USE OF SEQUENCE CHARACTERIZED AMPLIFIED REGION MARKERS IN MARKER ASSISTED SELECTION FOR COMMON BACTERIAL BLIGHT RESISTANCE IN BREEDING POPULATIONS OF COMMON BEAN

BY

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GREAT EAST ROAD

LUSAKA

DECLARATION

I, Lizzie Kalolokesya, declare that the work documented in this thesis represents my own work and has not been submitted or presented at any institution for the offer of academic or merit award.

Signature: …… Date: ……

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APPROVAL

This dissertation of Lizzie Kalolokesya is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Plant Breeding and Seed systems (University of Zambia).

Examiner's name Signature Date

1. …….

2. ……..

3. ……

SUMMARY

Common bacterial blight is a major seed-borne disease of common bean. Its resistance is complex because of the quantitative nature. The combination of molecular-marker technology (MAS) with traditional phenotypic selection greatly facilitates the selection of resistant lines. The molecular markers mostly used to identify QTL conditioning resistance to CBB are SCARs. SCAR markers BC420, SU91, and SAP6 are linked with three major QTL on B6, B8, and B10 respectively, and are being used for MAS.

The overall objective of this study was to select CBB resistant genotypes to be used as donor lines for production of disease free seed in order to increase bean productivity. The specific objectives were (1) Identify parental traits that support conventional breeding and MAS for CBB tolerance (2) Detect the presence of SCAR markers associated with CBB resistance in bean breeding populations (3) Assess the effectiveness of the SCAR markers SAP6, SU91 and BC420 in MAS

CBB was negatively correlated with some agronomic traits measured in the parental materials except for flower colour and pod length. There were significant ($p<0.001$) negative correlations between days to maturity and CBB ($r =-0.944$) and also between seed yield and CBB ($r = -0.917$).

There were significant differences ($p<0.001$) among the parental lines in their reaction to *Xf*260 and *Xf*410 isolates. The donor parents of Mesoamerican background VAX3 and VAX6 were most resistant with scores of 1 and 2 respectively. The donor parents of Andean origin (RMX lines) had intermediate response to CBB (scores of 4 and 5) and they possessed SAP6 marker. SCAR markers SU91 and SAP6 were present in VAX3 and VAX6 donors. Most of the susceptible parents also possessed SAP6 except RMA 71 (ALS donor parent) that possessed both SAP6 and SU91. None of the parental lines had BC420 marker.

Artificial inoculation of *Xanthomonas campestris pv. phaseoli* using *Xf*260 and *Xf*410 isolates showed significant differences among the $F_{4.6}$ ($p<0.001$) and $F_{3.5}$ ($p<0.001$) populations tested in the greenhouse and field. Correlation analysis for field and

greenhouse scores showed that there were no statistically significant relationship ($p \geq 0.10$) between field and greenhouse scores for both $F_{3.5}$ and $F_{4.6}$ generations.

MAS results indicated that SAP6 marker was detected in most of the progenies with intermediate reaction to CBB. SU91 was associated with phenotypic resistance though it was also detected in some intermediate and susceptible genotypes.

Correlation analysis showed highly significant relationship ($p<0.001$) between phenotypic and marker scores in some of the populations and insignificant ($p>0.005$) in some populations. Using direct selection, 51 of the $F_{3.5}$ progenies were resistant and 40 of these had the marker indicating successful transfer of the resistant QTL. In the $F_{4.6}$ generation, 46 progenies were resistant to the isolates and 44 of these had the marker band.

DEDICATION

To my beloved Mutisungilire!

