effort was made to treat samples the same in all years, any minor changes to the canning procedure likely influenced the quality attributes measured in this study. The only intentional modification in procedures was made between 2005 and 2006 during the soak prior to canning, so differences in color retention between those years may reflect the change in processing protocol. This change was made to reduce the amount of color lost from the beans, so the darker color values measured in the later years suggest this modification was effective. No other adjustments to the canning protocol were made from year to year.

Linkage Map

Common bean has 11 linkage groups, which correspond to the genome's 11 chromosomes that are estimated to cover a total genetic distance of 1200cM (Freyre et al., 1998). The current map consisting of 15 linkage groups spans 460cM representing 38% of the estimated genome size. Ten of the 11 linkage groups of the bean consensus map were anchored based on the placement of SSR markers (Blair et al., 2003), or mapping of SRAP and TRAP markers in the BAT93 x JaloEEP558 (BJ) core map population. Linkage group B9 was absent from the current map, although three small linkage groups remain unanchored and one may represent a small portion of B9. Linkage groups B1 and B4 were each represented by two un-joined linkage groups.

Low polymorphism levels are often observed in narrow crosses within a gene pool, race, or market class. A recent study by Blair et al. (2006b) examined polymorphism levels of 129 SSR markers in 44 common bean genotypes from both Middle American and Andean

gene pools. A higher inter gene pool polymorphism level of 59.6% was observed, while the intra gene pool level was 37.9%. Comparisons between two races or closely related cultivars were less polymorphic, suggesting comparisons between two black beans would result in an additional reduction of informative markers.

The low number of polymorphic SSR markers available in this study that resulted in only 38% coverage of the genome suggests the need for continued marker development to make this marker system widely applicable to variety development. Although SSR markers have been successfully used to detect numerous alleles at a locus in genetic diversity studies such as Blair et al. (2006b) or Gomez et al. (2004), the reality is that many loci will be fixed for the same allele within elite breeding germplasm. To overcome this limitation, breeders need access to a larger group of markers so that despite a lower polymorphism rate in closely related populations, upwards of 120 markers would still be informative. More of these molecular tools are currently available in other crops such as soybean (USDA, 2008), and help to provide improved coverage in linkage mapping studies.

Although developing new markers will require an investment of resources, substantial progress has been realized in other crops. Over 1000 SSRs have been placed on the consensus map of soybean (USDA, 2008). In wheat, over 500 SSRs have been developed and more than 300 placed on the consensus map (Song et al., 2005). Yu et al. (1999) concluded microsatellite sequences are abundant in the common bean genome. However, to date less than 200 of the 500 SSR markers developed have been mapped (BIC, 2008).

Recently, Buso et al. (2006); Benchimol et al. (2007); Campos et al. (2007); Grisi et al. (2007); and Hanai et al. (2007) have developed a large group of new SSR markers, more than doubling the number available at the beginning of the present study. A number of these markers were integrated into the current linkage map (Figure 2.2). However, if 1000 or more SSR markers were available, one could construct a map of a similar population with a single

co-dominant marker system, as is routinely done in other legumes such as soybean. This could lead to more uniform genome coverage by selecting evenly distributed markers rather than relying on non-species specific, dominant marker systems such as SRAP or TRAP that tend to cluster (Miklas et al., 2006).

Few published maps of bean have utilized TRAP markers with the exception of Miklas et al. (2006b), and no literature is available for SRAP markers in bean. Unpublished data indicate the polymorphism rate of SRAP markers was three times greater than either RAPD or AFLP markers, and TRAP markers were twice as polymorphic as those marker systems (V. Vallejo, personal comm.) In the present study, SRAP markers possessed three times the polymorphism rate of the SSR markers, which was equivalent to the rate observed with SSRs for intra gene pool comparisons (Blair et al., 2006). Similarly, TRAP markers were about twice as polymorphic as the SSRs, and the 1.5 markers generated per primer pair agreed closely with the results of Miklas et al. (2006b) who observed an average of 1.3 markers per primer combination within a race of the Mesoamerican gene pool in a 'Dorado' x XAN176 RIL population.

Segregation distortion

Linkage group B4b (Figure 2.2) contained six markers including the phenotypic marker for rust resistance. These markers all showed severe segregation distortion that favored the 115M allele. Although markers on other linkage groups in the current map differed from the expected 1:1 ratio, the observed differences were much less than those markers on B4b. These data were particularly interesting since they occurred near a known cluster of resistance genes (Miklas et al., 2006), suggesting this region of the 115M genome was favored throughout the population development process. Similarly, Blair et al. (2003b) found significant distortion on the same region of B4 in a cultivated by wild population. In that study, the cultivated allele was always favored, and the region was associated with the

architecture of the recurrent cultivated parent. Cichy et al. (2009) reported that a genomic region related to the determinate growth habit on B1 was favored over indeterminate plant types in a population derived from a determinate/indeterminate cross. In the present study, the distorted linkage group was associated with lodging, suggesting an association to plant architecture but the reason for the distortion remains unclear, unless unconscious selections were made for upright plant types during population development.

QTL

Twenty QTL were identified for ten traits in thirteen marker intervals across the genome when data was combined across the four environments (Table 2.12). Several additional QTL were identified in one or more single environments for some traits, while for other traits no QTL were detected in some years (Table 2.13). These results support the value of using a RIL population to conduct the experiment across a wide range of environments in order to determine which genomic regions consistently control the largest portions of the variation for each trait.

Although more agronomic traits were considered, fewer QTL and a lower percentage of total variability were explained per trait than detected for the seed quality traits (Table 2.13). The firm texture and poor color retention of 115M along with the softer texture and higher color retention of 'Jaguar' resulted in a wide distribution of lines in the population that facilitated more efficient detection of loci associated with quality characteristics (Table B.1, Table 2.13). In contrast, the two parents differed significantly only for a few agronomic traits including seed size, maturity, lodging, and desirability score, which resulted in a narrow distribution for these traits within the RIL population.

Yield

A single region of linkage group B10 was associated with 19% of the variation for yield in the combined environment. The allele from 115M had an additive effect of

127kg/ha. The detection of this QTL in only three of four individual environments was surprising, based on the high LOD score in the combined environment. In addition, the lack of a significant yield QTL in 2007 was unexpected. However, these varied results agree with those of Tar'an et al. (2002), who found only 25% (5 of 20) of the QTL detected across environments were detected in single environments. The study also found additive effects and approximate location in the genome varied from one environment to another, which agrees with the results of the current study.

The detection of QTL on B3, B5, and B11 in one or more years but not consistently in all years suggests that several genomic regions with relatively small effects are influencing yield in this population and their effect varies depending on the environmental conditions present that season. These results were not unexpected due to the variation in precipitation and other weather patterns among the four growing seasons in the study. In addition, the QTL detected on B3 was associated with the 'Jaguar' allele in 2004 but in 2006 a region 20cM from the same QTL was associated with the 115M allele. Previous studies identified QTL for yield on B3 and B5 in a navy bean breeding line (Beattie et al., 2003), and QTL on B5 and B10 were identified in the navy cultivar 'OAC 95-4' (Tar'an et al., 2002). Blair et al. (2006a) also identified two QTL on B3, one associated with a wild bean (G24404) and one with the Andean cultivar 'Cerinza'. The wild bean was collected in the same region of Colombia as G24423, the wild parent of 115M. This information suggests one or more regions of linkage group B3 are associated with enhanced yield in a range of both Middle American and Andean beans from diverse genetic backgrounds. These data also support the complex genetic nature of yield potential described by previous studies and suggest that limited improvements in yield are possible by selecting for any one QTL alone. A breeder would need to transfer positive alleles at several loci into a single cultivar to significantly improve yield and ensure stable increased yield potential across varied environments.

Introgression of all these QTL into other breeding lines may be challenging, but these minor QTL still represent a source of positive variation for yield.

In contrast, QTL with more significant effects, such as the B10 QTL that explained 19% of the variation in yield across four environments, represent loci that could have a larger individual influence on yield. The B10 QTL represents a region that could be targeted for MAS in black beans or for introgression into other classes of common bean. The cost of performing MAS for this region may influence whether genotypic or phenotypic selection is used to introgress this QTL into other lines, but RILs possessing this QTL certainly should be crossed with other elite breeding lines.

Seed Size

All QTL identified for seed size were associated with the 115M allele. These regions located on linkage groups B6 and B11 were identified in the combined environment and each increased seed size by 0.3g. These results were interesting as they suggest no negative effect of the small seeded wild bean, G24423, on seed size in 115M or the population. The average seed size of 115M was slightly larger than that of the recurrent parent 'Tacana' as well as 'Jaguar', which was not associated with any QTL for this trait. In every individual environment, one or more regions were associated with an increase in seed size, with additional QTL on B5 and B8 identified in one environment each. No QTL accounted for more than 15% of the variation for seed size, suggesting control of this trait resides at many genomic locations each with small effects. Blair et al. (2006a) identified a QTL for seed size on B6 associated with G24404, as well as on B8 and B11 associated with 'Cerinza'. Perez-Vega et al. (2008) located QTL for seed size on B6 and B8 in an Andean by Middle American population. Tar'an et al. (2002) also detected a QTL on B11 in a navy bean population, whereas Park et al. (2000) identified similar seed size QTL associated with Andean cultivar 'PC-50' on B5, B6, and B8.

Days to Flowering

QTL detected for days to flowering on B11 and LG2 were consistent among the combined and individual environments, although they were not detected in all single environments. The 'Jaguar' allele on B11 was associated with a slight increase in days to flower, while the 115M allele on LG2 had a similar effect. The occurrence of QTL for both days to flowering and maturity at the same location on the unanchored LG2 supports the results of Tar'an et al (2002) for navy bean and Blair et al. (2006a) for the wild bean G24404. Both of these studies showed co-localized QTL for days to flowering and maturity in populations derived from similar genetic backgrounds as the 'Jaguar'/115M RIL population. This information suggests LG2 could correspond to the same region of B9 where co-localized QTL for these traits were previously identified, but attempts to anchor this 15cM linkage group to the core map were unsuccessful, and B9 was not mapped in the current study.

Maturity

Linkage groups B5 and LG2 carried QTL associated with the 115M allele that delayed maturity in the combined environment. The QTL on LG2 was interesting as it co-localized with a QTL for days to flowering. As mentioned above, previous studies identified a region of B9 that controlled both of these traits, and although speculative, these results suggest LG2 could represent a portion of B9. Due to the absence of sufficient polymorphic markers on B9, we were unable to verify an association with LG2. Additional QTL were detected on B1, B3 and B7 and in each case the 'Jaguar' allele delayed maturity. These additional regions were each specific to a single environment. Blair et al. (2006a) associated similar regions of B5 and B7 with maturity in the Andean cultivar 'Cerinza'. In contrast, Beattie et al. (2003) and Tar'an et al. (2002) did not identify any similar regions associated with maturity in Middle American beans.

Lodging

Two QTL on B4 and B6 that increased lodging score were associated with the 115M allele. The effect of each of these regions on lodging was relatively small, although together they accounted for 28% of the variation in lodging score. Unlike most traits studied where various regions influenced a trait depending on the year, lodging was consistently associated with these regions in both individual years and the combined environments. Beattie et al. (2003) associated a similar region of B4 with lodging in a navy bean population while the QTL on B6 has not been identified in previous studies. The location of the B4b QTL was also interesting in that rust resistance in 115M mapped to the same region, and a higher than expected frequency of resistant lines was observed. Since all markers in this linkage group also showed a distorted segregation in favor of the 115M allele, the B4b QTL provides an explanation why the population more closely resembles 115M than 'Jaguar' in regard to lodging.

Height

Increased plant height was associated with the 115M allele in a region of linkage group B5 in the combined environment. Additional QTL associated with the 'Jaguar' allele in regions of B3, B6, and B11 were detected in one or more environments but not in the combined analysis, supporting the hypothesis that plant height is largely influenced by environmental conditions. In addition, a QTL from 115M was detected on linkage group B6 in 2004 at a distance of 18cM from the B6 QTL contributed by 'Jaguar' in 2006, suggesting that multiple alleles influencing plant height reside in close proximity to each other on linkage group B6. Similar QTL associated with increased plant height were reported by Checa and Blair (2008) on linkage groups B3 and B11 in an indeterminate Middle American climbing bean. Blair et al. (2006a) identified similar QTL on B6 that were derived from both

an Andean cultivar and a wild bean accession, while Tar'an et al. (2002) located a QTL for height in a similar region of B6 in a navy bean population. Both Tar'an et al. (2002) and Beattie et al. (2003) identified QTL for plant height in similar regions of B3 in different Middle American cultivars, suggesting that plant height is controlled by this region in a number of different genetic backgrounds.

Desirability

Increased desirability was associated with two 'Jaguar' alleles located on B5 and B6. Each locus had an equivalent effect on desirability score and no additional QTL were detected in any individual environment. No QTL for desirability were associated with 115M. This result was not unexpected due to the less desirable architecture of 115M compared with 'Jaguar', which has a more compact, upright growth habit. As increased desirability score reflects the sum of other phenotypic traits, such as early maturity, lodging resistance, and increased plant height, so QTL on B5 and B6 are likely associated with regions that control these traits.

Canning Traits

Eight QTL for quality traits were located at seven unique locations across the genome, suggesting that the contrast between 115M and 'Jaguar' for seed quality characteristics allowed for the efficient detection of regions influencing these traits. Except for the co-localization of QTL for color and visual canning score on B8, all QTL occurred in separate regions of the genome, supporting the complex, quantitative nature of these traits as established by previous studies (Hosfield et al., 2004). However, direct comparisons with previous studies were not possible due to unanchored linkage groups reported in previous QTL analyses. Six of the eight QTL detected in the current study were contributed by 115M. These results were interesting since this line was never selected for canning traits, but three

of the six QTL associated with 115M had a positive effect on canning quality. The lack of QTL detected from 'Jaguar' was surprising, but reasonable based on the resemblance of many of the lines in the population to 115M when canned.

Canned Bean Color

Although four regions were associated with color retention in the combined environment, within a single year one to three loci were detected, suggesting that environmental conditions in a given year largely influenced this trait. Previous studies have also implicated environmental factors as contributing to large differences in the results of canned bean quality evaluations, and suggested that results of quality evaluations are largely location and population specific (Walters et al., 1997; Posa-Macalincag et al., 2002). The QTL identified on B3, B5, and B8 each decreased color retention by 0.4 points and originated in 115M. The QTL on B11 decreased color retention similarly, but was associated with the 'Jaguar' allele. The QTL from 115M were not surprising based on the poor canning characteristics of that line, while the QTL from 'Jaguar' was not expected based on the acceptable canned bean color of that cultivar. Individually the four QTL accounted for 7-15% of the variation in bean color retention, but collectively they accounted for 46% of the variation in color. Posa-Macalincag et al. (2002) identified a similar region of B3 that was associated with improved canning quality in the Andean kidney bean cultivar 'Montcalm', but they did not detect any other QTL identified in the current study.

Texture

Together, the three QTL detected in two regions of linkage group B1 and on B6 accounted for 42% of the variation for texture. At each locus the 115M allele had a positive effect on texture ranging from 2.0-3.6kg force. The increase in texture influenced by the 115M allele was surprising based on the poor visual canning characteristics of that parent. However, similar increases in texture have been recorded for pinto beans with poor visual

appearance following canning. The total R^2 for all QTL detected for canned bean texture in this population was greater than in any of three populations examined by Walters et al. (1997) or two populations studied by Posa-Macalincag et al. (2002).

Visual Appearance

In the combined environment, a single QTL associated with the 'Jaguar' allele on linkage group B8 slightly increased visual appearance. However, in 2006, this region was associated with the 115M allele and increased visual appearance by 0.4, which suggests the 115M allele influenced visual appearance more than 'Jaguar' in that environment. An additional QTL influencing this trait was detected on linkage group B5 in 2005 and associated with the 'Jaguar' allele. Neither of these loci explained a large percentage of the variation for visual appearance, suggesting that QTL analysis for this trait was not as effective as it was for canned bean color or texture. These contrasting results could reflect the difference between the objective measures of color and texture and the subjective evaluation of visual appearance. Walters et al. (1997) also explained a lower percentage of the variation for visual appearance explained compared with other canning quality traits.

Washed-Drained Weight

QTL for washed-drained weight were identified only in 2006 where three QTL were detected on linkage groups B3 and B10. The 'Jaguar' allele increased washed-drained weight by 1.6g each on B3 and the upper end of B10, while on the lower end of B10 the 115M allele resulted in a similar increase. Together, these loci accounted for 33% of the variation for the trait. The reason these loci were detected only in a single year remains uncertain, as this was the only trait where QTL were inconsistent across multiple years. These results underscored the large effect environmental conditions had on this trait.

Co-localized QTL

QTL that co-localized at four locations in the genome were detected for the combined environment. Co-localized QTL often indicate the location of tightly linked loci, or a single locus with pleiotropic effects (Hittalmani et al., 2002). QTL co-localized on B6 for lodging and agronomic desirability, on B8 for canned bean color and visual appearance, and on LG2 for days to flowering and maturity. Since lodging score was a component of the desirability score, canned bean color contributed to visual appearance, and days to flowering influences maturity, these QTL detected in the same regions likely indicate a single locus controlling multiple traits. In contrast, the cluster of four QTL for maturity, plant height, desirability, and canned bean color on linkage group B5 represented the only instance of a seed quality QTL co-located with loci controlling agronomic traits. While maturity, plant height, and desirability were correlated with each other and are likely controlled by the same QTL, seed color was not correlated with any of those traits, suggesting it is influenced by another locus that is adjacent but distinct.

Combined and Individual Environments

In addition to the 20 QTL identified in the combined analysis, 18 other QTL were identified in one or more single environments. No additional QTL for days to flowering, lodging, or desirability were detected in any of the four environments considered. Only QTL for seed size and color were identified in every environment, while there were environments where no QTL for other traits were detected. Chaib et al. (2006) reported similar variation in a study comparing stability of quality QTL over years, generations, and genetic backgrounds using multiple QTL introgressed into various population structures and genetic backgrounds of tomato. Their results showed large differences in the number as well as magnitude and direction of individual QTL detected depending on environment, even when phenotype was

determined in a closely controlled greenhouse. Together, these results suggest there is value in conducting QTL studies over a number of diverse environments to detect as many of the different genomic regions influencing a trait as possible so as to identify stable QTL over years.

Conclusions

The QTL analysis for agronomic and seed quality traits across four contrasting environments identified desirable alleles from 115M that enhanced yield, seed size, plant height and canned bean texture. A single QTL accounted for 19% of the variation for yield across four environments, while in a single environment up to three QTL were identified that controlled 34% of the variation for yield. Likewise, 19%, 16%, and 42% of the variation for seed size, plant height, and canned bean texture, respectively, were accounted for in the combined environment. However, alleles with undesirable effects for days to flowering, maturity, lodging, overall desirability, as well as canned bean color and visual appeal were also detected.

The analysis was particularly useful for dissecting the genetics of canned bean color and texture. These two traits were controlled by loci on at least six chromosomes, suggesting that accumulating favorable alleles at all loci in a single line will remain difficult. Although the 115M allele was generally associated with an undesirable effect on canning quality, a few positive effects were noted. In the combined environment, the 115M allele for a QTL on B11 improved canned bean color, while in 2006, the 115M allele at a QTL on B8 improved visual appearance. The positive effects of the three 115M QTL associated with texture were also unexpected based on the undesirable visual appearance of 115M following canning. These loci demonstrate the potential of inferior parents to contribute positive alleles that result in desirable transgressive segregants.

One or more QTL were identified for all agronomic traits examined including yield, seed size, days to flower, maturity, lodging, plant height, and overall agronomic desirability score. Additional QTL were found for seed processing quality traits including canned bean color, texture, and visual appearance. Washed-drained weight was the only trait considered where no stable QTL across combined environments were identified. The total phenotypic variation explained for visual appearance exceeded that of four out of five previously studied populations. A total of 42% of the variation in texture was also explained by 3 QTL. A complex cluster of 4 QTL was identified in the middle of linkage group B5, while pairs of QTL for different traits co-localized on groups B6, B8 and LG2.

This group of black bean lines reflected the yield potential of 115M, and showed transgressive segregation for both high and low yield. Several lines that consistently exceeded the yield of 115M should be considered for use as parent material to enhance the yield and seed size of elite germplasm. Alleles that improve canned bean texture could be separated from those that confer color loss, based on the independence of these loci. These results support the use of TRAP markers for mapping and tagging QTL in common bean. However, conversion of closely linked TRAP or SRAP markers to more robust SCAR markers prior to implementing MAS would likely improve the efficiency of the selection process by facilitating the multiplexing of markers.

Continued research would provide additional details regarding the true breeding value of this germplasm. Although useful alleles were identified across diverse environments for key traits, the current study provides no information about the combining ability of these alleles with different genetic backgrounds. Crosses with a subset of elite lines from this population will provide additional insight into how these lines will combine with other breeding materials to improve yield of common bean.

Table 2.1. ANOVA table showing mean squares ($p \leq 0.0001$) for yield, 100 seed weight, days to flowering, plant height, lodging score, maturity, and agronomic desirability score for 96 recombinant inbred lines in the Jaguar/115M population combined across four environments (2004-2007) in Michigan.

