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USE OF MARKER-ASSISTED SELECTION TO BREED FOR RESISTANCE TO COMMON BACTERIAL BLIGHT IN COMMON BEAN

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Patrick Daniel O'Boyle

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James D. Kelly

Major Professor's Signature

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USE OF MARKER-ASSISTED SELECTION TO BREED FOR RESISTANCE TO COMMON BACTERIAL BLIGHT IN COMMON BEAN

By

Patrick Daniel O'Boyle

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ABSTRACT

USE OF MARKER-ASSISTED SELECTION TO BREED FOR RESISTANCE TO COMMON BACTERIAL BLIGHT IN COMMON BEAN

By

Patrick Daniel O'Boyle

Resistance to common bacterial blight (CBB) in common bean is a quantitative trait. Although previous attempts to breed CBB resistant bean cultivars have had limited success, progress in marker-assisted selection (MAS) has created new opportunities for breeding programs. The potential of the marker SU91 in MAS for CBB resistance was evaluated in field experiments. SU91 was correlated with lower CBB leaf scores in East Lansing (r = -0.50), and Saginaw (r = -0.59), and correlated with pod resistance in Saginaw (r = -0.48). SU91 exhibited a negative correlation with yield (r = -0.20) and a positive correlation with maturity (r = 0.22) in East Lansing, but showed no association in Saginaw. Selections carrying SU91 were crossed with additional sources of resistance to CBB and anthracnose. F_2 progeny were screened for the marker BC420, linked to a QTL for CBB resistance on bean linkage group B6. The effects of SU91 and BC420 were examined in two greenhouse studies. The presence of SU91 was negatively correlated with CBB disease ratings for leaves (r = -0.20) and pods (r = -0.27). Presence of BC420 was only correlated with pod ratings (r = -0.19) in experiment one, and CBB leaf resistance (r = -0.18) in experiment two. Presence of both markers resulted in lower levels of CBB resistance than provided by either marker alone.

DEDICATION

To all the wonderful family and friends who have been there with love, support, and friendship.

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Impacts of Common Bacterial Blight Infection

Common bacterial blight (CBB) is one of the most serious production problems of common bean (*Phaseolus vulgaris* L.) worldwide (Tar'an et al. 2001). CBB is a seedborne disease, caused by the bacterial pathogen *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*). CBB is often responsible for significant yield losses and entire crop failures (Vakili et al., 1975). Studies by Wallen and Jackson (1975) and Yoshii et al. (1976) determined that yield losses ranged from 38% to 45% in *Xap*-inoculated plots, and were 22% due to natural CBB infections (Yoshii et al., 1976). Losses are typically more severe in hot years, when average temperatures exceed 30°C (Saettler, 1989; Kelly and Copeland, 1996).

The seedborne nature of CBB has been a major constraint in commercial bean production, and has also influenced the distribution of the dry bean seed production industry. To attain certification, bean seed fields must be free of the disease, because infected seeds will transmit the infection to their progeny (Kelly and Copeland, 1996). In areas where CBB severity is high, the production of dry bean seed is not economical for producers, resulting in the displacement of seed production from areas that once had highly successful production programs. In-state production of certified, disease-free seed is ideal for Michigan growers because of reduced costs and assurance of sufficient quantity (Anderson et al., 1970). A 92% reduction in acreage (from 5,000 ha to 400 ha) dedicated to certified navy bean production in Michigan between 1974 and 1994 was primarily due to losses caused by CBB (Kelly and Copeland, 1996). Competition from

western states where seed is produced in relatively disease-free environments has resulted in displacement of the bean seed industry from Michigan.

In direct contrast to navy and black bean seed production in mid-Michigan, the successful production of certified dark red kidney bean seed in northern Michigan increased in acreage over the same time period (from 400 to 1,200 hectares). This increase was primarily due to two factors. The first is the geographic isolation of kidney bean seed production from infected commercial bean fields downstate, where other bean market classes are grown and produced. The second factor that accounts for the success of kidney bean seed production in Michigan is the use of moderately resistant cultivars, such as Montcalm (Kelly and Copeland, 1996). These factors have heavily impacted the success of the kidney seed production industry. Over the course of a 15-year period of observation, Kelly and Copeland (1996) noted a 2-4% average of seed lot rejection per year for certified kidney beans. During the same period, navy beans had seed lot rejection rates of as high as 50%. In 1994, 50% of all navy bean certified seed was rejected primarily due to CBB infection, although the seed borne fungal disease, bean anthracnose was also responsible for a portion of the rejections. CBB has played a significant role in the reduction of certified navy and black bean seed production in Michigan (Kelly and Copeland, 1996).

The seed borne fungal disease bean anthracnose is another serious threat to dry beans in Michigan. This disease is caused by the hemibiotrophic fungal pathogen *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib. In the early 1900's, anthracnose was considered as one of the most serious bean diseases in the United States (Zaumeyer and Thomas, 1957). Since that time, however, the deployment of genetic

resistance has decreased the impact of this disease in the dry bean industry. The combination of anthracnose resistance and CBB resistance in high yielding dry bean lines would greatly benefit growers and seed producers by allowing them access to certified seed with resistance to the two major seed-borne diseases of dry beans in Michigan.

Causal Organism Characteristics

Xanthomonas axonopodis pv. *phaseoli* is a gram-negative, rod-shaped bacterium that is aerobic and has a polar flagellum. Most isolates display mucoid growth on media, however, some have been known to have a grainy growth habit. The majority of isolates produce a yellowish carotenoid pigment (xanthomonadin) on nutrient media. An exudate produced by the bacteria (in cultures and in the host plant) is a high molecular weight extracellular polysaccharide that has a demonstrated role in symptom development and bacterial survival (Saettler, 1989). A study by Leach et al. (1957) examined the role of this polysaccharide in bacterial fitness. The results suggest that the exudate may play a role in tolerance to ultraviolet light. The exudates induced wilting symptoms in beans, but it also produced symptoms in non-host species. This indicates that the polysaccharide induces symptoms not by specific toxicity, but by blocking water transport in the plant. Examination of water uptake confirmed water movement disruption in tomato (*Lycopersicon esculentum* Mill.) and sunflower (*Helianthus annuus* L.) (Leach et al., 1957). The bacteria are capable of causing symptoms in a large number of *Phaseolus* and *Vigna* species, including common bean and cowpea (Saettler, 1989).

CBB Symptoms and Epidemiology

The first sign of *Xap* infection in beans is usually the appearance of tiny water-soaked spots on the underside of leaves. Eventually, these spots coalesce, forming necrotic spots surrounded by a border of lemon-yellow chlorotic tissue, noticeable on the upper leaf surface. Eventually, diseased leaves become necrotic, and plant defoliation may result. In some cases, leaves may become entirely necrotic and remain attached to the plant following maturity. This is true especially in cases of extremely severe infections (Saettler, 1989).

All aerial plant organs (leaves, pods, seeds, and stems) may exhibit CBB symptoms, although leaf symptoms are the most noticeable. Pod lesions originate as water-soaked areas that become dark and sunken as the disease progresses. The symptoms on pods can result in seed discoloration and/or shriveling, when infections are severe (Saettler, 1989). Although seed infection is more commonly observed when pod lesions are visible, Cafati and Saettler (1980c) reported infected seeds in symptomless pods. Seed symptoms can be difficult to observe on dark-colored seed coats. Symptoms on light colored seeds (e.g. navy beans) can easily be observed as yellow-brown spots (Zaumeyer and Thomas, 1957). Occasionally, the hilum of infected seeds becomes discolored. Stem lesions, although less common, can result in girdling of the entire stem, disrupting water and nutrient transport. This is more common in plants that originated from contaminated seeds, compared to plants infected during the course of the growing season (Zaumeyer and Thomas, 1957). Occasionally, yellow bacterial exudates can be observed in the vicinity of a lesion. These exudates contain living *Xap* cells, and serve as

a major source of secondary inoculum, as these bacterial populations may easily be moved, from infected leaves to healthy plants (Zaumeyer and Thomas, 1957).

Xap commonly enters the leaves through natural openings, such as wounds or stomata, and proceeds to occupy the intercellular spaces. As bacterial colonies form, they damage cells, and dissolve the middle lamella. The bacteria can also enter the stem, which can result in clogging of vascular tissue, causing the plant to wilt. The seed is also susceptible to infection, most commonly through the pod's vascular system, although the pedicel may also be an entry point for the pathogen (Saettler, 1989). The disease cycle is perpetuated because an infected seed holds considerable potential for transmission of the pathogen to the resulting seedling (Aggour, 1989). Seedlings originating from *Xap* infected seeds commonly exhibit lesions on cotyledons and primary leaves. These symptoms will typically appear earlier in the growing season, beginning around the time of flowering (Zaumeyer and Thomas, 1957). The translocation of *Xap* from infected to healthy leaves in susceptible genotypes, was observed by Cafati and Saettler (1980a). Translocation of bacteria was not observed in CBB resistant tepary bean selections, suggesting that the limitation of bacterial movement may be a consequence of host resistance (Cafati and Saettler, 1980a; Aggour et al., 1989).

Since CBB is a seed-transmitted disease, *Xap* infected seed may initiate an epidemic after planting. Infected seeds that do not exhibit visible external symptoms are regarded as the primary source of inoculum (Jung et al., 1997). Although the survival of *Xap* in contaminated bean seeds has not been fully examined, viable cells have been recovered from bean seeds stored for10 years (Zaumeyer and Thomas, 1957).

Xap is readily disseminated by wind-driven rain, mechanical transmission by cultivation, and human or animal passage through wet bean fields. According to Claflin et al. (1973), wind-blown soil can play a major role in the dissemination of *Xap* by wounding bean plants, creating an entry point for the pathogen, and the soil particles themselves may be responsible for moving bacteria. This study revealed that CBB infection increased with wind speed and exposure time. Steadman et al. (1975) determined the potential for *Xap* to spread via irrigation water in regions where bean crops are irrigated. Another factor which impacts the degree of infection is the pathogen's ability to persist in the field for an extended period of time (Anderson et al., 1970). Schuster (1970) noted that *Xap* can persist in bean straw for at least 10 months in Nebraska. After 22 months, however, no *Xap* had survived in the field. Saettler et al. (1986) concluded that *Xap* does not overwinter in Michigan. A collection of 20 isolates was investigated, to account for possible differences in the ability of distinct isolates to overwinter.

As with many plant pathogenic bacteria, *Xap* can live epiphytically on the leaves of non-host species for a short period of time. Viable *Xap* cells have been recovered from soybean (*Glycine max* L.), maize (*Zea mays* L.), cowpea (*Vigna unguiculata* L.), and several weed species (e.g. *Chenopodium album* and *Amaranthus retroflexus*). The methods of bacterial transmission from infected bean plants to healthy bean plants may also move *Xap* from non-host species to healthy beans (Cafati and Saettler, 1980b; Saettler, 1989).

Kaiser and Vakili (1978) examined the possibility of insect transmission of *Xap* from infected to healthy bean plants. They concluded that some insect species play a role

in transmission, however it is not considered to be a major source of spread in CBB epidemics. This research was conducted in Puerto Rico, and it is possible that the results obtained may not apply to other regions, depending on insect species present and climatic conditions. Unfortunately, similar studies have not been conducted in most major areas of dry bean production, so it is unknown whether these results apply in other regions.

An additional method by which *Xap* can spread is from seed to seed contact, during threshing. Bacteria on the exterior of seeds may become airborne upon threshing and can thus, be moved to uninfected seeds. These bacteria may serve as source of primary inoculum the following growing season in cases where seed is kept by the farmer for planting (Weller and Saettler, 1980). This is also a concern in the seed production industry, where it can result in apparently uninfected seeds being sold to farmers, thereby introducing the pathogen into bean fields the following year.

Environmental Conditions

Climatic conditions favoring CBB infection in bean fields have been studied. The disease is generally considered a high-temperature disease, although it may occur over a wide range of temperatures. Goss (1939) found that bean plants exhibited CBB symptoms over the entire range of temperatures tested (16 to 32°C); however, the higher temperatures favored more rapid symptom development. Symptoms occurred most rapidly at temperatures between 28 and 32°C. Inoculated plants grown at 32°C displayed symptoms at 6 days after inoculation, while symptoms were not noticeable in plants grown at 24°C until 14 days after inoculation. Plants grown at 16°C did not display CBB symptoms until placed at 28°C for 7 days (Goss, 1939).

The effect of moisture was also examined in the experiment by Goss (1939). A comparison of low and high humidity after inoculation revealed that symptoms developed significantly earlier and affected a greater area in conditions of low humidity. At least some moisture is required for symptom development, however, it seems the disease is favored by low to moderate humidity (Goss, 1939). Timely precipitation accompanied by wind during the period between flowering and maturity can also play an important role in disease development, as this is one of the major methods by which *Xap* spreads from plant to plant (Zaumeyer and Thomas, 1957).

Disease Management and Breeding for CBB Resistance

Various disease management strategies have been implemented to control CBB, but have achieved limited success. Some of the methods used have been: planting of disease free seed, bactericide seed treatment, crop rotation management, deep-plowing, avoiding the reuse of irrigation water from infected fields, and isolation of diseased fields (Zaumeyer and Thomas, 1957; Anderson et al., 1970). The most effective of these methods is to plant disease-free seed, however, this has not been practical in some locations due to insufficient supply of seed certified free of *Xap* (Saettler, 1973). The use of copper sprays and other chemical methods has also been widely examined in controlling CBB, with limited success (Saettler, 1971; Saetller, 1973). Saettler (1971) found that even after six applications of various bactericidal chemicals, the disease was still not controlled effectively. Many bactericide seed treatments (e.g. Streptomycin) have been effective in decreasing the amount of primary inoculum on the seed surface, but this does not reduce the titer of the bacterium within the seed itself (Saettler, 1989).

When possible, early planting may allow plants to escape CBB infection by reaching maturity before the infection becomes severe, as the bacteria typically thrive in the later, hotter portion of the growing season (Zaumeyer and Thomas, 1957).

Until recently, breeding for genetic resistance has not been a reasonable option for protecting against CBB infections. Genetic linkage with negative agronomic traits (in particular, grain yield) was prohibitory to the successful development of useful CBB resistant varieties (Kelly and Copeland, 1996). Well-adapted CBB resistant breeding lines in various bean market classes have now emerged from different bean breeding programs. These successes have been responsible for the emergence of CBB resistance breeding as the preferred option in preventing CBB epidemics (Singh and Munoz, 1999).

Due to the reasons discussed above, the development of resistant cultivars provides the single, most efficient method of CBB control (Singh and Munoz, 1999). However, the CBB resistance sources currently available only confer partial resistance. Resistant varieties still need to be planted in an integrated management system consisting of: crop rotation, weed control, avoiding passage through wet bean fields, seed treatment with bactericide slurry, isolation of known *Xap* infested fields, and deep-plowing of infected bean debris (Zaumeyer and Thomas, 1957; Saettler, 1989).

Genetic Nature of CBB Resistance

CBB resistance in common bean is a quantitatively inherited trait that exhibits low heritability, controlled by an unknown number of genes. Genetic inheritance studies conducted in various breeding programs with different genetic populations have found differing results regarding the genetic nature of CBB resistance. Most studies have

reported low (h^2= 0.14; Coyne and Schuster, 1974b) to moderate heritability for CBB resistance, but high heritability (h^2= 0.74) values have been reported (Tar'an et al., 2001). A range of values for narrow-sense heritability from h^2= 0.11 to 0.87 were reported for various plant organs in a study by Silva et al. (1989). Heritability for CBB resistance seems to vary greatly based on resistance source used and genetic background of the recipient.

Past research has also reported differing results regarding the expected number of genes involved in the expression of CBB resistance, as well as their mode of action. Data published by Adams et al. (1988) suggested that a single recessive gene from a snap bean line (A-8-40) conferred leaf resistance to CBB. The possibility exists, however, that modifying genes with minor effects could also be involved. The presence of additive gene effects for CBB resistance has been reported, while other studies have suggested incomplete dominant gene action. Coyne and Schuster (1974b) determined that CBB resistance was dominant in a cross between GN 1140 (susceptible parent) x PI 207262 (resistant parent), and that a small number of genes controlled the resistant reaction. Musaana et al. (1993) determined that the number of genes associated with resistance to CBB varied depending on the parents used in population development, and the stage at which the plants were evaluated. Leaf resistance was controlled by one to three genes at an early growth stage, one to four genes later in maturity, while pod resistance was conditioned by one to two genes.

Tepary bean (*Phaseolus acutifolius*), a relative of common bean that exhibits CBB resistance, has been useful for breeders attempting to enhance resistance present in *P. vulgaris* germplasm (Singh and Munoz, 1999). Resistance in tepary bean leaves and

pods appears to be explained by a single dominant gene (Drijfhout and Blok, 1987). When CBB resistance in tepary bean was introgressed into common bean, the resistance was found to be quantitatively inherited (Coyne and Schuster, 1965). CBB resistance introgressed from tepary bean was studied in three different crosses by Scott and Michaels (1988), and resistance was determined to be controlled by two dominant genes. A study of CBB resistance in four tepary x tepary crosses generally agreed with the results proposed by Drijfhout and Blok (1987), determining that CBB resistance was conditioned by one dominant gene, although in one cross, the interaction of two complementary dominant genes was responsible for CBB resistance (Urrea et al., 1999).

Scarlet runner bean (*Phaseolus coccineus*) is another donor of CBB resistance used in interspecific crosses with *P. vulgaris*. Resistance levels within this species are considered intermediate to the high levels in *P. acutifolius* and the low levels in *P. vulgaris* (Singh and Munoz, 1999). Welsh and Grafton (2001) examined the inheritance of CBB resistance in common bean cultivars, with resistance derived from *P. coccineus* and determined that resistance was controlled by a single recessive gene. Although this gene appeared to account for most of the genetic variation in phenotypic CBB ratings, the presence of minor modifying genes may also be involved.

Obstacles to the Development of Genetic Resistance to CBB in Dry Bean

Breeding for genetic resistance to CBB in common bean is complicated by many factors. The complex genetic nature of the host resistance is one obstacle to the development of cultivars with enhanced levels of CBB resistance. As previously mentioned, CBB resistance is a quantitatively inherited trait of low to moderate

heritability, controlled by an unknown number of genetic loci (Park et al., 1999). Although bean genotypes with CBB resistance have been identified, the resistance is only partial in nature, and as a result, does not provide complete protection from pathogen infection (Park et al., 1999). A second complication of quantitative disease resistance is that the genetic loci involved are subject to variation depending on environmental influences (Paterson et al. 1991). The level of expression of quantitative resistance in contrasting genetic backgrounds is another obstacle in breeding for CBB resistance.

A third consideration in breeding for CBB resistance is the differential response of plant organs to CBB infection. There is little correlation between seeds, pods, and leaves in their reaction to CBB (Arnaud-Santana et al., 1994). Valladares-Sanchez et al. (1979) demonstrated that many *Phaseolus* genotypes may exhibit resistance in leaves, while the pods of the same plant exhibited a susceptible reaction to CBB. In addition, there is an absence of correlation between seed and pod infections. There may be a differential reaction for the interior and exterior of pods within the same genotype, however, this difference is not as pronounced as the difference between pod and leaf reactions. In order to attain the desired level of resistance, it may be necessary for breeders to simultaneously breed for both pod and leaf resistance. An important consideration regarding pod resistance is that it can minimize the possibility of disease transmission to progeny (Valladares-Sanchez et al., 1979). Drijfhout and Blok (1987) observed high correlations between leaf and pod resistance in tepary bean. In two different populations (developed by an interspecific cross between *P. acutifolius* and *P. vulgaris*), leaf and pod reactions were positively correlated (r^2= 0.99).

A final problem in breeding for CBB resistance is the tendency of the resistance to be unstable. CBB resistance has been known to segregate after many generations of multiple selections and evaluations. This has complicated attempts to develop highly resistant, true breeding lines. The reasons for this instability of resistance are currently unknown (Singh and Munoz, 1999).

When breeding for quantitative traits (e.g. CBB resistance in bean), genes that have a negative impact in the plant may be inherited along with the trait, making quantitative trait breeding a challenging endeavor (Dudley, 1993). The association of resistance with negative traits, a concept known as linkage drag, has been repeatedly noted in attempts to breed for CBB resistance. A study conducted by Valladares-Sanchez et al. (1979) reported a linkage between CBB resistance and late maturity in common bean. CBB resistance was also associated with indeterminate plant growth habit in this study.