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ACRONYMS AND ABBREVIATIONS

ADD…….....Agricultural Development Divisions

ALS……...Angular Leaf Spot

ARC…….....Agricultural Research Council

BCMV……...Bean Common Mosaic Virus

CBB……..Common bacterial blight

CIAT…….International Center for Tropical Agriculture (Centro Internacional de Agricultura Tropica)

CTAB……Hexadecyltrimethylammonium bromide

Kg/ ha……Kilogram per hectare

LG…….Linkage group

MAS…….Marker Assisted Selection

PCR…….Polymerase chain reaction

PABRA.....Pan African Bean Research Alliance

QTL…….....Quantitative Trait Loci

SCAR……..Sequence Characterized Amplified Region

RAPD……..Random amplified polymorphic DNA

Xcp……*Xanthomonas campestris pv. phaseoli Synonyms: Xap (Xanthomonas axonopodis pv. phaseoli), X. campestris pv. phaseoli var. fuscans (Xcpf)*

YCD……Yeast-extract-dextrose-calcium-carbonate agar

CHAPTER ONE

INTRODUCTION

1.1. Nutritional and economic importance of common bean

The common bean (*Phaseolus Vulgaris* L.) stands out worldwide as one of the most important crops from both economic and nutritional point of view (Ferreira *et al.*, 2003). It is a major food crop for rural and urban populations in Latin America and Africa, and is gaining steady popularity in developed countries where the population is more concerned with healthier diets (Gallegos *et al.*, 2007). The dry seeds provide a good source of proteins (Table 1), calcium and iron (Miklas *et al.,* 2005). Though the quality of bean protein is low in methionine and cystine, sulphur - containing amino acids, it has higher levels of lysine, which is relatively deficient in maize, rice, and cassava. Consumption of common beans mixed with these carbohydrate staples provides a balanced diet (CIAT, 1981; CIAT, 2004; Gepts *et al*., 2008). It has been estimated that maize and beans should be consumed in a 2:1 ratio to achieve an optimum amino acid balance in the diet (Gallegos *et al.*, 2007).

Common bean can be eaten as green vegetable using the leaves or green pods which are good sources of vitamins and minerals (Table 1). Regular intake of beans has been observed to lower glyceric and cholesterolemic indices that in turn lower incidence of certain types of cancer (Singh, 1999; Gallegos *et al.*, 2007). The crop is also a rich source of zinc and iron, two micronutrients depleted from individuals with AIDS (Buys *et al.*, 2002).

Table 1: Nutritive value of different parts of bean plant (*Phaseolus vulgaris* L) per 100g

Plant part	Nutritional content					
	Energy (cal)	Protein (g)	Calcium (mg)	Iron (mg)	Vit A (I.U)	Vit C mg
Green immature pods	34	2.0	50	1.4	200	20
Green shelled seeds	104	7.0	40	2.0	150	25
Mature dry seeds	339	22	110	8.0	10	0

Adapted from CIAT, 1981

The common bean has high economic potential and good export market, hence providing opportunities for foreign currency (CIAT, 1981). World wide, the common bean has annual production value of over US $ 10 billion (Rao, 2001). In Africa, the common bean provides a significant and a growing source of income for rural households, with annual sales worth over US$ 580 million (PABRA, 2008). In some countries bean production through research has brought spectacular economic returns, for instance, the internal rate of return on research investments in Tanzania from 1985 to 2005 was estimated at 60%, while in Eastern DR Congo farmers' incomes from beans have increased fivefold (PABRA, 2008).

1.2. Bean production

Phaseolus dry beans are the leading grain legume crop taking up to 30% of the total pulse production and grown on more than 14 million hectares world wide (Singh, 2001). Total production exceeds 23 million metric tones (MT) of which 7 million MT are produced in Latin America and Africa (Broughton *et al*., 2003). African smallholders grow over 4 million hectares of beans annually providing food to more than 100 million people in Africa (PABRA, 2008) (Table 2).