Trait	YLD	SW	FLWR	HT	LDG	MTR	DS
Genotype (G)	139723.1	10.6	4.2	3.9	0.9	7.9	1.7
Environment (E)	26612095	755.9	3872.1	1307	44.8	2349	76
GxE	25018.5	1.6	0.89ns	2.3	0.3	2.1	0.5

ns= not significant at $p \leq 0.05$

YLD=Yield, SW=100-Seed Weight, FLWR=Days to Flowering, HT=Plant Height, LDG=Lodging Score, MTR=Maturity, DS=Agronomic Desirability

Table 2.2. Pearson correlation coefficients for agronomic and seed quality traits from 96 recombinant inbred lines developed from a 'Jaguar' by 115M cross grown in Michigan during 2004-2007.

	YLD	SW	FLWR	MTR	LDG	HT	DS	CLR	TXT	VA
SW	-0.16									
FLWR	0.26**	0.04								
MTR	0.16	0.10	0.59***							
LDG	0.38***	-0.01	0.45***	0.50***						
HT	0.42***	-0.02	0.60***	0.53***	0.27**					
DS	-0.10	-0.14	-0.47***	-0.66***	-0.70***	-0.34***				
CLR	0.28**	0.16	0.13	0.15	0.01	0.24**	-0.06			
TXT	0.05	0.14	-0.07	-0.17	0.20*	-0.08	-0.10	-0.09		
VA	0.04	0.02	0.04	-0.15	0.08	-0.14	0.08	-0.53***	0.13	
WDWT	-0.08	0.02	-0.13	-0.04	-0.17	-0.14	0.19	-0.21*	-0.50***	0.41***

*≤0.05
**≤0.01
***≤0.0001
YLD=Yield, SW=100-Seed Weight, FLWR=Days to Flowering, HT=Plant Height, LDG=Lodging Score, MTR=Maturity, DS=Agronomic Desirability, CLR=Canned Bean Color, TXT= Texture, VA=Visual Appearance, WDWT=Washed-drained Weight

Table 2.3. Phenotypic means and ranges for yield, 100 seed weight, days to flowering, maturity, lodging score, plant height, and desirability score; canned bean color, texture, visual appeal, and washed drained weight for 96 recombinant inbred lines in the Jaguar/115M population combined across four environments (2004-2007) in Michigan.

Trait	Parents		Recombinant inbred lines			Checks	
	Jaguar	115M	Mean	Range	LSD.05	Tacana	T-39
Agronomic Traits							
Yield (kg/ha)	3050	3350	3058	2249-3654	321	2915	2999
100 Seed Weight (g)	19.5	21.6	20.4	18.1-22.7	1.1	21.1	20.8
Days to Flower	47	47.5	47.4	45.8-50.1	1.1	46.3	47.2
Days to Maturity	95	96.5	96.3	94.5-99.4	1.4	95.8	95.3
Lodging Score	1.2	1.9	1.6	1.0-2.6	0.5	1.7	3.6
Plant Height (cm)	48.4	49.3	49.1	47.3-50.7	1.5	48.7	42.5
Desirability Score	5.3	3.9	4.4	3.1-5.4	0.7	4.25	2.9
Canning Traits							
Color (L)	14.9	17.2	16.0	13.7-20.0	1.3	16.3	14.6
Texture (kg-force)	48.4	71.5	59.3	44.6-79.0	9.6	84.1	48.5
Visual Appeal	3.9	2.3	2.4	1.6-3.2	0.6	2.4	3.3
Washed-Drained Weight (g)	253	246.1	248.6	234.9-260.5	6.8	245.8	254.7

Table 2.4. Four year average (2004-2007) seed yield and 100-seed weight of the top ten and bottom five recombinant inbred lines in the Jaguar/115M population ranked by seed yield.

			Rank based on Yield			
Line	Yield	100-Seed Weight	2004	2005	2006	2007
	kg/ha	g				
B04431	3654	22.3	5	6	2	2
B04404	3601	20.5	1	4	8	6
B04391	3553	22.6	39	16	1	1
B04444	3539	18.6	7	3	6	12
B04445	3527	21.1	2	11	7	8
B04411	3482	21.2	22	14	3	5
B04384	3474	19.8	6	9	46	3
B04429	3463	21.1	8	18	10	7
B04412	3393	20.2	31	2	17	27
B04443	3387	18.4	15	25	11	11
B04434	2642	22.2	91	80	74	98
B04392	2625	21.2	97	98	81	57
B04381	2532	20.8	77	99	97	99
B04425	2502	21.8	99	97	72	94
B04442	2249	22.7	100	100	100	100
Jaguar	3050	19.5	60	38	71	51
115M	3350	21.6	11	23	25	28
Tacana	2915	21.1	78	64	75	55
T-39	2999	20.8	10	77	80	87
Test Mean(100)	3058	20.4				
LSD(.05)	321	1.1				

Table 2.5. Flowering day, maturity, lodging score, plant height, and agronomic desirability score of the top ten and bottom five yielding recombinant inbred lines in the Jaguar/115M population ranked by average seed yield from 2004-2007.

	Flowering		Maturity		Lodging		Height		Desirability	
Line	Mean	Range	Mean	Range	Mean†	Range	Mean	Range	Mean‡	Range
	----------days----------						-------cm-----			
B04431	48	45-55	97	96-107	1.6	1.5-3.0	51	49-55	4.4	3.0-4.0
B04404	48	43-55	96	92-101	2.2	1.0-2.0	50	47-53	3.8	4.0-6.0
B04391	48	46-56	99	95-101	1.9	1.0-3.0	50	43-53	3.1	3.0-5.0
B04444	48	44-54	96	94-102	1.6	1.0-2.0	50	47-54	4.4	3.0-6.0
B04445	48	42-54	98	96-102	1.8	1.0-2.9	50	48-53	4.3	3.0-5.0
B04411	48	43-55	97	94-102	2.0	1.0-2.5	50	48-52	4.4	3.5-5.0
B04384	47	43-53	97	92-101	1.4	1.0-2.6	50	48-53	5.0	3.0-4.5
B04429	47	43-55	96	93-106	1.6	1.0-2.4	50	47-54	4.1	3.5-5.5
B04412	47	43-53	95	92-101	1.1	1.0-2.0	49	46-52	5.3	3.5-5.5
B04443	47	45-55	96	95-101	1.5	1.0-3.0	50	48-54	4.5	3.0-5.0
B04434	49	43-55	99	91-102	2.3	1.0-2.5	50	44-52	3.5	3.0-4.5
B04392	46	42-54	96	91-100	1.3	1.0-1.5	49	45-52	4.3	4.0-6.0
B04381	47	42-54	95	95-102	1.2	1.0-2.0	48	47-54	4.3	4.5-5.5
B04425	49	44-54	99	96-100	1.8	1.0-2.0	50	49-54	3.3	3.0-5.0
B04442	47	45-54	96	96-102	1.3	1.0-2.6	48	49-54	4.6	3.5-5.0
Jaguar	47	43-54	95	92-100	1.2	1.0-1.5	48	45-52	5.3	5.0-6.0
115M	48	44-54	97	94-102	1.9	1.5-2.5	49	46-54	3.9	3.5-4.5
Tacana	46	42-52	96	94-99	1.7	1.0-2.0	49	46-52	4.3	4.0-4.5
T-39	47	43-55	95	93-100	3.6	2-4.1	43	35-47	2.9	1.5-5.0
Test Mean(100)	47		96		1.6		49		4.4	
LSD(.05)	1.1		1.4		0.5		1.5		0.7	

† Lodging rated 1=erect to 5=prostrate

‡ Desirability rated 1=undesirable to 7=desirable

Table 2.6. Canned bean color, texture, visual appearance, and washed-drained weight of the top ten and bottom five yielding recombinant inbred lines in the Jaguar/115M population ranked by average seed yield from 2004-2007.

	Color		Texture		Visual Appearance		Washed-drained weight	
Line	Mean	Range	Mean	Range	Mean†	Range	Mean	Range
	--------L-value-------		----------kg----------				----------g----------	
B04431	16.0	14.8-17.8	57.7	53.0-57.1	2.9	2.6-3.5	251.3	243.8-254.8
B04404	15.6	13.6-18.4	63.6	56.4-77.5	2.5	1.8-3.3	244.7	235.6-251.5
B04391	15.4	13.6-17.1	58.6	52.7-63.9	2.6	2.1-3.4	250.1	247.2-253.5
B04444	15.5	14.5-16.9	45.5	43.2-49.0	2.9	2.4-3.4	260.5	258.6-262.2
B04445	14.4	12.2-15.8	56.7	49.3-61.2	2.2	1.4-3.1	248.2	243.0-253.6
B04411	15.8	14.4-16.6	49.2	48.3-49.6	2.5	1.9-3.4	252.7	249.4-258.4
B04384	17.1	15.3-19.0	70.7	63.9-76.2	2.1	1.5-2.9	234.9	230.1-242.7
B04429	15.7	13.6-16.8	65.3	49.3-84.0	2.4	1.5-3.4	248.1	244.6-255.1
B04412	17.6	16.7-18.5	65.4	57.5-80.9	2.1	1.9-2.3	251.2	244.6-249.8
B04443	15.8	13.8-17.7	66.0	59.5-70.7	2.7	2.2-3.4	244.1	241.0-248.4
B04434	15.7	13.4-17.8	54.2	49.6-62.9	2.3	1.4-3.5	246.2	244.1-248.5
B04392	16.0	14.6-16.7	67.5	63.6-70.7	3.1	2.8-3.5	254.0	253.2-255.7
B04381	17.0	15.5-18.8	55.2	47.9-63.9	2.0	1.7-2.6	251.0	244.4-259.8
B04425	18.4	15.7-22.0	68.5	62.2-79.6	1.6	1.2-2.4	238.2	226.9-249.4
B04442	16.0	15.3-16.4	64.9	51.3-74.8	2.7	2.1-3.6	249.2	243.6-254.0
Jaguar	14.9	12.8-16.3	48.4	42.2-53.0	3.9	3.4-4.4	253.0	249.8-256.2
115M	17.2	16.1-18.7	71.5	63.2-77.5	2.3	1.7-2.9	246.1	243.3-249.2
Tacana	16.3	13.5-20.3	84.1	76.2-92.5	2.4	1.8-3.1	245.8	237.7-254.7
T-39	14.6	13.6-15.8	48.5	44.9-51.7	3.3	2.5-3.7	254.7	251.2-259.9
Test Mean(100)	16.0		59.3		2.4		249.0	
LSD(.05)	1.3		9.6		0.6		6.8	

† Visual appearance rated 1=undesirable to 7=desirable

Table 2.7. Number of years recombinant inbred lines in the Jaguar/115M population ranked in the top 10% or bottom 5% based on seed yield.

Top 10%		
4 Years	3 Years	2 Years
B04431	B04384	B04366
B04404	B04429	B04391
	B04444	B04411
	B04445	
Bottom 5%		
B04442	B04381	B04392
		B04408

Table 2.8. Yearly trait means for 2004-2007 and corresponding least significant differences for the 'Jaguar'/115M recombinant inbred line population grown in Michigan.

Year	YLD	SDWT	FLWR	MTR	LDG†	HT	DS‡
	kg/ha	----g----	----d----	---d---		--cm--	
2004	2226	20.4	46.5	93.7	1	46.9	5.1
2005	3508	22.8	43.6	96	1.8	47.7	4.7
2006	3474	19	45	94.3	2.2	48.8	3.7
2007	3047	19.6	54.4	101.4	1.5	52.8	4
LSD(.05)	67	0.1	0.2	0.3	0.1	0.3	0.2

YLD=Yield, SW=100-Seed Weight, FLWR=Days to Flowering, HT=Plant Height, LDG=Lodging Score, MTR=Maturity, DS=Agronomic Desirability

† Lodging rated 1=erect to 5=prostrate

‡ Desirability rated 1=undesirable to 7=desirable

Table 2.9 Four year mean (2004-2007) yields for 96 recombinant inbred lines in the Jaguar/115M population, parents, and checks ranked by descending seed yield.

	Yield	Line	Yield	Line	Yield	
	kg/ha		kg/ha		kg/ha	
B04431	3648	B04396	3196	Tacana	2915	
B04404	3601	B04452	3196	B04419	2892	
B04391	3553	B04407	3190	B04436	2892	
B04444	3539	B04422	3173	B04377	2889	
B04445	3527	B04385	3170	B04417	2889	
B04411	3482	B04361	3159	B04401	2884	
B04384	3474	B04449	3151	B04432	2853	
B04429	3463	B04450	3128	B04426	2842	
B04412	3393	B04369	3126	B04379	2833	
B04443	3387	B04409	3117	B04437	2825	
115M	3350	B04372	3117	B04388	2822	
B04394	3350	B04447	3114	B04415	2822	
B04423	3345	B04440	3109	B04399	2819	
B04414	3322	B04454	3106	B04359	2799	
B04387	3317	B04441	3106	B04371	2797	
B04360	3311	B04382	3103	B04393	2797	
B04451	3308	B04420	3100	B04389	2797	
B04370	3297	B04397	3100	B04416	2780	
B04366	3286	B04406	3089	B04390	2763	
B04410	3274	B04421	3089	B04438	2749	
B04376	3244	B04418	3083	B04395	2743	
B04446	3241	B04403	3081	B04448	2726	a
B04386	3230	B04402	3075	B04424	2718	a
B04453	3224	B04364	3066	B04375	2710	a
B04383	3215	B04367	3061	B04427	2676	a
B04400	3215	B04363	3050	B04380	2667	a
B04398	3215	Jaguar	3050	B04408	2645	a
B04374	3210	B04362	3019	B04434	2642	a
B04433	3207	B04373	3007	B04392	2625	a
B04439	3207	B04368	3007	B04381	2532	a
B04435	3207	T-39	2999	B04425	2502	a
B04413	3204	B04428	2991	B04442	2249	a
B04405	3199	B04378	2951			
B04365	3196	B04430	2915			

a= Significantly lower yield than 'Jaguar' (LSD(.05)=321kg/ha).

Table 2.10. Analysis of variance for canning quality traits of a 'Jaguar' by 115M RIL population including canned bean color, texture, washed-drained weight, and visual appearance.

Trait	Color	Texture	Washed-drained weight	Visual Appearance
Genotype (G)	6.7	279.4	50.9	0.4
Environment (E)	566.5	5682.4	2823.7	32.5
G x E	1.3	72.5		
Mean	16	59.3	248.6	2.4
LSD	0.8	2.5	6.7	0.6

Table 2.11. Mean values by year for canned bean color, texture, washed-drained weight, and visual appearance for 2005-2007.

YEAR	CLR	TXT	VA	WDWT
2005	17.6	63.9	2.2	243.3
2006	14.2	60.8	3.1	248.9
2007	16.2	53.5	2.0	253.9
LSD(.05)	0.4	2.5	0.1	1.5

CLR=Canned Bean Color, TXT= Texture, VA=Visual Appearance, WDWT=Washed-drained Weight

Table 2.12. Putative QTL for agronomic and seed quality traits identified in the combined environment from 96 recombinant inbred lines developed from a 'Jaguar'/115M cross and evaluated in Michigan during 2004-2007.

Agronomic Trait	Linkage group	Nearest† marker	LOD‡ score	LOD Threshold	R2 CIM §	Additivity¶
Yield	B10	F2R2.575	5.4	2.6	0.19	127
Seed Size	B6	ME3/5.375	2.8	2.8	0.08	0.31
	B11	F7R8.620	3.6		0.11	0.34
Days to Flowering	B11	F17R8.420	3.7	2.5	0.15	-0.35
	LG2	ME3/4.470	2.9		0.11	0.31
Maturity	B5	F10R8.150	5.1	2.6	0.19	0.46
	LG2	ME3/4.470	4.2		0.19	0.45
Lodging	B6	PVBR14	4.1	2.8	0.15	0.14
	B4b	IAC66	4.0		0.13	0.16
Height	B5	FJ16	3.5	2.7	0.14	0.26
Desirability	B5	F10R8.150	3.1	2.7	0.12	-0.16
	B6	PVBR20	4.3		0.15	-0.18
Canning Trait						
Color	B3	F9R1.150	5.3	2.7	0.15	0.42
	B5	F12R7.250	5.1		0.13	0.39
	B8	F1R9.400	4.2		0.11	0.36
	B11	F5R10.475	2.9		0.07	-0.29
Texture	B1	PVBR233	3.6	2.7	0.12	2.40
	B6	ME4/3.1200	2.9		0.08	2.01
	B1b	IAC28	4.1		0.22	3.64
Visual Appearance	B8	F1R9.400	2.7	2.7	0.14	-0.14

† Primer information for SRAP and TRAP markers located in Tables C.8 and C.9, SSR markers online at: http://www.css.msu.edu/bic/PDF/Bean SSR Primers 2007.pdf.

‡ LOD: Log of odds, all LOD scores meet or exceed the genome wide empirical threshold of 1000 permutations at p = 0.05.§ Proportion of the phenotypic variance explained by the QTL at peak LOD using composite interval mapping (CIM).

¶ Effect of substituting a single allele from one parent to another. Positive values indicate allele from 115M and negative from 'Jaguar'.

Table 2.13. Putative QTL for agronomic and seed quality traits identified in the combined and individual environments from 96 recombinant inbred lines developed from a 'Jaguar'/115M cross and evaluated in Michigan during 2004-2007.

Agronomic Trait	Linkage group	Nearest† marker	LOD‡ Score	LOD Threshold	R2 § CIM	Additivity¶	LOD# QTLxE
Yield							
Combined	B10	F2R2.575	5.4	2.6	0.19	127	2.6
2004	B3	ME1/6.550	3.2	2.7	0.11	-168	1.3
	B10	PVBR181	3.2		0.11	144	3.2
	B11	ME3/3	3.3		0.12	41	1.7
2005	B5	F13R5.220	4.6	2.6	0.15	141	3.7
	B10	F17R10.320	7.8		0.28	192	4.6
2006	B3	PVat008	2.7	2.7	0.08	77	1.7
	B10	F2R2.575	4.4		0.16	110	1.5
Seed Size							
Combined	B6	ME3/5.375	2.8	2.8	0.08	0.31	2.6
	B11	F7R8.620	3.6		0.11	0.34	3.3
2004	B6	F3R9.875	3.6	2.7	0.12	0.51	2.4
	B8	TE1/6.340	2.7		0.11	0.40	1.4
2005	B6	ME3/5.325	3.1	2.7	0.10	0.42	3.0
	B11	F7R8.600	3.2		0.12	0.45	3.2
2006	B11	F7R8.600	3.9	2.7	0.13	0.39	3.9
2007	B5	F10R8.150	2.6	2.5	0.09	0.36	1.8
	B6	ME3/5.375	3.6		0.15	0.48	4.6
Days to Flowering							
Combined	B11	F17R8.420	3.7	2.5	0.15	-0.35	2.2
	LG2	ME3/4.470	2.9		0.11	0.31	1.3
2004	B11	F17R8.420	3.4	2.7	0.12	-0.37	1.7
	LG2	ME3/4.470	4.2		0.19	0.47	2.2
2006	B11	F17R8.420	3.0	2.6	0.14	-0.43	1.0
	LG2	ME3/4.470	3.7		0.16	0.48	1.9
2007	B11	F17R8.420	3.3	2.7	0.15	-0.50	1.5

Table 2.13 (Cont'd).

Agronomic Trait	Linkage group	Nearest† marker	LOD‡ Score	LOD Threshold	R2 § CIM	Additivity¶	LOD# QTLxE
Maturity							
Combined	B5	F10R8.150	5.1	2.6	0.19	0.46	2.3
	LG2	ME3/4.470	4.2		0.19	0.45	1.7
2005	B7	F1R9.650	2.9	2.6	0.12	-0.26	1.7
2006	B3	ME1/6.550	3.4	2.8	0.11	-0.58	1.2
	B5	F10R8.150	4.3		0.22	0.78	1.8
	LG2	ME3/3.545	4.0		0.17	0.71	1.3
2007	B1	PVBR233	2.7	2.4	0.11	-0.41	0.8
	B5	F10R8.150	4.4		0.14	0.49	2.7
	LG2	ME3/4.470	3.0		0.11	0.41	1.6
Lodging							
Combined	B6	PVBR14	4.1	2.8	0.15	0.14	3.6
	B4b	IAC66	4.0		0.13	0.16	1.8
2005	B6	PVBR14	3.3	2.6	0.16	0.18	1.5
	B4b	F15R10.580	2.7		0.09	0.35	1.8
2006	B6	PVBR14	2.9	2.6	0.14	0.27	1.4
2007	B6	PVBR5	2.8	2.7	0.10	0.16	2.1
	B4b	Rust	3.1		0.11	0.19	1.0
Height							
Combined	B5	FJ16	3.5	2.7	0.14	0.26	2.9
2004	B3	ME1/6.550	3.9	2.8	0.15	-0.64	0.3
	B6	PVBR163	3.7		0.13	0.54	1.9
	B11	F17R8.420	3.6		0.17	-0.62	1.1
2006	B6	IAC47	2.7	2.5	0.10	-0.33	3.3
2007	B5	F20R4.250	4.3	2.8	0.17	0.37	2.7

Table 2.13 (Cont'd.)