Pathogen variability further complicates development of CBB-resistant dry bean lines. Bacteria are prone to frequent mutations, which can result in their overcoming resistance. Mutations are not the only consequence of pathogen variability that impact attempts to breed for resistance to CBB. Because many different strains may be present or absent in regions where beans are grown, the possibility of the accidental introduction of a strain from one region to another can affect the development of CBB-resistant lines (Schuster and Coyne, 1975).

Pathogen Variability and Resulting Complications for Resistance Breeding

Most of the characterization of *Xap* has been conducted by inoculating multiple bean cultivars. Isolates differing in pathogenicity (the capability to cause disease) across bean genotypes are classified by bean pathologists as different strains (or descendents of a single isolation), but a well characterized structure of genetic variability has not yet been determined for *Xap*. The lack of information regarding variability of this pathogen is an obstacle in the application of host genetic resistance.

One trait that has been examined in *Xap* characterization is the production of a yellowish polysaccharide believed to be the symptom-inducing agent. Corey and Starr (1957) examined the polysaccharide production of four *Xap* variants, and determined that the amount of this toxic polysaccharide produced was correlated with lesion size. Isolates of *Xap* differ greatly in the production of this polysaccharide, suggesting that this may be a useful characteristic to use in isolate differentiation. Isolates that produced this compound were formerly classified as *X. phaseoli* var. *fuscans*, but are no longer categorized separately from *X. campestris* pv. *phaseoli*, the former name of X. *axonopodis* pv. *phaseoli* (Schuster and Coyne, 1975).

Pathogenicity has been more commonly used to differentiate between isolates of *Xap*. Schuster and Coyne (1971) reported new strains isolated from Colombian bean seeds that were pathogenic on the CBB resistant breeding line GNN #1 Sel. 27. This breeding line had previously demonstrated tolerance to many North American *Xap* strains, however, it was susceptible to both Colombian strains tested. Other bean lines tested in this experiment also displayed differing disease reactions, depending on the *Xap* isolate used in inoculations. Ekpo and Saettler (1976) noted significant variation in the

pathogenicity of 15 isolates tested on a set of differential bean lines. Observations from this experiment indicated that highly used resistant lines, such as the CBB resistant breeding line GNN #1 Sel. 27, varied from highly resistant to highly susceptible, depending on the isolate used. Although this line was resistant to the majority of isolates, it was very susceptible to specific isolates, receiving a rating of 4.0 in response to inoculation with four different isolates. A 1-4 scale of visual CBB rating was used: 1 = tolerant, few necrotic flecks, 2 = slightly susceptible, <20% of inoculated leaf necrotic, 3 = moderately susceptible, 20-50% of inoculated leaf necrotic, and 4 = severely susceptible, >50% of inoculated leaf necrotic. The isolates used in screening were obtained from various locations in North America, South America, and Africa. Such examples illustrate the importance of pathogen variability as it pertains to breeding for genetic resistance. Resistance of a bean line to a specific *Xap* isolate does not guarantee that it will be resistant to all isolates that may be present in the area where the line will be grown. This issue is obviously of great concern to breeders who wish to develop resistant varieties for production in a specific region (Schuster and Coyne, 1975). Ekpo and Saettler (1976), proposed the use of multiple resistance sources to resolve issues related to differential pathogenicity of *Xap*.

Sources of CBB Resistance

The naturally occurring resistance to CBB found in *P. vulgaris* is limited. Sources of resistance are limited, with only intermediate levels of resistance. However, resistance sources present in various other *Phaseolus* species have been identified and utilized in bean breeding programs. In addition to quantitative resistance present in *P.*

vulgaris, resistance is also found in the genomes of related species *P. acutifolius* and *P. coccineus* (tepary and scarlet runner beans, respectively). Scarlet runner bean is a part of the secondary gene pool of common bean, which means that it can be crossed with common bean without the need for embryo rescue. Tepary bean is part of the tertiary gene pool of common bean, which means that crosses between the two species require embryo rescue (Singh, 2001). Breeding lines have been successfully developed through interspecific hybridization of both *P. vulgaris* x *P. acutifolius* (Singh and Munoz, 1999) and *P. vulgaris* x *P. coccineus* (Freytag et al., 1982). The development of gene pyramids consisting of resistance from various backgrounds has been suggested as a strategy for the development of CBB-resistant varieties (Singh and Munoz, 1999). Gene pyramiding and interspecific hybridization have not yet led to the development of lines that possess a level of resistance equal to that found in *P. acutifolius*. These lines have, however, shown marked improvement over naturally occurring resistance present in *P. vulgaris* (Singh and Munoz, 1999). The first interspecific hybridization between *P. vulgaris* and *P. acutifolius* reported by Honma (1956), involved Montana No. 5 (the common bean parent) and Tepary 4 (the *P. acutifolius* parent) and resulted in a line, Great Northern Nebraska #1 Sel. 27 (or GNN #1 Sel. 27), that was widely used as a donor of CBB resistance in breeding programs. Recent evidence, however, has questioned whether an interspecific hybridization was made in this experiment (Miklas et al., 2003). Interspecific crosses with *P. coccineus* have also yielded CBB resistant breeding lines, such as XR-235-1-1 (Freytag et al., 1982).

Currently, the majority of breeding for CBB resistance has implemented three major sources of resistance: GNN #1 Sel. 27, XAN 159, and OAC 88-1. The resistance

present in GNN #1 Sel. 27 originated from *P. vulgaris* (Miklas et al., 2003), whereas the resistance in both XAN 159 and OAC 88-1 was introgressed from *P. acutifolius* (Thomas and Waines, 1984; Bai et al., 1997; Singh and Munoz, 1999).

GNN #1 Sel. 27 was selected from a cross between Montana No. 5, a great northern cultivar (*P. vulgaris*) and Tepary #4 (*P.* acutifolius). This cross was made by Honma (1956), resulting in Great Northern Nebraska #1, however, it was Coyne et al. (1963), who identified and selected GNN #1 Sel. 27 for CBB resistance breeding purposes. It was originally assumed that the resistance in GNN #1 Sel. 27 was derived from the tepary parent, simply because of the large amount of CBB resistant tepary germplasm (as opposed to the limited amount present in *P. vulgaris*). Recent evidence has contradicted these assumptions, confirming that the CBB resistance was donated by the common bean parent, Montana No. 5 (Miklas et al., 2003).

One example of successful tepary-derived resistance through interspecific hybridization is the breeding line XAN 159, derived from the tepary source PI 319443. This line originated from a number of *P. vulgaris* x *P. acutifolius* crosses, followed by embryo rescue (Thomas and Waines, 1984; McElroy, 1985; Jung et. al. 1997). Another instance of tepary-derived resistance is present in the line OAC 88-1, that carries resistance obtained through the interspecific hybridization of *P. vulgaris* and the tepary line PI 440795.

Anthracnose Resistance

Resistance to anthracnose in common bean is conditioned as a simply inherited, monogenic trait, with both recessive and dominant resistance genes. Anthracnose has a

highly characterized race structure, and known host resistance genes confer resistance in a race-specific fashion. Additionally, multiple alleles are characterized at three of the known anthracnose resistance loci (*Co-1, Co-3, Co-4*). An allele at the *Co-4* locus, *Co-4*^{2}, provides the most desirable, broad-based resistance, conferring resistance to 33 of 34 *C. lindemuthianum* races tested by Balardin and Kelly (1998). The ideal strategy, however, is to combine resistance genes from two gene pools, Andean and Middle American, in a single genotype. Such a combination is proposed to provide the most durable resistance (Kelly et al., 1994). The *Co-1* gene was historically the most widely utilized anthracnose resistance gene and is of Andean origin. The *Co-1*^{2} allele, found in the differential cultivar Kaboon, is the most useful Andean anthracnose resistance gene for North America (Melotto and Kelly, 2000).

Molecular Markers and Quantitative Trait Loci (QTL) in CBB Resistance

In recent years, the ability to identify molecular markers linked to quantitative traits has given breeders a useful tool for examining these complex traits. Molecular markers allow the identification and genetic linkage mapping of quantitative trait loci (QTL). QTL are regions of the genome that account for a portion of the total phenotypic variation of a quantitative trait. QTL analysis allows breeders to identify loci associated with a trait, verify its novelty (based on the location to which the QTL maps in a genetic linkage map), and determine the contribution of the locus to a phenotypic trait (reported as an r^2 value). The number of genes within a QTL region is unknown, as are their effects. It is likely that the QTL region identified by a molecular marker contains multiple genes, which may not all be related to the trait being studied (Castro et al.,

2003a). The substantial progress made in molecular marker technology holds considerable promise in the use of breeding for genetic resistance to CBB. Molecular markers provide breeders with an opportunity to avoid unreliable direct screening techniques that are not effective in selection for quantitative traits that are significantly affected by environmental factors. Several reliable QTL are now available that provide breeders with a useful tool to use in breeding for CBB resistance, but their efficiency in new genetic backgrounds requires testing (Singh and Munoz, 1999; Miklas et al., 2000).

Data from QTL studies of CBB resistance in bean has typically agreed with the conclusions from previous genetic studies. A small number of major-effect QTL, each explaining a portion of the phenotypic variation has commonly been observed. For example, Tar'an et al. (1998) examined two molecular markers linked to QTL for CBB resistance, discovering that in combination, they accounted for 22% of the genetic variation. A study of resistance to CBB in a cross between BAT93 and Jalo EEP558 revealed the presence of 4 QTL that explained 75% of the variation for CBB resistance. Because one of these putative QTL accounted for a third of the variation (32%), these results agree with the majority of previous genetic studies, that CBB resistance in common bean appears to be controlled by a major effect QTL with a small number of minor loci also affecting the trait (Nodari et al., 1993). Jung et al. (1997) found the number of QTL associated with tepary-derived CBB resistance in a common bean population varied from one to four (explaining 18% to 53% of the phenotypic variation) depending on the pathogen isolate used in screening and the plant organ used for phenotypic evaluation. A QTL linked to the RAPD marker $BC420_{900}$ explained 62% of the variation for CBB resistance in an experiment by Yu et al. (2000). Such data supports

the hypothesis of a single major locus with several contributing minor QTL controlling the resistance reaction. The presence of four and five markers linked to QTL for leaf and pod CBB resistance, respectively, was reported by Ariyarathne et al. (1999). The observation that one locus controlled 44% of the variation for leaf resistance and 41% of the variation for pod resistance support the theory that CBB resistance is conditioned by one major locus and a small number of minor loci contribute to that resistance.

The identification of molecular markers linked to QTL provides an opportunity for breeders to screen for the presence of specific molecular markers. This type of approach can reduce the number of lines that require direct screening for a quantitative trait. Widespread screening of breeding materials for the presence or absence of specific markers, a process known as marker-assisted selection (MAS), has received considerable attention recently. MAS is the logical result of QTL and marker discovery studies, however it is not without its share of limitations and challenges. Unfortunately, the majority of research has focused on the identification of genomic regions associated with quantitative traits, and less on the application of QTL in breeding programs (Ribaut and Hoisington, 1998).

Some serious challenges are presented when working with QTL in disease resistance breeding. The expression of QTL between distinct genetic populations can vary, limiting their usefulness in breeding programs (Castro et al., 2003b). Since QTL are not associated with the complete phenotypic variation of a trait, the resistance provided by a QTL is only partial. In order to confer high levels of resistance to a cultivar, more than one QTL may be required. Since quantitative resistance displays continuous variation and is subject to environmental influences, large-scale field

evaluations are needed to confirm the presence and effects of QTL (Castro et al., 2003b). Additionally, Beavis (1998) suggested that the small population sizes used in QTL identification studies have caused problems by overestimating the effects of QTL and underestimating the number of loci involved in a quantitative trait. Although breeding for quantitative traits has experienced modest success in comparison to breeding for major gene resistance, it provides breeders with the only alternative for developing improved cultivars, where monogenic resistance does not exist.

Quantitative Trait Loci (QTL) in CBB Resistance Sources

The characterization of QTL in various CBB resistance sources has been investigated. One report has given breeders insight regarding the origin of the resistance QTL carried by the CBB resistant breeding line GNN #1 Sel. 27. It has been proposed that the resistance found in GNN #1 Sel. 27 originated from *P. vulgaris*, not *P. acutifolius*, as previously considered (Miklas et al., 2003). In field studies, Montana No. 5 (the common bean parent of GNN #1 Sel. 27) appeared to have partial CBB resistance, similar to GNN #1 Sel. 27 (Miklas et al., 2002). Perhaps the most compelling evidence of this theory regarding the origin of the CBB resistance in GNN #1 Sel. 27, is the molecular marker data. The availability of molecular markers linked to CBB resistance in common bean has resulted in new opportunities for addressing questions regarding the origins of specific genomic regions. The SCAR marker $SAP6_{820}$, which is linked to the major resistance QTL present in GNN #1 Sel. 27, was used for screening, and the marker present in GNN #1 Sel. 27 can be amplified in the Montana No. 5 cultivar, but not in Tepary #4. Additionally, cluster analysis using 169 molecular markers did not reveal any

relationship between Tepary #4 and GNN #1 Sel. 27. The evidence presented is insufficient to determine whether an interspecific hybridization ever occurred between Montana No. 5 and Tepary #4 (Miklas et al., 2003). A re-examination of the F_3 data presented by Honma (1956) seems to suggest that a cross pollination did not occur. Based on the results of other attempts at similar cross hybridizations, Miklas et al. (2003) concluded that the probability of obtaining a cultivar with the seed quality of GN #1 without at least one backcross to *P. vulgaris* is highly unlikely.

It seems apparent, however, that the major-effect QTL linked to SAP6 (on bean linkage group B10), which is present in GNN #1 Sel. 27, did originate from the Montana No. 5 parent. The higher levels of resistance consistently observed in GNN #1 Sel. 27 indicate the presence of additional minor loci that are involved in genetic resistance to CBB in this population (Miklas et al., 2003). The presence of transgressive segregants identified from a GNN #1 Sel. 27/Montana No. 5 cross, also suggests the presence of minor-effect QTL that are not expressed in Montana No. 5 (Miklas et al., 2003). The nature and origin of these additional resistance loci are yet to be determined.

The bean breeding line XAN 159 is now widely used in breeding programs as a source of CBB resistance, because it carries two major QTL for CBB resistance, mapped to linkage groups B6 and B8 of the integrated bean linkage map (Freyre et al., 1998). Both QTL are linked to molecular markers that can be used for indirect screening. The sequence-characterized amplified region (SCAR) markers linked to these two QTL are referred to as SU91 and BC420, respectively (Pedraza et al., 1997; Miklas et al., 2000; Yu et al., 2000). SCAR markers, which are derived from random amplified polymorphic DNA (RAPD) markers, are more specific, typically amplifying only one fragment.

RAPD markers, which utilize single 10 nucleotide primers, are much less specific. This results in a larger number of bands amplified, which in turn, makes them more difficult to score and use for selection. The development of SCAR markers from RAPDs involves sequencing the fragment amplified by the original RAPD primer, and developing longer (and thus, more specific) primers. These primers (approximately 24 base pairs in length) result in the amplification of a single, specific product, scored as the presence or absence of a single band on an agarose gel (Paran and Michelmore, 1993).

The CBB resistant breeding line OAC 88-1 has also been examined in QTL studies. The resistance present in this line appears to be conferred by at least two major QTL, linked to the SCAR markers R7313 and R4865. The R7313 QTL has been mapped to linkage group B8, while the other QTL remains unmapped (Bai et. al., 1997; Miklas et. al., 2000). The QTL in OAC 88-1 may be the same QTL in XAN 159. The SCAR markers SU91 and R7313 are mapped to the same location on bean linkage group B8, which provides support for the idea that these markers identify the same QTL for CBB resistance (Tar'an, 1998; Miklas et al., 2000). This example outlines the need for QTL mapping in determining the novelty of a resistance source. Because QTL identified in diverse germplasm may actually be the same QTL, determining their novelty based on their location on a genetic linkage map can help ensure that research efforts are not duplicated, prior to the initiation of crossing with the resistance source (Kelly and Vallejo, in press).

Marker-Assisted Selection and Gene Pyramiding for CBB Resistance

The opportunity to pyramid QTL (as compared to using a single QTL) for resistance to CBB offers several distinct advantages to breeders attempting to develop bean cultivars with genetic resistance. Due to pathogen isolate variability and the resulting potential isolate specificity of CBB resistance sources, the pyramiding of multiple resistance QTL would provide a broader resistance base than the use of a single resistance locus. The potential durability of genetic resistance is also higher when multiple QTL or genes are pyramided, as it decreases the likelihood of a pathogen overcoming the resistance (Jung et al., 1999; Castro et al., 2003a; Castro et al., 2003b). Since the partial nature of the resistance conferred by individual QTL for CBB resistance, the use of multiple QTL potentially provide higher levels of resistance (Singh et al. 2001; Castro et al., 2003a). The demonstrated organ specificity of known CBB resistance QTL illustrates another potential advantage of pyramiding QTL for resistance. Because certain QTL are known to confer organ specific resistance, the use of multiple QTL would help ensure that resistance levels are increased in all affected plant organs. The opportunity currently exists to begin pyramiding various QTL associated with CBB resistance, and this approach clearly demonstrates advantages over the use of single loci (Jung et al., 1999; Park et al., 1999; Singh et al., 1999; Miklas et al., 2000).

Molecular markers linked to a trait can be easily used to screen for genetic determinants of the trait. One promising approach to developing CBB resistant bean cultivars is to utilize MAS to facilitate the introgression of gene pyramids into agronomically acceptable lines, followed by the confirmation of resistance by extensive greenhouse and field disease screening (Singh and Munoz, 1999). Progress has been

made in pyramiding resistance from various *Phaseolus* species. Pinto bean accession G 17341, pinto bean breeding line NY 79-3776-1, and white bean breeding line Wilkinson 2 are the result of combining resistance derived from *P. vulgaris*, *P. coccineus*, and *P. acutifolius*. These genotypes exhibited improved CBB resistance when compared to that of the three parental lines GNN #1 sel. 27, XAN 159, and OAC 88-1 (Singh and Munoz, 1999). Another group of resistance sources with pyramided CBB resistance is the VAX lines, derived from an interspecific hybridization between common bean (the cultivar ICA Pijao) and tepary bean (accession G 40001). The original cross was conducted by Mejia-Jimenez et al. (1994) and resulted in the breeding lines VAX 1 and VAX 2. Four additional VAX lines (VAX 3-VAX 6) were derived from these first two lines using multiple CBB resistant parents, and have been utilized in a number of breeding programs. XAN 159 was used in the development of only VAX 4 and VAX 5. The common bean cultivar 'Tara' was used in developing VAX 3 and VAX 6. All six VAX lines have resistance introgressed from the CBB resistance source PI 207262. Due to the use of additional resistance sources in the VAX 3-VAX 6 lines, their CBB resistance is greater than the resistance in VAX 1 and VAX 2. The mean ratings for CBB reaction of the VAX 3 – VAX 6 lines ranged from 1.5 to 2.7, based on the CBB visual disease rating described by Schoonhoven and Pastor-Corrales (1987), where: 1 = no visual symptoms, 3 = approximately 2% of the leaf area has lesions, 5 = approximately 5% of the leaf area has lesions, 7 = approximately 10% of the leaf area has lesions, 9 = more than 25% of the leaf area has lesions. These mean CBB ratings for VAX 3 – VAX 6 rank among the best of the available CBB resistant breeding lines (Singh and Munoz, 1999).

Use of Marker-Assisted Selection to Improve Phenotypic Selection

As with most plant pathogens, CBB infection most commonly occurs within specific ranges of temperature and humidity. CBB epidemics generally require temperatures above 28°C. In addition to this, humidity is often required for CBB infection to occur (Saettler, 1989). Environmental conditions are not the only factors that impact the susceptibility of dry beans to *Xap*. The growth stage of the plant also plays a large role in plant-pathogen interactions. As a general rule, plants that are late maturing due to either genetic or environmental conditions are more likely to escape infection. This is complicated by the differential response of various bean plant organs to CBB, because of independent genetic control of resistance in the different plant organs. Plant organs are not all susceptible at the same developmental stage in the growing season. This differential reaction can have an enormous impact on CBB incidence in different years, as well as attempts at breeding resistant cultivars (Coyne and Schuster, 1974). Although an optimal breeding strategy would include the development of plants with both leaf and pod/seed resistance, Valladares-Sanchez et al. (1979) suggested that pathogen spread could be significantly reduced with sufficient pod resistance, since it would reduce the primary source of inoculum.