Table 2: Bean production in Africa

Region	Area (%)	Area(ha×10^3)
Eastern Africa – highland and mid-altitude- (Burundi, DR Congo, Ethiopia, Kenya, Rwanda, Tanzania, Uganda)	62	2490
Southern Africa- (Lesotho, Madagascar, Malawi, Mozambique, South , Swaziland, Tanzania, Zambia, Zimbabwe)	31	1290
Western Africa (Angola, Cameroon, Cape Verde, Togo)	3	135
Lowlands-winter season (Algeria, DR Congo, Egypt, Mali, Malawi, Mauritius, Morocco, Nigeria, Sudan, Tunisia)	4	200
Total	100	4025

Adapted from Broughton *et al*., 2003

Average bean yields in Malawi are about 200 kg/ha and 600 kg/ha under intercropping and pure stands, respectively, creating a sharp contrast with potential yield of 1500 kg/ha – 3000 kg/ha obtained by researchers (Pachico, 1993**).** The yield obtained by farmers in all ADDs of Malawi is by far much below the genetic potential of beans and do not meet consumption requirements (Mloza-Banda, 2000).

1.3. Constraints to Common bean production

Phaseolus vulgaris L. is a short season crop usually maturing from 65-110 days after planting (Buruchara, 2008). Despite the importance of common bean and its short duration, the crop suffers from several biotic and abiotic constraints.

1.3.1 Abiotic constraints

The abiotic constraints include low soil fertility in general, particularly deficiency of nitrogen and phosphorus and aluminum and manganese toxicity which are the most widely distributed (Wartmann *et al.*, 1989). Water stress or drought is another phenomenon of abiotic constraint being prevalent in most bean production regions (Singh, 1999). The drought effect on bean production is quite huge that it marginally exceeds the effect of bean diseases globally (Wartmann *et al.*, 1989).

1.3.2 Biotic constraints

1.3.2.1 Insects

Bean fly (*Ophiomyia phaseoli* Tryon) is by far the most damaging insect of beans in Africa (Wartman *et al.*, 1989). Leafhopper (*Empoasca kraemeri* Ross and Moore*)* is the most widely distributed in the tropics and subtropics (Singh, 1999).

1.3.2.2 Diseases

Angular leaf spot, Anthracnose and Rust are considered among the most widely distributed foliar fungal diseases that cause severe yield losses of common bean in Latin America, Africa and other parts of the world (Sing, 1999). Viral diseases that cause severe yield losses in susceptible bean cultivars include Bean Common Mosaic, Bean Golden Mosaic and Bean yellow Mosaic (Sing, 1999).

Common bacterial blight (CBB) amongst the bacterial diseases is the most widespread problem from tropical to temperate bean growing environments (Sing,

1999). The disease has been reported in 19 of the 20 bean producing countries in Eastern and Southern Africa, and is considered as one of the five most important biotic constraints of dry bean production in sub-Saharan Africa (Fourie, 2002).

CBB is a systemic, seed-transmitted disease caused by *Xanthomonas campestris pv. phaseoli* (Smith) Dye (*Xcp*). Contaminated seed is typically the primary source of inoculum for both local and global distribution of CBB disease (Vandemark *at al.*, 2008).

1.4. Strategies for management of Common Bacterial Blight

Effective CBB disease management involves the use of certified seed, crop rotation, and sanitation e.g. deep ploughing or removal of debris and weed management, (Mkandawire *et al*., 2004). In Africa, most farmers do not have access to certified seed; and sanitation, weed management and rotation options are limited. Thus, development of cultivars with durable CBB resistance offers the most promising long term and economical means of disease management and facilitates the production and distribution of pathogen-free seed (Mkandawire *et al*., 200; Singh *et al.,* 1999).

CBB resistance in common bean is a quantitative trait that exhibits low to moderate heritability, and has been reported to be an excellent candidate for marker assisted selection (O'Boyle *et al.*, 2007). Despite the complexity of its inheritance, the resistance has been introgressed into bean breeding lines and several authors suggest the possibility of using molecular markers through marker assisted selection (MAS) as a way to improve the efficiency of breeding, evaluating and selecting common bean cultivars with bacterial blight resistance (Jung *et al.,* 1999; O'Boyle *et al.*, 2007).