Agronomic Trait	Linkage group	Nearest† marker	LOD‡ Score	LOD Threshold	R2 § CIM	Additivity¶	LOD# QTLxE
Desirability							
Combined	B5	F10R8.150	3.1	2.7	0.12	-0.16	1.8
	B6	PVBR20	4.3		0.15	-0.18	3.8
2004	B6	PVBR14	5.6	2.6	0.21	-0.26	4.0
2005	B6	PVBR5	3.0	2.7	0.11	-0.21	3.0
Canning Trait							
Color							
Combined	B3	F9R1.150	5.3	2.7	0.15	0.42	3.2
	B5	F12R7.250	5.1		0.13	0.39	2.2
	B8	F1R9.400	4.2		0.11	0.36	2.9
	B11	F5R10.475	2.9		0.07	-0.29	1.0
2005	B3	F9R1.150	7.0	2.6	0.21	0.75	4.0
	B5	IAC96	3.5		0.10	0.51	2.9
	B8	TE1/6.340	5.1		0.16	0.65	3.3
2006	B5	F22R1.400	3.4	2.7	0.13	0.44	2.8
2007	B1	F3R1.235	3.0	2.6	0.10	0.30	2.0
	B8	TE1/6.340	3.1		0.11	0.29	1.9
	B11	F5R10.475	3.0		0.09	-0.26	2.4

Table 2.13 (Cont'd).

Agronomic Trait	Linkage group	Nearest† marker	LOD‡ Score	LOD Threshold	R2 § CIM	Additivity¶	LOD# QTLxE
Texture							
Combined	B1	PVBR233	3.6	2.7	0.12	2.40	2.3
	B6	ME4/3.1200	2.9		0.08	2.01	1.8
	B1b	IAC28	4.1		0.22	3.64	3.1
2005	B11	F17R8.420	3.0	2.7	0.11	2.85	2.4
2006	B6	F8R2.350	3.8	2.6	0.12	3.53	4.1
Visual Appearance							
Combined	B8	F1R9.400	2.7	2.7	0.14	-0.14	2.3
2005	B5	IAC96	2.7	2.7	0.12	-0.21	0.8
2006	B8	TE1/6.340	4.1	2.8	0.19	0.37	3.6
Washed-Drained Weight							
2006	B3	ME1/6.550	3.3	2.6	0.12	-1.65	3.2
	B10	F2R2.575	3.2		0.11	-1.62	1.5
	B10	F18R2.775	3.1		0.10	1.48	1.2

† Primer information for SRAP and TRAP markers can be found in Tables C.8 and C.9, SSR markers online at: http://www.css.msu.edu/bic/PDF/Bean SSR Primers 2007.pdf.

‡ LOD: Log of odds, all LOD scores meet or exceed the genome wide empirical threshold of 1000 permutations at p = 0.05.

§ Proportion of the phenotypic variance explained by the QTL at peak LOD using composite interval mapping (CIM).

¶ Effect of substituting a single allele from one parent to another. Positive values indicate allele from 115M and negative from Jaguar'.

LOD score for the presence of a genotype by environment interaction

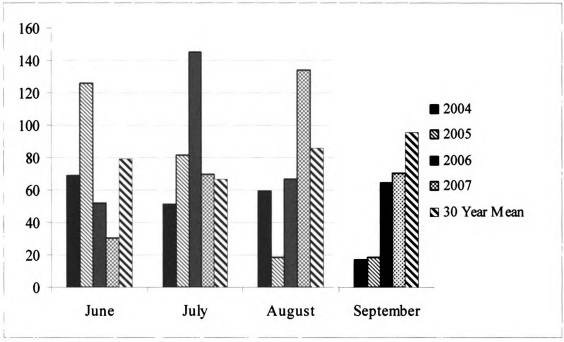

Figure 2.1. Monthly precipitation (mm) measured from June to September 2004-2007 at the Saginaw Valley Bean and Beet Research Farm, Saginaw, MI.

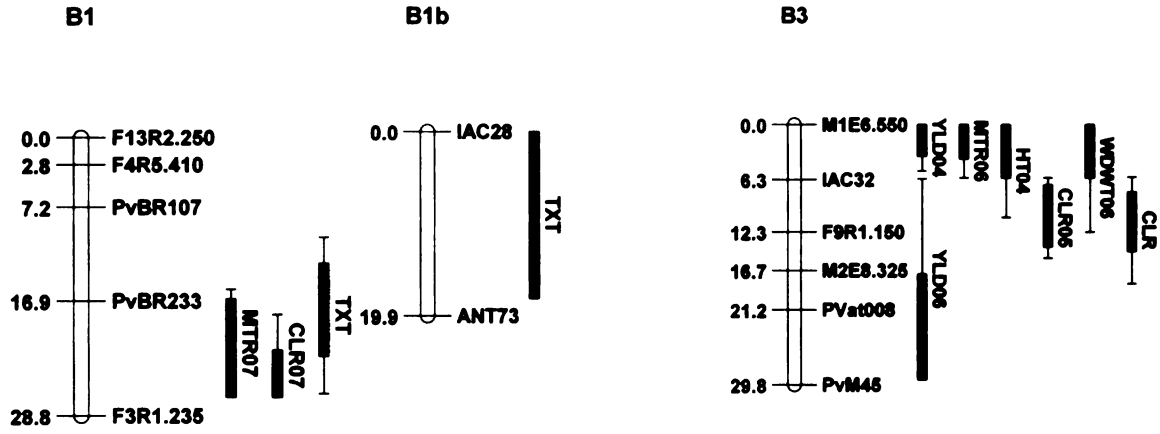

Figure 2.2. Linkage map of 'Jaguar'/115M RIL population and QTL locations for seed yield (YLD), seed size (SDWT), days to flowering (FLWR), plant height (HT), days to maturity (MTR), lodging (LDG), agronomic desirability (DS), and canned bean visual appearance (VA), color (CLR), texture (TXT), and washed-drained weight (WDWT). QTL are further identified by the last two digits of year (04-07) and QTL with no year specified were detected in the 4-year combined environment.

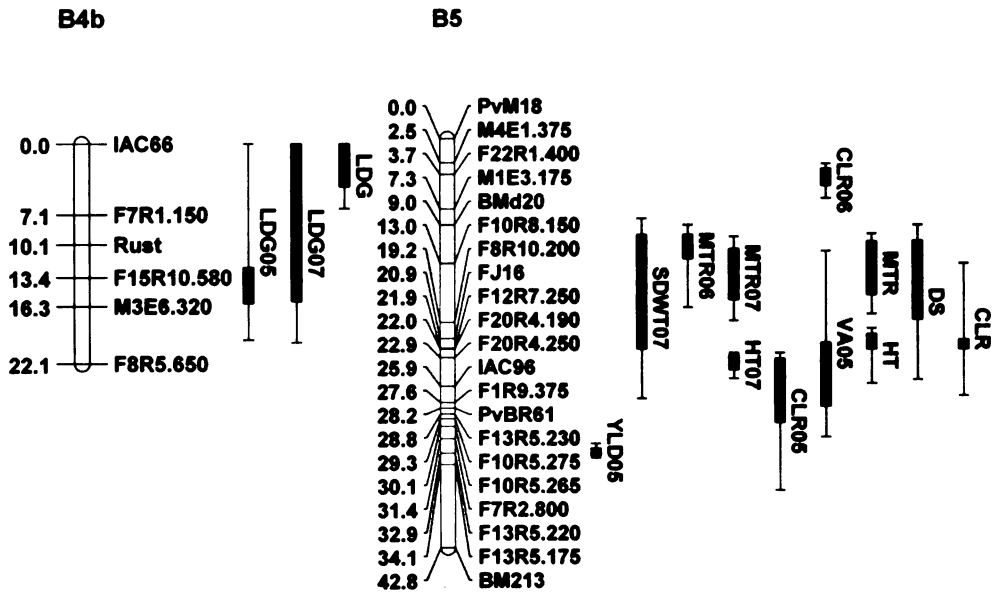

Figure 2.2 (Cont'd.)

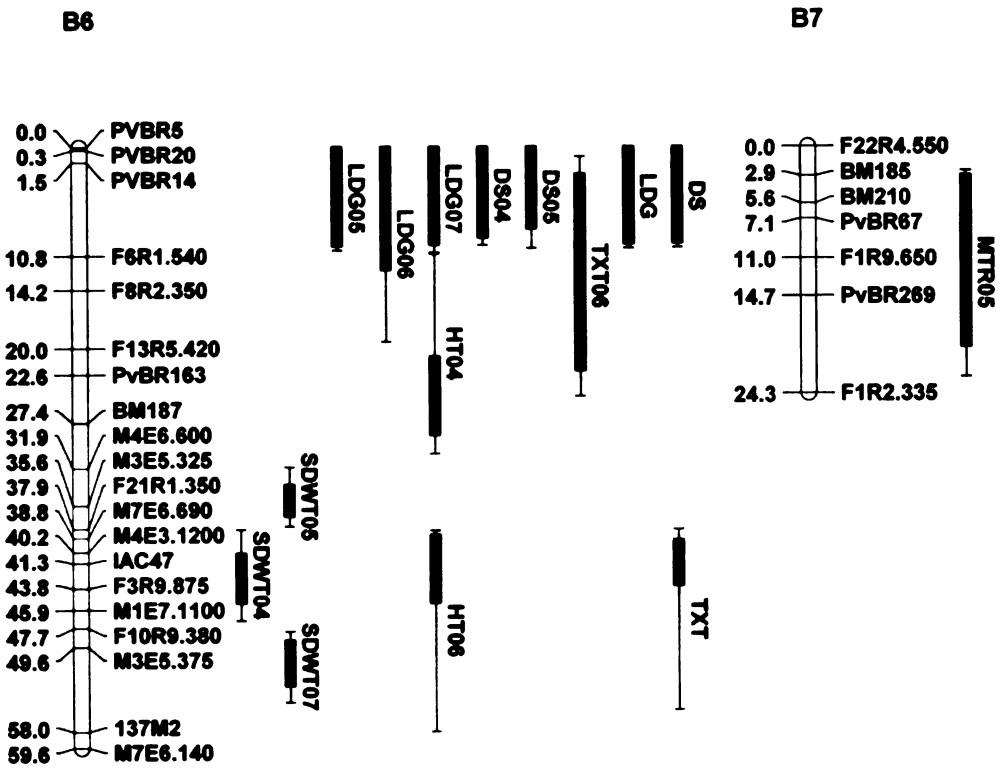

Figure 2.2. (Cont'd.).

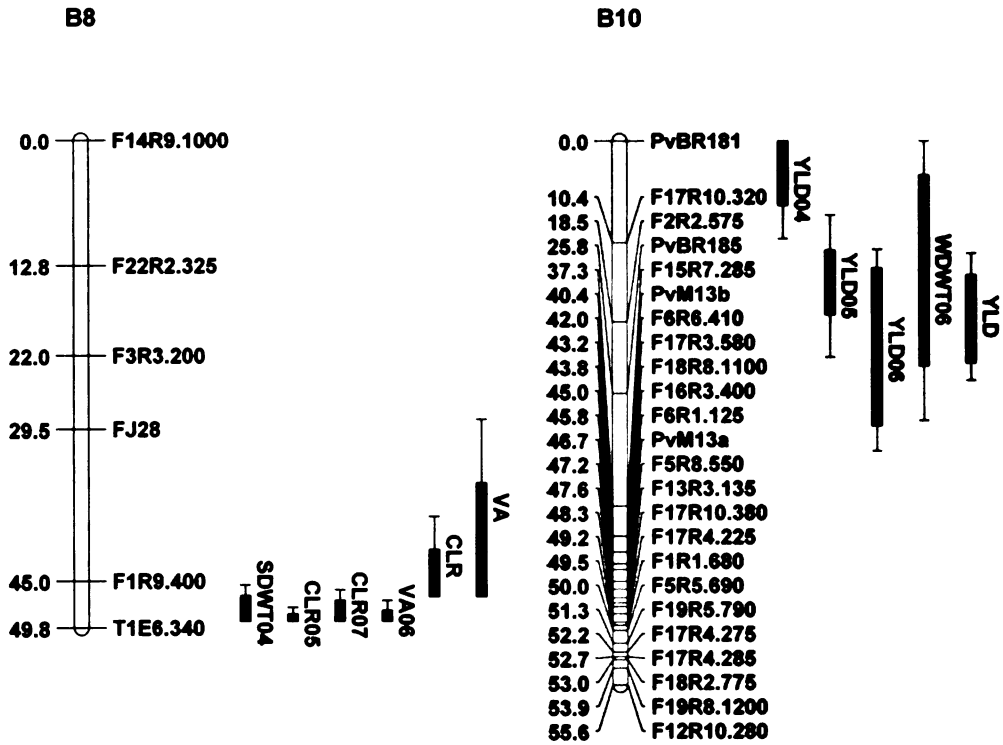

Figure 2.2. (Cont'd.).

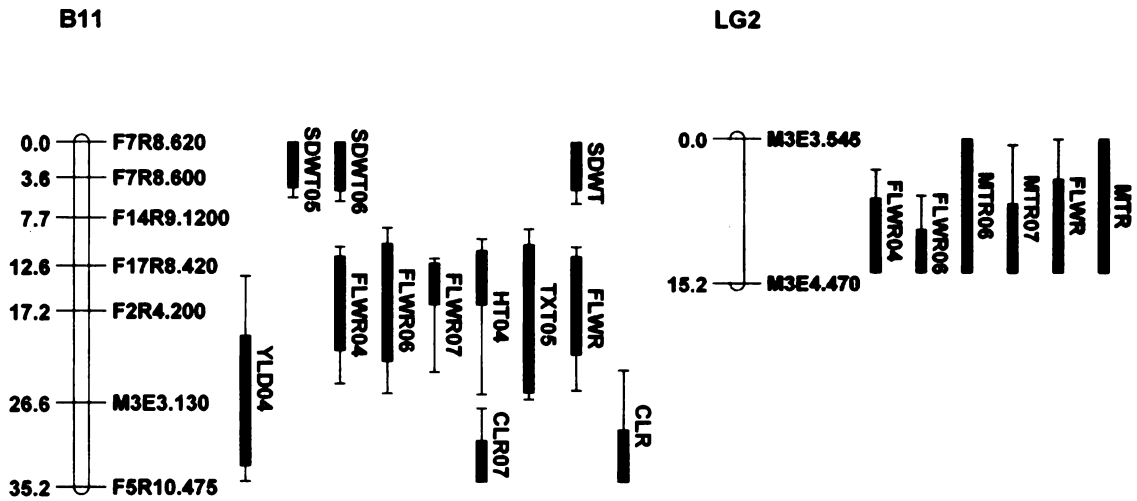

Figure 2.2. (Cont'd.).

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Chapter 3: Use of TRAP markers to map resistance to a new race of common bean rust in Michigan

Abstract

A recombinant inbred line (RIL) population from the cross of the black bean cultivar 'Jaguar' and the breeding line 115M was used to map resistance to a new race of common bean rust (*Uromyces appendiculatus*). The pathogen was isolated during 2007 from a cultivar possessing *Ur-3*, a gene which previously conditioned resistance to all indigenous races of bean rust in Michigan. A differential series was used to characterize the isolate and a total of 96 RILs were inoculated in the greenhouse and their reaction to the pathogen was evaluated based on pustule size. SSR, TRAP, SRAP, and phenotypic markers were used to create a genetic map of the population, including a locus that conditioned rust resistance. The isolate was characterized as race 3:22 and its virulence against *Ur-3* was confirmed by the susceptible reaction of the differential cultivar 'Aurora'. In the RIL population, rust resistance was associated with 115M and mapped to linkage group B4. The locus was flanked by two TRAP markers, both at an approximate distance of 3 cM. These results support the utility of TRAP markers to tag disease resistance loci and provide a valuable source of rust resistance in a black bean adapted to Michigan.

Introduction

Common bean rust caused by the hypervariable fungal pathogen *Uromyces appendiculatus* (Pers.:Pers.) Unger severely limits common bean (*Phaseolus vulgaris* L.) production worldwide (Stavely and Pastor-Corrales, 1989). Bean rust has the potential to reduce yields by 25 to nearly 100 percent in susceptible cultivars (Mmbaga et al., 1996b), severely limiting dietary protein and nutrients in developing countries and causing economic losses in developed areas (Broughton et al., 2003). Strategies to manage this pathogen include crop rotation, residue management, adjustment of planting date, use of fungicides, and host plant resistance (Mmbaga et al., 1996b). However, due to the varied effectiveness of cultural practices and expense or inaccessibility of fungicides, genetic resistance to rust remains the preferred management strategy to prevent crop losses (Steadman et al., 2002).

Virulence diversity in *U. appendiculatus* was first described by Harter et al. (1935, as cited by Stavely et al., 1994). Since that time, over 300 races have been reported worldwide (Mmbaga et al., 1996a). Following the adoption of an international differential series (Stavely et al., 1983), 90 unique races have been verified and catalogued (Stavely, 2000). Currently, a revised differential series of twelve bean cultivars, six each from the two bean gene pools, is used to classify the variability of the rust pathogen (Steadman et al., 2002). Each cultivar in this series corresponds to a binary value, and the binary values of the differentials susceptible to a particular isolate are summed to describe its virulence. This classification system more accurately reflects the gene pool differences of rust isolates and resistance genes than the previous differential series used to describe pathogenic variation of the rust pathogen.

Specific races of rust exhibit patterns of virulence that mirror the division between the Andean and Middle American bean gene pools, suggesting a history of co-evolution between

the rust pathogen and its host (Pastor-Corrales, 2004; Acevedo et al., 2008). Molecular analysis of the pathogen has also confirmed this relationship (Araya et al., 2004).

Breeding for genetic resistance to rust is complicated by the variability of rust populations and the rapid breakdown of major resistance genes deployed in cultivars. Pyramiding multiple resistance genes originating in both the Andean and Middle American gene pools into a single cultivar provides the broadest and most durable resistance to this pathogen (Stavely, 2000). Incorporation of varied resistance characteristics (specific, slow rusting, reduced pustule size, age-dependent resistance, and pubescence) also decreases the likelihood that the rust pathogen will defeat a resistance gene (Miklas et al., 2005). Despite the development of adapted germplasm which possesses two resistance genes from each gene pool with complementary resistance spectrums, many commercial cultivars carry only single resistance genes (Pastor-Corrales et al., 2005).

Nine named resistance genes and four unnamed genes have been characterized, tagged with RAPD or SCAR markers, and mapped to five linkage groups of common bean (reviewed by Miklas et al., 2006a). Pastor-Corrales et al. (2008) mapped and tagged an additional unnamed gene from PI260418 that confers resistance to all but one known race of *U. appendiculatus*. At least one rust resistance gene (*Ur-5*) is inherited as a cluster of five tightly linked loci (Stavely, 1984), and others such as *Ur-3* may consist of similar complex clusters of tightly linked genes (Miklas et al., 2006a). To date, all rust resistance genes characterized are dominantly inherited.

Previously, the *Ur-3* gene conditioned rust resistance to all rust races found in the state of Michigan (Pastor-Corrales et al., 2007). However, during 2007, rust was observed on the leaves and stems of the cultivars 'Jaguar', 'Merlot', and 'Vista' which possess the *Ur-3* gene. The objectives of the present study were to 1) characterize the new isolate of rust collected in 2007 using the rust differential series 2) validate the reaction of current bean

cultivars including 'Jaguar' and 115M, the parents of a RIL mapping population, to the new rust isolate 3) map the source of rust resistance in 115M using the RIL population and 4) identify TRAP markers associated with rust resistance.

Materials and Methods

Samples of infected leaves with sporulating pustules were collected from the cultivars 'Jaguar', 'Merlot', and 'Vista' by G.V. Varner in Tuscola county, MI in the fall of 2007. A spore suspension was prepared from these samples and used to inoculate the same three cultivars, along with the susceptible cultivar 'Othello' and the breeding line 115M in the MSU greenhouse according to the methods of Stavely et al. (1983). Spores collected from 'Jaguar', 'Merlot', and 'Vista' were used to inoculate 'Othello' to obtain additional inoculum to facilitate screening the differential cultivars for rust reaction.

Preliminary characterization of this unknown race of common bean rust was accomplished by inoculating the differential series of twelve cultivars proposed by Steadman et al. (2002). This series included six Andean and six Middle American genotypes that together possessed the characterized rust resistance genes *Ur-3* thru *Ur-13* (Table 3.1). The rust evaluation scale proposed by Stavely et al. (1983) was used to rate the reaction of cultivars to the rust pathogen on a scale of 1=immune to 6=very susceptible. If more than one pustule size was observed, the most common pustule size is reported first, followed by the less frequent pustule size. The binary values associated with each susceptible (predominant presence of grade 4 or greater pustule size) differential were summed for both the Andean and Middle American differentials to determine the race of the isolate as described by Steadman et al. (2002).

A population of 96 RILs derived from the cross 'Jaguar' (susceptible) by 115M (resistant) was also inoculated to enable mapping of the rust resistance segregating in this black bean population. A minimum of four plants were inoculated per RIL. The genetic map

constructed with JoinMap 3.0 for this population consisted of 119 loci including 62 TRAP, 19 SRAP, 36 SSR, and 2 phenotypic markers spanning 460cM of the common bean genome.