The variability of the bean plant's reaction to CBB makes resistant cultivar development using visual selection alone impractical, because many factors can result in susceptible genotypes appearing resistant in the field. The use of MAS, alone, or in conjunction with conventional screening helps to overcome the difficulties associated with environmental conditions (Yu et al., 2000; Tar'an et al., 2001). MAS may hold greater promise for disease resistance than for other traits, because it helps alleviate the

difficulties associated with experimental systems involving multiple living organisms. It allows breeders to screen a large number of genotypes in absence of the pathogen, determining their resistance potential by using molecular markers linked to disease resistance loci (Kelly and Miklas, 1999). The preliminary screening of genotypes in the absence of the pathogen is useful if normal selection for resistance is complicated by excessive variability in the pathogen, in experimental conditions, or if there is a need for conducting the research in a location where the pathogen (or a particular strain) is not naturally present. In the latter case, pathogen escape would be a concern in the use of inoculations (Kelly and Miklas, 1999).

There are some limitations to the usefulness of MAS, despite its obvious advantages. One of the major complications is the possible population specificity of some molecular markers. When attempting to use markers in different populations from those in which the marker was developed, the original linkage between the marker and the trait of interest may be lost (Bai et al., 1997). A RAPD marker ($OA14_{1100}$) linked to a rust resistance gene in beans, was only useful in the Middle American gene pool and provided no value in breeding Andean beans (Miklas et al., 1993).

In conclusion, the strategy with the most potential for identifying CBB resistant genotypes seems to be through the combination of molecular marker-assisted selection and traditional phenotypic selection in the field and greenhouse. The use of both direct and indirect selection helps to avoid some of the problems associated with using either method of selection alone.

GENERAL OBJECTIVES

Common bacterial blight can be devastating for both growers and seed producers of dry bean. The adequate control of this pathogen requires the deployment of bean cultivars with high levels of resistance. Currently, none of the cultivars available to Michigan dry bean growers have sufficiently high levels of CBB resistance. To identify and improve potentially resistant genotypes, an efficient method of selection must be developed. This is complicated by pathogen variability and environmental effects. Marker-assisted selection provides an opportunity to overcome some of the limitations of traditional phenotypic selection for resistance to CBB. The objectives of this research were to: 1) examine the potential for using previously developed molecular markers linked to CBB resistance in a marker-assisted selection strategy, 2) determine the effects on CBB resistance of previously discovered QTL in genetic backgrounds suited for Michigan, 3) determine the potential for combining multiple sources of CBB resistance, and 4) combine CBB resistance with specific genes conferring anthracnose resistance in new bean germplasm.

Chapter 1 addresses the potential of using a previously developed molecular marker originating from another bean breeding program to select for a QTL linked to CBB resistance in a different genetic background than the background in which the QTL was discovered. Chapter 2 examines the use of MAS to pyramid diverse CBB resistance sources with anthracnose resistance sources to develop cultivars with resistance to both seed-borne common bean pathogens.

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CHAPTER ONE

THE USE OF MARKER-ASSISTED SELECTION TO ENHANCE THE LEVEL OF RESISTANCE TO COMMON BACTERIAL BLIGHT IN COMMON BEAN LINES

ABSTRACT

Common bacterial blight (CBB) is a destructive disease of common bean (*Phaseolus vulgaris* L.), caused by the bacterial pathogen *Xanthomonas axonopodis* pv. *phaseoli*. Previous attempts to develop highly resistant cultivars have not succeeded due to complications including: pathogen variability, complexity of host genetic resistance, and lack of reproducibility in direct screening with the pathogen. In this study, the potential for utilizing marker-assisted selection (MAS) was examined, to determine if this approach could be used to overcome the limitations of phenotypic CBB evaluations.

Field studies were established in East Lansing and Saginaw to compare the CBB resistance of bean genotypes with or without the sequence characterized amplified polymorphism (SCAR) marker SU91 (linked to a QTL for CBB resistance on bean linkage group B8). Presence of SU91 was correlated with enhanced CBB leaf resistance at both locations ($p<0.0001$ and $p<0.0001$, respectively). Presence of SU91 was correlated with enhanced CBB pod resistance only at the Saginaw location ($p<0.0001$).

The presence of negative correlations between SU91 and important agronomic traits was also examined. Presence of SU91 was not correlated with late maturity and was correlated with reduced yields in the East Lansing experiment ($r = 0.22$ and $r = -0.20$*, respectively). Despite this negative association between the SU91 marker and

seed yield, the selection of early-maturing high-yielding, CBB resistant genotypes was achieved. Presence of SU91 was not correlated with late maturity or reduced yields in the Saginaw experiment ($p= 0.24$ and $p= 0.08$, respectively).

These results indicate that SU91 is an effective molecular marker for the selection of bean lines with enhanced CBB resistance from Michigan's dry bean breeding and genetics program. These findings are encouraging, because multiple reports suggest population specificity and linkage drag problems are associated with tepary-derived CBB resistance sources in common bean.

INTRODUCTION

Common bacterial blight (CBB), caused by the bacterial pathogen *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*) is a destructive disease of common bean (*Phaseolus vulgaris*). CBB infections generally produce negative impacts on various agronomic traits, such as: lodging, maturity, pods per plant and grain yield (Tar'an et al., 2001). In severe cases, CBB may reduce bean yields, with losses potentially exceeding 60% (Zaumeyer and Thomas, 1957). Perhaps the most significant consequence of CBB epidemics has been the impact on the bean seed production industry. CBB infection is especially problematic for seed producers because of the seed borne nature of the disease (Kelly and Copeland, 1996). Seed producers contend with potential yield losses due to CBB and also the rejection of certified seed beans. Certified seed fields are inspected for several criteria, one of which is the absence of CBB symptoms. Since *Xap* is able to survive in the seed, transmission to seedlings is common (Weller and Saettler, 1980). CBB infection in certified bean seed is not permissible. In years with serious CBB epidemics, the rejection rate in Michigan has approached 50% (Kelly and Copeland, 1996).

Due to favorable climatic conditions for CBB development in Michigan, there has been a dramatic decrease of in-state production of certified seed. From 1974 to 1994, the area devoted to certified navy bean seed production in Michigan decreased from 5,000 hectares to 400 hectares. This reduction, a 92% decrease, was primarily due to CBB. Interestingly, kidney bean seed production in Michigan has increased during this time period. Isolation of kidney bean seed production from commercial fields, as well as the

use of moderately resistant cultivars (such as 'Montcalm') has played a significant role in this growth (Kelly and Copeland, 1996).

Many cultural and chemical controls have been used to protect bean crops from CBB, none of which have proven successful (Zaumeyer and Thomas, 1957; Saettler, 1971). The use of genetic resistance would be the most desirable method of disease prevention. Previous attempts to develop bean cultivars with CBB resistance have not been successful (Singh and Munoz, 1999).

Many factors account for the inability to breed resistant bean cultivars. The most notable factor is due to the nature of the host resistance. Resistance to CBB in common bean is a quantitatively controlled trait, which exhibits low to moderate heritability. The inability to identify discrete genetic loci conditioning CBB resistance in beans has been a limiting factor in attempts to develop resistant cultivars. The expression of quantitative traits, such as CBB resistance, is typically complicated by environmental interactions that make it difficult to accurately identify genotypes with CBB resistance (Yu et al., 2000). Another major complication of traditional approaches to breeding for CBB resistance has been the inaccuracy of using *Xap* in direct screening. Problems with reproducibility in direct screening with the pathogen are partially due to fluctuations in environmental conditions (Tar'an et al., 2001). There are two major reasons for this: 1) sensitivity of *Xap* to temperature and moisture, and 2) variability in virulence among isolates between experiments; which combine to adversely impact the successful selection of resistant genotypes (Goss, 1939). The variability of *Xap* is relatively uncharacterized, and because the usefulness of resistance sources may be strain specific, pathogen variability presents a problem for resistance breeding (Schuster and Coyne, 1971; Schuster and Coyne, 1975;

Ekpo and Saettler, 1976). A genotype identified as resistant in a particular experiment may not exhibit the same resistance level in a different experiment using a different *Xap* isolate. An additional constraint to breeding for CBB resistance in common bean is the association of resistance with undesirable agronomic traits. Linkage of a quantitative trait and undesirable traits, or linkage drag, is a common complication in breeding quantitative traits (Dudley, 1993). Beebe (1989) reported linkage of CBB resistance to both negative seed traits (brilliance and color instability) and low bean yield. Linkage between CBB resistance and late maturity was also reported by Valladares-Sanchez et al. (1979).

The recent introduction of molecular marker technology has created additional opportunities for disease resistance breeding strategies, and the potential to overcome these challenges is now available (Yu et al., 2000). The use of MAS has the potential to overcome the major limitations of traditional phenotypic selection for CBB resistance. Molecular markers can be used to identify specific genomic regions linked to a quantitative trait, and provide the opportunity to screen for the presence of quantitative trait loci (QTL), which are genetic loci associated with the quantitative trait-of-interest (Lande and Thompson, 1990). MAS also resolves problems associated with reproducibility of direct screening, due to environmental effects. Plants may be selected in absence of the pathogen, which alleviates the concerns of working with both biological organisms, although direct inoculations may be required to confirm results of MAS (Kelly and Miklas, 1999).

Despite the obvious advantages over direct screening, MAS has certain limitations. The issues of pathogen genetic variability and potential specificity of

bacterial isolates by resistance loci are not resolved by using MAS. Ekpo and Saettler (1976) reported that the widely used CBB resistance breeding line GNN #1 Sel. 27 exhibited a highly susceptible reaction when screened with specific isolates. Verification of resistance provided by specific resistance sources to local *Xap* isolates would help ensure that a desired resistance source is useful for that particular region. Another limitation to the use of MAS in breeding programs is the expression of QTL in different genetic backgrounds. The usefulness of a molecular marker in a genetic background that is different from the background in which the QTL was identified is not guaranteed. The performance of QTL linked to the marker must be examined to ensure that it confers the desired phenotype (Dudley, 1993; Yu et al. 2000). Prior to the widespread adoption of MAS for disease resistance, usefulness of markers developed by other research programs in successfully predicting resistance must be verified. Yu et al. (2000) reported a successful attempt to use a molecular marker linked to CBB resistance in common bean population differing genetically from that in which it was developed. Jung et al. (1999) examined a SCAR marker linked to a different CBB resistance QTL, and determined that it was associated with resistance in all four of the genetic populations tested. In contrast, Paterson et al. (1991), determined that only four of 29 QTL for fruit size and quality traits of tomato were associated with the same trait in three environments, while 10 QTL were stable across two environments.

The practical application of MAS in selection for disease resistance, including CBB, has been examined, with notable successes and failures (Yu et al., 2000). Research conducted by Yu et al. (2000) determined that selection based on SCAR marker BC420 was 92.5% as effective as direct selection in identifying resistant bean genotypes, and

was also more cost-effective. Various CBB resistance sources are available for use in bean breeding programs, and robust molecular markers have been developed to screen for CBB resistance (Singh and Munoz, 1999).

The SCAR marker SU91 is linked to a QTL for CBB resistance on common bean linkage group B8 (Pedraza et al., 1997). This QTL originated from tepary bean (*Phaseolus acutifolius*) and was introgressed into common bean through an interspecific hybridization. The original resistance source from which SU91 was characterized is XAN 159, a breeding line that has been used extensively in breeding for CBB resistance. SU91, which amplifies a single 700 bp fragment, was derived from the RAPD marker U9 (Pedraza et al., 1997).

The SCAR markers SAP6 and BC409 are both linked to the same QTL for CBB resistance on common bean linkage group B10 (Miklas et al., 2000; Jung et al., 1999). The original source from which this QTL was identified is the CBB resistant breeding line GNN #1 Sel. 27. SAP6 exhibits a tighter linkage with the QTL than BC409 (Miklas et al., 2000). SAP6 and BC409 were derived from the RAPD fragments AP6 and BC409, and amplify single fragments of 820 and 1250 bp, respectively (Miklas et al, 2000; Jung et al., 1999).

The primary objective of this research was to develop black and navy bean lines with enhanced levels of CBB resistance for production in Michigan, using the SCAR marker SU91 in a marker-assisted selection program as a complement to traditional direct screening using local *Xap* isolates. A second objective was to evaluate the potential for using SU91 in a genetic background that is different from the original one in which it was

identified and evaluate the association between CBB resistance linked to the SU91 marker and important agronomic traits in common bean.

MATERIALS AND METHODS

Parental Material and Population Development. The resistant parents (99L91-45 and 99L91-47) initially used for the development of CBB resistant populations in this study were developed from VAX 5, a breeding line developed at CIAT (International Center for Tropical Agriculture), from an interspecific hybridization between *P. vulgaris* and *P. acutifolius* (Singh and Munoz, 1999). VAX 5 has CBB resistance derived from both XAN 159 and GNN #1 Sel 27, two of the most widely used resistance sources. VAX 5 is a small-seeded, type II (indeterminate upright), black bean with high levels of CBB resistance (Singh and Munoz, 1999). The resistance found in VAX 5 (as well as the other VAX lines) is lower than the level of resistance found in *P. acutifolius*, however, it is superior to the resistance found in most resistant *P. vulgaris* accessions.

The MSU black bean breeding line B98311 was crossed with VAX 5 to develop two breeding lines (99L91-45 and 99L91-47). These breeding lines were crossed with a commercial cultivar ('Phantom') and elite breeding lines (N99219, B98306, and B00136) to combine disease resistance with favorable agronomic characteristics (Frahm, 2002). A summary of the crosses made in this study is included in Figure 1.1.

In total, eight F_2 populations were selected for advancement via the single seed descent (SSD) breeding method (Johnson and Bernard, 1962). One pod was harvested from each F_2 individual within each population from the field in the fall of 2001. The F_3 plants were grown in the greenhouse during the fall/winter 2001-02, in 6-inch clay pots and sterile potting soil. Two seeds were planted for each genotype, and thinned to one seedling/genotype after emergence. Upon maturity, 1 pod from each plant was again harvested for SSD. Advancement of the F_4 generation was conducted in the same

manner as the F_3 generation during the spring of 2002. Tissue was collected from young trifoliate leaves of F_3 plants, for use in DNA extraction (as described below). All individuals were screened for presence of the SCAR marker SU91. The F_5 populations were planted on June 10, 2002 at the Saginaw Valley Bean and Beet Farm in one-row plots. The plots were space-planted (ranging from 2-25 seeds planted per plot, with variable spacing) and fertilized with 200 lbs of 33-11-0 at planting. These five F_5 populations consisted of a total of 454 F_5 lines that were evaluated based on evaluated agronomic traits (e.g. time to flowering, pod set, plant architecture). These lines were compared to parental lines (99L91-45, 99L91-47, B00136, B98306, N99219, and Phantom) and check lines (HR45, Jaguar, N97774, VAX 3, VAX 5, VAX 6, Vista, and XAN 159). A select group of 38 F_5 lines were chosen and harvested for further study (for flowering data on these selections, see Appendix A3).

Molecular Marker-Assisted Selection. The SCAR markers used in selection for CBB resistance were easily scored as the presence or absence of a single band on an agarose gel. The PCR protocol used was described by Melotto et. al. (1996), except Invitrogen *Taq* polymerase was used (Invitrogen, Carlsbad, CA). The SCAR marker SU91, linked to a QTL for CBB resistance on bean linkage group B8, was used in screening. A SCAR marker (SAP6) associated with a QTL for CBB resistance on bean linkage group B10 was used to screen parental material. This marker was present in the susceptible parents, so it was not used in screening the F_3 populations. A SCAR marker (BC409), linked to the same CBB resistance QTL as SAP6 (on bean linkage group B10) was used to screen the parental materials. This marker was present in the susceptible parents, and was not

used to screen the F_3 populations. The SCAR marker BC420, which is linked to a CBB resistance QTL on bean linkage group B6, was not present in any parental materials, and was not used to screen the F_3 populations. All reactions were completed with a final step of 5 minutes at 72°C, and samples remained at 4°C until their preparation for agarose gel electrophoresis. A summary of PCR profiles for this research is presented in Table 1.1.

Figure 1.1 Order of crosses made in the development of populations with increased CBB resistance. **A)** The resistant parent VAX 5 was crossed with the MSU elite black bean breeding line B98311. **B)** Two of the resulting lines that were verified to carry the SCAR marker SU91 were used in crosses with 'Phantom' (black bean cultivar), N99219 (navy bean breeding line), B98306 (black bean breeding line), and B00136 (black bean breeding line). Crosses were performed in both directions, using each parent as pollen parent and female parent in separate crosses. The 99L91-45 and 99L91-47 parents were used in crosses as $F_{3:5}$ plants.

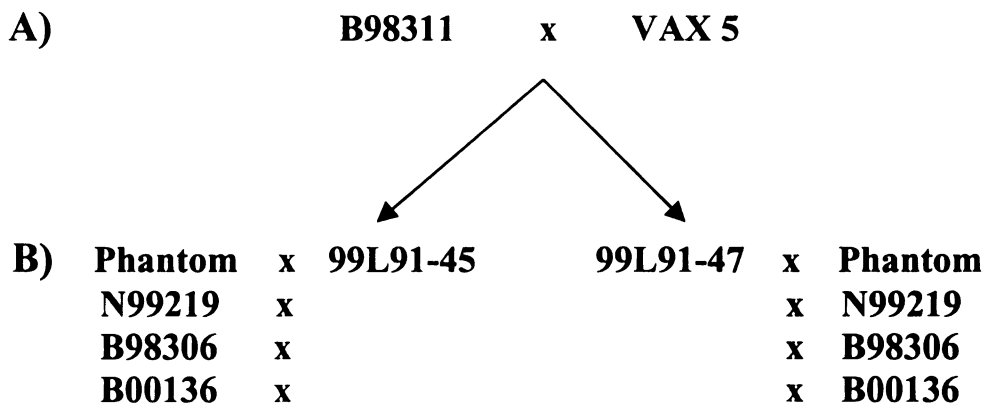

Table 1.1 A summary of PCR profiles used in screening for the SCAR markers SU91, SAP6, BC409, BC420.

SCAR	PROFILE
SU91	94°C/10", 58°C/40", 72°C/2' 34 cycles; 72°C/5', 4°C soak
SAP6	94°C/10", 55°C/40", 72°C/2' 34 cycles; 72°C/5', 4°C soak
BC409	94°C/10", 70°C/1', 72°C/2' 34 cycles; 72°C/5', 4°C soak
BC420	94°C/30", 50°C/30" , 72°C/1' 35 cycles; 72°C/5', 4°C soak

Multiplex PCR was used in some instances to screen genotypes for SU91, BC420, and SAP6. For this multiplex reaction, the SAP6 profile was used, which has an annealing temperature intermediate to that of SU91 and BC420. For each reaction, the components were present in the same concentrations suggested by the aforementioned protocol (Melotto et. al., 1996), with the following exceptions: 1.5 µl (10 ng/µl) of each primer, 16.35 µl H_20.

PCR results were analyzed using a 1.4% agarose gel stained with ethidium bromide (0.02 µg/mL). Bands present on the gel were compared by size to a 100 bp ladder (Invitrogen, Carlsbad, CA). When multiplex PCR was utilized, SU91 (700 bp), BC420 (900 bp), and SAP6 (820 bp) were distinguishable by size using ultra-violet light fluorescence.

Collection and Culture of Bacterial Isolates. Diseased plant tissue was collected from the field for the purpose of isolating *Xap* for use in field and greenhouse inoculations. Twelve samples of infected bean leaves were collected from diverse genotypes, locations, and market classes at the Saginaw Valley Bean and Beet Farm. An additional 12 samples of infected bean leaves were collected at the Montcalm Research Farm.