The substantial progress made in molecular marker technology for common bean holds considerable promise for breeding for genetic resistance to CBB. Molecular markers allow breeders to avoid direct screening techniques that may be less effective in selection of quantitative traits that are significantly affected by environmental factors (O'Boyle *et al.*, 2007).

Molecular markers are more effective in selecting for quantitative and low heritability traits than using conventional methods (Mohan *et al.,* 1996). MAS is rapid, it saves time of releasing a variety as selection among segregating materials can be done in early generations. Thus, with MAS, it is possible for the breeder to conduct many rounds of selection in a year without depending on the natural occurrence of the pest or pathogen (Mohan *et al.*, 1996).

The molecular markers mostly used to identify QTL conditioning resistance to CBB are Sequence Characterized Amplified Region (SCARs). They are robust and reliable; they improve selection because of multiplex polymerase chain reaction (PCR) and relative ease of scoring for complex inherited CBB resistance (Mutlu *et al.,* 2005). SCAR markers BC420, SU91, and SAP6 linked to three major QTL on chromosomes, B6, B8, and B10, respectively, and are being used for MAS of CBB resistance to validate QTL present in resistant lines selected by phenotypic selection (Milklas *et al*, 2005).

Most research scientists have reported the use of phenotypic and genotypic data for resistance selection for CBB. Regarding the complexity of CBB resistance inheritance, both phenotypic and genotypic data should be used in selecting resistance genotypes (Blair *et al.*, 2007). Phenotypic selection is needed to retain minor effect QTL and select for epistatic interactions that contribute to improved resistance (Miklas *et al.*, 2005).

This study was carried out to evaluate the reaction of $F_{3.5}$ and $F_{4.6}$ (Andean/Meso American and Andean/Andean) progenies to CBB, and validate the presence of SCAR marker $SU91_{700,}$ $SAP6_{820}$ and $BC420_{900}$ using MAS.

1.5 Research objectives

The overall objective of the study was to select CBB resistant genotypes to be used as donors for production of disease free seed in order to increase bean productivity.

Specifically, the research aimed at identifying parental traits that support conventional breeding and MAS for CBB tolerance, detect the presence of SCAR markers

associated with CBB resistance in bean breeding populations and assess the effectiveness of the SCAR markers SAP6, SU91 and BC420 in MAS.

1.6 Research hypothesis

This research tested the hypothesis that parental traits/characters can provide useful information to be used in both molecular and conventional breeding for CBB tolerance. It also tested the hypothesis that SCAR marker are tightly linked to the QTLs conferring resistance to CBB and that closely linked QTLs conferring resistance to CBB can effectively be used in MAS

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1The common bean plant-General description and gene pools

Phaseolus vulgaris L. is the most important and widely cultivated species following a process of agricultural domestication and adaptation, and is the most important grain legume species in Eastern and Southern Africa (Buruchara, 2008). The common bean belongs to the family *Fabaceae*, sub-family *Faboideae* alternately *Leguminosae*, Genus and species; *Phaseolus vulgaris*. The genus *Phaseolus* is a member of the tropical tribe Phaseoleae, which also includes cowpea, pigeon pea, and soybean. The Phaseoleae tribe is part of the Phaseoloid-Millettioid clade, which diverged some 45–50 million years ago from the Hologalegina clade, which contains most temperate crop legumes, such as pea, alfalfa (and *Medicago truncatula*), chickpea, and lentil (Gepts *et al*., 2008).

Phaseolus is a diploid genus with most species having 2n = 2x = 22 chromosomes and genome size of 580 Mbp/haploid (Gepts *et al.*, 2008). There are 55 known species in the genus *Phaseolus*, five of these have been domesticated; that is; the common bean (*P. vulgaris* L), the year bean (*P. dumosus* Macfad), the runner bean (P. *coccineus* L), the tepary bean (*P.acutifolius* A. Gray) and the lima bean (*P. lunatus* L). Among the five domesticated species, *P.vulgaris* accounts for more than 90% of cultivated crop worldwide and is by far the most widely consumed grain legume in the world (Gallegos *et al.*, 2007).