Results

Reaction of cultivars

This isolate of common bean rust produced a susceptible, large pustule reaction in the cultivars 'Jaguar', 'Merlot', and 'Vista' (Table 3.2). These cultivars carry the *Ur-3* resistance gene and have previously been resistant to all known races of rust in the state of Michigan. 'Aurora', the rust differential that possesses *Ur-3*, and the susceptible check 'Othello' both showed a similar large pustule reaction following inoculation with this isolate (Table 3.1, 3.2). These results confirmed the virulence of this isolate on cultivars with resistance conditioned by *Ur-3,* representing the first report of the breakdown of this resistance source in the state of Michigan. 'Tacana', the recurrent parent of 115M, exhibited a resistant small pustule reaction to this isolate. Additional cultivars screened for rust resistance were susceptible except the black bean cultivar 'Shania' which was heterogeneous and exhibited both resistant and susceptible plants (Table 3.2).

Characterization

When the complete differential series proposed by Steadman et al. (2002) was inoculated with this isolate, five of the twelve cultivars displayed a susceptible reaction (pustule size ≥ 4; Table 3.1). The Middle American resistance genes *Ur-3* and *Ur-7* conditioned susceptible and moderately susceptible reactions, respectively. The Andean resistance genes *Ur-6* and *Ur-13* also produced moderately susceptible or susceptible reactions when challenged with this isolate. 'Montcalm', an important dark red kidney variety grown in Michigan, was also susceptible. Conversely, *Ur-5* and *Ur-11* were immune to this isolate. The remaining cultivars in the series were moderately resistant with varying frequencies of small pustule reaction. The isolate was classified as race 3:22 based on the

summation of the binary values associated with the two Middle American and three Andean susceptible differentials.

Mapping and Tagging

Race 3:22 conditioned a small pustule resistant reaction on 115M and a highly susceptible large pustule reaction on 'Jaguar'. The RIL population ('Jaguar'/115M) and genetic map provided an opportunity to map the resistance present in 115M. 96 RILs were evaluated; 63 were resistant, 18 were heterogeneous with both resistant and susceptible plants and 15 were susceptible (Table 3.3). These data significantly differed ($p<.0001$) from the expected 1:1 resistant to susceptible ratio for a single gene trait.

Using the data (Table 3.3) as a phenotypic marker, resistance to race 3:22 was mapped to linkage group B4b, which corresponds to the lower end of linkage group B4 of the bean consensus map (Freyre et al., 1998). Rust resistance was flanked by two TRAP markers, 3cM from F7R1.150 and 3.3cM from F15R10.580 (Table 3.3, Figure 3.1). F7R1 amplified a 150bp fragment that co-segregated in coupling phase with rust resistance while F15R10 produced a 580bp fragment that co-segregated in repulsion phase. All markers on this linkage group exhibited skewed segregation ratios that favored the 115M allele.

Discussion

Reaction of cultivars

The susceptible reaction of 'Jaguar', 'Merlot', and 'Vista' to the isolate of *U. appendiculatus* confirmed the virulence of the rust pathogen has evolved from that previously reported in Michigan. These cultivars possess *Ur-3,* a resistance gene that previously conditioned resistance to all common bean rust found in the United States (Pastor-Corrales et al., 2007). This discovery suggests commonly grown bean cultivars are vulnerable to rust infection and significant yield reductions could occur in the future if environmental conditions favor disease development. This isolate was collected from a single field late in

the 2007 season, so no conclusions can be drawn about the distribution of the race in the state. However, no other sources have reported rust on cultivars possessing *Ur-3*, suggesting the distribution of this race is limited. Collecting additional isolates from several fields in the region in future years would provide additional information about the persistence and distribution of this new race.

Characterization

The complete characterization of the rust isolate with a differential series revealed five of the twelve cultivars were susceptible to race 3:22, but others including those possessing *Ur-5* or *Ur-11* were immune or resistant. These results supported the virulence of this isolate against *Ur-3*, the resistance gene present in the differential cultivar 'Aurora'. This gene is used almost exclusively in rust resistant cultivars grown in Michigan, leaving the bean crop vulnerable to losses from rust.

The immunity conferred by *Ur-5* or *Ur-11* was encouraging as these genes have been widely effective against numerous rust races in previous studies (Stavely, 2000). The current results indicate these were the most effective resistance genes when challenged with race 3:22. *Ur-5* represents a tightly linked block of single dominant genes (Stavely, 1984) that confers resistance to 70 of 90 rust isolates in the USDA-ARS rust collection (Stavely, 2000). *Ur-11* confers resistance to all but one isolate, race 108, and is linked to *Ur-3* which conditions resistance to 44 races including race 108 (Stavely, 2000). *Ur-5* has been tagged with RAPD (Haley et al., 1993) and SCAR (Melotto and Kelly, 1998) markers that are effective for MAS in a range of genetic backgrounds while RAPD markers linked to Ur-11 (Johnson et al., 1995) have been less reliable (Kelly and Miklas, 1999). Despite the availability of reliable markers, *Ur-5* has been underutilized in breeding for rust resistance while *Ur-11* has been more widely deployed in recent years (Kelly et al., 2003). Based on their resistance to a broad range of rust races including race 3:22, bean breeders should

consider pyramiding one or both of these additional resistance genes into their breeding programs to maintain complete resistance to all rust races in Michigan.

Mapping and Tagging

Resistance to race 3:22 was mapped to linkage group B4b in the 'Jaguar'/115M RIL population. Based on alignment of this linkage group with the bean consensus map, this location corresponds to a region on the lower end of linkage group B4 where multiple disease resistance genes have previously been identified (Miklas et al., 2006b; Pastor-Corrales et al., 2008). This location suggests the Ur-gene conditioning resistance to race 3:22 was inherited from 115M, since rust resistance in 'Jaguar' is conditioned by *Ur-3* which resides on linkage group B11 (Kelly et al., 2001; Miklas et al., 2006b).

Two rust resistance genes, *Ur-5* and *Ur-Dorado-108* reside in this region of B4 (Miklas et al., 2000) and could condition the resistance observed in 115M. However, the immune reaction of the cultivar 'Mexico309' which carries only *Ur-5* was not consistent with the small pustule resistance of 115M. This suggests that *Ur-5* may not condition the resistant reaction of 115M to race 3:22. 'Dorado', the original source of *Ur-Dorado-108* (Miklas et al., 2000), also proved susceptible to race 3:22 (Table 3.2). 'Dorado' appears in the pedigree of 'Tacana' (Lopez-Salinas et al., 1997), the recurrent parent of 115M, suggesting *Ur-Dorado-108* could have been inherited by 'Tacana' and subsequently 115M. However, the susceptible reaction of 'Dorado' suggests it does not confer the resistance observed in the RIL population. Thus the small pustule resistance exhibited by both 'Tacana' and 115M suggests rust resistance is conditioned by the same Ur-gene or genes in both cultivars, but further work will be necessary to precisely identify this locus.

The skewed ratio of resistant to susceptible lines was unexpected. This ratio suggests either the presence of a more complex, multi-locus resistance or an unintentional selection bias that inadvertently favored resistant genotypes. Closer examination of the genotypic data

for each of the markers mapped to linkage group B4b revealed that all markers were skewed in favor of the 115M allele and the segregation ratios were similar to that observed for rust resistance. In contrast, markers residing on linkage group B4 of the 'Jaguar'/115M map, which corresponds to the upper end of linkage group B4 on the consensus map, are not skewed significantly. The reason for the skewed segregation ratio at one end of the linkage group but not the other remains unclear. A QTL for lodging co-located with rust resistance (Table 2.13, Figure 2.2) raises further questions about the implications of the increased frequency of the 115M allele. The location of that QTL for lodging in a genomic region skewed toward 115M agrees with the increased frequency of RILs that lodge similarly to 115M.

The tagging of the resistance source present in 115M with flanking TRAP markers supports the conclusion of Miklas et al. (2006b) who suggested the utility of TRAP markers to tag disease resistance genes of common bean. These markers present a useful tool to use for indirect selection of rust resistance. F7R1.150, linked in coupling phase with rust resistance at a distance of 3.1cM, would be the best marker to use for marker assisted selection. Only three recombinants were observed when comparing the presence of F7R1.150 with rust resistance. Six recombinants were observed between F15R10.580 and rust resistance. This suggests F15R10.580 is less tightly linked to the resistance loci, although still valuable when used in addition to F7R1.150 to flank the region surrounding the resistance gene.

Since several disease resistance genes have been mapped to the same region of B4, these markers may be useful in selecting for other resistance genes within the cluster if their linkage can be verified. For example, F7R1.150 was also present in 'Mexico309', which implies the marker is linked to *Ur-5*. The SI19 SCAR marker linked to *Ur-5* (Melotto and Kelly, 2000) was present in 115M, but did not segregate in the RIL population, which

prevented the mapping of both F7R1.150 and SI19 markers in relation to each other. Screening additional 115M plants with SI19 revealed the marker was not present in all cases, suggesting 115M is heterogeneous at this marker locus. A previous study by Miklas et al. (2000) attempted to map *Ur-5* in relation to Ur-Dorado-108, but found that only the SI19 marker and not the *Ur-5* gene segregated in a 'Dorado' by XAN159 population. These results underscore the difficulty in reconciling phenotypic and genotypic data at complex resistance gene clusters. Additional markers mapped to this region in the future will help verify the relationship between genes at this locus. Allelism tests between 115M, 'Dorado', and 'Mexico309'(*Ur-5*) will also be necessary to determine the precise relationship among the resistance loci in these cultivars.

Conclusions

Breakdown of previously effective resistance to any pathogen presents a challenge to breeders to identify alternative solutions. Although the *Ur-3* gene was overcome in Michigan bean fields during the 2007 growing season by a new rust race 3:22, the long-term implications of this discovery remain uncertain. This knowledge should serve as a reminder that pathogen populations are continually evolving, and maintaining successful genetic resistance requires continual effort. Further work is needed to determine the precise identity of the resistance gene conditioning resistance in 115M. Allelism tests with *Ur-5* and *Ur-Dorado-108* should be performed in the future. Additionally, TRAP markers F7R1.150 and F15R10.580 that flanked and co-segregated with rust resistance should be considered for use in marker assisted selection to incorporate resistance to the new rust race 3:22 in future bean cultivars for production in Michigan.

Table 3.1. Reaction of 12 differential common bean cultivars inoculated with race 3:22 of common bean rust collected from Tuscola county, MI.

Middle American Genotype	Binary Value	R-Gene†	Reaction‡§	Andean Genotype	Binary Value	R-Gene†	Reaction‡§
GN1140	(1)	*Ur-7*	3,4 (S)	Early Gallatin	(1)	*Ur-4*	2,3
Aurora	(2)	*Ur-3*	6,5 (S)	Redlands Pioneer	(2)	*Ur-13*	4,3 (S)
Mexico 235	(4)	*Ur-3+*	3	Montcalm	(4)	unknown	4,5 (S)
Mexico 309	(8)	*Ur-5*	1	PC 50	(8)	*Ur-9, Ur-12*	2,3
CNC	(16)	*Ur-CNC*	2,3	Golden Gate Wax	(16)	*Ur-6*	5,6 (S)
PI181996	(32)	*Ur-11*	1	PI260418	(32)	unknown	2,3

†Rust resistance gene present in differential cultivar according to Steadman et al. (2002).

‡Reactions scored from 1=resistant to 6=susceptible according to Steadman et al. (2002).

§Most frequent reaction, followed less frequent reaction if present. Values ≥ 4 are recorded as susceptible. Race is identified based on summation of binary values associated with susceptible cultivars (Middle American:Andean).

Table 3.2. Reactions of selected bean cultivars to inoculation with *U. appendiculatus* race 3:22.

Cultivar	Reaction	Class
Eclipse	5,6	Black
Jaguar	5,6	Black
Shania	3/5*	Black
Zorro	5,6	Black
Vista	5	Navy
Matterhorn	5,4	Great Northern
Lapaz	5,6	Pinto
Othello	5,6	Pinto
Merlot	5,4	Red
Dorado	5,6	Red

***Heterogeneous: Four resistant plants and two susceptible plants**

Table 3.3. Reactions of 96 RILs from a 'Jaguar' by 115M population to *U. appendiculatus* rust race 3:22 and presence or absence of two TRAP markers (F7R1, F15R10).

RIL	RUST	F7R1	F15R10	RIL	RUST	F7R1	F15R10	RIL	RUST	F7R1	F15R10
1	H	P	A	34	R	P	A	67	S	A	P
2	R	P	A	35	R	P	A	68	R	P	A
3	R	P	A	36	H	A	P	69	R	P	A
4	R	A	P	37	H	A	A	70	R	P	A
5	R	P	A	38	R	P	A	71	H	A	P
6	R	P	A	39	R	P	A	72	S	A	P
7	S	P	A	40	R	P	P	73	R	P	A
8	S	P	A	41	R	P	A	74	R	P	A
9	R	P	A	42	S	A	P	75	S	A	P
10	R	P	A	43	R	P	A	76	R	P	A
11	H	A	P	44	H	P	P	77	S	A	P
12	H	P	A	45	R	P	A	78	R	P	A
13	R	P	A	46	R	P	A	79	R	P	A
14	R	P	A	47	R	P	A	80	R	P	A
15	H	P	P	48	R	P	A	81	H	P	A
16	R	-	P	49	R	P	A	82	R	P	A
17	S	A	P	50	R	P	A	83	R	P	A
18	R	P	A	51	R	P	A	84	H	P	A
19	S	A	P	52	R	P	A	85	H	P	A
20	H	P	A	53	H	P	P	86	R	P	A
21	H	P	P	54	R	P	A	87	R	P	A
22	R	P	A	55	S	A	P	88	R	P	A
23	R	P	A	56	S	A	A	89	R	P	A
24	R	P	A	57	R	P	A	90	H	P	P
25	R	P	A	58	S	A	P	91	R	P	A
26	R	P	A	59	R	P	A	92	H	A	P
27	R	P	A	60	R	P	A	93	R	P	A
28	R	P	A	61	R	P	A	94	S	A	P
29	H	A	P	62	R	P	A	95	R	P	A
30	R	P	A	63	R	P	A	96	H	A	P
31	R	P	A	64	R	P	A	115M	R	P	A
32	S	A	P	65	R	P	A	Jaguar	S	A	P
33	R	P	A	66	S	A	P	Tacana	R	P	A

A=Absent, P=Present, R=Resistant, S=Susceptible, H=Heterogeneous

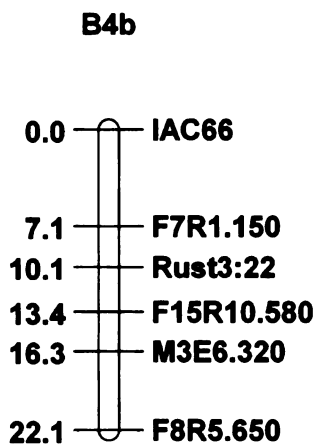

Figure 3.1. Linkage group B4b of the 'Jaguar' by 115M recombinant inbred line population which contains the resistance locus for *U. appendiculatus* race 3:22.

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Appendix A: Indirect screening for color loss in two black bean populations

Introduction

Black bean (*Phaseolus vulgaris* L.) is especially prone to loss of seed coat color during the thermal processing prior to canning (Bushey and Hosfield, 2000; 2004). This color leaching results in a canned bean product that appears brown or washed-out, and visually unappealing to consumers. Prior work undertaken to better understand the physiology and genetics associated with this leaching suggests the value of a rapid screen to detect differences between black bean breeding lines at an early generation when seed quantities are limited. Lu et al. (1996), Ruengsakulrach et al. (1991) and Shellie and Hosfield (1991) proposed screening methods that evaluated various physical or chemical characteristics of a small seed sample and correlated the results with those of traditional canning protocols. To date, none of these early generation selection methods have been widely implemented, suggesting the need for continued research in this economically important aspect of black bean breeding.

Another indirect screening method, the soak water color test, was recently developed by Bushey and Hosfield (2007). Their method requires ten seeds per line, along with minimal lab facilities and time, to indirectly screen for color retention in black bean. The objectives of the current study were twofold. The first was to purify and re-establish the original populations used by Bushey and Hosfield (2007) to develop this screening technique as a genetic resource to facilitate future study of black bean color retention. The second was to verify the reproducibility of the technique in other germplasm for future use in breeding for color retention in black beans.

Materials and Methods

Seed of two black bean populations previously established by G.L Hosfield was obtained from USDA-ARS. Population 1 ('Black Magic' x 'Shiny Crow') consisted of 93 recombinant inbred lines (RILs), while population 2 ('Black Magic' x 'Raven') consisted of 106 RILs. 'Black Magic' and 'Raven' are black beans with a dull seed coat luster and 'Shiny Crow' has a shiny seed coat. Several of the bulks in population 1 segregated for both shiny and dull seed within a line, so a single seed descent purification process was immediately undertaken for each line in the MSU greenhouse during spring 2007. At maturity, single plant rows were established at the Saginaw Valley Bean and Beet Research Farm near Saginaw, MI. Rows were harvested as bulks and data collected on each line included: total seed weight, seed coat (dull or shiny), 100-seed weight, and dry seed color (measured with HunterLab LabScanXE, Reston, VA.). Two samples of 10 seeds each were then taken from each line and tested using the soak water color test as described by Bushey and Hosfield (2007). Soak water color was determined both as a luminosity (L) value using a HunterLab UltraScanXE and as a visual rating from 1=clear to 5=very dark.

In addition, eleven of the lines in population 1 that were segregating for shiny and dull seed coats were randomly chosen for use in creating a group of near isogenic lines (NILs) differing in seed coat luster. The only differences in procedure from that described above were three shiny and three dull seeds for each of the eleven lines were planted in the greenhouse and then bulked prior to planting in the field.

Results and Discussion

The amount of seed obtained for each line within the populations varied from 53 to 963g, with most lines producing sufficient seed to facilitate future work in replicated field plots. The few lines that produced little seed were the result of plant rows containing very few plants. Seed size, measured as 100-seed weight, ranged from 15.7 to 27.0g. On average,

population 1 had larger seed size, with a mean of 21.2g, while population 2 was slightly smaller with a mean of 19.7g (Table A.1). As expected, population 1 segregated by line for seed coat luster; 39 lines had shiny seed coats, while 54 were dull. All lines in population 2 had dull seed coats, as expected.

Dry seed color was equivalent between RILs in the two populations. However, differences between shiny versus dull seed coats became apparent in the soak water color test. In this test, a lower luminosity value for the soak water indicates more color loss from the bean, thus a higher luminosity value is more desirable. Population 1, where 39 lines had shiny seed coats, had an average luminosity of 75.6 and visual rating of 3.1 (Table A.1). In contrast, population 2, with all dull seed coats, had an average luminosity of 61.3 and visual rating of 4.5. As shown in Table A.1 both populations had similarly low luminosity and visual values, but population 1 had higher values reflecting the presence of lines with shiny seed coats that did not leach as much color. As expected, the lines with dull seed coat luster in population 1 lost more color than those with shiny seed coats. However they retained more color than the lines in population 2 which all had dull seed coat luster. These data suggest that selecting for dull seed coat luster from progeny derived from crosses between dull and shiny black beans may improve color retention.

Similar trends were observed in seed color when comparing the 11 NILs differing only in seed coat luster. Dry seed color was very similar between the two groups, while soak water color was much lighter in the shiny group that leached less color (Table A.2). The range in soak water color resembled the range in values measured in population 1, suggesting that the NILs reflect the range in color retention of the RIL population. Seed size varied from an average of 22.2 to 25.6g/100-seed for the shiny and dull groups of NILs, respectively. This was unexpected, since the mean seed sizes for the dull and shiny groups of RILs derived from population 1 were the same, and smaller than either group of NILs. One explanation for

these differences is that the initial lines used for development of NILs were chosen at random from population 1, with no selection based on seed size. Since only eleven of 93 lines were used, if several larger seeded lines were chosen, they would have easily increased the mean of the NILs, whereas the average for the RILs represents the full variability of the entire population. However, there is no apparent reason for the difference between the shiny and dull groups of NILs, and the results for the larger group of RILs suggests there is not a relationship between seed size and seed coat luster.

Conclusions

These data demonstrate that much of the variation for color loss originally present in two populations was maintained throughout the process of purification and renewal. The genetic variation for black bean color retention presents a unique opportunity for continued study of this economically important trait. Individuals in population 1 that have a dull seed coat facilitating water uptake, but a high luminosity value for soak water color would be particularly interesting to breeders (Table A.1). These lines possessed improved color retention when compared with the average color retention of population 2. These results suggest that black beans with a shiny seed coat luster are useful for improving color retention in black beans with dull seed coat luster and this strategy may provide an opportunity to improve processing. In contrast, the luminosity and visual scores in population 2 underscore the difficulty that breeders must confront in retaining processed seed color when crossing two black bean lines with dull seed coats.

The group of NILs developed represent a useful genetic tool for studying other changes associated with differences in seed coat luster. The range in luminosity among these lines suggest the NILs reflect the variability for color retention present in population 1. While it is evident that shiny or dull seed coats cause beans to take up water differently and therefore influence their color retention, future analysis at the molecular level is needed to

elucidate additional genetic differences. Such studies will provide practical knowledge useful for breeding future black bean cultivars with improved processing characteristics.