The protocol used for bacterial isolation was as described by Claflin et. al. (1987), with some minor modifications. Approximately 0.5 g sample of tissue removed from the margin of chlorotic regions was ground using an autoclaved mortar and pestle. The extraction buffer was autoclaved prior to use in grinding. A 50 µl aliquot of each sample was plated on the semi-selective media MXP (Claflin et. al., 1987). Petri plates were immediately covered with aluminum-foil to prevent photodegredation of antibiotics in the

MXP media. The plates were incubated at 28°C for approximately 48 hours, and were stored in a refrigerator at 4°C. For long-term storage, bacterial cultures were stored in 50% glycerol at -80°C.

Field Inoculations and Disease Evaluation. Field plots were established at the Agronomy Farm of Michigan State University in East Lansing, Michigan on May 28, 2003. All plots were fertilized at planting with 46 kg/ha of 19-19-19, which was banded. Weed control was achieved by applying 0.38 L/ha ethafluralin and 0.25 L/ha metolachlor prior to planting. Plots were treated with 0.19 L/ha dimetholate for insect control as needed during the growing season. The experiment consisted of 42 entries, with each plot consisting of two 15-foot rows and 3 replications arranged in a 6 x 7 lattice design. Each replication included an additional block consisting of 12 rows of 'Midland' as a spreader, to increase disease pressure. The 42 entries consisted of 30 experimental F_6 lines (chosen from the 38 F_5 lines selected for evaluation of CBB resistance), parental lines (N99219, 99L91-45, 99L91-47, B98306, 'Phantom', and B00136), resistant checks (VAX 5, HR45, XAN 159), and susceptible checks ('Othello' and 'Midland'). The 30 experimental F_6 lines were selected on the basis of SU91, to ensure that 15 lines carried the marker, and 15 did not. 'Midland' seed infected with *Xap* was used to facilitate the spread of bacterial inoculum. The 'Midland' seed was harvested from diseased field plots grown at the Montcalm Research Farm the previous year.

Field plots were inoculated with a *Xap* solution (10^6 cfu/ml) of the isolate 9712-3 (obtained from the University of Nebraska) on July 16 and July 24, 2003. A power sprayer was used to apply the bacterial solution at approximately 150 psi

and the inoculum was prepared in 76 liter batches. Plants were sprayed until water-soaking occurred and upper leaf surfaces were completely covered with inoculum.

The field plots were evaluated for various agronomic traits. Days until flowering (at least 50% of the plants in a plot had a minimum of one open flower) was recorded for each plot. Leaf disease reaction was evaluated on August 6 and 13, 2003 (13 and 20 days after the 2nd inoculation, respectively), using the 1-9 scale described by Schoonhoven and Pastor-Corrales (1987). This rating scale was described by the following guidelines: 1 = no visible disease symptoms, 3 = 2% of the leaf surface has visible lesions, 5 = 5% of the leaf surface has visible lesions, 7 = 10% of the leaf surface has visible lesions, 9 = More than 25% of the leaf surface has visible lesions (Schoonhoven and Pastor-Corrales, 1987). Pod disease reaction was recorded separately from leaf reaction on a scale of 0 to 3 (using intervals of 0.5). The scale was described as follows: 0 = no pod lesions, 1 = approximately one to three pod lesions, 2 = approximately four to eight pod lesions, 3 = more than eight pod lesions. Agronomic traits evaluated prior to harvest were: height, lodging, maturity, and desirability (DS). Height was recorded in centimeters (cm). Lodging was evaluated on a 1 to 5 scale: 1 = no lodging, 3 = intermediate lodging, and 5 = excessive lodging. Maturity was recorded as days after planting (DAP) with respect to the day that the plot was entirely mature and ready to be harvested. DS was determined as a visual rating of overall agronomic desirability. This rating used a 1 to 9 scale with the following guidelines: 1-4 = above average agronomic traits, 5 = average (compared to commercial checks), 6-9 = unacceptable agronomic traits. Those entries with a DS of below 5 are typically selected, with some selections made within those genotypes receiving a rating of 5. Plants were mechanically harvested using the bean knife method

on September 5, 2003. Yield data was calculated by harvesting 4.57 meters of the center 2-rows for each 4-row plot.

Experimental Design and Statistical Analysis. Analysis of variance (ANOVA) was determined for each experiment, using the PROC GLM function of SAS (SAS Institute Inc., 2000). Traits showing association ($p>0.05$) with SU91 based on F-values of single-factor ANOVA were analyzed using Fisher's Protected LSD ($\alpha=0.05$). The PROC REG function of SAS was used for regression analysis of all traits, and r-values were determined. Correlations were examined using PROC CORR of SAS.

RESULTS

Genotypes Evaluated in East Lansing and Saginaw Field Studies. The list of genotypes included in the East Lansing experiment was presented in Table 1.2. This experiment included 15 SU91+ F_6 lines, 15 SU91- F_6 lines, and 12 check lines. The Saginaw field experiment included additional SU91- lines that were grown as part of the preliminary yield trial. These additional lines were included in molecular marker analysis. A list of genotypes grown in Saginaw was presented in Table 1.3.

Use of the SCAR Marker SU91 in Marker-Assisted Selection. In the East Lansing field experiment, the 1st rating of leaves for CBB resistance was significantly different between SU91+ and SU91- genotypic classes ($p<0.0001$), as was the 2nd rating of leaves for CBB resistance ($p<0.0001$). The mean leaf ratings for SU91+ lines were 3.37 and 3.48 for the 1st and 2nd leaf ratings, respectively. Comparatively, the mean leaf ratings for the SU91- lines were 4.26 and 4.71. No significant differences were detected at the 0.05 significance level between genotypic classes in either the 1st or 2nd rating of pods for CBB resistance ($p=0.09$ and $p=0.052$, respectively). A comparison between phenotypic disease ratings between lines with or without SU91 is summarized in Table 1.4.

The results obtained from the PYT at the Saginaw Valley Bean and Beet Research Farm differed in that the presence of SU91 was significantly associated with both leaf ($p<0.0001$) and pod ($p<0.0001$) phenotypic disease ratings. Only one rating each of leaves and pods was recorded at this location. The SU91+ genotypes had a mean leaf

disease rating of 4.67, compared to a mean of 6.56 for those lines without SU91. The mean pod disease ratings were 0.53 and 1.05 for SU91+ and SU91- lines, respectively (Table 1.4).

Mean CBB leaf ratings for each of the 42 entries included in the East Lansing experiment were presented (Table 1.2). Means were listed in rank, beginning with the lowest mean CBB leaf rating (2.30) and ending with the highest (7.63). SU91 genotypic class was included for each entry (SU91+ or SU91-). Mean CBB pod ratings were not included, because pod ratings were not significantly associated with SU91.

Mean CBB leaf ratings for each of the 72 entries included at the Saginaw study were presented in Figure 1.2. Pod ratings were significantly associated with SU91 in the Saginaw experiment. The mean CBB pod ratings are presented by genotypic class in Figure 1.3.

SU91 and Yield. A significant negative correlation ($r = -0.20^*$) was observed between SU91 and yield at the East Lansing location. The mean yield for SU91+ and SU91- lines were 2.05 ± 0.06 and 2.20 ± 0.09 T/ha, respectively, which were significantly different ($p= 0.02$). The presence of SU91 was not significantly associated with reduced yields at the Saginaw location ($p= 0.08$), where the mean yields were 3.08 ± 0.11 and 2.96 ± 0.07 T/ha for SU91+ and SU91- lines, respectively (Figure 1.4).

Table 1.2 Common Bacterial Blight Leaf and Pod Ratings of 30 F_6 lines and check cultivars or breeding lines, East Lansing (2003).

Genotype†	SU91‡	Leaf§	Pod¶	Genotype†	SU91‡	Leaf§	Pod¶
HR45	+	2.30	0.89	N03616	-	3.78	1.00
B03644	-	2.90	1.00	B03647	-	3.81	1.05
B03637	+	3.02	1.00	B03622	+	3.85	0.92
99L91-45	+	3.03	1.22	B03625	+	3.96	0.92
B03633	+	3.06	0.94	B98306	-	3.99	1.00
B03645	-	3.06	1.00	B03623	-	4.05	1.00
VAX 5	+	3.06	1.08	N99219	-	4.10	1.00
N03611	+	3.12	1.00	B03646	-	4.11	1.00
XAN 159	+	3.20	0.94	B03628	+	4.35	0.75
B03632	+	3.34	1.00	B03631	+	4.41	0.83
99L91-47	+	3.34	0.92	N03618	-	4.49	1.17
B03639	+	3.37	1.00	Phantom	-	4.50	1.00
B03635	-	3.38	0.92	B03613	-	4.52	1.00
B03643	+	3.38	0.92	B00136	-	4.60	1.08
B03630	+	3.43	0.94	B03638	-	4.63	1.00
B03641	-	3.43	1.00	B03620	-	4.73	1.00
N03614	+	3.47	1.17	B03629	-	4.84	1.00
B03642	+	3.55	1.17	B03636	-	4.87	1.08
B03627	-	3.57	1.00	Othello	-	5.99	0.67
N03617	+	3.60	1.00	Midland	-	7.30	2.00
B03634	+	3.74	1.00	Midland	-	7.63	2.50

† = Genotypes beginning with "B" and "N" denote black and navy bean breeding lines, respectively. Also included are check genotypes (HR45, 99L91-45, VAX 5, XAN 159, 99L91-47, N99219, Phantom, Othello, and Midland).

‡ = "+" denotes bean genotypes which carry the SCAR marker SU91.
"-" denotes bean genotypes which do not carry the SCAR marker SU91.

§ = Indicates mean CBB leaf rating for specified bean genotype. The rating scale used was a 1-9 rating of visual CBB symptoms (1= no visible disease symptoms, 3= Approximately 2% of leaf surface has lesions, 5= Approximately 5% of leaf surface has lesions, 7= Approximately 10% of leaf surface has lesions, 9= More than 25% of leaf surface has lesions).

¶ = Indicates mean CBB pod rating for specified bean genotype. The ratings scale used was a 0-3 rating of visual CBB symptoms (0= no pod lesions, 1= small pod lesions, 2= large pod lesions, 3= multiple large pod lesions).

Table 1.3 List of genotypes evaluated in preliminary yield test in Saginaw, MI (2003).

Genotype†	SU91‡	Leaf§	Pod§	Genotype†	SU91‡	Leaf§	Pod§
N03614*	+	3.0	0.0	N00729	-	6.0	1.0
N03617*	+	3.5	0.0	B03613*	-	6.5	1.0
B03632*	+	3.5	0.0	B03624	-	6.5	1.5
B03633*	+	3.5	0.5	B03640	-	6.5	0.5
B03637*	+	4.0	0.5	B00136	-	6.5	1.0
B03639*	+	4.0	0.0	I03361	-	6.5	1.5
B03643*	+	4.0	0.5	I03366	-	6.5	1.0
N02234	-	4.0	1.0	I03370	-	6.5	1.0
I03373	-	4.0	0.0	N00792	-	6.5	1.0
B03622*	+	4.5	0.5	N00838	-	6.5	1.0
B03647*	-	4.5	0.5	I03390	-	6.5	1.0
N02250	-	4.5	1.0	B03619	-	7.0	1.5
I03367	-	4.5	1.0	B03620*	-	7.0	1.0
N03611*	+	5.0	0.5	B03623*	-	7.0	1.0
N03618*	-	5.0	1.5	B03626	-	7.0	1.0
B03630*	+	5.0	1.0	B03636*	-	7.0	0.5
B03634*	+	5.0	0.5	B03638*	-	7.0	1.0
B03642*	+	5.0	0.5	B03641*	-	7.0	1.5
N02247	-	5.0	1.0	Jaguar	-	7.0	1.0
B98306	-	5.0	1.0	Condor	-	7.0	1.0
I03362	-	5.0	1.0	Vista	-	7.0	1.0
B03612	-	5.5	1.0	I02521	-	7.0	1.0
B03621	-	5.5	1.0	N00756	-	7.0	1.5
B03625*	+	5.5	1.0	B03615	-	7.5	1.5
B03635*	-	5.5	1.0	N03616*	-	7.5	1.5
I03371	-	5.5	1.0	B03627*	-	7.5	1.5
B03631*	+	6.0	1.0	B03629*	-	7.5	1.0
B03644*	-	6.0	1.0	B03645*	-	7.5	1.0
B03646*	-	6.0	1.5	T-39	-	7.5	1.0
N02249	-	6.0	1.0	I03364	-	7.5	1.0
N99219	-	6.0	1.0	N00760	-	7.5	1.0
115-M	-	6.0	1.0	N00794	-	7.5	1.0
I03363	-	6.0	1.5	I01794	-	7.5	1.0
I03365	-	6.0	1.5	B03628*	+	8.0	1.5
I03369	-	6.0	1.0	I03368	-	8.0	1.0
I03372	-	6.0	1.0	Seahawk	-	8.0	1.0

* = Denotes genotypes evaluated in both East Lansing and Saginaw.

† = Genotype names beginning with "B" or "N" are black or navy bean lines, respectively. Genotype names beginning with "I" are bean breeding lines from other breeding programs.

‡ = "+" denotes bean genotypes which carry the SCAR marker SU91. "-" denotes bean genotypes which do not carry the SCAR marker SU91.

§ = "Leaf" denotes the mean CBB leaf rating for the genotype. Leaf ratings were recorded on a 1-9 scale, with 1 = uninfected and 9 = severe necrotic regions. "Pod" denotes the mean CBB pod rating for the genotype. Pod ratings were recorded on a 0-3 scale, with 0 = uninfected and 3 = severe pod infection.

¶ =Denotes susceptible bean cultivars or breeding lines included as checks for yield comparisons.

Table 1.4 Mean leaf and pod common bacterial blight (CBB) ratings for SU91+ and SU91- F_6 lines from field plots at East Lansing and Saginaw, MI (2003).

Location	SU91†	Leaf1‡	Leaf2‡	Mean-Leaf	Pod1§	Pod2§	Mean-Pod
East Lansing	+	3.37	3.48	3.43	0.95	1.01	0.98
	-	4.26	4.71	4.49	1.05	1.17	1.11
$LSD_{0.05}$¶		0.40*	0.38*	0.39*	0.11	0.16	0.14
Saginaw#	+	4.67	-	-	0.53	-	-
	-	6.56	-	-	1.05	-	-
$LSD_{0.05}$¶		0.64*	-	-	0.23*	-	-

* = Denotes significance at the 0.05 probability level.
† = "+" represents the class of genotypes which carry the SCAR marker SU91.
= "-" represents the class of genotypes which do not carry the SCAR marker SU91.
‡ = Denotes mean CBB leaf ratings 1 and 2 (13 and 20 days after the final inoculation, respectively) in East Lansing (2003). Leaf ratings were recorded on a 1-9 scale, with 1 = uninfected and 9 = severe necrotic regions.
§ = Denotes mean CBB pod ratings 1 and 2 (13 and 20 days after the final inoculation, respectively) in East Lansing (2003). Pod ratings were recorded on a 0-3 scale, with 0 = uninfected and 3 = severe pod infection.
¶ = Fisher's Least Significant Difference (LSD)
# = Only one CBB leaf rating and one CBB pod rating were taken at the Saginaw location. This experiment was not inoculated, natural infection was sufficient for screening.

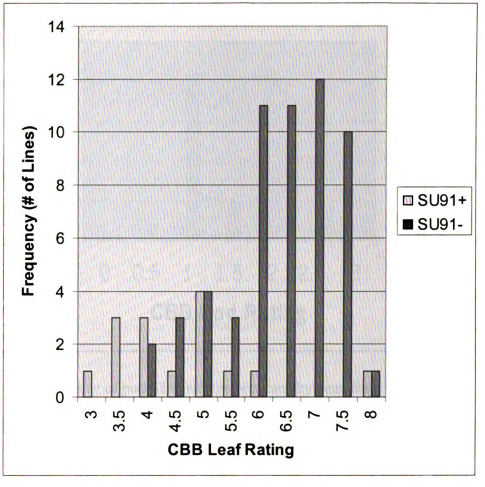

Figure 1.2. Frequency of leaf CBB ratings between bean lines with SU91 (SU91+) and those without SU91 (SU91-). All 72 entries from the navy and black bean preliminary yield trials are represented. The rating scale used was the previously described 1-9 scale (1 = no infection, 9 = severe necrotic lesions). The minimum and maximum mean leaf ratings for this study were 3 and 8, respectively.

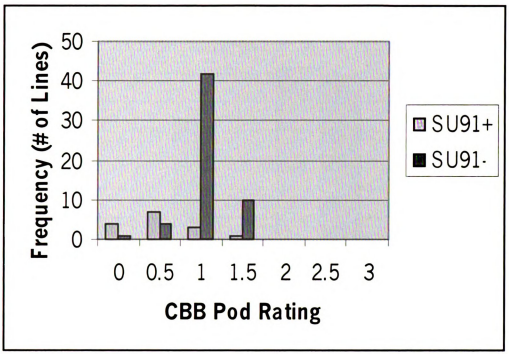

Figure 1.3. Frequency of pod CBB ratings between dry bean lines with SU91 (SU91+) and those without SU91 (SU91-). All 72 entries from the navy and black bean preliminary yield trials are represented. The rating scale used was the previously described 0-3 scale (0 = no pod lesions, 3 = severe pod lesions). The minimum and maximum mean pod ratings for this study were 0 and 1.5, respectively.

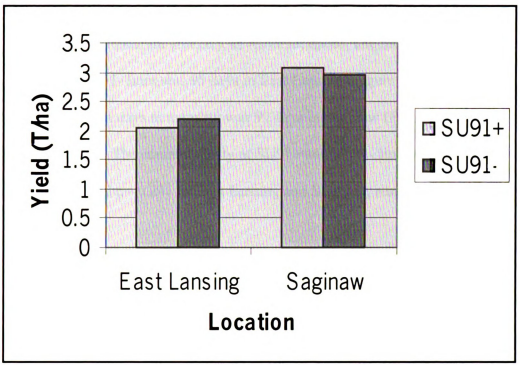

Figure 1.4. Comparison of mean yield between lines with (SU91+) and without (SU91-) the SCAR marker SU91. The mean yields at East Lansing of 2.05 and 2.20 T/ha for SU91+ and SU91- lines, respectively, were significantly different (p= 0.02). Mean yields at Saginaw of 3.08 and 2.96 T/ha, for SU91+ and SU91- lines, respectively, were not significantly different (p= 0.08).

SU91 and Maturity. The presence of SU91 was not associated with later maturity (compared to lines without the marker), in either location. The mean days to maturity for both SU91+ and SU91- lines was 95 days in East Lansing (Figure 1.5), ranging from 91 to 97 days. The mean days to maturity was 99 days in Saginaw (Figure 1.6), ranging from 97 to 103 days. The mean maturity of SU91+ and SU91- lines was not significantly different ($p= 0.22$ and $p= 0.24$) for East Lansing and Saginaw, respectively.

SU91 and Seed Weight. The presence of SU91 was not significantly associated with bean seed weight at either location. The mean seed weight for SU91+ and SU91- lines in East Lansing was 21.7 g/ 100 seeds and 20.9 g/ 100 seeds, respectively. These means were not significantly different. The mean seed weight for SU91+ and SU91- lines in Saginaw was 18.7 g/100 seeds and 18.0 g/100 seeds, respectively. These means were not significantly different ($p= 0.065$).

Yield and Phenotypic Disease Ratings. To evaluate the impact of CBB resistance on yield, the visual disease ratings were correlated to yield at both locations. For the East Lansing experiment, only the 2nd pod rating was associated with yield. A negative correlation was observed ($r = -0.26^{**}$) between yield and the 2nd pod rating. In contrast, an examination of the Saginaw study illustrated an association between yield and leaf ratings ($r = -0.35^{**}$), while there was no significant association between yield and pod ratings.

Leaf and Pod Disease Ratings. Leaf and pod ratings were compared to examine potential differences in tissue susceptibility (Table 1.2 and Table 1.3). For the East Lansing study, the 1st leaf rating showed a positive correlation with the 1st pod rating (r = 0.39***). The 2nd leaf rating was also positively correlated with the 2nd pod rating (r = 0.59***). The two leaf ratings were strongly correlated (r = 0.77***), as were the two pod ratings (r = 0.52***). The leaf and pod ratings were also correlated in the Saginaw study as well (r = 0.65***).