The bean plants are polymorphic, meaning that they can have a variety of different forms. They may be erect and bushy (up to 60cm in height) or climbing (with stems up to 3m long). The growth habit is classified into four (Table 3). Bushy beans are the most predominant types grown in Africa. However, climbing beans, originally restricted to small pockets of higher and more fertile soil in northern Rwanda, north east DR Congo and Malawi are now spreading to other areas and countries, particularly those where land is limiting and human population density is high (Bruchara, 2008).

Table 3: Growth habit classifications and descriptions of common bean

Growth habit	Description
Type I	Determinate growth habit, terminal bud reproductive, terminal guide absent, pods distributed along the length of the stem
Type II	Indeterminate growth habit, terminal bud vegetative, stems and branches erect. Terminal guide absent or medium, pods distributed along the length of the stem
Type III	Indeterminate growth habit, terminal bud vegetative, stems and branches prostrate with little or no climbing ability. Terminal guide small or long. Pods distributed mainly in the basal portion
Type IV	Indeterminate growth habit, terminal bud vegetative, stems and branches twining with strong climbing ability. Terminal guide long or very long. Pods distributed along the length of the stem or mainly in the upper portion

Source: Bean breeding scales- Beaver (2007)

Beans have compound leaves, with three smooth-edged oval leaflets that taper to a point. Small variegated, white, pink or purplish flowers, about 1cm long, develop in clusters. Flowering continues for two to three weeks, so pods develop at different times within the same plant, the pods range in shape from cylindrical to flat and usually contain from two to four seeds. The seeds come in a variety of shapes, size and colour (Singh, 2002).

The bean crop needs up to four months of warm weather and are not frost tolerant, they do poorly in wet or humid tropical climates because of susceptibility to bacterial and fungal diseases (Singh, 2002). The common bean needs well-drained soils with a pH between 6.5 and 7 and is sensitive to deficiencies or high levels of minerals in the soil (Singh, 2002). Seeds vary in colour, shape and size. CIAT (1987) reported standard seed colour scale (Table 4). Bean seed size is often reported as the weight (g) of 100 seed.

Table 4: Seed colour scale for common bean

Seed color group	Seed colour
1	White
2	Cream-beige
3	Yellow
4	Brown-maroon
5	Pink
6	Red
7	Purple
8	Black
9	Others

Source: CIAT (1987)

Phaseolus vulgaris is considered noncentric crop with at least two centers of domestication, and a wide geographical distribution of its wild relatives in middle and South America (Gallegos *et al.*, 2007). In the case of domesticated *P.vulgaris*, two large gene pools that pre-existed domestication are recognized in Meso-America and Andes. The wild and domesticated forms of common bean display higher variation in Meso-American as compared to Andean populations (Gepts, 1998). Within the two domesticated gene pools, several ecogeographic races have been identified within each gene pool based on the plant morphology, geographic and ecological distribution, and isozyme and molecular information (Gallegos *et al.*, 2007).

The Middle American (small seeded) cultivars often yield significantly higher than their Andean (medium to large seeded) counterparts. They also possess favorable alleles and QTL imparting higher level of resistance to both biotic and abiotic stress (Asensio *et al.,* 2005). In addition to higher yields, small seeded cultivars from Middle America, in comparison with their Andean counterparts, possess different genes or mechanisms and higher levels of resistance to drought stress, bacterial blight, angular leaf spot, anthracnose, rust, Bean golden yellow mosaic virus, and Bean common mosaic virus, among other traits (Singh *et al.,* 2002). This situation provides strong justification for broadening the genetic base of cultivars and for seeking gains from selection through interracial and inter-gene pool hybridization (Singh *et al.*, 2002). Genetic variation has potential for the development of improved cultivars (Gallegos *et al.*, 2007). Thus, for broadening the genetic base and breeding for higher yielding, multiple stress resistant in Andean cultivars use of inter-gene pool populations is essential (Asensio *et al.,* 2005).