Table A.1. Trait means and ranges for five traits in two populations segregating for color retention based on the 'Soak Water Color Test'.

	Population 1: Black Magic x Shiny Crow					Population 2	
	Overall	Dull		Shiny		Black Magic x Raven	
Trait	Mean	Mean	Range	Mean	Range	Mean	Range
Dry Seed Color (L)	19.3	19.0	17.2-23.4	19.8	17.2-21.4	19	16.2-22.6
Soak Water Color (L)	75.6	68.2	46.5-90.8	85.9	53.9-97.0	61.3	43.1-75.5
Soak Water Color-Visual	3.1	3.9	1.5-5.0	2.0	1.0-5.0	4.5	2.5-5.0
100-Seed Weight (g)	21.2	21.2	15.7-27.0	21.2	17.0-26.8	19.7	15.1-24.7

Visual rating: 1=clear 5=very dark (Bushey and Hosfield, 2007)

Table A.2. Trait means and ranges for five traits measured on 11 pairs of NILs selected from population 1 (Black Magic x Shiny Crow) on basis of dull or shiny seed coat.

	Shiny		Dull	
Trait	Mean	Range	Mean	Range
Dry Seed Color (L)	19.8	18.0-22.2	19.2	18.2-20.3
Soak Water Color (L)	85.5	73.8-98.5	61.9	51.8-78.7
Soak Water Color-Visual	1.9	1.0-3.5	4.5	3.0-5.0
100-Seed Weight (g)	22.2	17.8-26.1	25.6	22.2-31.0

Visual rating: 1=clear 5=very dark (Bushey and Hosfield, 2007)

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Appendix B: Validation of the soak water color test in the 'Jaguar'/115M RIL population

Introduction

The soak water color test was developed by Bushey and Hosfield (2007) as an indirect screening method to predict color loss from black beans that inevitably occurs during the thermal processing associated with canning. Loss of black pigment during canning results in processed black beans that appear brown and washed out, which makes them unappealing to consumers, and therefore processors (Bushey et al., 2000). Due to the importance of color retention, potential black bean cultivars with superior agronomic traits will be discarded if they fail to produce an acceptable quality canned product (Posa-Macalincag et al., 2002). The soak water color test provides a means to screen breeding lines at an early generation before several years are invested to generate enough seed to perform traditional canning evaluations (Bushey et al., 2004). This method uses as little as 10 seeds (<3g) and two hours of time, while at least 100g of seed are needed to evaluate a line by traditional canning protocols that require more time, supplies, and specialized equipment. Since the soak water color test had only been used in the two related populations where it was initially developed, the objective of this study was to determine the reproducibility of the method in different genetic backgrounds, and assess the correlation between this indirect method and canning scores, both within a population and among a group of unrelated breeding lines.

Materials and Methods

Seed from plots grown at the Saginaw Valley Bean and Beet Research Farm (Saginaw, MI) in 2005 was evaluated using both the soak water color test as described by Bushey and Hosfield (2007) and by the canning procedure described in detail at the BIC website: (http://www.css.msu.edu/bic/PDF/Bean%20Processing.pdf). Seed from the same

plot was used for both methods and was free of splits or cracks in the seed coat. The 96 RILs derived from the cross of 'Jaguar'/115M were used to compare the methods within a population. Similarly, 32 of the top yielding lines from the standard black yield trial grown at the same location in 2006 were used to represent a group of genetically diverse black bean breeding lines. The soak water color test was performed on two occasions with two replications each, while visual appearance was based on the average score assigned to a single can by a group of panelists rated on a scale of: 1=undesirable to 7=desirable. Proc GLM (SAS, 2000) was used to calculate significant differences in color loss due to genotype and replication. Pearson correlation coefficients for luminosity measured by the soak water color test and canning score were calculated with Proc Corr (SAS, 2000) using the average luminosity (L-value) obtained from the two replications.

Results and Discussion

Both the RIL population and the group of breeding lines showed significant ($p<0.0001$) variation for color loss and visual appearance, suggesting these groups were suitable for evaluating a range in seed quality by both the soak water color test and canning methodologies. Transgressive segregation was observed in the population using both methods, although none of the RILs had higher visual appearance scores than 'Jaguar', even though several had values lower than 115M (Table B.1, Table B.2). In the group of breeding lines, considerable variability was also noted, with some lines higher and others lower than the parents of the RIL population (Table B.3). Unexpectedly, 115M had a higher visual appearance score than 'Jaguar' in 2006. This result was unexpected since 115M generally has very poor canned bean quality, although inconsistent results in canning quality have occurred occasionally in other years (data not shown).

The correlation between the results of the soak water color test and the visual appearance showed different relationships in the two groups. In the population, there was a

significant negative correlation between the two methods (Table B.2). This result seems counterintuitive as it suggests a higher (more desirable) visual appearance score is associated with a lower soak water luminosity value, and therefore more leaching of color in the soak water. Intuitively, leaching more color when soaked in hot brine would be an indicator of color loss that will be exacerbated by the increased temperature and pressure associated with the canning process. These results support the hypothesis that different breeding lines have different quantities of anthocyanin pigment in the seed coat, and therefore could leach more color but still retain a darker seed color following processing.

Salinas-Moreno et al. (2005) compared dry seed luminosity with quantity of anthocyanins in the seed coat for a diverse group of black beans. Their results indicated a significant range in anthocyanin content ranging from 10.1 to 18.1 mg/g; however the difference in dry seed luminosity for these two genotypes was insignificant (L=17.9 and 18.1). Although only anthocyanins were measured, this study suggests that genotypes with very similar luminosity values vary greatly in pigment content, so estimating color loss by measuring the total amount of pigment that is leached into a standard volume of brine may not be an appropriate measure of actual canned bean color. Further measurements of variation for anthocyanins within seeds of the same genotype and luminosity of soaked seeds in a similar type of study would be useful.

Color of the canned bean is only one component of the visual appearance score. Other factors such as splitting, overall texture, or starchiness of the brine were all considered by panelists when rating canned samples. These factors were not considered by the soak water color test, and may explain the lack of a strong correlation with the visual appearance score. A significant positive correlation between the luminosity values from the two separate evaluations of the population suggests that this method reproducibly detects differences in color of the soak water (Table B.2).

In the group of diverse breeding lines from various genetic backgrounds, the results of the two methods did not correlate significantly ($p=0.24$), although the relationship between the two results was still inverse. This result supports the inverse relationship between luminosity of the soak water and canning score observed in the population but it does not disprove the hypothesis that pigment content varies by genotype. However, since the results of the two methods do not significantly correlate, the soak water color test may not be suitable for comparing genetically diverse germplasm.

In practice, comparing a wide range of unrelated germplasm would represent the main application of this rapid screening method. However, results with a sample of unrelated germplasm (Table B.3) suggest this screening method may not reliably predict the canning quality under these conditions. One of the breeding lines (B04644) that leached the most color (Table B.3) has been used as a parent to improve canning quality, as the visual color of the cooked beans is much blacker than other entries, despite leaching more color into the soak water. This would suggest that certain lines possess higher pigment levels and appear to leach more into the soak water, while retaining satisfactory cooked color. An objective reading of cooked bean color is not possible as the meter gives erroneous results based on light reflectance from the surface of moist cooked beans. Cooked bean color is rated visually and panelists have noted the 'blacker' color of cooked samples of the B04644 breeding line when compared with other black bean lines, based on higher score for visual appearance. Other lines that leached less (higher L-values) have been discarded based on poor canning quality as the cooked bean is brown in color and any black pigments in the seed coat have been lost in the soak water.

Recently van der Merwe et al. (2006) suggested that canning evaluations in the laboratory very closely predict performance under commercial canning procedures. Due to the number of factors influencing the quality of canned beans, and the consequences of

bringing a new cultivar with inferior quality to the market, canning should be viewed as a solid investment for breeding programs. The results of the current study indicate the soak water color test was not appropriate for accurately comparing diverse black bean breeding lines, and should not be considered for screening diverse germplasm for color loss during processing.

Conclusions

The limited evaluation of the soak water color test showed this method detects significant variation in color loss, both within and among genetic backgrounds of black bean. This method may have some predictive value for evaluating lines within a population, and the measured luminosity is inversely related to visual canning score. However, the moderate strength of the correlation suggests caution should be exercised when interpreting the results of the soak water color test, and canning score should still be considered more informative since it encompasses all components of canning quality, not just color retention.

A weak and insignificant negative correlation was observed when a group of 32 diverse breeding lines were evaluated with both methods. Among diverse genetic backgrounds, the soak water color test cannot be considered predictive of color loss during canning. Many of the lines with above average canning characteristics lost the most color when measured by this method, suggesting that they had increased levels of black seed coat pigment. Overall, these results suggest canning should remain the preferred method of evaluating color retention in black bean breeding programs.

Table B.1. Phenotypic values for seed of the 96 'Jaguar'/115M recombinant inbred lines evaluated by both the soak water color test and by visual evaluation of canned bean samples.

	Mean	Jaguar	115M	Range
L-value 1	76.4	56.4	75.6	55.5-90.8
L-value 2	67.6	58.3	83.6	42.6-87.0
Canning Score	2.2	3.8	1.7	1.1-3.8
Dry Seed Color	15.9	15.5	16.3	14.8-18.9

Table B.2. Phenotypic correlations between visual canning score and Hunter L value of leachate from the soak water color test in a population of 96 recombinant inbred lines developed from the cross 'Jaguar'/115M grown in Saginaw, MI in 2005.

	L-value 1	L-value 2	Canning Score
L-Value 1			
L-Value 2	0.86**		
Canning Score	-0.41**	-0.33**	
Dry Seed Color	0.19	0.14	0.01

**Indicates significance at P<0.001.

Table B.3. Phenotypic values for seed of the 32 diverse black bean genotypes evaluated by the soak water color test and by visual evaluation of canned bean samples during 2006.

Black Bean Genotypes	L-value	Visual Appearance§
B04596	90.8	2.2
B05069	90.3	2.8
Raven†	90.3	NA
B01793	89.6	3.0
B04585	89.3	3.2
Domino	88.4	2.3
B05024	87.6	2.7
B05051	86.9	2.6
B05065	85.0	2.9
B05066	85.0	2.8
B03622	84.8	3.0
B04607	84.6	3.6
B04227	84.0	2.7
B01741	84.0	2.8
B04610	83.4	3.1
Condor	82.9	4.3
B05070	82.4	3.8
B04561	82.2	2.3
B04591	81.5	4.2
Zorro	81.1	4.0
B05055	80.7	3.6
115M	80.6	3.8
B04587	79.2	2.8
Jaguar	78.1	3.2
B05054	78.1	3.0
B05041	77.9	3.6
Raven	77.8	2.7
B05040	77.5	3.9
B04260	76.4	2.6
B05039	76.3	3.3
B04644‡	76.3	4.1
T-39	75.4	3.1
B04644	71.8	2.6
Eclipse	69.1	2.7

† Grown at Montcalm Research Farm

‡ Grown in Presque Isle, MI

§ Canning scores range from 1=undesirable to 4=neither undesirable nor desirable to 7=desirable

Table B.4. Phenotypic values for all of the 96 RILs 'Jaguar'/115M RILs evaluated by the soak water color test and by visual evaluation of canned bean samples in 2005.

Entry	AVG L	VA	Entry	AVG L	VA	Entry	AVG L	VA
B04399	87.03	1.7	B04385	72.49	1.7	B04387	62.64	1.5
B04392	86.81	1.9	B04415	72.48	2.1	B04454	62.52	3.1
B04370	83.67	1.8	B04390	72.45	1.9	B04401	62.45	1.8
B04375	83.38	1.3	B04359	72.21	2.1	B04411	62.29	1.6
B04417	81.60	2.6	B04422	71.91	1.5	B04427	62.07	2.3
B04380	81.45	1.7	B04443	71.83	1.6	B04430	62.00	2.5
B04389	81.10	1.7	B04405	71.43	2.9	B95556	61.97	3.8
B04448	80.59	2.0	I01892	71.35	1.7	B04386	61.90	2.4
I98402	80.50	1.8	B04423	71.16	2.5	B04412	59.79	2.7
B04379	80.42	2.4	B04406	71.03	2.4	B04451	59.78	2.2
B04396	80.06	2.1	B04361	70.67	2.7	B04432	59.03	2.3
B04372	79.80	2.1	B04384	70.39	1.7	B04426	58.78	3.0
B04438	79.35	2.5	B04376	69.67	1.4	B04381	58.14	2.2
B04382	78.79	2.4	B04400	69.66	2.5	B04393	56.19	2.0
B04365	78.55	1.3	B04368	69.13	2.2	B04440	55.56	3.6
B04428	78.08	1.4	B04394	69.11	2.3	B04436	55.46	2.7
B04373	78.04	2.3	B04403	68.80	1.5	B04416	55.19	2.3
B04369	76.83	2.5	B04374	68.22	2.1	B04445	54.85	2.4
B04441	75.87	3.6	B04452	68.07	2.6	B04362	54.52	2.4
B04366	75.46	2.3	B04418	67.83	3.1	B04378	53.69	2.0
B04431	75.26	2.3	B04447	67.34	3.0	B04410	53.65	1.9
B04397	74.91	1.9	B04433	67.30	1.9	B04383	53.26	2.1
B04398	74.78	2.2	B04420	67.15	2.0	B04429	53.01	1.9

Table B.4 (cont'd.)

Entry	AVG L	VA	Entry	AVG L	VA	Entry	AVG L	VA
B04409	74.65	1.7	B04414	67.01	2.2	B04453	52.69	1.9
B04437	74.15	2.0	B04395	66.64	3.0	I81066	52.06	3.7
B04360	73.89	1.1	B04435	66.63	1.6	B04404	51.84	2.4
B04391	73.73	1.5	B04388	66.41	2.0	B04446	51.56	2.2
B04413	73.68	2.0	B04408	66.03	3.1	B04419	50.75	3.0
B04363	73.42	2.1	B04377	65.75	2.6	B04450	50.37	2.4
B04367	73.41	2.4	B04371	63.82	2.5	B04425	50.09	3.5
B04421	73.25	2.0	B04434	63.60	2.7	B04442	48.72	2.0
B04402	73.15	2.3	B04364	63.55	1.3	B04439	42.64	2.4
B04407	72.96	1.8	B04449	63.51	2.5			
B04444	72.70	2.5	B04424	63.39	2.2			

AVG L= Average luminosity value (L), VA=Visual Appearance

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Appendix C: Supplemental Data Collected from the 'Jaguar' by 115M RIL population from 2004-2007.

Table C.1. 2004 Agronomic and canning data for the 'Jaguar' by 115M RIL population.

Line	Entry	VA†	YLD	SDWT	FLWR	MTR	LDG	HT	DS
			kg/ha	g	days	days		cm	
B04404	46	2.6	3013.1	20.2	47.0	94.5	1.0	48.1	4.5
B04445	87	2.4	2990.6	20.1	47.0	96.0	1.0	49.0	5.0
B04407	49	2.5	2968.1	20.4	47.0	92.0	1.0	47.6	6.0
B04422	64	2.4	2956.9	21.9	46.0	94.5	1.0	48.0	5.0
B04431	73	2.7	2866.9	22.2	48.0	96.5	1.0	50.1	5.0
B04384	26	2.1	2833.2	20.5	46.0	95.0	1.0	47.1	5.0
B04444	86	3.1	2833.2	17.9	47.0	94.0	1.0	48.5	5.5
B04429	71	2.5	2822.0	21.9	46.0	93.5	1.0	48.5	5.0
B04374	16	2.5	2788.2	21.0	46.0	94.0	1.5	47.1	5.5
T-39	13	3.2	2777.0	19.9	47.0	93.0	2.0	47.0	5.0
115M	100	2.4	2754.5	22.2	47.0	94.0	1.5	48.5	4.5
B04394	36	3.4	2743.3	21.7	48.0	95.5	1.0	48.5	5.0
B04383	25	2.7	2743.3	19.6	46.0	94.0	1.0	46.6	5.0
B04403	45	2.3	2720.8	18.5	46.0	94.0	1.0	47.5	5.0
B04443	85	2.5	2653.3	17.7	47.0	93.9	1.0	47.0	5.0
B04452	94	3.5	2653.3	19.5	48.0	94.0	1.0	48.5	6.0
B04439	81	3.5	2653.3	22.3	45.0	92.0	1.0	47.9	5.0
B04433	75	3.4	2653.3	18.8	47.0	92.0	1.0	47.1	5.5
B04387	29	2.9	2653.3	20.7	45.0	96.0	1.0	48.6	4.5
B04361	3	2.7	2642.1	19.0	48.0	94.5	1.0	47.6	5.0
B04362	99	3.0	2630.8	19.0	45.0	92.5	1.0	46.0	5.5
B04411	53	2.1	2630.8	20.6	47.0	95.0	1.0	48.5	6.0
B04453	95	2.5	2619.6	21.8	46.0	93.5	1.0	47.0	5.5
B04360	2	3.0	2585.9	19.8	49.0	95.5	1.0	48.6	4.5
B04367	9	3.0	2563.4	20.9	47.0	94.0	1.0	47.0	5.0
B04370	12	2.5	2552.1	19.9	47.0	95.0	1.0	49.1	5.5
B04441	83	3.1	2540.9	20.7	47.0	94.0	1.0	47.0	5.5
B04406	48	3.5	2540.9	19.0	45.0	93.5	1.0	47.0	5.0
B04451	93	3.0	2507.2	21.0	46.0	95.0	1.0	47.0	5.0
B04414	56	2.9	2495.9	22.3	47.0	93.9	1.0	48.0	5.0
B04412	54		2484.7	19.5	46.0	92.0	1.0	46.4	6.0
B04385	27		2462.2	21.7	48.0	95.5	1.0	48.6	4.5
B04399	41		2451.0	19.9	46.0	92.0	1.0	47.0	6.0
B04418	60		2451.0	21.4	46.0	94.4	1.0	47.5	5.0
B04376	97		2451.0	19.5	48.0	93.0	1.0	48.0	6.0
B04420	62		2417.2	20.6	46.0	92.5	1.0	46.5	5.0
B04410	52		2372.3	20.2	48.0	96.5	1.0	50.0	5.0
B04435	77		2372.3	19.0	47.0	95.5	1.0	48.4	5.5

Table C.1 (cont'd.)

B04391	33		2372.3	24.0	47.0	95.5	1.0	49.0	4.5
B04382	24		2372.3	20.2	47.0	93.5	1.0	46.5	5.5
B04409	51		2349.8	19.8	45.0	95.5	1.0	47.5	4.5
B04398	40		2349.8	21.3	46.0	95.0	1.0	48.1	6.0
B04369	11		2338.5	20.1	46.0	92.0	1.0	47.4	5.5
B04436	78		2327.3	22.1	46.0	93.0	1.0	47.0	5.0
B04359	1		2304.8	20.9	47.0	94.0	1.0	47.4	5.0
B04397	39		2304.8	20.4	46.0	92.5	1.0	46.5	5.5
B04365	7		2304.8	20.5	46.0	93.5	1.0	47.4	5.0
B04402	44		2293.6	18.4	47.0	94.0	1.0	46.5	5.0
B04373	15		2282.3	17.4	47.0	94.5	1.0	47.0	5.0
B04421	63		2271.1	20.6	46.0	93.0	1.0	46.5	5.0
B04450	92		2259.8	19.8	45.0	91.5	1.0	44.0	5.5
B04413	55		2259.8	20.4	47.0	94.5	1.0	45.6	5.0
B04363	5		2259.8	19.7	47.0	94.5	1.0	46.0	5.0
B04449	91		2248.6	19.4	46.0	94.5	1.0	47.5	5.0
B04400	42		2248.6	19.5	47.0	94.0	1.0	47.5	4.5
B04423	65		2226.1	19.4	49.0	95.0	1.0	48.5	5.0
B04447	89		2226.1	21.1	47.0	94.4	1.0	47.9	6.0
B04372	14		2214.9	21.2	48.0	95.5	1.0	48.1	5.0
B04405	47		2214.9	19.4	47.0	91.5	1.0	45.5	5.0
Jaguar	4	4.4	2169.9	19.5	47.0	92.0	1.0	45.0	5.0
B04396	38		2169.9	19.1	46.0	94.0	1.0	45.6	4.5
B04368	10		2158.6	19.3	48.0	93.5	1.0	48.4	5.5
B04366	8		2124.9	20.2	47.0	92.5	1.0	47.0	5.5
B04454	96		2124.9	20.6	45.0	91.0	1.0	46.4	6.0
B04428	70		2113.7	19.8	48.0	96.0	1.0	48.5	5.0
B04393	35		2057.4	19.1	48.0	96.4	1.0	48.9	4.0
B04430	72		2023.7	22.3	45.0	93.1	1.0	45.1	5.0
B04446	88		2012.5	19.2	47.0	94.5	1.0	47.6	5.0
B04438	80		1990.0	21.7	47.0	93.5	1.0	45.5	5.0
B04415	57		1990.0	21.3	47.0	92.0	1.0	46.0	5.5
B04440	82		1978.7	20.0	45.0	92.5	1.0	46.5	5.0
B04378	20		1967.5	20.6	45.0	91.5	1.0	45.0	5.5
B04389	31		1956.3	18.9	45.0	92.5	1.0	44.5	5.0
B04386	28		1956.3	19.3	46.0	91.5	1.0	44.0	5.5
B04401	43		1933.8	20.4	46.0	91.5	1.0	45.0	5.0
B04408	50		1933.8	18.1	46.0	94.5	1.0	45.5	4.5
B04381	23		1922.5	20.7	46.0	91.5	1.0	45.9	5.0
Tacana	18		1911.3	22.5	47.0	94.1	1.0	46.0	4.5
B04364	6		1900.0	20.5	46.0	95.0	1.0	46.5	4.0
B04419	61		1877.6	20.6	47.0	91.5	1.0	46.0	5.5

Table C.1 (cont'd.)