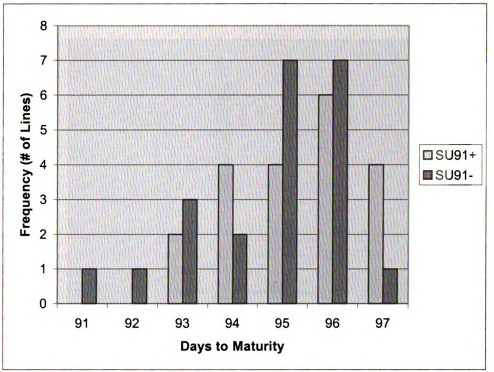

Figure 1.5 Maturity is compared between SU91+ and SU91- lines at East Lansing, 2003. Days to maturity was recorded as the average for 3 replications of each line. The overall mean maturity for the experiment was 95 days. The mean maturity for both SU91+ and SU91- genotypes was also 95 days.

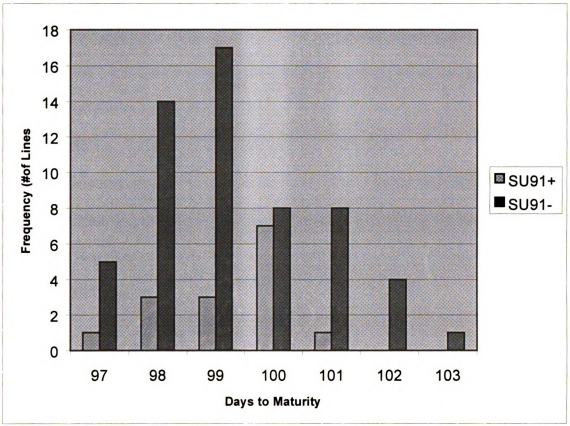

Figure 1.6. Maturity is compared between SU91+ and SU91- lines at Saginaw, 2003. Days to maturity was recorded as the average for 3 replications of each line. The overall mean maturity for the experiment was 99 days. The mean maturity for both SU91+ and SU91- genotypes was also 99 days.

Yield Results from CBB Resistance Screening Study – East Lansing 2003

The 30 genotypes included in the CBB screening experiment in East Lansing are listed in Table 1.2. Of the 30 lines, 15 had the SU91 marker, while the remaining 15 did not. Also included were 11 checks (N99219, 99L91-45, 99L91-47, B98306, 'Phantom', B00136, 'Othello', VAX 5, HR45, XAN 159, and 'Midland').

Yield summaries of the SU91+ and SU91- genotypes are presented in Table 1.6. Check genotypes are included for a yield comparison. Yields of this experiment (across genotypes) ranged from 1.44 to 2.73 T/ha, respectively and the overall mean yield for this study was 2.13 T/ha.

Table 1.6 Yield (T/ha) of bean genotypes with or without the SCAR marker SU91 compared to check varieties and cultivars, East Lansing, MI (2003).

Rank †	Genotype‡	SU91	Yield (T/ha)
1	B03625	+	2.73
2	B03645	-	2.59
3	B03613	-	2.58
4	B03629	-	2.51
5	Phantom¶	-	2.49
6	B00136	-	2.45
7	B03635	-	2.41
8	B03623	-	2.39
9	B03638	-	2.35
10	B03630	+	2.33
11	B03631	+	2.33
12	B03644	-	2.32
13	N99219§	-	2.26
14	B98306	-	2.20
15	B03637	+	2.20
16	Othello§	-	2.18
17	B03633	+	2.18
18	B03642	+	2.18
19	VAX 5¶	+	2.18
20	99L91-47¶	+	2.17
21	B03647	-	2.13
22	B03643	+	2.08
23	B03641	-	2.08
24	N03618	-	2.07
25	N03616	-	2.06
26	B03634	+	2.05
27	B03620	-	2.04
28	N03614	+	2.03
29	B03627	-	2.03
30	B03632	+	2.02
31	99L91-45¶	+	2.00
32	N03611	+	1.98
33	B03622	+	1.96
34	B03639	+	1.93
35	B03628	+	1.92

Table 1.6, continued.

Rank†	Genotype‡	SU91	Yield (T/ha)
36	B03646	-	1.91
37	B03636	-	1.83
38	XAN 159¶	+	1.78
39	N03617	+	1.73
40	HR45¶	+	1.65
41	Midland§	-	1.62
42	Midland§	-	1.44
	Overall Mean		2.13
	LSD (0.05)		0.39
	CV		11.4

† = Indicates rank by yield (T/ha) of genotype among 42 entries.
‡ = Genotypes beginning with "B" and "N" denote black and navy bean breeding lines, respectively.
§ = Denotes susceptible (SU91-) check varieties or breeding lines.
¶ = Denotes resistant (SU91+) check varieties or breeding lines.

Yield Results from Preliminary Yield Trial (PYT) – Saginaw 2003

All lines evaluated in the East Lansing study were also evaluated in the test in Saginaw. Additional lines were included in the Saginaw experiment for the purpose of yield comparison. A summary of relevant genotypes evaluated in the Saginaw field study is presented in Table 1.3.

Among the 37 experimental F_6 lines tested, 15 had the SU91 marker (SU91+), while 22 lines did not have the marker (SU91-). Of the 15 SU91+ lines, 10 yielded above the average yield of the test, which was 2.92 T/ha. Additionally, 5 of these lines yielded higher than all of the checks included for yield comparison. The yields of the 15 SU91+ lines are summarized in Table 1.7 in comparison to the check genotypes (B98306, Condor, 115-M, Vista, B00136, Jaguar, N99219, Seahawk and T-39). The yield data is presented by rank among the 72 total entries for the test. The minimum and maximum yields of the study were 2.08 and 3.65 T/ha, respectively.

In contrast, only 11 of the 22 SU91- lines yielded higher than the mean yield of 2.92 T/ha. A summary of the yields for these lines is also presented in Table 1.7. The yield data for the check genotypes is included for comparison. Four of these SU91- lines (B03623, B03621, B03627, and B0361) yielded higher than the highest-yielding check (Condor, which yielded 3.16 T/ha).

Table 1.7 Yield (T/ha) of bean genotypes with or without the SCAR marker SU91 compared to check cultivars, Saginaw, MI (2003).

Genotype†	SU91‡	Yield (T/ha)
B03625	+	3.65
B03623	-	3.60
B03637	+	3.55
B03643	+	3.51
B03621	-	3.47
B03639	+	3.41
B03622	+	3.40
I03363	-	3.30
B98306	-	3.29
B03627	-	3.25
B03641	-	3.20
Condor	-	3.16
B03635	-	3.15
B03634	+	3.12
I03369	-	3.12
N03614	+	3.10
B03642	+	3.08
N03618	-	3.08
B03647	-	3.08
B03626	-	3.06
I03390	-	3.05
115-M§	-	3.05
B03636	-	3.03
B03631	+	3.01
Vista§	-	3.00
B03613	-	3.00
B03640	-	2.99
I02521	-	2.98
B00136	-	2.96
I03368	-	2.96
B03632	+	2.95
I03364	-	2.93
B03624	-	2.90
I03366	-	2.90
Jaguar§	-	2.90

Table 1.7, continued.

Genotype†	SU91‡	Yield (T/ha)
N99219§	-	2.89
B03633	+	2.88
B03620	-	2.86
B03630	+	2.83
Seahawk§	-	2.81
N03616	-	2.79
I03365	-	2.77
N03617	+	2.75
N03611	+	2.74
B03615	-	2.74
B03629	-	2.73
B03612	-	2.71
B03619	-	2.71
B03645	-	2.68
I03362	-	2.66
B03638	-	2.64
I01794	-	2.63
B03646	-	2.57
B03628	+	2.54
I03361	-	2.53
I03371	-	2.52
B03644	-	2.51
I03370	-	2.47
I03367	-	2.43
T-39§	-	2.43
I03373	-	2.42
I03372	-	2.08
Overall Mean		2.92
LSD (0.05)		0.37
CV %		11.4

† = Genotypes beginning with "B" and "N" denote black and navy bean breeding lines, respectively.
‡ = Denotes presence (+) or absence (-) of the SCAR marker SU91
§ = Denotes check cultivars or genotypes used in yield comparison.

DISCUSSION

In the current study, the association between the SU91 SCAR marker and CBB field resistance was examined to address the situation of population specificity of marker-trait associations. Additionally, because linkage drag has been reported for a number of CBB resistance sources, SU91 was examined to determine if it was significantly associated with negative agronomic traits (Valladares-Sanchez et al., 1979; Beebe et al., 1989).

The use of MAS as a tool in the selection of quantitative traits is ideal in cases where traditional phenotypic selection to improve the trait has proven inadequate. MAS can provide an alternative for selection of traits that exhibit low heritability, and are not responsive to visual selection (Dudley, 1993). Resistance to CBB in common bean is a quantitative trait that exhibits low to moderate heritability, and should be an excellent candidate for MAS (Yu et al., 2000). In order for MAS to be useful in a breeding program, however, several factors must be considered. Since QTL-marker associations in breeding programs may be population specific, the usefulness of a marker in selecting for the quantitative trait must be verified in other populations (Dudley, 1993; Yu et al., 2000). Based on field experiments at two locations in 2003, presence of the SCAR marker SU91 was clearly associated with significant differences in CBB foliar resistance in the populations under study, but the association was less clear in the pods. The marker was associated with high levels of leaf resistance in both experiments, and was also associated with pod resistance in one of the two experiments. The results from the Saginaw study were especially encouraging, because the difference in mean CBB leaf

ratings was approximately 2 units on the 1-9 scale used in CBB leaf evaluations, which was a significant difference.

It is interesting to note that the CBB-resistant black bean check VAX 5, which was the source of the resistance in these lines, received an average CBB leaf rating of 3.1. As no other resistant parents were used to derive these lines, this CBB leaf rating gives an approximation as to the lowest ratings that should be expected. Although values less than this were observed, they were not significantly different.

The lack of significant association between SU91 and pod disease resistance in the East Lansing study may be a result of the rating scale used. The rating scale of 0-3 may not successfully discriminate between severities of pod infections. To better differentiate between CBB resistant and susceptible bean pods, a 1-9 scale complementary to the scale used for leaf disease ratings was adopted for future field evaluations. Although the rating scale used in leaf disease ratings, as proposed by Schoonhoven and Pastor-Corrales (1987), allowed for simultaneous leaf and pod ratings, the scale was not used in this manner. Previous studies have determined that differences in tissue susceptibility exist between genotypes (Aggour et al., 1989; Valladares-Sanchez et al., 1979), and that a scale with only one value for leaves and pods is not satisfactory. This scale will continue to be used, however, for the evaluation of leaf disease reaction in both field and greenhouse experiments. Given past reports of differences in tissue susceptibility within bean cultivars (Aggour et al., 1989; Valladares-Sanchez et al., 1979) it is possible that the SU91 QTL does not confer adequate levels of resistance to CBB in the pods of the population used in this study. This suggests an alternative explanation for the lack of association between the marker and CBB pod resistance.

Relationships between important agronomic traits in common bean and SU91 were examined at both locations. Previous research has indicated a potential for linkage drag associated with CBB resistance. Late maturity and low yield are two important traits that have been problematic in past bean breeding efforts (Valladares-Sanchez et al., 1979). The possibility of linkage between CBB resistance and unfavorable agronomic traits must be examined prior to the development of a successful marker-assisted selection program.

The SU91 marker was weakly correlated with lower bean yields in the East Lansing study. No such correlation was evident for the Saginaw experiment. Despite the negative implications of the association in East Lansing, high yielding SU91+ lines with high levels of field CBB resistance were observed. The highest yielding line in East Lansing, which carried the SU91 marker, was also the highest yielding line in the test at Saginaw. The test in Saginaw was direct-harvested, while the East Lansing CBB screening study was knife-pulled prior to threshing. Based on this preliminary evidence, this black bean line (B03625) seems to be well-suited for either type of harvest method. Although a slight negative correlation between SU91 and yield was apparent in East Lansing, the identification of genotypes such as B03625 is promising for the development of high-yielding cultivars with enhanced levels of CBB resistance. The observation of yield drag associated with SU91+ in East Lansing may indicate a yield penalty caused by the presence of the QTL that can only be observed in cases of low disease pressure, as the disease pressure was lower at that location. The CBB resistance conferred by SU91 may have obscured this effect in environments where the disease pressure is high. Yield comparison of these lines in the complete absence of the disease

will be required to determine whether the presence of SU91 is associated with linkage drag in these genotypes. The correlation between SU91 and reduced yield in the location with lower disease pressure indicates that such an association between SU91 and linkage drag may exist.

Late maturity is another negative agronomic trait that has commonly been reported as being associated with CBB resistance in beans (Coyne and Schuster, 1974). This linkage has been broken in the past by other research projects (Coyne and Schuster, 1973), however it is a concern that must be addressed. SU91 was not associated with late maturity in either field location. This information is encouraging for the development of bean cultivars with suitable levels of CBB resistance, as it allows for the selection of early-maturing, high-yielding, CBB resistant bean lines.

The presence of SU91 was not correlated with the other agronomic traits evaluated in either location (days to flowering, lodging, height, and seed weight). The lack of linkage between CBB resistance and these important agronomic traits indicates that selection of CBB resistant genotypes with favorable agronomic characteristics is feasible in the genetic backgrounds described in the present study.

In conclusion, although the use of MAS will not completely eliminate the need for direct selection for CBB resistance, it can be applied in earlier generations to reduce the number of lines that require direct screening. Potential CBB resistant breeding materials could be screened for molecular markers, such as SU91, in the F_2 generation. Although MAS is more expensive than direct screening for many traits, a study by Yu et al. (2000) determined that MAS is more cost effective than conventional screening in selecting CBB resistant lines. The primary reason for this is the inefficiency of direct screening

using *Xap*, because of the effects of environment on the screening results. By eliminating genotypes that do not have SU91 from the screening process, direct screening programs can be optimized. Inclusion of exclusively SU91+ lines in field and greenhouse screening will increase the possibility of selecting highly resistant genotypes. The direct screening in the greenhouse and field could begin in later generations, for those genotypes which carry the marker, as determined by the indirect selection in the F_2 generation. Conducting the direct screening in later generations is ideal, as the seed supply at that point would allow for replicated testing, whereas seed supply in early generations may be insufficient for replicated studies. Screening of potentially resistant materials in several locations and multiple years in the field would be desirable. Although it may be difficult to screen breeding materials with multiple *Xap* isolates in the field, this portion of the CBB resistance verification could be performed in the greenhouse.

The results reported here are based on data from two field locations in 2003. It would be important to confirm these results across multiple years and locations, as suggested above. Because of the unreliability of visual phenotypic ratings for CBB resistance, these additional evaluations would validate the usefulness of screening for SU91 in a MAS program.

Advanced materials selected from this research are currently being utilized to introgress CBB resistance into other market classes of dry bean. Suitable levels of resistance are not currently present in commercial cultivars of the major bean market classes cultivated in the United States. Highly resistant black and navy bean lines

identified in this research may be useful parents in the introduction of resistance into other market classes, where resistance is lacing.

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CHAPTER TWO

THE USE OF MARKER-ASSISTED SELECTION TO PYRAMID RESISTANCE TO COMMON BACTERIAL BLIGHT AND ANTHRACNOSE IN COMMON BEAN (*Phaseolus vulgaris* L.)

ABSTRACT

The used of marker-assisted selection to develop dry bean lines with resistance to common bacterial blight (CBB) and anthracnose was examined. Sources of CBB and anthracnose resistance were combined in 4-way crosses with previously developed CBB resistant bean breeding lines. Selections that carried the SCAR marker SU91 (linked to a CBB resistance QTL on bean linkage group B8), which were identified in a previous study, were crossed to breeding lines with the anthracnose resistance gene *Co-4*^{2}. The resulting F_1 progeny were crossed with F_1 plants from the cross HR45/Kaboon to generate 4-way crosses. HR45 is a CBB resistant small white navy bean used as a donor parent of a QTL for CBB resistance (BC420) mapped to bean linkage group B6. Kaboon is an Andean anthracnose differential cultivar that carries the anthracnose resistance gene *Co-1*^{2} that mapped to bean linkage group B1.

The F_2 populations originating from the 4-way crosses were screened for SCAR markers SU91, BC420, and SAS13, linked to CBB resistance QTL (on bean linkage groups B8 and B6) and anthracnose resistance loci (on bean linkage group B8). Potential resistance conferred by the CBB resistance QTL linked to SU91 (on linkage group B8) and BC420 (on linkage group B6), was examined in both the field and greenhouse. The SAS13 marker was used to screen for the *Co-4*^{2} Middle American anthracnose resistance gene. Results from anthracnose screening are based only on presence of molecular

markers, as direct screening for anthracnose resistance was not included in this study because a differentiating race of the pathogen was unavailable.

Neither SU91 nor BC420 was associated with CBB resistance in the field experiment. This was unexpected, as the results obtained in Chapter One illustrated the potential for SU91 to identify CBB resistant bean genotypes. The level of disease incidence in this study may have been inadequate for a comparison of marker genotypes.

In two greenhouse experiments, however, both SU91 and BC420 were associated with increased CBB resistance. SU91 was associated with both leaf (r= -0.20**) and pod (r= -0.17*) resistance in experiment 1. The results were similar in experiment 2, as SU91 was associated with both leaf (r= -0.15**) and pod (r= -0.27***) resistance to CBB. The marker BC420 was only associated with pod resistance (r= -0.19*) in experiment 1, and leaf resistance (r= -0.18**) in experiment 2. Unexpectedly, the presence of both SCAR markers in a single genotype conferred levels of CBB resistance inferior to that of genotypes with either SU91 or BC420 alone.

INTRODUCTION

Common bacterial blight (CBB), caused by the bacterial pathogen *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*), is a destructive disease of common bean (*Phaseolus vulgaris* L.). Resistance to CBB is controlled as a quantitative trait exhibiting low to moderate heritability (Arnaud-Santana et al., 1994; Coyne and Schuster, 1974), although partial resistance exhibiting high ($h^2 = 0.87$) heritability has been observed in some genetic studies of CBB resistance in common bean (Silva, 1989).

Research conducted in various bean breeding programs, using diverse genetic backgrounds have differed greatly in estimating the number of genes or QTL controlling resistance to CBB. Resistance to CBB was controlled by only a single dominant gene in one genetic study, however, that resistance was obtained from tepary bean (*Phaseolus acutifolius*), not common bean (Drijfhout and Blok, 1987). McElroy (1985) determined that one major gene and several minor genes were responsible for the CBB resistance in XAN 159, a CBB resistant breeding line derived from a cross between *P. vulgaris* and *P. acutifolius*. Eskridge and Coyne (1996) found that as many as five genes may control CBB resistance in common bean. Additionally, molecular information has suggested the association of up to six distinct QTL with CBB resistance (Nodari et al., 1993; Jung et al., 1996). Between two and six QTL were associated with CBB resistance, depending on the isolate used for evaluating disease reaction and the bean tissue (leaves, pods, or seeds) inoculated (Jung et al., 1996). The polymorphic markers associated with resistance in analysis by Jung et al. (1996) each explained 14% to 34% of the total phenotypic variation, depending on the combination of isolate and bean tissue. Nodari et al. (1993) determined that at least four independent QTL were involved in CBB resistance.

Together, these four putative QTL explained 75% of the total phenotypic variation for CBB resistance in common bean.

One possible explanation for the different numbers of estimated genes or QTL controlling quantitative traits was proposed by Paterson (1995). Based on quantitative trait data in rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*), such traits are controlled by a small number of major-effect genes and a large number of minor-effect genes that make extremely small contributions to the overall phenotypic variance. This scenario provided an explanation of the results obtained in studies of CBB resistance in common bean. A small number of QTL responsible for a large proportion of the total phenotypic variance for CBB resistance, have been discovered. At least four major-effect QTL conditioning CBB resistance have been identified. These four QTL have been mapped to bean linkage groups B6, B7, B8, and B10, respectively (Nodari et al., 1993; Pedraza et al., 1997; Ariyarathne et al., 1999; Miklas et al., 2000). These QTL do not entirely account for the total phenotypic variance, and it is therefore possible that a large number of minor QTL are also involved. There is limited information, however about the individual genes involved in CBB resistance, or the mechanism by which they confer resistance.