Portuguese traders introduced the bean crop to the East African coast in the sixteenth century and quickly became established as a food crop in many environments of Africa (Bruchara, 2008). Beans reached Malawi from the east coast of Africa more than 300 years ago under the influence of traders and merchants (Mughogho, 1972). Presently, it is the second most important source of human dietary protein and the third most important source of calories for over 100 million people in rural and poor urban communities in Africa (Bruchara, 2008). Its protein is cheaper than animal protein making it highly competitive and important in dietary regimes of poor people in Africa. Many plant parts are cooked; leaves, green pods, green seed, but dry grain is the most important product (Asensio *et al.,* 2005).

2.2 Common Bacterial Blight-CBB

2.2.1 Economic importance

CBB caused by *Xanthomonas campestris* pv. *phaseoli* (*Xcp*), is one of the most destructive bean diseases when environmental conditions are favorable for the pathogen (Dursun *et al.*, 2002). CBB was first recognized by Beach in 1892 in New York, USA (Gilbertson *et al*., 1991)**.** It is wide spread and part of a complex *Xanthomonas* bacterial pathogens attacking many broadleaf and vegetable crops (Gilbertson *et al*., 1991)**.**

Outbreaks of bacterial disease reduce yield and quality of the crop. Losses range from a trace to complete crop failure. In Africa, yield losses of up to 220,000 t/y are experienced; of which 146,000 t are lost in Eastern Africa and nearly 70,000 t/y in Southern Africa (Wortmann *et al.,* 1998).

2.2.2 Disease development, dissemination and survival

The disease typically develops when contaminated seed is planted, when plantings are made in fields with a history of the disease, and when the climate is consistently hot and wet or humid (Pernezny and Jones, 2008). Rainfall or overhead irrigation is usually needed for disease development and progression in the field (Gilbertson *et al*., 1991). Cells of *Xcp*. enter bean plants through stomates (breathing pores) in leaves and other plant organs and hydathodes (vein endings) at margins of leaves, vascular bundles may also be invaded and result into plant wilting (Fourie, 2002). Research

has shown that wind-blown soil particles create wounds that are very important as entry points for blight bacteria (Pernezny and Jones, 2008).

The disease occurs 'on' and 'in' seed, rendering seed as one of the mechanisms for disease transmission (Allen *et al.,* 1998). Dissemination of blight bacteria can be viewed in two ways; long distance and short distance. Contaminated seed is an extremely efficient means of long distance spread and the widespread distribution of *Xcp* (Gilbertson *et al*., 1990). Successful spread of the *Xcp* within the field (short distance) is dependent on the presence of water especially when field plants are wet, and also insects such as whiteflies, leaf miners, and beetles which may transmit the bacterium from plant to plant (Zhang *et al.*, 2002).

Xcp may survive in seed, crop debris and weeds for up to 10 years (O Boyle *et al.,* 2007; Zhang *et al.,* 2002) and prolonged survival of up to thirty six years has also been observed (Fourie, 2002). Seed contamination which may be internal or external and even symptomless has serious implications for seed certification schemes (Fourie, 2002).

2.2.3 Symptoms of CBB

Common blight is primarily a foliar disease, but symptoms can also occur on stems and pods (Zhang *et al.,* 2002). The most characteristic and diagnostic symptoms are on trifoliate leaves, but they also occur on primary leaves (Gilbertson *et al*., 1991). The first disease symptoms of Bacterial blight are brown nectrotic lesions surrounded by a bright yellow hallow at the leaf margin or interior of the leaf, if the disease is severe, leaflets are killed, and premature defoliation will result (Fourie, 2002).