B04377	19	1866.3	19.4	47.0	92.5	1.0	45.0	5.0
B04432	74	1821.3	19.7	46.0	92.5	1.0	47.0	5.0
B04390	32	1810.1	21.6	48.0	94.0	1.0	46.1	4.5
B04375	17	1787.6	20.0	47.0	92.5	1.0	43.0	4.5
B04448	90	1776.4	21.6	45.0	95.5	1.0	47.9	5.0
B04426	68	1753.9	18.8	46.0	93.0	1.0	44.5	5.0
B04371	98	1753.9	20.5	45.0	92.5	1.0	45.5	4.5
B04424	66	1742.6	20.7	45.0	92.5	1.0	47.0	5.0
B04379	21	1708.9	19.3	45.0	94.5	1.0	45.9	5.0
B04416	58	1686.4	21.6	45.0	91.0	1.0	43.0	4.0
B04434	76	1630.2	23.4	49.0	96.0	1.5	49.0	4.0
B04417	59	1607.7	19.0	47.0	94.0	1.0	45.5	5.0
B04388	30	1551.5	19.0	47.0	93.0	1.0	46.4	5.0
B04437	79	1529.0	19.5	46.0	92.4	1.0	44.4	4.5
B04395	37	1472.8	22.1	47.0	91.0	1.0	44.5	6.0
B04427	69	1450.3	24.5	47.0	93.5	1.0	45.9	5.0
B04392	34	1439.1	20.6	45.0	93.5	1.0	45.9	5.0
B04380	22	1270.4	22.9	47.0	92.4	1.0	45.4	5.0
B04425	67	1214.2	21.7	47.0	99.0	1.0	49.4	3.0
B04442	84	1214.2	24.0	46.0	94.0	1.0	46.9	5.0
MEANS		2237.3	20.4	46.5	93.7	1.0	46.9	5.1
LSD (p=.05)		607.1	1.7	0.0	0.9	0.1	1.3	0.5
LSD (p=.01)		787.0	2.2	0.0	1.2	0.2	1.7	0.6

VA=Visual Appearance, YLD=Yield, SDWT=100-seed Weight, FLWR=Days to Flowering, MTR=Maturity, LDG=Lodging Score, HT=Plant Height, DS=Agronomic Desirability

† Only a subset of the population was evaluated for visual appearance in 2004.

Table C.2. 2005 Agronomic and canning data for the 'Jaguar' by 115M RIL population.

Line	Entry	VA	YLD	SDWT	FLWR	MTR	LDG	HT	DS
			kg/ha	g	days	days		cm	
B04366	8	2.3	4171.1	22.9	42.0	95.0	2.0	48.0	4.5
B04412	54	2.7	4159.9	23.3	42.0	96.4	1.0	48.0	5.5
B04444	86	2.5	4137.4	20.6	45.0	95.6	1.5	48.5	4.5
B04404	46	2.4	4137.4	23.6	43.0	96.0	2.5	47.5	3.5
B04449	91	2.5	4092.4	20.9	45.0	95.0	2.0	47.5	5.0
B04431	73	2.3	4081.2	23.5	45.0	96.0	2.0	48.5	5.0
B04410	52	1.9	4047.4	22.4	44.0	96.9	2.0	48.0	5.0
B04386	28	2.4	4013.7	22.1	43.0	96.0	1.0	47.5	6.0
B04384	26	1.7	3991.2	21.7	42.0	96.0	2.0	48.0	5.5
B04365	7	1.3	3923.8	21.8	43.0	97.0	2.0	49.0	4.0
B04445	87	2.4	3912.5	22.9	45.0	96.6	2.0	48.5	5.0
B04433	75	1.9	3912.5	21.5	43.0	96.0	1.5	49.5	5.5
B04394	36	2.3	3912.5	24.6	46.0	97.0	3.0	42.5	3.0
B04411	53	1.6	3901.3	23.4	44.0	95.6	2.0	47.5	4.5
B04396	38	2.1	3878.8	21.0	42.0	97.5	2.0	47.0	4.0
B04391	33	1.5	3867.6	25.1	45.0	98.1	2.0	48.0	3.0
B04423	65	2.5	3856.3	20.7	46.0	96.5	2.0	50.0	4.5
B04429	71	1.9	3856.3	24.5	43.0	95.5	1.5	49.0	4.0
B04446	88	2.2	3856.3	20.3	45.0	95.9	2.0	48.0	5.0
B04360	2	1.1	3822.6	21.5	45.0	97.0	2.0	48.5	4.0
B04363	5	2.1	3822.6	22.5	42.0	95.1	2.0	48.5	5.0
B04451	93	2.2	3788.9	24.6	45.0	95.9	2.0	46.5	5.0
115M	100	1.7	3777.6	23.6	44.0	96.0	2.5	46.0	3.5
B04376	97	1.4	3755.1	23.2	43.0	96.1	1.0	49.0	5.0
B04443	85	1.6	3743.9	20.2	43.0	96.0	1.5	48.0	5.0
B04387	29	1.5	3743.9	24.2	43.0	95.9	2.5	44.5	4.0
B04435	77	1.6	3732.6	20.6	45.0	95.9	2.0	48.5	4.0
B04369	11	2.5	3732.6	23.7	42.0	95.0	2.0	47.5	5.5
B04374	16	2.1	3721.4	24.4	43.0	96.9	3.0	46.0	4.0
B04368	10	2.2	3721.4	21.1	46.0	95.5	2.0	47.0	4.0
B04414	56	2.2	3710.2	21.8	43.0	96.0	1.0	49.0	4.0
B04453	95	1.9	3687.7	23.8	44.0	96.1	2.0	48.5	4.5
B04413	55	2.0	3687.7	24.3	44.0	97.0	1.0	48.5	5.0
B04452	94	2.6	3687.7	20.9	45.0	96.0	2.0	49.0	4.5
B04405	47	2.9	3676.4	21.8	42.0	95.0	1.0	46.9	6.0
B04417	59	2.6	3676.4	22.0	43.0	95.0	1.5	46.5	5.0
B04454	96	3.1	3676.4	24.1	42.0	95.5	1.5	47.0	5.0
Jaguar	4	3.8	3665.2	21.5	43.0	95.5	1.5	48.5	6.0
B04447	89	3.0	3642.7	23.5	44.0	96.4	2.0	46.5	4.5
B04398	40	2.2	3631.5	23.4	44.0	96.0	2.0	47.5	4.5

Table C.2 (cont'd.)

B04409	51	1.7	3631.5	21.9	45.0	96.1	2.0	48.5	4.5
B04397	39	1.9	3631.5	22.7	43.0	96.0	2.0	49.0	4.5
B04370	12	1.8	3631.5	21.8	45.0	96.1	2.0	48.0	4.5
B04407	49	1.8	3609.0	23.5	43.0	95.0	2.0	46.5	5.5
B04383	25	2.1	3597.7	23.1	43.0	96.5	2.0	46.5	5.0
B04388	30	2.0	3564.0	21.9	43.0	95.5	1.5	49.0	5.0
B04372	14	2.1	3564.0	22.0	46.0	96.0	2.0	47.5	5.0
B04403	45	1.5	3564.0	20.7	45.0	95.5	2.0	49.0	5.0
B04400	42	2.5	3541.5	22.1	46.0	97.5	2.0	49.0	4.0
B04364	6	1.3	3507.8	23.8	45.0	96.0	2.0	48.0	4.5
B04420	62	2.0	3507.8	23.6	43.0	95.5	1.0	49.0	6.0
B04428	70	1.4	3496.5	23.1	44.0	96.0	1.5	50.0	4.0
B04450	92	2.4	3496.5	23.2	43.0	95.5	2.0	46.5	6.0
B04441	83	3.6	3485.3	22.6	45.0	96.0	2.0	48.0	5.5
B04373	15	2.3	3485.3	22.7	45.0	96.5	1.5	49.0	5.0
B04402	44	2.3	3485.3	21.4	46.0	97.5	1.5	48.0	4.0
B04385	27	1.7	3485.3	22.8	45.0	95.5	2.0	47.5	5.0
B04440	82	3.6	3474.1	21.9	43.0	95.0	1.5	47.0	6.5
B04419	61	3.0	3474.1	26.3	41.0	95.0	1.5	47.5	5.0
B04418	60	3.1	3474.1	22.7	45.0	95.5	2.0	47.0	4.5
B04379	21	2.4	3462.8	21.6	42.0	95.5	2.0	48.5	4.5
B04378	20	2.0	3451.6	22.4	42.0	96.5	2.0	47.5	4.5
B04439	81	2.4	3451.6	24.3	43.0	95.5	2.0	47.5	4.0
Tacana	18	1.8	3451.6	22.9	42.0	95.5	2.0	48.0	4.0
B04432	74	2.3	3384.1	22.6	43.0	95.9	2.0	47.0	5.5
B04406	48	2.4	3384.1	21.2	43.0	95.5	2.0	50.0	5.0
B04361	3	2.7	3361.6	20.9	44.0	95.4	2.0	48.5	5.0
B04421	63	2.0	3361.6	22.1	43.0	95.5	2.0	48.5	5.0
B04362	99	2.4	3350.4	22.0	42.0	96.0	2.0	43.5	4.0
B04382	24	2.4	3327.9	22.8	45.0	96.5	2.0	49.5	4.5
B04367	9	2.4	3316.7	22.8	45.0	96.5	2.0	48.0	4.5
B04422	64	1.5	3305.4	24.1	45.0	96.5	2.0	47.5	5.0
B04427	69	2.3	3282.9	24.4	46.0	95.5	1.5	48.5	5.0
B04380	22	1.7	3282.9	24.2	43.0	95.0	1.0	48.0	5.0
B04377	19	2.6	3271.7	22.2	44.0	95.5	1.0	48.0	6.0
B04430	72	2.5	3238.0	23.3	43.0	95.6	2.0	47.5	5.0
T-39	13	3.7	3226.7	24.3	43.0	95.5	4.0	35.0	3.0
B04359	1	2.1	3204.2	22.8	43.0	95.0	2.0	48.0	4.0
B04438	80	2.5	3181.7	23.3	43.0	96.5	1.5	47.0	4.5
B04434	76	2.7	3170.5	23.8	45.0	98.0	2.5	47.0	4.0
B04424	66	2.2	3159.3	21.8	44.0	95.6	2.0	47.0	4.5
B04416	58	2.3	3159.3	24.2	44.0	95.0	1.0	49.0	4.5

Table C.2 (cont'd.)

B04399	41	1.7	3148.0	22.2	42.0	98.0	1.5	46.5	4.5
B04401	43	1.8	3136.8	22.7	42.0	96.5	1.0	46.5	6.0
B04426	68	3.0	3114.3	20.6	45.0	95.5	1.0	48.0	4.5
B04437	79	2.0	3114.3	22.3	43.0	95.5	2.0	47.5	4.5
B04371	98	2.5	3103.0	23.4	44.0	95.4	2.0	48.5	5.0
B04395	37	3.0	3103.0	26.3	42.0	95.6	1.0	46.0	5.5
B04436	78	2.7	3046.8	23.2	44.0	95.0	1.5	48.5	4.5
B04415	57	2.1	3046.8	25.2	43.0	95.5	1.5	47.0	4.5
B04448	90	2.0	3024.3	22.1	42.0	95.5	2.0	48.0	4.5
B04393	35	2.0	3013.1	21.8	43.0	97.0	2.0	49.0	4.0
B04389	31	1.7	3013.1	22.9	42.0	96.5	1.0	45.9	5.5
B04390	32	1.9	2979.4	23.2	42.0	95.5	1.5	47.5	5.0
B04375	17	1.3	2979.4	22.1	42.0	95.5	1.0	47.0	5.5
B04408	50	3.1	2911.9	20.7	43.0	96.5	2.0	46.0	5.0
B04425	67	3.5	2855.7	23.1	45.0	97.5	2.0	48.5	4.0
B04392	34	1.9	2810.7	24.5	42.0	95.5	1.0	47.5	5.0
B04381	23	2.2	2743.3	24.0	43.0	95.0	1.5	48.0	4.0
B04442	84	2.0	2473.4	24.8	43.0	95.0	2.0	46.0	4.5
MEANS		31.2	3507.8	22.8	43.6	95.9	1.8	47.6	4.7
LSD (p=.05)			314.8	1.2	0.0	0.7	0.4	1.2	0.6
LSD (p=.01)			404.7	1.5	0.0	0.9	0.5	1.6	0.8

VA=Visual Appearance, YLD=Yield, SDWT=100-seed Weight, FLWR=Days to Flowering, MTR=Maturity, LDG=Lodging Score, HT=Plant Height, DS=Agronomic Desirability

Table C.3. 2006 Agronomic and canning data for the 'Jaguar' by 115M RIL population.

Line	Entry	VA	YLD	SDWT	FLWR	MTR	LDG	HT	DS
			kg/ha	g	days	days		cm	
B04391	33	3.5	4261.1	18.9	44.9	98.5	2.6	47.9	2.0
B04431	73	2.9	4013.7	21.2	46.0	95.1	2.0	49.9	4.0
B04411	53	2.9	3957.5	19.8	46.1	95.8	3.0	48.6	3.0
B04398	40	2.5	3912.5	18.8	46.4	94.8	2.1	48.4	4.0
B04370	12	3.1	3912.5	18.6	45.0	97.2	3.5	48.4	3.0
B04444	86	2.9	3867.6	16.7	45.9	93.2	2.0	49.4	4.5
B04445	87	3.6	3856.3	20.7	45.0	96.5	2.6	49.4	3.5
B04404	46	3.7	3856.3	19.0	46.4	92.0	2.5	49.1	4.0
B04446	88	3.4	3856.3	16.3	46.0	96.1	3.0	47.5	2.5
B04429	71	3.4	3822.6	18.8	44.0	92.2	2.0	49.0	4.0
B04443	85	2.6	3822.6	17.1	44.0	94.2	2.0	50.1	4.0
B04423	65	3.4	3822.6	18.0	48.0	94.3	3.5	48.9	3.0
B04414	56	3.4	3800.1	18.1	44.0	95.9	2.5	49.1	3.5
B04400	42	3.3	3766.4	18.1	44.4	94.7	2.1	48.9	4.0
B04386	28	3.3	3755.1	20.1	43.0	91.8	1.4	50.1	5.0
B04397	39	2.7	3732.6	19.8	44.4	95.9	2.0	48.1	4.0
B04412	54	3.6	3721.4	19.2	43.5	92.6	1.5	50.6	4.5
B04450	92	3.7	3721.4	19.4	44.0	93.3	1.6	48.0	4.5
B04451	93	3.4	3721.4	20.3	44.4	93.5	2.0	48.0	4.0
B04376	97	3.3	3710.2	18.7	44.5	94.3	2.0	48.5	4.0
B04410	52	3.2	3710.2	19.4	45.0	97.0	3.0	48.5	3.0
B04435	77	2.8	3698.9	17.3	45.0	94.7	2.5	48.0	3.5
B04440	82	3.2	3698.9	17.7	44.0	92.2	1.5	48.5	4.5
B04364	6	2.4	3687.7	20.3	45.5	93.5	1.5	52.5	4.0
115M	100	2.9	3665.2	19.8	45.0	94.2	2.0	49.0	3.5
B04396	38	2.7	3665.2	19.0	43.0	96.0	3.0	48.5	3.0
B04387	29	2.9	3653.9	19.3	44.5	91.9	2.4	48.1	3.5
B04405	47	3.7	3642.7	19.2	43.5	93.3	1.5	50.0	5.5
B04437	79	3.5	3642.7	19.6	44.1	91.1	2.5	48.5	4.0
B04409	51	3.0	3631.5	19.0	47.4	95.2	3.0	49.0	3.0
B04402	44	2.7	3620.2	16.0	45.5	96.0	2.0	49.5	4.0
B04428	70	2.4	3575.2	20.1	44.0	95.9	2.0	50.5	3.0
B04413	55	3.0	3575.2	18.6	45.5	93.8	2.5	47.9	3.0
B04433	75	3.6	3564.0	17.5	45.0	94.9	2.0	49.9	4.0
B04382	24	3.6	3564.0	19.2	44.6	95.1	2.0	50.0	4.0
B04371	98	3.4	3564.0	19.0	44.0	93.3	2.0	48.9	4.5
B04420	62	2.7	3541.5	18.8	45.0	93.7	2.0	48.4	4.0
B04368	10	3.6	3530.3	18.1	46.1	93.4	2.6	48.4	3.0
B04360	2	3.1	3530.3	18.8	48.1	95.9	3.0	46.9	3.0
B04365	7	2.2	3519.0	19.2	44.7	93.2	2.0	49.0	4.0

Table C.3 (cont'd.)

B04366	8	2.7	3507.8	18.6	44.1	94.3	2.0	49.0	3.5
B04447	89	3.4	3507.8	19.0	45.1	96.2	2.0	47.9	3.5
B04372	14	2.7	3507.8	20.5	45.9	95.4	2.0	49.1	3.5
B04361	3	3.2	3507.8	18.6	45.5	97.1	2.5	48.0	2.5
B04406	48	3.1	3507.8	18.0	45.1	93.4	3.0	48.5	3.5
B04384	26	2.6	3496.5	18.0	44.5	94.7	1.5	49.5	4.5
B04454	96	2.7	3485.3	19.2	44.0	91.5	2.5	48.1	3.5
B04416	58	2.9	3485.3	19.3	44.4	94.0	1.0	51.0	5.0
B04439	81	3.8	3485.3	19.2	44.6	92.8	3.0	46.0	2.5
B04421	63	3.9	3474.1	18.7	44.4	92.0	2.0	48.5	4.0
B04388	30	3.2	3462.8	17.8	44.6	91.4	2.0	50.5	5.0
B04385	27	2.8	3440.3	18.8	48.5	93.8	3.4	47.6	3.0
B04369	11	3.4	3429.1	20.1	44.1	92.4	1.9	49.1	4.5
B04378	20	3.7	3429.1	19.0	44.0	95.1	2.1	48.5	4.0
B04367	9	3.1	3417.8	19.1	44.8	95.5	2.0	49.0	3.5
B04362	99	3.2	3417.8	17.2	44.4	93.2	2.6	49.0	3.5
B04453	95	3.7	3417.8	20.4	45.9	94.7	2.1	48.9	4.0
B04418	60	3.3	3417.8	20.9	45.9	95.4	3.0	48.1	4.0
B04374	16	3.0	3417.8	19.4	44.6	96.6	3.5	47.5	2.5
B04417	59	2.9	3417.8	18.6	44.1	91.0	2.0	49.0	3.5
B04394	36	3.4	3406.6	20.0	46.0	95.6	3.0	48.5	3.0
B04401	43	2.8	3395.4	19.2	44.1	94.4	2.0	49.1	4.0
B04422	64	2.6	3372.9	20.1	46.4	95.8	2.0	49.4	3.5
B04419	61	3.5	3361.6	18.6	44.1	92.0	1.9	48.5	4.0
B04383	25	2.8	3361.6	18.8	44.5	94.2	1.4	49.2	5.5
B04436	78	3.1	3361.6	19.2	44.4	95.4	1.5	49.0	4.0
B04426	68	2.8	3350.4	18.6	44.5	93.8	1.0	50.0	4.0
B04395	37	3.5	3339.1	21.1	44.0	91.6	1.1	48.9	5.5
B04389	31	2.9	3339.1	18.9	44.1	94.5	2.0	48.5	4.0
B04441	83	3.7	3327.9	19.1	45.8	95.4	2.0	48.9	3.5
Jaguar	4	3.9	3316.7	18.6	44.0	92.4	1.5	48.5	5.0
B04425	67	3.7	3305.4	19.1	46.6	95.5	1.9	49.6	3.0
B04379	21	2.6	3271.7	19.4	44.1	93.0	2.0	48.9	3.0
B04434	76	3.5	3271.7	19.4	47.0	96.3	3.0	49.0	3.0
Tacana	18	3.1	3260.4	19.6	44.5	94.8	1.9	48.6	4.5
B04432	74	3.4	3260.4	20.4	44.5	95.5	2.4	49.0	3.5
B04377	19	3.2	3260.4	18.9	44.4	92.4	1.5	49.5	4.5
B04438	80	2.6	3238.0	19.6	45.0	93.9	1.0	49.6	4.0
B04393	35	3.0	3238.0	17.5	45.1	96.4	2.6	47.0	3.0
T-39	13	3.7	3238.0	18.8	44.4	92.5	4.1	46.0	1.5
B04392	34	2.4	3238.0	20.5	44.4	94.9	2.0	49.0	4.0
B04449	91	3.4	3226.7	17.9	45.0	91.7	2.0	48.0	3.5

Table C.3 (cont'd.)