Colletrichium lindemuthianum (the causal agent of bean anthracnose), is a destructive seed-borne pathogen of common bean localized to humid production areas in North America. Two highly virulent races (7 and 73) are present in Michigan (Kelly et al., 1994). The availability of resistance sources with dominant R-genes conditioning resistance to specific races of the pathogen have reduced the impact of anthracnose in North America. The *Co-4*^{2} anthracnose resistance gene, identified in the Middle

American cultivar G 2333, provides a broad spectrum of resistance to the majority of known *C. lindemuthianum* races (Young et al., 1998). The *Co-1*^{2} gene, an anthracnose resistance gene of Andean origin is another valuable source of genetic resistance (Balardin and Kelly, 1998). Introgression of both an Andean and a Middle American anthracnose resistance gene into single cultivars is the most effective means of developing broad-based protection to anthracnose (Young and Kelly, 1996; Melotto and Kelly, 2000).

The combination of anthracnose and CBB resistance in a single cultivar would be a valuable contribution to the development of improved bean cultivars. A small white navy bean HR45, with CBB resistance derived from XAN 159 was released in 1994 (Park and Dhanvantari, 1994). High levels of CBB pod resistance have been observed in this line. Although HR45 also exhibited leaf resistance, the level of leaf resistance was lower than that exhibited by XAN 159. HR45 was used to introgress an additional QTL for CBB resistance into lines selected from the research presented in Chapter One of this thesis. The Andean differential cultivar Kaboon was utilized as a donor of the *Co-1*^{2} anthracnose gene, an allele of the *Co-1* gene identified in Michigan Dark Red Kidney (Melotto and Kelly, 2000). Two small-seeded black bean lines developed at Michigan State University, Phantom*2/SEL 1308 or Jaguar*2/SEL 1308, were used to introduce the *Co-4*^{2} anthracnose resistance gene (Middle American). The donor of *Co-4*^{2} was SEL 1308, which is a bean breeding line with anthracnose resistance derived from the highly resistant cultivar G 2333 (Young et al., 1998).

Breeding strategies involving quantitative traits, such as CBB resistance, create challenges for breeders that may not be encountered when breeding qualitative traits.

With disease resistance, for example, the partial resistance conferred by a single QTL may not provide adequate resistance. The pyramiding of multiple resistance QTL in a single genotype offers a possible solution. Gene pyramiding for quantitative disease resistance may not provide total protection against a pathogen, however the level of resistance may be higher than that of a single QTL (Castro et al., 2003).

Gene (or QTL) pyramiding became much more feasible with the introduction of molecular marker technology. The phenomenon of epistasis, or interaction between multiple loci that control expression of a phenotype, made gene pyramiding for disease resistance difficult prior to the advent of linked markers. Traditional phenotypic evaluation may not adequately separate the effects of QTL or individual genes. QTL pyramiding of multiple loci associated with disease resistance allows not only the development of lines with increased resistance, but also the opportunity to study the effects of QTL in a novel genetic background. Interactions with the environment and other QTL can also be examined in populations with pyramided resistance (Castro et al., 2003).

Multiplex PCR provides an advantage in pyramiding multiple loci for resistance in a single cultivar, as it allows for the simultaneous screening of multiple molecular markers in a single reaction (Miklas et al., 2000). For this approach to be useful, however, the annealing temperature of the primers must be similar. The SU91 and BC420 primers used in selection for this research, have annealing temperatures of 58 and 50°C, respectively. Another SCAR linked to CBB resistance in common bean, SAP6, has an intermediate annealing temperature of 55°C (Miklas et al., 2000). Although this marker was monomorphic in our genetic population, and is therefore not useful for

selection, its PCR profile is compatible for amplification of SU91 and BC420 in multiplex PCR. Using this program in PCR for both SU91 and BC420 can reduce the cost and time involved in conducting marker-assisted selection (MAS). SAP6 is useful for selection of CBB resistance in some genetic backgrounds, such as the kidney bean cultivar 'Montcalm' (see Appendix A2).

The marker SU91 is linked to a QTL on bean linkage group B8 that confers partial resistance to CBB in both leaves and pods (Pedraza et al., 1997). This marker was derived from the random amplified polymorphic DNA (RAPD) marker $U9_{700}$. XAN 159 was the original source of the resistance conferred by the QTL linked to SU91. Another SCAR marker R7313, derived from the RAPD marker BC73 (also known as R7313), is believed to be linked to the same QTL (Bai et al., 1997; Beattie et al., 1998; Miklas et al., 2000). In this case, the resistance source was the tepary bean selection PI 440795, however, the resistance among tepary beans may be similar (Bai et al., 1997).

The SCAR marker BC420, which is linked to a QTL for CBB resistance on bean linkage group B6 (Miklas et al., 2000), was derived from the fragment amplified by the RAPD marker $BC420_{900}$ (Yu et al., 2000). In a recombinant inbred (RI) population originating from the cross HR67/W1744d, the BC420 SCAR marker was responsible for 62% of the phenotypic variation ($R^2 = 0.62$). Additionally, greenhouse results determined that BC420 is 94.2% accurate in the identification of resistant genotypes (Yu et al., 2000). Although the usefulness of this marker across different genetic populations has not been previously examined, the original RAPD marker, $BC420_{900}$, has been studied. In three different genetic populations, this marker explained 8% to 30% of the total phenotypic variation for CBB leaf resistance (Park et al., 1999). This range in

variation accounts for differences between the genetic populations used, the *Xap* isolates used in screening, and both greenhouse and field studies. $BC420_{900}$ was not associated with pod resistance to CBB in any of the populations studied by Park et al. (1999).

The dominant SCAR marker SAS13, developed by Young et al. (1998) is linked to the *Co-4*^{2} allele. This marker was derived from the $OAS13_{950}$ RAPD fragment, both of which are very tightly (0.39 cM) linked to *Co-4*^{2} (Young et al., 1998). This resistance allele was identified in the breeding line SEL 1308, and confers resistance to 33 of 34 races of *C. lindemuthianum* tested (Balardin and Kelly, 1998).

The primary objective of this research was to develop pyramided resistance to common bacterial blight and anthracnose, based on the use of indirect selection of various combinations of molecular markers. Selections were made for SCAR markers linked to two QTL for CBB resistance a single anthracnose resistance gene. A second objective was to evaluate the effects of the SCAR markers SU91 and BC420 on resistance in novel genetic backgrounds, using field and greenhouse inoculations with local isolates of *X. axonopodis* pv. *phaseoli*. The combination of the SU91 and BC420 markers in the same genotype was examined to determine its effectiveness in enhancing CBB resistance compared to single QTL resistance sources.

MATERIALS AND METHODS

Parental Material and Population Development. A series of crosses were conducted, with the purpose of combining in single genotypes, higher levels of resistance to anthracnose and CBB. One set of crosses resulted in 11 F_1 progeny derived from a cross between HR45 and Kaboon. A second set of crosses involved 15 F_5 black and navy bean lines with SU91 crossed with either Phantom*2/SEL 1308 or Jaguar*2/SEL 1308 (donors of the *Co-4*^{2} anthracnose resistance gene). The resulting single cross F_1 progeny from these two sets of crosses were inter-mated to develop 40 four-way F_1 populations, which were self-pollinated to produce the F_2 populations. Approximately one-half the seed from each F_2 population was planted (due to greenhouse space limitations), resulting in a total of 432 F_2 plants. Some F_2 individuals were lost in the greenhouse due to late maturity, genetic incompatibility, and failure to produce seed. A total of 320 $F_{2:3}$ lines were grown as single-row plots in the field (East Lansing) during the summer of 2003. The one-row plots were space-planted and fertilized with 113 kg of 19-19-19, which was banded at planting. The F_3 lines were evaluated for agronomic characteristics (days to flowering, lodging, re-greening and agronomic desirability). Days to flowering was recorded as the number of days after planting that at least 50% of the plants in a plot had a minimum of one open flower. Lodging was evaluated on a one to five scale: 1) no lodging, 3) intermediate lodging, and 5) excessive lodging. Re-greening was recorded as the presence (1) or absence (0) of re-greening at maturity. Desirability (DS) was determined as a visual rating of overall agronomic desirability. This rating used a 1 to 9 scale with the following guidelines: 1-4 = above average agronomic traits, 5 = average (compared to commercial checks), 6-9 = unacceptable agronomic traits. The F_3 lines

were also inoculated and evaluated for CBB resistance. Inoculations were conducted as described in Chapter One, on July 17, July 24, and August 6, 2003. The *Xap* isolate that was used for inoculations was '9712-3', an isolate from Nebraska that had originally been isolated from infected bean seed grown in Michigan. Two CBB ratings (15 and 22 days after the final inoculation) were recorded for the field experiment. Single plant selections were made within the most desirable 93 of these 320 F_3 lines based on agronomic traits, marker data, and disease reaction.

Greenhouse Inoculations and Disease Evaluation. Greenhouse experiments were conducted at Michigan State University. Plants were grown in 15.2 cm clay pots, with sterile potting soil used as growth medium. Slow-release fertilizer was added to each pot at the primary leaf stage. Plants were watered as needed. Inoculation experiments consisted of four replications, each composed of 93 F_3 genotypes selected from the 320 $F_{2:3}$ lines in the field in addition to four control genotypes of known phenotypic reaction to CBB inoculation. The four control genotypes were present twice in each replication (as inoculated and non-inoculated controls). This resulted in a total of 101 entries per replication.

Two greenhouse inoculation experiments were conducted. Trifoliate leaves were inoculated twice. The first inoculation was when all plants had at least one flower, and the second was one week later. Flowering was delayed in these experiments due to slow emergence. The first inoculation was at 65 and 66 days after planting (DAP) in the first and second experiment, respectively, which was when the majority of plants had begun flowering. Leaves were evaluated for visual CBB symptoms when they became

apparent. The leaf ratings for the first experiment were recorded at 7 days after the final inoculation and at 14 days after the final inoculation for the second experiment. Two pods were inoculated per plant with *Xap*, during the flat pod stage of pod development. Pods were only inoculated once, and were evaluated 14 days after inoculation in the first study. Ratings were delayed in the first study, due to slow disease development. Pods were evaluated 9 days after the pod inoculation in the second experiment.

Polymerase Chain Reaction (PCR) and Molecular Marker Screening.

DNA extraction, PCR, and agarose gel electrophoresis were conducted as previously described in Chapter One of this thesis. When multiplex PCR was used for simultaneous screening of the SCAR markers SU91 and BC420, the thermal cycler profile for a marker with an intermediate (55°C) annealing temperature (SAP6) was used. This profile is described in Chapter One (Table 1.1).

Screening for the SCAR marker SAS13, linked to the *Co-4*^{2} anthracnose resistance gene, was conducted as previously described for SCAR markers. The PCR profile used was as described by Young et al. (1998), and consisted of 34 cycles of: 10 seconds at 94°C, and 2 minutes and 40 seconds at 72°C. The reactions were completed by a final step of 5 minutes at 72°C. The single resulting band, an amplified fragment of approximately 950 base pairs (bp), was visualized as previously described using fluorescence.

RESULTS

A total of 93 lines were selected from the 320 lines examined as F_3 lines in a field study at East Lansing (2003). These lines were selected on the presence of molecular markers (SU91 and BC420), overall agronomic suitability, and low phenotypic ratings for CBB in the field. A summary of the 93 lines selected is presented in Table 2.1. These lines were screened for SAS13 marker, which is linked to the *Co-4*^{2} anthracnose resistance gene. All 93 resulting F_4 lines were also evaluated for CBB resistance in the greenhouse by direct inoculations.

Field Results – East Lansing (2003)

The 320 F_3 lines in the field study consisted of 4 genotypic classes, based on the presence or absence of molecular markers: SU91+/BC420+, SU91+/-, -/BC420+, and -/-. The effects of both markers (SU91 and BC420) were examined alone and in combination for relationships with various phenotypic traits. The traits evaluated in this study included: days to flowering, CBB leaf rating 1 (14 days after inoculation), CBB leaf rating 2 (21 days after inoculation), CBB pod rating 1 (14 days after inoculation), CBB pod rating 2 (21 days after inoculation), re-greening, lodging and overall agronomic desirability score (DS).

Neither SU91 nor BC420 were significantly associated with CBB leaf or pod resistance in the field study. The results of this study are summarized in Table 2.2. These findings disagree with previous data from Chapter One, where presence of the SU91 marker was associated with increased CBB resistance.

A relationship was noted, however between both markers and days to flowering. Presence of the SU91 and BC420 markers were both negatively correlated with days to flowering ($r = -0.13^{*}$ and -0.18^{**} for SU91 and BC420, respectively). In combination, SU91 and BC420 were also negatively correlated with days to flowering ($r = -0.22^{**}$). Data for days to flowering was presented in Appendix A4.

BC420 was associated ($r = 0.15^{*}$) with re-greening of plants late in the growing season. This trait complicates harvest and reduces the overall quality of potential bean cultivars. SU91 was not, however, associated with re-greening in this study. Re-greening data from this study was reported in Appendix A4. Because of extensive re-greening in this experiment, actual maturity dates were not recorded, and therefore could not be included in the analysis.

Table 2.1 Marker data, phenotypic CBB ratings on leaves and pods, and agronomic desirability of 93 F_3 selections from a field study established in East Lansing, 2003.

Genotype †	SU91‡	BC420‡	Leaf1§	Leaf2§	Pod1§	Pod2§	DS¶
03T-9037	-	-	3	3	1	1	4
03T-9056	-	-	3	3	1	1	4
03T-9098	-	-	3	3	1	1	4
03T-9165	-	-	4	3	1	1	4
03T-9291	-	-	3	2	1	1	4
03T-9306	-	-	1	2	1	1	4
03T-9195	-	+	3	3	1	1	5
03T-9199	-	+	3	3	1	1	5
03T-9204	-	+	3	3	1	1	5
03T-9019	+	-	4	3	1	1	6
03T-9026	+	-	3	3	1	1	5
03T-9048	+	-	3	3	1	1	5
03T-9049	+	-	3	3	1	2	4
03T-9058	+	-	3	3	1	1	5
03T-9061	+	-	3	4	1	1	4
03T-9062	+	-	2	3	1	1	5
03T-9067	+	-	2	3	1	1	5
03T-9076	+	-	4	3	1	1	5
03T-9079	+	-	3	3	1	1	5
03T-9082	+	-	3	3	1	1	5
03T-9092	+	-	2	3	1	1	5
03T-9093	+	-	3	3	1	1	5
03T-9099	+	-	3	3	1	1	5
03T-9104	+	-	3	3	1	1	5
03T-9111	+	-	2	2	1	1	5
03T-9113	+	-	3	3	1	1	5
03T-9115	+	-	3	3	1	1	4
03T-9119	+	-	3	4	1	1	4
03T-9124	+	-	3	3	1	1	5
03T-9126	+	-	3	3	1	1	5
03T-9132	+	-	3	4	1	1	5
03T-9133	+	-	4	4	1	1	5
03T-9137	+	-	3	3	1	1	5
03T-9139	+	-	3	3	1	1	5
03T-9142	+	-	4	3	1	1	5
03T-9146	+	-	5	5	1	1	5
03T-9148	+	-	4	4	1	1	4
03T-9153	+	-	3	3	1	1	5
03T-9154	+	-	3	3	1	1	5
03T-9156	+	-	3	3	1	1	5
03T-9163	+	-	4	4	1	1	5
03T-9166	+	-	3	3	1	1	5
03T-9169	+	-	3	3	1	1	5
03T-9172	+	-	2	3	1	1	5

Table 2.1 Continued.

Genotype †	SU91‡	BC420‡	Leaf1§	Leaf2§	Pod1§	Pod2§	DS¶
03T-9178	+	-	2	3	1	1	4
03T-9180	+	-	3	3	1	1	5
03T-9184	+	-	3	3	1	1	5
03T-9185	+	-	3	3	1	1	5
03T-9186	+	-	3	3	1	1	5
03T-9217	+	-	3	3	1	1	5
03T-9241	+	-	4	2	1	1	5
03T-9245	+	-	3	3	1	1	5
03T-9247	+	-	3	3	1	1	5
03T-9250	+	-	3	3	1	1	4
03T-9259	+	-	3	3	1	1	5
03T-9263	+	-	3	4	1	2	5
03T-9267	+	-	3	3	1	1	5
03T-9268	+	-	3	3	1	1	5
03T-9277	+	-	3	3	1	1	5
03T-9281	+	-	3	3	1	1	5
03T-9288	+	-	1	2	0	1	4
03T-9299	+	-	2	2	1	1	5
03T-9319	+	-	1	2	1	1	5
03T-9007	+	+	3	4	1	1	5
03T-9013	+	+	4	4	1	1	6
03T-9029	+	+	3	4	1	1	5
03T-9036	+	+	4	4	1	1	6
03T-9046	+	+	4	3	2	1	6
03T-9050	+	+	2	3	1	1	5
03T-9051	+	+	4	3	1	1	5
03T-9055	+	+	3	3	1	1	5
03T-9059	+	+	2	3	1	1	6
03T-9060	+	+	2	3	1	1	6
03T-9090	+	+	3	3	1	1	5
03T-9112	+	+	3	3	1	1	5
03T-9116	+	+	3	4	1	1	5
03T-9128	+	+	4	4	1	1	5
03T-9136	+	+	3	3	1	1	5
03T-9141	+	+	4	3	1	1	5
03T-9187	+	+	3	3	1	1	6
03T-9188	+	+	3	3	1	1	5
03T-9189	+	+	3	3	1	1	5
03T-9190	+	+	3	3	1	1	5
03T-9191	+	+	3	3	1	1	6
03T-9201	+	+	3	3	1	1	5
03T-9202	+	+	3	3	1	1	5
03T-9207	+	+	3	3	1	1	5
03T-9222	+	+	3	3	1	1	5

Table 2.1 Continued.

Genotype †	SU91‡	BC420‡	Leaf1§	Leaf2§	Pod1§	Pod2§	DS¶
03T-9269	+	+	3	3	1	1	6
03T-9270	+	+	4	4	1	1	6
03T-9271	+	+	3	4	1	1	5
03T-9305	+	+	2	2	1	1	5
03T-9309	+	+	2	2	1	1	4

† = Indicates the F_3 genotype screened for CBB resistance using direct inoculations and molecular markers.
‡ = SU91 and BC420 are SCAR markers linked to CBB resistance. "+" and "-" indicate presence or absence of the marker.
§ = Bean leaves and pods were rated twice for visual CBB symptoms. "Leaf1" and "Leaf2" refer to CBB leaf ratings, recorded 15 and 22 days after inoculation, respectively. Leaf ratings were recorded on a scale of 1-9 (1= no symptoms, 9= severe symptoms. "Pod1" and "Pod2" refer to CBB pod ratings, recorded 15 and 22 days after inoculation, respectively. Pod ratings were recorded on a 0-3 scale (0= no symptoms, 3= severe symptoms).
¶ = DS represents overall agronomic desirability (1= superior, 5= average, 9= poor).

Table 2.2. Relationship of the SCAR markers SU91 and BC420 with leaf and pod resistance in field inoculations of 320 $F_{2:3}$ lines (East Lansing, 2003).

	Field – Rating 1§		Field – Rating 2§	
Marker†	CBB-Leaf‡	CBB-Pod‡	CBB-Leaf‡	CBB-Pod‡
SU91 +	3.13 NS	1.02 NS	3.19 NS	1.01 NS
SU91 -	3.21 NS	1.03 NS	3.23 NS	1.04 NS
BC420 +	3.13 NS	1.02 NS	3.26 NS	1.04 NS
BC420 -	3.18 NS	1.02 NS	3.20 NS	1.02 NS
SU91+/BC420+	3.17 NS	1.03 NS	3.23 NS	1.00 NS
SU91-/BC420-	3.22 NS	1.03 NS	3.24 NS	1.03 NS

NS = Not significant

† = "SU91+" and "SU91-" designate means for genotypes carrying or lacking the SU91 marker, linked to a CBB resistance QTL mapped to bean linkage group B8.
"BC420+" and "BC420-" designate means for genotypes carrying or lacking the BC420 marker, linked to a CBB resistance QTL mapped to bean linkage group B6.

‡ = CBB-Leaf ratings were recorded on a 1-9 scale (1= no symptoms, 9 = severe symptoms).
CBB-Pod ratings were recorded on a 0-3 scale (0= no symptoms, 3= severe symptoms).