B04375	17	2.2	3215.5	18.7	44.1	94.4	0.9	50.6	5.5
B04390	32	3.0	3193.0	19.4	45.5	95.2	2.0	48.9	3.5
B04415	57	2.3	3193.0	20.1	44.1	91.8	0.9	48.1	4.5
B04452	94	3.5	3170.5	18.6	47.4	95.3	3.0	48.4	3.5
B04399	41	2.6	3159.3	18.8	43.9	95.9	1.5	49.0	3.5
B04373	15	2.9	3159.3	20.0	46.6	97.5	2.0	50.1	2.5
B04448	90	3.1	3148.0	20.5	45.5	95.9	2.9	47.6	3.0
B04363	5	3.2	3136.8	17.6	43.4	95.4	3.5	49.0	3.0
B04380	22	2.7	3125.5	20.4	45.6	91.6	1.0	50.0	5.5
B04407	49	3.3	3125.5	19.1	44.4	92.7	2.0	49.5	4.0
B04430	72	3.3	3114.3	20.7	45.0	93.6	1.5	50.0	3.5
B04403	45	3.4	3103.0	17.8	47.6	94.5	2.5	47.0	3.0
B04424	66	2.4	3091.8	18.8	44.0	94.8	2.0	49.5	3.5
B04408	50	3.1	3058.1	16.2	44.0	94.9	2.0	47.5	4.0
B04381	23	3.2	3001.9	18.9	45.0	91.5	1.1	47.5	4.0
B04359	1	3.0	2990.6	18.0	43.9	92.2	4.0	47.5	2.0
B04427	69	3.1	2990.6	19.8	45.1	94.6	1.5	48.9	4.0
B04442	84	2.7	2900.7	20.4	45.2	94.1	1.0	48.5	5.0
MEANS			3474.1	19.0	45.0	94.2	2.2	48.8	3.7
LSD (p=.05)		303.6	1.4	1.1	1.4	0.5	1.1	0.7	
LSD (p=.01)		393.5	1.8	1.4	1.9	0.7	1.4	0.9	

VA=Visual Appearance, YLD=Yield, SDWT=100-seed Weight, FLWR=Days to Flowering, MTR=Maturity, LDG=Lodging Score, HT=Plant Height, DS=Agronomic Desirability

Table C.4. 2007 Agronomic and canning data for the 'Jaguar' by 115M RIL population.

Line	Entry	VA	YLD	SDWT	FLWR	MTR	LDG	HT	DS
			kg/ha	g	days	days		cm	
B04391	33	1.2	3710.2	22.2	55.5	103.1	2.1	54.0	3.0
B04431	73	1.7	3631.5	22.3	54.9	101.6	1.5	54.0	3.5
B04384	26	1.8	3575.2	18.8	54.5	101.5	1.1	53.5	5.0
B04423	65	2.2	3474.1	18.9	57.4	102.6	2.0	54.5	3.1
B04411	53	1.6	3440.3	21.1	56.1	101.9	1.9	53.5	4.0
B04404	46	2.0	3395.4	19.2	54.5	101.5	2.6	53.5	3.0
B04429	71	1.9	3350.4	19.3	54.5	101.5	2.0	52.5	3.5
B04445	87	2.1	3350.4	20.6	54.1	101.4	1.5	53.5	3.5
B04366	8	1.9	3339.1	18.7	54.1	101.5	1.5	54.0	3.5
B04394	36	2.1	3339.1	21.3	55.5	100.6	2.6	53.0	3.0
B04443	85	2.2	3327.9	18.7	55.1	101.4	1.5	53.5	4.0
B04444	86	2.2	3316.7	19.2	55.0	101.5	2.0	54.0	3.0
B04360	2	2.2	3305.4	20.4	55.4	102.1	2.5	53.5	3.5
B04400	42	2.1	3305.4	19.5	55.0	100.6	2.1	53.5	3.5
B04385	27	1.8	3294.2	20.7	55.0	101.9	2.1	53.5	3.5
B04413	55	2.1	3294.2	19.7	54.5	101.1	1.1	53.1	4.4
B04414	56	1.9	3282.9	20.3	54.6	101.9	0.9	52.5	4.5
B04430	72	2.8	3282.9	19.9	54.1	101.0	1.7	52.5	3.5
B04440	82	2.5	3282.9	19.0	53.5	100.1	1.1	52.5	4.5
B04452	94	3.1	3271.7	19.2	55.9	101.5	1.9	54.0	3.1
B04405	47	1.9	3260.4	18.8	54.5	99.9	0.9	51.0	5.0
B04421	63	2.8	3249.2	17.8	53.9	101.4	1.9	53.4	4.0
B04439	81	3.1	3238.0	19.8	54.6	101.4	1.5	52.5	3.5
B04446	88	2.4	3238.0	19.3	54.9	100.8	1.9	53.9	3.5
B04387	29	2.0	3215.5	19.7	52.5	100.8	2.6	52.5	3.0
B04451	93	2.4	3215.5	20.4	53.4	99.9	1.4	51.5	4.0
B04412	54	2.3	3204.2	18.7	54.9	100.1	1.0	52.0	5.0
115M	100	2.3	3204.2	20.8	54.0	102.0	1.6	53.5	4.0
B04386	28	2.6	3193.0	19.2	52.9	100.5	1.0	51.5	5.0
B04372	14	2.2	3181.7	18.8	56.1	101.9	1.6	53.0	4.0
B04364	6	3.0	3170.5	19.3	53.0	100.6	1.1	51.6	3.9
B04453	95	1.6	3170.5	22.2	55.5	101.5	2.0	53.5	3.5
B04377	19	1.8	3159.3	18.8	53.6	100.5	0.9	53.0	5.0
B04383	25	2.6	3159.3	18.6	52.6	100.9	1.1	52.5	4.0
B04382	24	2.7	3148.0	19.5	55.4	101.1	1.6	53.6	3.9
B04426	68	2.4	3148.0	19.5	54.4	100.6	1.1	51.1	5.0
B04454	96	2.3	3136.8	20.4	54.5	101.0	1.0	52.0	4.0
B04361	3	1.9	3125.5	18.7	54.5	101.1	1.5	53.0	4.4
B04373	15	1.8	3103.0	20.4	55.1	103.0	2.0	52.6	3.9
B04370	12	2.0	3091.8	19.4	55.0	100.9	2.0	53.0	3.6

Table C.4 (cont'd.)

B04447	89	2.4	3080.6	20.4	54.0	102.0	1.9	54.0	3.0
B04390	32	2.2	3069.3	21.1	53.4	102.0	0.9	53.5	4.6
B04396	38	2.4	3069.3	18.2	52.0	101.9	0.9	52.9	4.1
B04401	43	2.4	3069.3	19.1	52.5	100.4	1.0	51.9	4.6
B04441	83	2.5	3069.3	20.3	55.0	101.0	1.0	52.5	4.5
B04376	97	2.8	3058.1	17.8	54.5	100.9	0.9	53.5	4.5
B04395	37	2.4	3058.1	20.2	54.1	100.0	1.5	51.5	4.0
B04407	49	1.9	3058.1	18.4	53.6	101.0	1.6	52.5	4.0
B04415	57	2.5	3058.1	20.6	53.0	101.0	2.0	52.5	3.5
B04422	64	2.5	3058.1	19.7	54.9	101.5	1.4	53.5	4.1
Jaguar	4	3.4	3046.8	18.5	54.0	99.8	0.9	51.5	5.0
B04365	7	2.8	3035.6	18.6	54.5	102.6	1.0	53.5	4.0
B04449	91	2.0	3035.6	18.6	53.4	101.4	2.0	52.9	3.5
B04450	92	2.8	3035.6	19.3	52.0	99.6	1.1	50.5	4.4
Tacana	18	2.3	3035.6	19.3	51.6	98.9	1.7	52.0	4.0
B04435	77	1.8	3024.3	19.3	55.6	102.6	1.4	54.0	3.0
B04392	34	2.3	3013.1	19.3	54.0	102.0	1.0	53.5	3.0
B04437	79	1.4	3013.1	18.4	55.1	100.5	2.0	52.0	3.5
B04369	11	1.9	3001.9	19.2	52.5	101.1	2.1	52.5	4.0
B04380	22	1.3	2990.6	20.7	54.5	99.5	0.9	51.5	5.0
B04418	60	1.9	2990.6	20.3	55.1	101.9	1.5	53.0	3.5
B04363	5	1.9	2979.4	18.9	55.0	100.0	1.4	52.5	4.5
B04427	69	2.0	2979.4	21.4	54.6	104.1	1.0	51.5	3.0
B04398	40	1.3	2968.1	20.5	55.0	101.6	1.5	54.0	4.0
B04410	52	1.1	2968.1	20.0	55.5	101.6	2.0	54.0	4.0
B04378	20	1.4	2956.9	19.1	54.5	100.5	1.9	52.5	4.0
B04448	90	1.7	2956.9	19.6	53.6	102.0	1.5	53.4	4.0
B04367	9	1.4	2945.6	19.2	54.0	102.0	0.9	52.5	4.0
B04432	74	1.5	2945.6	20.2	54.9	106.0	1.5	54.0	5.0
B04403	45	1.4	2934.4	17.7	55.0	101.1	2.1	53.0	3.4
B04420	62	1.7	2934.4	19.1	55.0	100.4	0.9	53.0	4.6
B04406	48	1.5	2923.2	18.0	55.0	100.5	1.5	52.5	4.5
B04374	16	1.6	2911.9	20.2	54.4	101.5	2.5	52.5	2.9
B04402	44	1.7	2900.7	18.0	54.9	103.0	1.0	52.1	3.5
B04379	21	1.6	2889.4	18.8	54.5	101.6	0.9	52.5	4.5
B04389	31	2.0	2878.2	18.8	53.5	101.0	1.5	52.0	4.5
B04393	35	2.0	2878.2	20.4	55.0	103.6	2.0	53.0	3.0
B04424	66	1.6	2878.2	19.6	55.1	101.5	0.9	52.0	4.0
B04375	17	1.3	2855.7	19.3	54.0	100.0	1.2	51.1	5.0
B04409	51	2.7	2855.7	20.8	55.6	102.4	1.0	54.0	3.5
B04417	59	2.8	2855.7	17.7	50.9	100.5	1.0	52.0	5.0
B04419	61	1.9	2855.7	20.0	54.0	99.4	1.0	50.9	5.0

Table C.4 (cont'd.)

B04436	78	2.1	2833.2	20.3	54.0	101.4	0.9	53.0	4.0
B04416	58	1.5	2788.2	19.2	53.1	100.0	0.9	52.0	4.5
B04428	70	1.2	2777.0	21.4	54.0	99.9	1.0	53.9	4.1
B04371	98	1.4	2765.8	18.9	54.9	101.0	1.5	53.0	4.0
T-39	13	2.5	2754.5	20.1	54.5	100.1	4.1	42.0	2.0
B04397	39	1.5	2732.0	18.6	53.5	101.9	0.9	54.0	4.0
B04388	30	1.9	2709.5	18.3	55.0	101.0	1.0	53.0	5.0
B04359	1	1.7	2698.3	20.1	55.0	102.5	1.4	52.5	4.0
B04433	75	1.7	2698.3	17.3	55.4	100.4	0.9	51.9	5.1
B04362	99	2.1	2675.8	17.7	53.0	101.0	2.0	53.0	3.5
B04408	50	1.5	2675.8	17.3	54.4	102.4	0.9	51.5	4.0
B04425	67	2.4	2630.8	23.1	55.9	103.1	2.1	53.6	3.0
B04368	10	1.2	2619.6	18.2	55.1	101.6	1.5	53.0	4.0
B04438	80	1.1	2585.9	19.3	54.4	102.4	0.9	52.4	3.6
B04399	41	1.7	2518.4	18.3	53.1	99.9	0.9	52.0	5.0
B04434	76	2.6	2495.9	22.3	55.5	107.1	2.0	54.5	3.0
B04381	23	1.9	2462.2	19.5	53.5	100.4	1.0	51.5	4.0
B04442	84	1.7	2406.0	21.4	53.1	102.1	1.0	52.0	4.0
MEANS			3046.8	19.6	54.3	101.3	1.5	52.7	4.0
LSD (p=.05)			348.5	1.1	0.9	1.1	0.4	1.0	0.5
LSD (p=.01)			449.7	1.4	1.2	1.4	0.6	1.2	0.7

VA=Visual Appearance, YLD=Yield, SDWT=100-seed Weight, FLWR=Days to Flowering, MTR=Maturity, LDG=Lodging Score, HT=Plant Height, DS=Agronomic Desirability

Table C.5. Three year averages for processed bean color (Hunter L-value), texture (Kg-force), and washed-drained weight (g) measured in the 'Jaguar' by 115M RIL population.

Line	CLR	Line	TXT	Line	WDWT
B04398	20.0	Tacana	84.1	B04447	260.5
B04391	18.5	B04363	79.0	B04441	256.3
B04428	18.4	B04359	72.9	B04374	256.0
B04420	18.2	B04385	71.6	B04377	255.7
B04396	17.7	115M	71.5	B04440	255.5
B04415	17.6	B04387	70.7	T-39	254.7
B04431	17.5	B04451	70.5	B04369	254.4
B04429	17.5	B04419	68.9	B04395	254.0
B04413	17.5	B04453	68.7	B04417	253.5
B04435	17.3	B04428	68.5	B04361	253.1
B04375	17.2	B04421	68.3	Jaguar	253.0
B04372	17.2	B04424	67.6	B04405	252.9
115M	17.2	B04395	67.6	B04414	252.7
B04385	17.2	B04408	67.3	B04365	252.5
B04409	17.1	B04362	67.1	B04399	252.4
B04387	17.1	B04404	67.1	B04438	252.0
B04384	17.0	B04454	66.8	B04379	251.8
B04374	17.0	B04439	66.6	B04400	251.8
B04402	16.8	B04413	66.5	B04450	251.3
B04380	16.8	B04449	66.1	B04419	251.3
B04370	16.7	B04366	66.0	B04434	251.3
B04403	16.7	B04446	66.0	B04415	251.2
B04397	16.7	B04360	65.7	B04444	251.2
B04417	16.7	B04415	65.4	B04402	251.0
B04405	16.6	B04432	65.3	B04449	251.0
B04365	16.6	B04445	64.9	B04384	251.0
B04443	16.5	B04425	64.5	B04409	250.8
B04360	16.5	B04389	64.3	B04382	250.8
B04449	16.4	B04386	64.2	B04412	250.7
B04438	16.4	B04382	64.0	B04378	250.7
B04444	16.3	B04429	64.0	B04439	250.6
Tacana	16.3	B04420	64.0	B04425	250.6
B04364	16.3	B04430	63.9	B04418	250.6
B04416	16.3	B04407	63.6	B04436	250.5
B04400	16.3	B04368	62.9	B04388	250.5
B04410	16.3	B04390	62.5	B04392	250.3
B04382	16.2	B04380	62.1	B04394	250.1
B04388	16.2	B04396	61.9	B04423	250.0
B04411	16.2	B04376	61.8	B04364	249.9

Table C.5 (cont'd.)

B04453	16.2	B04411	61.4	B04422	249.8
B04379	16.1	B04379	60.9	B04443	249.8
B04366	16.1	B04452	60.5	B04442	249.7
B04442	16.1	B04367	60.3	B04372	249.7
B04371	16.1	B04450	60.2	B04371	249.5
B04376	16.1	B04423	60.1	B04404	249.4
B04383	16.0	B04364	60.0	B04396	249.4
B04392	16.0	B04369	59.5	B04386	249.4
B04437	16.0	B04437	59.4	B04381	249.3
B04445	16.0	B04431	58.9	B04367	249.3
B04395	16.0	B04435	58.8	B04445	249.2
B04406	16.0	B04427	58.8	B04383	249.2
B04368	15.9	B04394	58.6	B04370	249.1
B04404	15.9	B04412	58.4	B04410	249.1
B04423	15.9	B04397	57.9	B04362	249.0
B04418	15.9	B04403	57.9	B04368	248.9
B04419	15.8	B04410	57.7	B04451	248.7
B04414	15.8	B04388	57.7	B04431	248.6
B04390	15.8	B04381	57.1	B04366	248.5
B04446	15.8	B04448	56.7	B04373	248.3
B04427	15.8	B04391	56.7	B04406	248.3
B04401	15.8	B04426	56.6	B04454	248.3
B04432	15.7	B04405	56.6	B04448	248.2
B04369	15.6	B04392	56.3	B04432	248.1
B04424	15.6	B04434	56.1	B04416	248.1
B04407	15.6	B04418	56.0	B04413	248.1
B04447	15.6	B04375	55.9	B04390	247.9
B04389	15.5	B04384	55.2	B04426	247.9
B04434	15.5	B04401	55.0	B04408	247.7
B04451	15.5	B04436	55.0	B04389	247.7
B04433	15.4	B04370	54.9	B04401	247.6
B04454	15.4	B04378	54.9	B04391	247.6
B04394	15.4	B04400	54.7	B04398	247.2
B04367	15.4	B04373	54.5	B04427	247.1
B04362	15.4	B04422	54.3	B04393	246.9
B04399	15.4	B04433	53.6	B04452	246.9
B04430	15.3	B04409	53.2	B04433	246.5
B04422	15.3	B04416	52.8	B04397	246.3
B04377	15.3	B04393	52.7	B04437	246.2
B04393	15.3	B04443	52.5	B04420	246.1
B04450	15.2	B04444	52.5	115M	246.1
B04386	15.2	B04365	52.3	B04421	245.9

Table C.5 (cont'd.)

B04436	15.2	B04441	52.1	Tacana	245.8
B04441	15.2	B04406	52.0	B04453	245.2
B04452	14.9	B04417	51.9	B04375	245.1
Jaguar	14.9	B04442	51.8	B04360	244.8
B04408	14.9	B04374	51.6	B04407	244.7
B04440	14.8	B04372	51.5	B04424	244.6
B04412	14.7	B04399	51.5	B04430	244.5
B04421	14.6	B04377	50.7	B04380	244.3
T-39	14.6	B04371	50.7	B04446	244.1
B04378	14.6	B04440	50.7	B04359	244.0
B04361	14.5	B04383	50.6	B04435	243.4
B04381	14.5	B04402	49.3	B04363	243.4
B04363	14.5	B04414	49.2	B04376	243.0
B04373	14.4	T-39	48.5	B04429	241.9
B04448	14.4	Jaguar	48.4	B04385	241.8
B04425	14.2	B04398	48.4	B04411	238.4
B04359	14.2	B04438	46.2	B04428	238.2
B04439	14.1	B04361	45.7	B04403	235.2
B04426	13.7	B04447	45.5	B04387	234.9
Mean	16.0		59.4		248.7
LSD (.05)	1.3		9.6		6.8
LSD (.01)	1.7		12.7		8.9

CLR=Canned bean color, TXT=Texture, WDWT=Washed-drained weight

Table C.6. Mean values by year for processed bean color, texture, and washed-drained weight measured in the 'Jaguar' by 115M RIL population during 2005-2007.

YEAR	CLR	TXT	WDWT
2005	17.6	63.9	243.3
2006	14.2	60.8	248.9
2007	16.2	53.5	253.9
LSD (.05)	0.4	2.5	1.5
LSD (.01)	0.5	3.3	2.0

CLR=Canned bean color, TXT=Texture, WDWT=Washed-drained weight

Table C.7. Reaction of 96 'Jaguar' by 115M RILs following inoculation with race 73 of *C. lindemuthianum* in the greenhouse. (S=susceptible, R=resistant).

	Plants			Plants			Plants	
Accession	S	R	Accession	S	R	Accession	S	R
B04359	7		B04404	3	2	B04449	5	
B04360	6		B04405		7	B04450		7
B04361		6	B04406		7	B04451	2	4
Jaguar		13	B04407		8	B04452	1	4
B04363	9		B04408	3	2	B04453	6	
B04364	5		B04409		7	B04454		9
B04365	6		B04410	9		B04376		5
B04366	10		B04411	5	2	B04371		7
B04367		6	B04412		10	B04362		7
B04368	5		B04413	11		115-11M	13	
B04369		11	B04414	1	5	Blackhawk	6	
B04370		7	B04415	8				
T-39	11		B04416	7				
B04372		5	B04417	3	4			
B04373		6	B04418		5			
B04374		6	B04419	7				
B04375	2	6	B04420	6				
Tacana	5		B04421	3	2			
B04377		7	B04422		7			
B04378		5	B04423	6	1			
B04379	5	1	B04424	4	2			
B04380	6		B04425		7			
B04381		11	B04426	6				
B04382	6		B04427		7			
B04383		5	B04428	6				
B04384		5	B04429	6	1			
B04385	9		B04430		7			
B04386	7		B04431	7				
B04387		7	B04432		6			
B04388		5	B04433		6			
B04389	2	7	B04434		7			
B04390		6	B04435	1	6			
B04391	1	6	B04436	3	3			
B04392		9	B04437		6			
B04393		6	B04438		7			
B04394		7	B04439	7				
B04395		6	B04440	7				
B04396		6	B04441		7			
B04397	5	2	B04442		6			
B04398		6	B04443		7			
B04399	3	4	B04444	5				
B04400	4	3	B04445		5			
B04401		6	B04446	6				
B04402	4	1	B04447		7			
B04403		10	B04448	7				

Table C.8. SRAP primer sequences used in pairwise combinations to screen for genomic polymorphisms in a 'Jaguar'/115M RIL population. Sequence information based on Li and Quiros (2001) TAG 103:455-461.

Code	Forward Primer	Code	Reverse Primer
M1	TGA GTC CAA ACC GGA TA	E1	GAC TGC GTA CGA ATT AAT
M2	TGA GTC CAA ACC GGA GC	E2	GAC TGC GTA CGA ATT TGC
M3	TGA GTC CAA ACC GGA AT	E3	GAC TGC GTA CGA ATT GAC
M4	TGA GTC CAA ACC GGA CC	E4	GAC TGC GTA CGA ATT TGA
M5	TGA GTC CAA ACC GGA AG	E5	GAC TGC GTA CGA ATT AAC
M6	TGA GTC CTT TCC GGT AA	E6	GAC TGC GTA CGA ATT GCA
M7	TGA GTC CTT TCC GGT CC	E7	GAC TGC GTA CGA ATT CAA
T1	TGT GTG GTT AAT ATG AGC	E8	GAC TGC GTA CGA ATT CAC

Table C.9. TRAP primer sequences used in pairwise combinations to screen for genomic polymorphisms in a 'Jaguar'/115M RIL population. Sequence information based on Hu and Vick (2003) Plant Mol. Bio. Rept. 21:289-294.

Code	Forward Primer	Code	Reverse Primer
F1	CAA CCG AAA ACC AGC AAT	R1	GCG AGG ATG CTA CTG GTT
F2	CGA TCT AGA ATC CAA GCC	R2	CTA TCT CTC GGG ACC AAA C
F3	CGA ATC TCC ACT AAA CCC	R3	TTC TAG GTA ATC CAA CAA CA
F4	CCG AGT TGG TAT GCT TGT	R4	TTA CCT TGG TCA TAC AAC ATT
F5	ATC AGT TCA TTA GGG CAC	R5	TTC TTC TTC CCT GGA CAC AAA
F6	GGA ACA TTT GTC TCT CGC	R6	TCA TCT CAA ACC ATC TAC AC
F7	CTT CAG CAG TGT CTC TCC	R7	GGA ACC AAA CAC ATG AAG A
F8	CTC GAT AAC ATC CTC CCA	R8	CCA AAA CCT AAA ACC AGG A
F9	TGG ATT TTC ACC AGC GTC	R9	CAC AAG TCG CTG AGA AGG
F10	GAA ATT AAC GGG GTT GGA	R10	ATA AGA ATC AGC AGA CGC AT
F11	GCT TCA ATT GGC CCT TAC		
F12	CAG AAC TTG TTG GTG GTG		
F13	CAT CGC ATA CTG ATG GAG		
F14	GCA GAC ATC GGT AGA AAG		
F15	AGT TGT TCC CAG ATG GAG		
F16	GTG GGA ACC TAG AAA TGG		
F17	CCT AAA TGG GAG GAA GTG		
F18	AAG ATC ACA CCT TGT CCC		
F19	AAT CTC AAG GAC AAA AGG		
F20	GCT TCA GAG CAT TGA AGT		
F21	GAA AGA CGA AGG AAC AGG		
F22	CGT TTA TTT CCT CGC CTC		

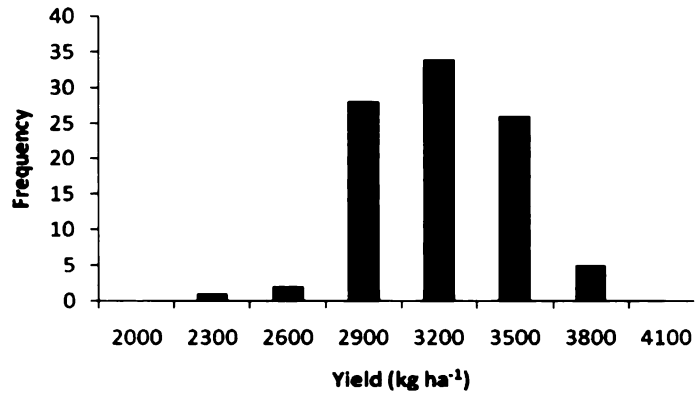
Table C.10. Reactions of 96 'Jaguar' by 115M recombinant inbred lines to *U. appendiculatus* race 3:22 and presence or absence of two TRAP markers.

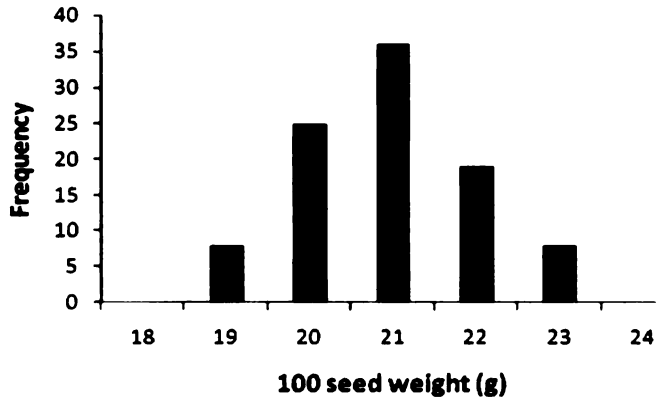
Accession	RUST	F15R10	F7R1	Accession	RUST	F15R10	F7R1	Accession	RUST	F15R10	F7R1
B04359	H	A	P	B04392	R	A	P	B04425	S	P	A
B04360	R	A	P	B04393	R	A	P	B04426	R	A	P
B04361	R	A	P	B04394	H	P	A	B04427	R	A	P
B04362	R	P	A	B04395	H	A	A	B04428	R	A	P
B04363	R	A	P	B04396	R	A	P	B04429	H	P	A
B04364	R	A	P	B04397	R	A	P	B04430	S	P	A
B04365	S	A	P	B04398	R	P	P	B04431	R	A	P
B04366	S	A	P	B04399	R	A	P	B04432	R	A	P
B04367	R	A	P	B04400	S	P	A	B04433	S	P	A
B04368	R	A	P	B04401	R	A	P	B04434	R	A	P
B04369	H	P	A	B04402	H	P	P	B04435	S	P	A
B04370	H	A	P	B04403	R	A	P	B04436	R	A	P
B04371	R	A	P	B04404	R	A	P	B04437	R	A	P
B04372	R	A	P	B04405	R	A	P	B04438	R	A	P
B04373	H	P	P	B04406	R	A	P	B04439	H	A	P
B04374	R	P	.	B04407	R	A	P	B04440	R	A	P
B04375	S	P	A	B04408	R	A	P	B04441	R	A	P
B04376	R	A	P	B04409	R	A	P	B04442	H	A	P
B04377	S	P	A	B04410	R	A	P	B04443	H	A	P
B04378	H	A	P	B04411	H	P	P	B04444	R	A	P
B04379	H	P	P	B04412	R	A	P	B04445	R	A	P
B04380	R	A	P	B04413	S	P	A	B04446	R	A	P
B04381	R	A	P	B04414	S	A	A	B04447	R	A	P

Table C.10 (cont'd.)

Accession	RUST	F15R10	F7R1	Accession	RUST	F15R10	F7R1	Accession	RUST	F15R10	F7R1
B04382	R	A	P	B04415	R	A	P	B04448	H	P	P
B04383	R	A	P	B04416	S	P	A	B04449	R	A	P
B04384	R	A	P	B04417	R	A	P	B04450	H	P	A
B04385	R	A	P	B04418	R	A	P	B04451	R	A	P
B04386	R	A	P	B04419	R	A	P	B04452	S	P	A
B04387	H	P	A	B04420	R	A	P	B04453	R	A	P
B04388	R	A	P	B04421	R	A	P	B04454	H	P	A
B04389	R	A	P	B04422	R	A	P	115M	R	A	P
B04390	S	P	A	B04423	R	A	P	Jaguar	S	P	A
B04391	R	A	P	B04424	S	P	A	Tacana	R	A	P

R= Resistant, S= Susceptible, H= Heterogeneous (both resistant and susceptible plants present), P= Present, A= Absent





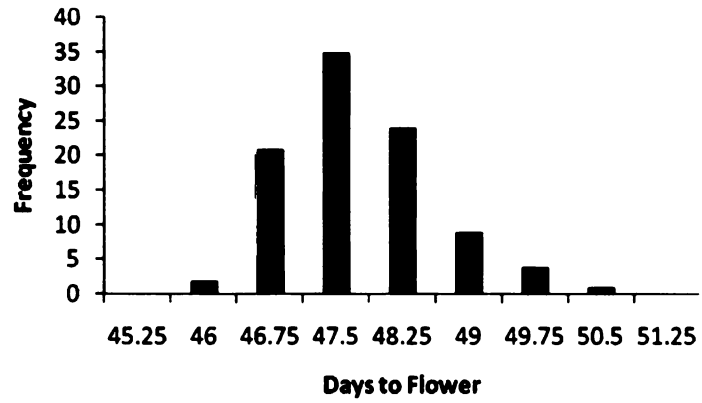
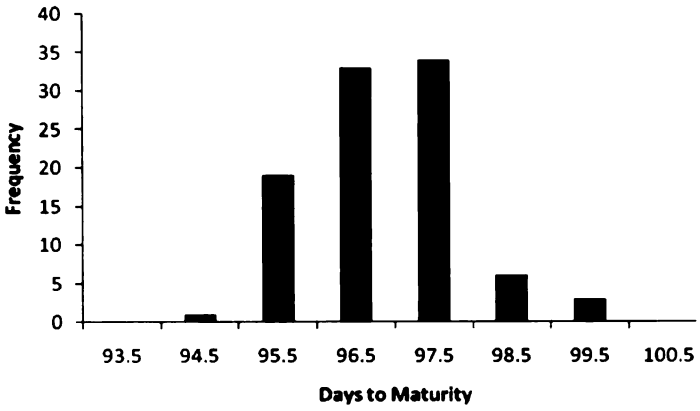
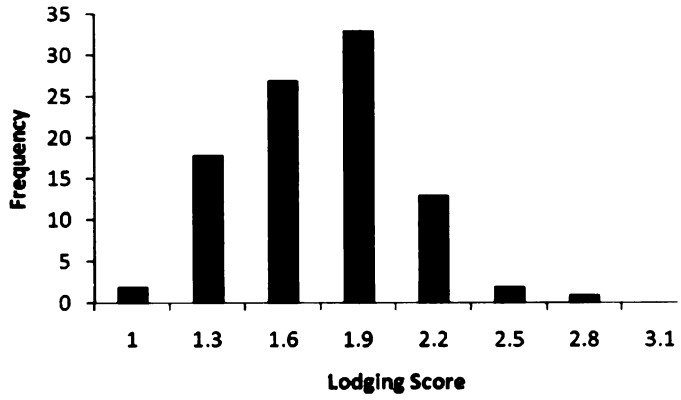


Figure C.1. Frequency distributions for agronomic and canning quality traits in the 'Jaguar' by 115M RIL population.





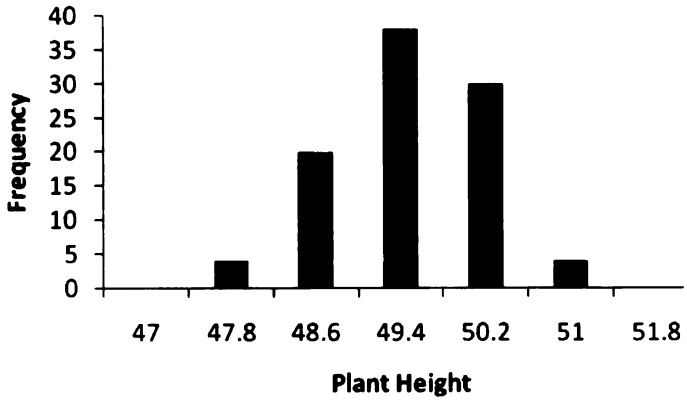
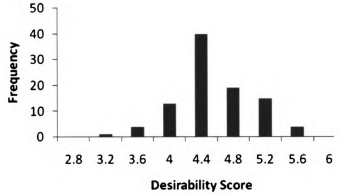
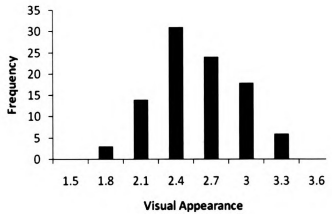


Figure C.1 (cont'd).





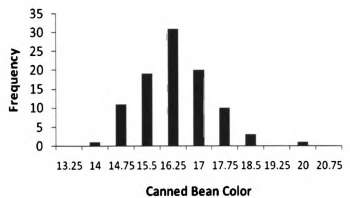
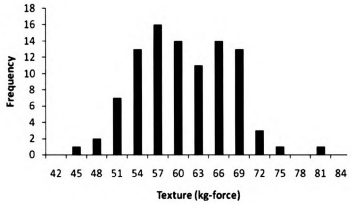


Figure C.1 (cont'd).



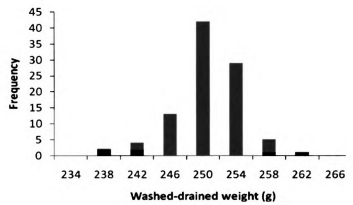


Figure C.1 (cont'd).

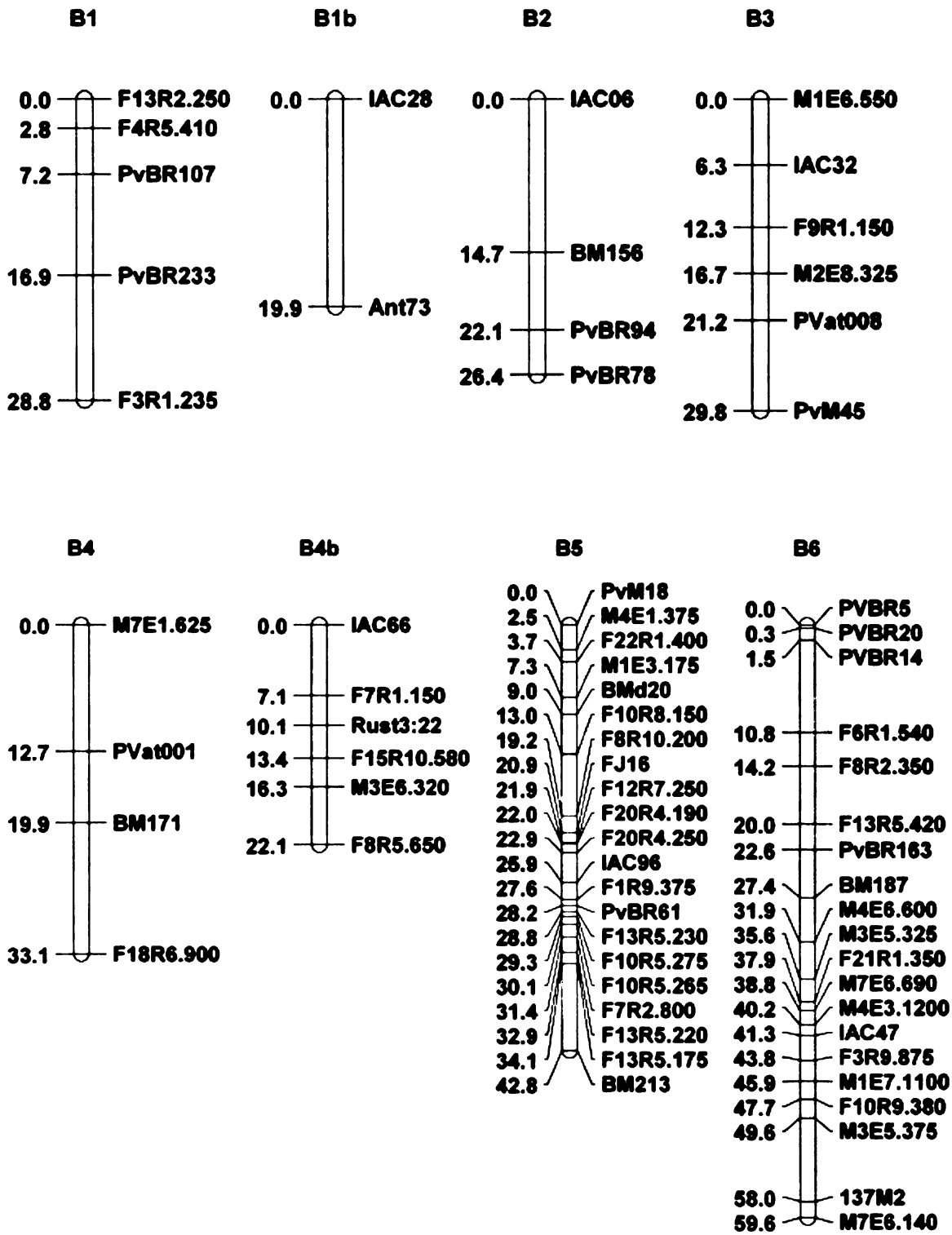


Figure C.2. Linkage map of 'Jaguar'/115M RIL population consisting of 119 SSR, SRAP, TRAP, and phenotypic markers placed on 15 linkage groups covering a combined distance of 460cM.

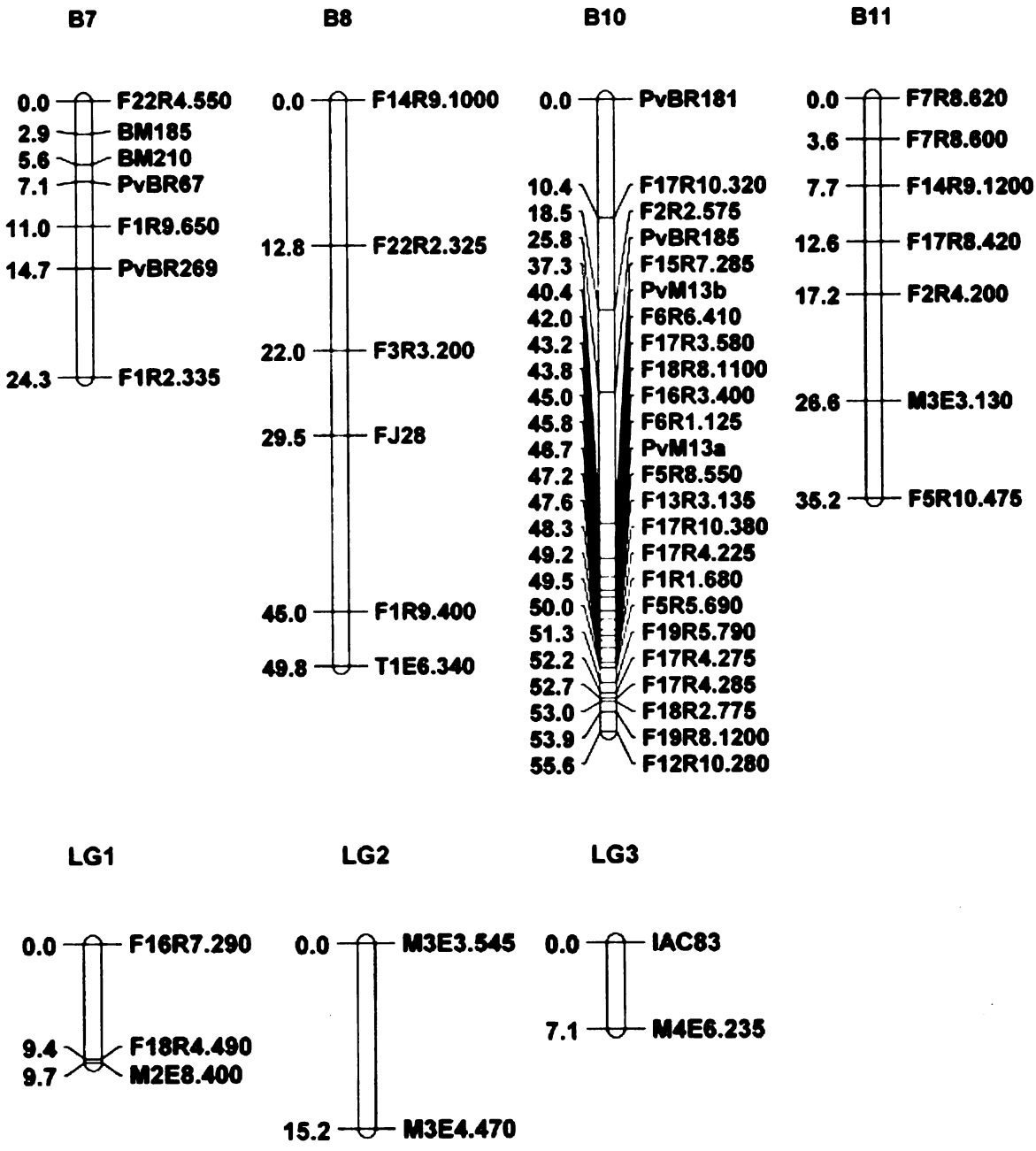


Figure C.2 (cont'd.)