§ = Rating 1 and Rating 2 refer to visual CBB ratings taken 15 and 22 days after inoculation, respectively.

Greenhouse Results – 2003

Greenhouse inoculations, which consisted of four replications of 101 (93 F_3 selections and 8 controls) entries, were conducted as two separate experiments one week apart. A summary of each entry included in the experiment is listed in Table 2.3. The information included for each entry consists of: presence or absence of BC420 and SU91, mean leaf rating (1st experiment), mean pod rating (1st experiment), mean leaf rating (2nd experiment), and mean pod rating (2nd experiment). VAX 5, HR45, 'Othello' and 'Midland' are included as controls, because they have well characterized responses to *Xap* infection. It is not known whether the resistant checks (VAX 5 and HR45) carry the *Co-4*^{2} gene for anthracnose resistance. The checks are included once as non-inoculated controls, and once as inoculated controls for disease reaction.

SU91 was significantly associated with both leaf and pod resistance in greenhouse experiments, although the correlation was not as strong as was observed in Chapter One. The effects of this marker were evaluated both alone and in combination with the SCAR marker BC420. The presence of SU91 alone was correlated with leaf resistance in experiment 1 (r = -0.20*) and experiment 2 (r = -0.15**). Additionally, SU91 was correlated with CBB pod resistance in experiment 1 (r = -0.17*) and experiment 2 (r = -0.27***). In the greenhouse, BC420 was associated with only pod resistance in experiment 1 (r = -0.19*) and CBB leaf resistance in experiment 2 (r = -0.18**). A summary of the statistical analysis of SU91 and BC420 for both greenhouse experiments is presented in Table 2.4.

Despite the associations between CBB resistance (leaf and pod) and the SCAR markers (SU91 and BC420), genotypes with both markers (SU91+/BC420+) did not

perform as expected. CBB leaf ratings for both experiments, and pod ratings for experiment 2 were actually higher than ratings from genotypes with either marker alone (SU91+/- or -/BC420+). A graphical interpretation of these results is shown in Figure 2.1.

Table 2.3. Molecular marker data and greenhouse inoculation results (2003-04). Data were presented by genotype. All bean genotypes except controls are $F_{3:4}$ lines (93 entries) and were selected in the field (2003). CBB leaf and pod ratings were averaged across 4 replications.

Genotype	SU91†	BC420†	SAS13†	Leaf1‡	Pod1‡	Leaf2‡	Pod2‡
03T-9056	-	-	-	3.0	4.0	4.5	2.3
03T-9098	-	-	-	2.3	1.0	2.3	2.7
03T-9291	-	-	-	2.5	1.0	2.0	2.0
03T-9306	-	-	-	4.3	3.0	3.0	1.7
03T-9026	.	.	-	6.8	2.5	4.8	2.3
Midland§	-	-	N/A*	1.0	1.0	1.0	1.0
Midland¶	-	-	N/A*	4.8	7.0	7.3	7.3
Othello§	-	-	N/A*	1.0	1.0	1.0	1.0
Othello¶	-	-	N/A*	7.3	3.0	5.8	3.8
03T-9014	-	-	+	3.0	2.5	3.5	2.3
03T-9037	-	-	+	4.5	2.5	4.8	2.7
03T-9165	-	-	+	6.0	6.0	5.0	3.0
03T-9195	-	+	-	2.8	1.0	2.5	1.0
03T-9204	-	+	-	4.3	6.0	2.8	1.3
03T-9199	-	+	+	1.0	1.0	2.0	2.0
03T-9058	+	-	-	3.3	4.0	5.3	2.7
03T-9067	+	-	-	1.3	1.0	1.3	1.3
03T-9079	+	-	-	2.0	2.0	2.5	4.5
03T-9093	+	-	-	5.8	4.0	3.8	2.7
03T-9099	+	-	-	3.0	1.0	2.5	3.0
03T-9113	+	-	-	2.0	2.5	2.8	2.3
03T-9119	+	-	-	1.5	2.0	2.3	2.3
03T-9132	+	-	-	2.0	1.5	1.3	2.3
03T-9146	+	-	-	4.5	6.5	5.0	3.5
03T-9148	+	-	-	5.8	5.0	5.8	3.0
03T-9153	+	-	-	2.5	3.0	3.0	3.0
03T-9166	+	-	-	2.5	1.0	2.0	1.3
03T-9169	+	-	-	1.7	1.0	2.8	2.0
03T-9172	+	-	-	1.5	2.5	2.8	2.0
03T-9184	+	-	-	4.0	5.0	4.3	2.0
03T-9241	+	-	-	3.0	3.0	2.8	2.3
03T-9247	+	-	-	1.5	4.5	2.3	4.0
03T-9250	+	-	-	2.0	4.0	1.8	1.5
03T-9263	+	-	-	1.5	1.0	2.0	2.0
03T-9267	+	-	-	2.3	1.0	3.0	2.0
03T-9277	+	-	-	1.0	2.0	1.5	1.5
03T-9281	+	-	-	1.5	5.0	2.0	1.7
03T-9288	+	-	-	3.7	1.0	4.0	3.3

Table 2.3 Continued.

Genotype	SU91†	BC420†	SAS13†	Leaf1‡	Pod1‡	Leaf2‡	Pod2‡
03T-9299	+	-	-	1.3	1.0	1.3	2.0
03T-9319	+	-	-	3.3	7.0	2.7	2.0
03T-9019	+	-	+	1.8	5.0	2.5	2.3
03T-9048	+	-	+	1.8	2.5	2.0	1.0
03T-9049	+	-	+	1.3	1.0	3.0	1.5
03T-9061	+	-	+	1.3	1.5	2.5	2.0
03T-9062	+	-	+	1.0	2.5	2.7	2.0
03T-9076	+	-	+	3.3	1.5	2.8	1.5
03T-9082	+	-	+	2.0	2.0	3.0	1.0
03T-9092	+	-	+	1.3	.	3.3	3.0
03T-9111	+	-	+	1.7	1.0	4.0	1.0
03T-9115	+	-	+	4.3	1.5	5.0	1.3
03T-9124	+	-	+	3.3	2.0	2.0	1.0
03T-9126	+	-	+	3.0	2.0	3.0	1.0
03T-9133	+	-	+	6.8	.	5.0	1.5
03T-9137	+	-	+	2.3	2.0	2.3	1.0
03T-9139	+	-	+	1.8	3.0	1.8	1.7
03T-9142	+	-	+	3.5	2.5	3.0	2.0
03T-9154	+	-	+	4.5	3.5	6.5	2.7
03T-9156	+	-	+	1.3	6.0	1.8	1.3
03T-9163	+	-	+	3.3	9.0	2.0	2.0
03T-9178	+	-	+	2.3	1.0	2.0	2.3
03T-9180	+	-	+	2.0	3.5	2.3	1.5
03T-9185	+	-	+	1.5	2.0	2.5	2.3
03T-9217	+	-	+	2.3	3.0	3.0	2.5
03T-9245	+	-	+	1.5	2.0	2.3	1.7
03T-9259	+	-	+	2.0	3.0	2.8	1.3
03T-9268	+	-	+	2.0	2.0	2.3	1.0
VAX 5¶	+	-	N/A*	1.0	1.5	2.0	1.5
VAX 5§	+	-	N/A*	1.0	1.0	1.0	1.0
03T-9007	+	+	-	2.7	1.5	4.8	3.0
03T-9036	+	+	-	2.8	3.0	4.3	1.0
03T-9050	+	+	-	1.8	1.0	4.8	2.3
03T-9090	+	+	-	3.0	1.0	2.5	1.7
03T-9112	+	+	-	1.3	3.0	2.8	2.3
03T-9116	+	+	-	2.5	1.0	2.8	1.7
03T-9128	+	+	-	2.8	2.0	3.3	1.5
03T-9136	+	+	-	3.0	4.5	3.7	3.3
03T-9141	+	+	-	1.3	1.0	2.3	1.0
03T-9187	+	+	-	2.5	4.5	6.0	2.7
03T-9188	+	+	-	4.3	1.0	6.7	1.7
03T-9202	+	+	-	4.0	4.5	5.5	2.0
03T-9222	+	+	-	2.3	4.0	4.0	2.0
03T-9271	+	+	-	3.7	1.5	4.5	4.3
03T-9305	+	+	-	2.8	1.0	3.0	1.5

Table 2.3 Continued.

Genotype	SU91†	BC420†	SAS13†	Leaf1‡	Pod1‡	Leaf2‡	Pod2‡
03T-9309	+	+	-	1.5	1.0	2.0	1.0
03T-9013	+	+	+	2.3	1.0	4.0	2.0
03T-9029	+	+	+	4.0	1.0	3.8	1.5
03T-9046	+	+	+	3.3	3.5	4.0	1.5
03T-9051	+	+	+	4.3	2.0	3.5	1.0
03T-9055	+	+	+	3.5	1.0	2.8	1.5
03T-9059	+	+	+	4.0	3.0	5.0	3.0
03T-9060	+	+	+	1.3	1.5	4.0	1.7
03T-9186	+	+	+	5.7	4.0	5.5	3.0
03T-9189	+	+	+	1.5	2.0	2.5	1.0
03T-9190	+	+	+	5.0	3.0	5.3	3.0
03T-9191	+	+	+	1.7	3.0	6.0	2.7
03T-9201	+	+	+	6.3	1.0	3.3	1.7
03T-9207	+	+	+	3.5	1.0	3.0	2.0
03T-9269	+	+	+	2.0	2.0	2.7	1.3
03T-9270	+	+	+	4.0	4.0	4.8	3.7
HR45§	+	+	N/A*	1.0	1.0	1.0	1.0
HR45¶	+	+	N/A*	1.3	2.0	2.5	1.5

* = N/A indicates "not available."

† = Presence or absence of SCAR markers is reported as "+" and "-", respectively.
SU91 = SCAR marker linked to CBB resistance QTL on bean linkage group B8.
BC420 = SCAR marker linked to CBB resistance QTL on bean linkage group B6.
SAS13 = SCAR marker linked to *Co-4*^{2} anthracnose resistance gene on bean linkage group B8.

‡ = Leaf1 and Leaf2 indicate visual CBB leaf ratings recorded 7 and 14 days after inoculation, respectively. Leaves were rated for CBB symptoms on a 1-9 scale (1= no symptoms, 9= severe symptoms).
Pod1 and Pod2 indicate visual CBB pod ratings recorded 7 and 14 days after inoculation, respectively. Pods were rated for CBB symptoms on a 0-3 scale (0= no symptoms, 3=severe symptoms).

§ = Designates resistant (VAX 5 and HR45) and susceptible (Othello and Midland) controls inoculated with water.

¶ = Designates resistant (VAX 5 and HR45) and susceptible (Othello and Midland) controls inoculated with *Xap*.

Table 2.4. Correlation coefficients of the SCAR markers SU91 and BC420 with leaf and pod resistance in two greenhouse inoculation experiments with 101 entries.

	Greenhouse – Experiment 1		Greenhouse – Experiment 2	
Marker†	CBB-Leaf‡	CBB-Pod‡	CBB-Leaf‡	CBB-Pod‡
SU91	-0.1979**	-0.1724*	-0.1477**	-0.2713***
BC420	0.0600 NS	-0.1924*	-0.1833**	-0.0592 NS

NS, *, **, *** = non-significant, significant at the 5%, 1%, and 0.1% probability levels, respectively.
† = SU91 is a SCAR marker linked to a CBB resistance QTL on bean linkage group B8.
BC420 is a SCAR marker linked to a CBB resistance QTL on bean linkage group B6.
‡ = CBB-Leaf and CBB-Pod refer to visual CBB ratings based on a 1-9 scale for leaves (1= no symptoms, 9= severe symptoms) and a 0-3 scale for pods (0= no symptoms, 3= severe symptoms). Correlation coefficients (r) are presented between the SCAR markers and the visual disease ratings.

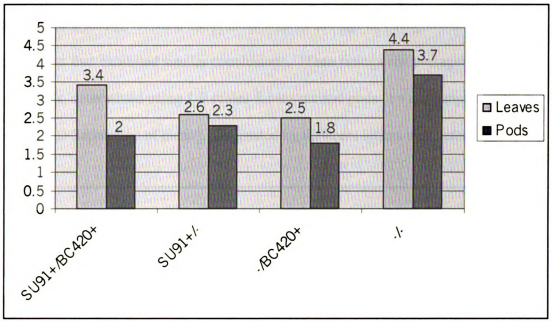

Figure 2.1. A comparison of the mean CBB leaf and pod ratings for various molecular marker combinations. Results are presented as an average of two greenhouse experiments that exhibited similar conclusions. Mean ratings are averages of 4 replications, determined using 1-9 scales for leaves and pods (1= no symptoms, 9= severe symptoms).

Table 2.5. A comparison of the mean CBB leaf and pod ratings for various molecular marker combinations. Results are presented as an average of two greenhouse experiments that exhibited similar conclusions. Mean ratings are averages of 4 replications, determined using 1-9 scales for leaves and pods (1= no symptoms, 9= severe symptoms). Separation of means, designated by different letters was determined using $LSD_{0.05}$.

Marker Combination	Leaves	Pods
-/-	4.4a	3.7a
SU91+/-	2.6b	2.3b
-/BC420+	2.5b	1.8b
SU91+/BC420+	3.4c	2.0b

Screening for the SCAR marker SAS13. The 320 F_3 lines evaluated in the field were also screened for the SCAR marker SAS13, linked to the anthracnose resistance gene *Co-4*[2]. The results of screening the 93 lines selected based on CBB resistance for SAS13 (described above) are presented in Table 2.3.

Of the 93 lines selected for CBB resistance, 45 (48%) carried the SAS13 marker, while the remaining 48 did not. The 45 lines that carried SAS13 were grouped into the following categories of molecular marker combinations (illustrated in Figure 2.2): SU91+/-/SAS13+ (26 lines), -/BC420+/SAS13+ (1 line), -/-/SAS13+ (3 lines), SU91+/BC420+/SAS13+ (15 lines).

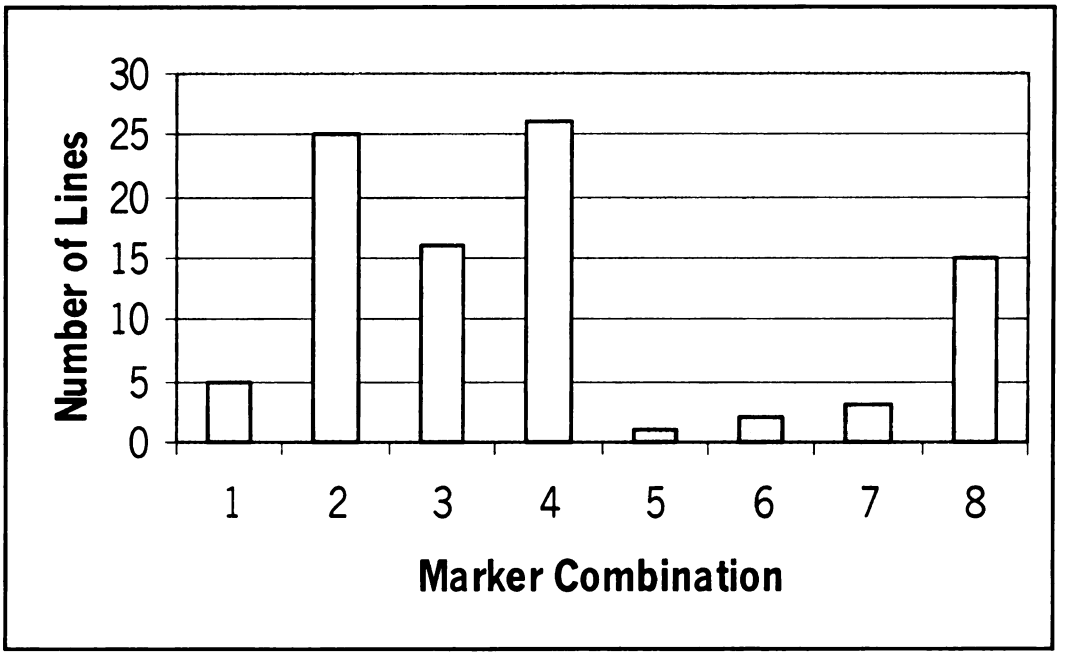

Figure 2.2. A summary of the molecular marker composition of the 93 F_3 lines selected from the field (East Lansing, 2003). SU91 is a SCAR marker linked to a CBB resistance QTL on bean linkage group B8. BC420 is a SCAR marker linked to a CBB resistance QTL on bean linkage group B6. SAS13 is linked to the Middle American anthracnose resistance gene *Co-4*^{2}. Marker combinations (1-8) correspond to the following: 1= -/-/-, 2= SU91+/-/-, 3= SU91+/BC420+/-, 4= SU91+/-/SAS13+, 5= -/BC420+/SAS13+, 6= -/BC420+/-, 7= -/-/SAS13+, 8= SU91+/BC 420+/SAS13+.

DISCUSSION

The potential of combining multiple sources of resistance to CBB and anthracnose was examined using a system of indirect selection in common bean. The combination of resistance to both diseases in a common genetic background would facilitate the development of commercial bean cultivars with an improved spectrum of disease resistance. Since resistance to CBB in common bean is controlled quantitatively, the level of resistance conferred by a single QTL is partial (Nodari et al., 1993; Eskridge and Coyne, 1996; Jung et al., 1996). Because of the availability of more molecular markers linked to quantitative traits, the pyramiding of multiple QTL is now possible. Pyramiding using direct screening was not previously feasible, due to epistatic interactions that prohibited the ability to distinguish the effects of distinct genetic loci (Ribaut and Hoisington, 1998). Development of breeding lines with pyramided QTL from various resistance sources could help increase the levels of partial resistance beyond that conferred by a single QTL (Castro et al., 2003).

A group of F_3 lines with differing combinations of the CBB resistance QTL SU91 and BC420 were evaluated for resistance to CBB in the field. Neither marker was associated with increased levels of CBB resistance in the field, which was unexpected, based on the results of previous studies (Chapter One). The results obtained in this field experiment were possibly due to inadequate establishment of disease (infection levels were low even in susceptible checks). Very little variation was observed in CBB ratings (most notably in pods), which may be a reason why it was difficult to discriminate between resistance and susceptibility. This field study was space planted to facilitate selection, which results in a lower planting density. The greater distance between plants

may have played a role in the inability to establish CBB pressure in this experiment, as the movement of bacteria from plant to plant may have been reduced in comparison to higher planting densities. The mean CBB leaf rating in this field experiment was only 3.05, on a scale of 1-9, and the maximum CBB leaf rating was 7.0. It is possible that the level of infection was not sufficient to effectively discriminate between susceptible and resistant plants.

The potential for linkage between CBB resistance and agronomic traits was also studied in the field experiment. Negative correlations between both markers for CBB resistance (SU91 and BC420) and flowering were observed in this experiment, which agreed with previous reports of delayed maturity linked to CBB resistance (Tar'an et al., 2001). BC420 was also associated with delayed maturity in this study. The effect of SU91 and BC420 on yield was not determined, as yield data was not available for this experiment, because of insufficient seed for replications. Based on previous results (Chapter One), it seems likely that early-maturing, high-yielding, CBB resistant lines can be recovered from this selection program, despite the negative associations with resistance loci.

The results of the two greenhouse direct inoculation experiments contrasted the field study. SU91 was correlated with enhanced levels of CBB resistance in leaves and pods in both greenhouse experiments. The presence of BC420 was also correlated with CBB resistance in leaves (experiment two) and pods (experiment one). Previous experiments have revealed that BC420 confers differing levels of resistance in pods and leaves (with pod resistance being higher). This may account for the differing results of lines carrying BC420 in greenhouse experiments. When the markers were combined

within a single genotype, however, the resistance levels were intermediate to the resistance conferred by either marker alone and that of genotypes with no known resistance loci. The difficulty in establishing a consistent method of evaluating common bean for resistance to CBB using direct inoculations has been documented (Singh and Munoz, 1999). The unreliability of direct inoculations could be one reason why the results obtained in this study were so unexpected. In the 1st greenhouse experiment, the susceptible check 'Midland' was rated a mean CBB leaf rating of only 4.75 (using the previously described 1-9 scale), although the other susceptible check 'Othello' was rated a mean CBB leaf rating of 7.3. Additionally, the mean CBB leaf rating of greenhouse experiment 1 was only 2.8. In the 2nd greenhouse experiment, which was conducted under similar conditions, 'Midland' had a mean CBB leaf rating of 7.25, while 'Othello' received a mean rating of 5.75. The mean CBB leaf rating for the 2nd greenhouse experiment was 3.3. These results illustrate the difficulty of establishing high levels of disease pressure using inoculations with the bacterium. There is variation in disease reaction even within the cultivars included as checks, possibly due to fluctuations in greenhouse environmental conditions.

The presence of both markers in this genetic background does not appear to be beneficial, based on the results obtained in the greenhouse. The potential failure of QTL pyramiding in this particular situation warrants further investigation. The potential benefits of pyramiding resistance loci have been previously documented, contradicting the results obtained in this research (Castro et al., 2003). QTL pyramiding allows breeders to combine the genetic components of a quantitative trait and have a larger impact on a phenotype that can be achieved by use of a single QTL. Future field

experiments need to be conducted to gain additional information regarding the apparent failure of QTL pyramiding in this study.

The results of the greenhouse inoculations provide further evidence that the field results from this study are less reliable, as SU91 was significantly associated with CBB resistance in leaves and pods (in two separate greenhouse experiments). SU91 was also associated with CBB resistance in previous field studies (Chapter One), also contrasting the field results in this experiment. Further greenhouse and replicated field evaluations using various isolates in different environmental conditions will be needed to confirm the CBB resistance potential of these 93 lines.

In conclusion, 93 F_4 lines with various combinations of resistance to common bacterial blight and anthracnose, were generated from this study. The molecular marker combination present in each genotype was determined, and the disease reaction of each genotype to direct inoculation with *Xap* was observed. Although it would be useful to confirm the results of MAS by screening the selected lines for anthracnose resistance, no races of the pathogen are currently available in our laboratory that would discriminate the effects of $Co\text{-}1^2$ and $Co\text{-}4^2$.

The selections derived from this material may be useful to introgress pyramided CBB and anthracnose resistance into other market classes of beans. No commercially available cultivar in any market class has adequate levels of CBB resistance for production in Michigan. Introgressing CBB resistance into any of the major market class of common beans would be beneficial.

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APPENDIX A1

COLLECTION OF *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) ISOLATES FROM MICHIGAN DRY BEAN FIELDS

ABSTRACT

Twenty-four samples of common bacterial blight (CBB) infected bean leaf tissue were collected from two Michigan State University Agricultural Experiment Station research farms in Saginaw and Montcalm counties, respectively. *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*) cultures were isolated from 19 of 24 samples and plated on MXP media (Claflin et al., 1987). Isolates showed variability in growth habit (grainy vs. mucoid) and the production of a yellow polysaccharide suspected of being associated with virulence (Corey and Starr, 1957; Ekpo and Saettler, 1976; Steadman et al., 2002). Additional *Xap* isolates were obtained from other Universities throughout the United States. These cultures were maintained for the eventual characterization of relative virulence. The identification of virulent Michigan isolates would facilitate the efficient screening of bean genotypes for CBB resistance.

INTRODUCTION

Common bacterial blight (CBB) is a seed-borne disease that affects common bean (*Phaseolus vulgaris* L.) crops worldwide. The causal agent, *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*), is a gram-negative bacteria (Saettler, 1991). Due to the ineffectiveness of chemical and cultural controls, the development of genetic resistance is the preferable method of preventing CBB epidemics. The variability of *Xap* has serious implications in the development of a successful CBB resistance screening protocol. The use of molecular marker-assisted selection (MAS) for disease resistance is a promising method of identifying resistant genotypes (Yu et al., 2000). When using molecular markers developed by other breeding programs, however, the markers must be evaluated to ensure their usefulness in genetic populations different from those in which the marker was developed (Dudley, 1993). Additionally, because bacterial isolates used in inoculations may differ between breeding programs, putative resistant plants should be tested for resistance to local *Xap* isolates. This would help ensure the potential of novel resistance sources to confer resistance levels suitable for the desired area of bean production.

Isolates of *Xap* differ greatly in their production of a yellow polysaccharide, ranging from bright yellow to white in color. Those isolates producing copious amounts of the yellow polysaccharide are referred to as 'Fuscans' isolates. This polysaccharide has been implicated in playing a role in the relative virulence of an isolate (Corey and Starr, 1957). Additional researchers also observed higher virulence of 'Fuscans' isolates compared to 'Non-Fuscans' isolates (Ekpo and Saettler, 1976; Steadman et al., 2002). When the two types of isolates were used in direct inoculations on a set of CBB resistant

bean breeding lines, the plants were consistently more virulent to isolates of the 'Fuscans' category.

The objective of this research was to obtain a collection of *Xap* isolates for use in screening potentially CBB resistant materials, with the purpose of developing resistant varieties for production in Michigan. Recent establishment of a *Xap* greenhouse inoculation procedure will allow the future screening of these isolates for relative virulence.

MATERIALS AND METHODS

CBB infected plant tissue was collected from the field for the purpose of isolating *Xap* for use in field and greenhouse inoculations. Twelve samples were collected from diverse genotypes, locations, and market classes at the Saginaw Valley Bean and Beet Farm. An additional 12 samples were collected at the Michigan State University Montcalm Potato Research Station.

The protocol used for bacterial isolation was as described by Claflin et al. (1987), with some minor modifications. All isolates collected from Saginaw and Midland, Michigan, and Washington State were recovered using the method previously described in Chapter One. The isolates received from Colorado State University (B434, B439, B455, and B458) were sent as active bacterial cultures in petri plates with YDC media. The isolate from The University of Nebraska (9712-3) was sent as a lyophilized culture. Prior to use, approximately 0.008 grams of the stock sample was rehydrated for 10-20 minutes on YDC media, using 50 µl of sterile water.

RESULTS

Cultures of *Xap* isolates were extracted from 24 samples of disease plant tissue. These isolates, which were collected from infected dry beans at Saginaw and Montcalm, varied greatly in colony growth habit and color. Samples were cultured on the semi-selective media MXP (Claflin et al., 1987). Viable bacterial cells were recovered from 19 of the 24 samples. Six additional samples were received from other locations, and were cultured according to the procedure described above. Four of these isolates are from Colorado State University. One sample was also collected from a CBB infested dry bean field in Washington. A final isolate was received from The University of Nebraska, however, it was isolated from CBB infected seeds of the pinto cultivar 'Tomahawk' originating from a Michigan bean field. Collection details and notes regarding isolate characteristics are summarized in Table A1.1.

Isolates collected were predominately of the Fuscans-type colonies, with mucoid growth and copious production of a yellow polysaccharide. Of the 20 samples from which live bacterial cultures were isolated, 15 yielded isolates of the Fuscans-type. The other samples resulted in the recovery of bacteria that were either Non-Fuscans (white and grainy) or intermediate in growth.

Table A1.1 Summary of information regarding *Xap* isolates collected from infected bean tissue at Saginaw and Montcalm research farms, in Michigan, in 2002.

Sample†	Source‡	Pigment§	Notes
S1	G00540	Fuscans	Runny
S2	G97905	Unknown	Did not survive culture
S3	B02581	Fuscans	Turned media brown
S4	B02525	Fuscans	Turned media brown
S5	N01504	Unknown	Did not survive culture
S6	N02233	Unknown	Did not survive culure
S7	N00810	Intermediate	Possibly two distinct isolates
S8	B02554	Fuscans	Bright yellow, runny
S9	I02522	Fuscans	Dull yellow, turned media brown
S10	B02540	Fuscans	Gradient, yellow to dull yellow
S11	P00233	Fuscans	Bright, viscous
S12	P99120	Fuscans	Runny
M1	Montcalm	Non-Fuscans	White, grainy
M2	Montcalm	Unknown	Did not survive culture
M3	Montcalm	Fuscans	Grainy, similar to non-fuscans
M4	Montcalm	Fuscans	Bright, viscous, turned media black
M5	Montcalm	Unknown	Did not survive culture
M6	Montcalm	Fuscans	Thick growth
M7	Montcalm	Non-Fuscans	Off-white, grainy
M8	Montcalm	Intermediate	Growth similar to non-fuscans
M9	Montcalm	Fuscans	Bright, shiny yellow
M10	Montcalm	Fuscans	Turned media dark
M11	Montcalm	Intermediate	Growth similar to non-fuscans
M12	Montcalm	Fuscans	Grainy, similar to non-fuscans
B434	Azufrado	Fuscans	Shiny, collected from Colorado
B458	Bill Z	Fuscans	Shiny, collected from Colorado
B439	Burke	Fuscans	Shiny, collected from Kansas
B455	Enola	Fuscans	Shiny, collected from Nebraska
W1	Washington	Fuscans	Collected from WA state
9712-3	Tomahawk	Fuscans	Received from Nebraska, collected from MI

† = Isolates designated with "S" were collected from the Saginaw Valley Bean and Beet Research Farm. Isolates designated with "M" were collected from the Montcalm County Research Station. Isolates designated with "B" were received from Colorado State University.

‡ = The variety from which the isolate was collected is presented, when available. Information regarding the variety from which an isolate was collected is unavailable for the isolates from Montcalm and Washington.

§ = "Fuscans" isolates produce a yellow pigment on media, "Non-Fuscans" isolates are off-white in color.

DISCUSSION AND POSSIBLE FUTURE RESEARCH

The development of CBB resistant cultivars requires the establishment of a successful disease resistance screening procedure, using isolates that will adequately identify genotypes with potential CBB resistance. A basic characterization of the isolates collected in this research will facilitate the development of an optimal CBB screening protocol. The isolates described above reflect a sample of local *Xap* populations present in infected Michigan bean fields. The identification of a small number of virulent isolates would be useful in both greenhouse and field screening. In combination with MAS, this would increase the efficiency of selection by reducing the frequency of susceptible genotypes.

Comparison of isolate relative virulence would be most easily studied under controlled greenhouse conditions. Conducting a study of this nature in the field could result in complications due to bacterial movement from weather events. Greenhouse inoculations allow the relative containment of bacterial isolates, provided that plants inoculated with different isolates are not grown immediately adjacent to each other. A study of this nature was not initiated as planned, due to unforeseen complications in the development of a reliable greenhouse screening protocol. The virulence of these isolates should be evaluated and compared on a small number of known highly resistant (VAX 5 and XAN 159) and highly susceptible ('Midland' and 'Othello') genotypes is a possible direction of future research. Virulent isolates identified from such an analysis would be useful for direct inoculations in the field and greenhouse.

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APPENDIX A2

CHARACTERIZATION OF RESISTANCE TO COMMON BACTERIAL BLIGHT (CBB) BETWEEN THE KIDNEY BEAN CULTIVARS 'MONTCALM' AND 'REDHAWK'

ABSTRACT

Observations in the field have suggested that differences in resistance to common bacterial blight (CBB) exist between the kidney bean cultivars 'Montcalm' and 'Redhawk'. DNA was extracted from these cultivars and screened for previously described molecular markers linked to CBB resistance. Only the SCAR marker SAP6 was polymorphic between these two cultivars. Greenhouse inoculations with the causal agent *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*) were used to examine the possible differences in resistance reported from field observations. Greenhouse inoculations confirmed that 'Montcalm' does have significantly higher resistance to CBB ($p= 0.0067$) than 'Redhawk'. This has possible implications for kidney bean growers who have experienced problems due to CBB infections and are seeking cultivars with higher levels of resistance to CBB.

INTRODUCTION

Common bacterial blight (CBB) is a destructive seed-borne disease of common bean, caused by the bacterial pathogen *Xanthomonas axonopodis* pv. *phaseoli*. Most cultivars grown commercially in Michigan display no resistance to CBB. Such cultivars would be highly beneficial to bean growers and seed producers. Several Michigan bean farmers have noted a difference in field resistance between the kidney bean cultivars 'Montcalm' and 'Redhawk' (Dr. James Kelly, personal communication).

The purpose of this research was to examine the level of CBB resistance in 'Montcalm'. Of the four SCAR markers linked to CBB resistance that were screened (BC409, BC420, SAP6, and SU91), only SAP6 was polymorphic between the two cultivars. This marker is linked to a QTL for CBB resistance that has been mapped to bean linkage group B10 (Miklas et al., 1998; Miklas et al., 2000). This QTL was identified in the CBB resistant breeding line GNN #1 Sel. 27, with the resistance originating from within *Phaseolus vulgaris* (Miklas et al., 2003).

MATERIALS AND METHODS

DNA was extracted from 'Montcalm' and 'Redhawk' cultivars according to the protocol described in Chapter One of this text. Both cultivars were screened with the following SCAR markers linked to resistance loci for CBB: BC420, SAP6, BC409, and SU91. The PCR profile of these markers is listed in Table 1.1.

Greenhouse inoculations were used to compare the level of CBB resistance between the two cultivars. Eight plants of each cultivar were planted in 6-inch pots. Inoculations followed the protocol described in Chapter One. Visual ratings were taken on three leaves per plant at 14 days after inoculation. The previously described 1-9 scale (1= no infection, 9= severe infection) was used for comparisons of CBB resistance in this study. Statistical analysis was conducted using the PROC GLM function of SAS.

RESULTS AND DISCUSSION

The SAP6 primer amplified a band that was polymorphic between the cultivars 'Montcalm' and 'Redhawk'. Because SAP6 is linked to a major CBB resistance QTL on B10 (Miklas et al., 2000), it would be expected that 'Montcalm' would display higher resistance to CBB.

Greenhouse inoculations with *X. axonopodis* pv. *phaseoli* confirmed higher levels of resistance in 'Montcalm', compared to 'Redhawk'. The mean leaf rating for 'Montcalm' was 3.0, compared to 4.5 in 'Redhawk'. The p-value for this comparison was 0.0067. SAP6 was responsible for 17.5 % of the total phenotypic variation in CBB leaf ratings.

This research suggests that higher levels of CBB resistance are present in the kidney bean cultivar 'Montcalm', compared to 'Redhawk'. It would appear that this resistance is due to the presence of the CBB resistance QTL linked to the SCAR maker SAP6, on bean linkage group B10. One of the Great Northern Nebraska #1 lines (with CBB resistance derived from Montana No. 5) was a parent of 'Montcalm' (Copeland and Erdmann, 1977). This would explain the presence of the QTL on bean linkage group B10 in 'Montcalm', as Montana No. 5 carries this QTL for CBB resistance. 'Montcalm' may be useful as a parent for introducing this resistance into other large-seeded market classes of dry bean. It also may be a useful alternative to cultivars with less CBB resistance in areas where severe CBB infections have occurred.

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APPENDIX A3

DAYS TO FLOWERING DATA FOR 38 F_5 SELECTIONS FROM SAGINAW, MI 2002.

Table A3.1 Summary of marker genotype (SU91+ or SU91-) and days to flowering for 38 F_5 selections from Saginaw, MI 2002.

Genotype†	SU91‡	Flowering§
02T-9111	+	49
B03622	+	45
B03625	+	43
B03628	+	44
B03630	+	49
B03631	+	49
B03632	+	45
B03633	+	50
B03634	+	49
B03637	+	50
B03639	+	48
B03642	+	45
B03643	+	51
N03611	+	48
N03614	+	47
N03617	+	44
B03612	-	46
B03613	-	46
B03615	-	48
B03619	-	47
B03620	-	44
B03621	-	45
B03623	-	45
B03624	-	48
B03626	-	50
B03627	-	45
B03629	-	44
B03635	-	44
B03636	-	55
B03638	-	49
B03640	-	47
B03641	-	46

Genotype†	SU91‡	Flowering§
B03644	-	51
B03645	-	46
B03646	-	51
B03647	-	45
N03616	-	44
N03618	-	45

† = "B" indicates black bean genotypes, "N" indicates navy bean genotypes, 02T-9111 is a black bean genotype.

‡ = "+" denotes presence of the SU91 marker in the genotype.
"-" denotes absence of the SU91 marker in the genotypes.

§ = days to flowering was recorded as the number of days from planting at which a minimum of 50% of the plants of the genotype had at least one open flower.

APPENDIX A4

DAYS TO FLOWERING DATA AND RE-GREENING DATA FOR F_3 LINES, EAST LANSING 2003.

Table A4.1 Summary of marker genotype (SU91+, SU91-, BC420+, and BC420-), days to flowering, and re-greening data for 38 F_5 selections from Saginaw, MI 2002.

Genotype†	SU91‡	BC420‡	Flowering§	Re-greening¶
03T-9007	+	+	53	+
03T-9013	+	+	50	+
03T-9019	+	-	51	+
03T-9026	+	-	50	+
03T-9029	+	+	50	+
03T-9036	+	+	50	+
03T-9037	-	-	48	-
03T-9046	+	+	50	+
03T-9048	+	-	52	-
03T-9049	+	-	56	-
03T-9050	+	+	45	+
03T-9051	+	+	47	+
03T-9055	+	+	53	+
03T-9056	-	-	47	-
03T-9058	+	-	50	+
03T-9059	+	+	47	+
03T-9060	+	+	58	+
03T-9061	+	-	49	-
03T-9062	+	-	51	-
03T-9067	+	-	52	-
03T-9076	+	-	48	-
03T-9079	+	-	48	-
03T-9082	+	-	51	+
03T-9090	+	+	51	-
03T-9092	+	-	50	-
03T-9093	+	-	51	-
03T-9098	-	-	51	-
03T-9099	+	-	50	-
03T-9104	+	-	49	+
03T-9111	+	-	51	-
03T-9112	+	+	47	+
03T-9113	+	-	48	+
03T-9115	+	-	48	-

Genotype†	SU91‡	BC420‡	Flowering§	Re-greening¶
03T-9116	+	+	48	+
03T-9119	+	-	48	+
03T-9124	+	-	53	-
03T-9126	+	-	47	+
03T-9128	+	+	46	-
03T-9132	+	-	48	+
03T-9133	+	-	48	-
03T-9136	+	+	47	+
03T-9137	+	-	47	+
03T-9139	+	-	47	+
03T-9141	+	+	46	+
03T-9142	+	-	47	+
03T-9146	+	-	51	-
03T-9148	+	-	46	-
03T-9153	+	-	48	+
03T-9154	+	-	47	-
03T-9156	+	-	48	-
03T-9163	+	-	51	-
03T-9165	-	-	52	-
03T-9166	+	-	52	+
03T-9169	+	-	48	-
03T-9172	+	-	53	-
03T-9178	+	-	50	+
03T-9180	+	-	48	+
03T-9184	+	-	45	-
03T-9185	+	-	50	-
03T-9186	+	-	46	+
03T-9187	+	+	45	+
03T-9188	+	+	47	-
03T-9189	+	+	47	+
03T-9190	+	+	45	+
03T-9191	+	+	46	+
03T-9195	-	+	47	+
03T-9199	-	+	47	+
03T-9201	+	+	46	-
03T-9202	+	+	47	-
03T-9204	-	+	51	+
03T-9207	+	+	47	+
03T-9217	+	-	48	+
03T-9222	+	+	48	+
03T-9241	+	-	45	+
03T-9245	+	-	47	+
03T-9247	+	-	46	+
03T-9250	+	-	47	-
03T-9259	+	-	47	+
03T-9263	+	-	47	+
03T-9267	+	-	46	+
03T-9268	+	-	47	+

Genotype†	SU91‡	BC420‡	Flowering§	Re-greening¶
03T-9269	+	+	47	+
03T-9270	+	+	47	+
03T-9271	+	+	47	+
03T-9277	+	-	45	+
03T-9281	+	-	48	+
03T-9288	+	-	43	+
03T-9291	-	-	51	-
03T-9299	+	-	49	-
03T-9305	+	+	48	-
03T-9306	-	-	51	+
03T-9309	+	+	50	-
03T-9319	+	-	48	+

† = Genotypes are F_3 selections from the field study (East Lansing, 2003).
‡ = "+" denotes presence of the marker (SU91 or BC420) in the genotype.
"-" denotes absence of the SU91 marker in the genotypes.
§ = days to flowering was recorded as the number of days from planting at which a minimum of 50% of the plants of the genotype had at least one open flower.
¶ = "+" indicates that the genotype exhibited post-maturity re-greening.
"-" indicates that the genotype did not exhibit post-maturity re-greening.