During warm, wet conditions the lesions rapidly enlarge and merge, as they develop, the centers become dry, brown, and surrounded by a distinct, narrow, zone of yellow tissue (Gilbertson *et al*., 1990). In highly susceptible varieties, the lesions continue to expand until the leaves appear scorched or sun scalded (Plate 1). Such leaves soon become ragged and torn by wind and rain.

Plate 1. Advanced lesion of common bacterial blight on top surface of bean leaves

The disease begins early in the development of the plant but symptoms are not obvious until about blossom (Hart and Seattler, 1981). Pod lesions begin as water-soaked spots that may produce yellow bacterial exudates during moist weather (Plate 2). They can enlarge and become sunken brown spots that are circular to irregular in shape (Hart and Seattler, 1981). Internal seed infections occur when pods become infected, and this seed then serves as source of infection the following season (Fourie, 2002).

Plate 2. CBB on fresh pods

2.2.4 Management practices

One of the reasons that common blight continues to be the most important bean disease worldwide is that it is difficult to control. This is due to the seed borne nature of the bacteria and also lack of high levels of disease resistance in *P.vulgaris* (Allen *et al*., 1998). Clean seed programs, chemicals, cultural practices, and genetic resistance are used to control this disease (Miklas *et al.,* 2005). Ultimately, the best method to manage the disease will be development of resistant cultivars.

2.2.4.1 Chemical control

Various chemicals have been applied to seed and/or foliage in attempts to control common blight, some success has been achieved using copper based chemicals though no complete control was attained (Dursun, 2002). Heavy use of copper based chemicals to control diseases caused by *Xanthomomas* can result in selection of copper resistant strains of the bacteria (Seater, 1991). Efficacy of CBB chemical control is limited and resultant yield increases are minimal (Fourie, 2002).

Development of resistance to chemicals among *Xc*p strains, cost involved and efficacy are the known drawbacks of chemical applications (Fourie, 2002). Thus, use of bean cultivars resistant to *Xcp* is economically and technically the most practical method for effective management of CBB. Yu *et al.,* (1998) indicated that, the complete ineffectiveness of chemical control for this pathogen leaves genetic resistance as the most effective method of control.

2.2.4.2 Cultural practices

Various disease management methods, including the use of clean seed, crop rotation, and deep ploughing of infected straw, have been proposed, but none is fully effective under conditions highly favourable for the disease (Mutlu *et al.,* 2008). Use of disease free seed does not guarantee disease control as other inoculum sources may exist and crop rotation may be less effective if epiphytic bacteria survive on non-host rotation plants (Fourie, 2002; Allen *et al*., 1998), as such, the cultural practices may not offer an effective control on CBB.

2.2.4. Biological control

No biological control strategies have been commercialized for common bacterial blight (Howard *et al.*, 2007). However, there have been reports that resistance in susceptible plants induced by inoculation with virulent isolates is known to exist, (Fourie, 2002).

2.2.5 Genetic resistance to CBB

Studies involving DNA based (RAPD, RFLP) markers support the existence of two to six quantitative trait loci (QTLs) responsible for CBB resistance in common bean (Singh and Munoz, 1999). Currently, five to eight major and minor alleles and QTL are estimated to control resistance to CBB in common bean (Mutlu *et al.,* 2008).

Breeding for CBB resistance is complicated by pathogen variability, linkage drag of undesirable traits and different genes conditioning resistance in leaves, pods, and seeds (Yu *et al*., 2000). A further complication of quantitative disease resistance is that the genetic loci involved are subject to environmental influence (Mutlu *et al.*, 2005). The expression of quantitative resistance in contrasting genetic backgrounds or environmental conditions may vary and complicate breeding efforts. In addition, the differential response of plant organs to CBB infection and lack of correlation in reaction to CBB between leaves, pods and seeds further complicates breeding for resistance (O'Boyle *et al.,* 2007).

The most serious problem in CBB resistance breeding has been the instability of resistance, often after more than a dozen generations of selfing, CBB-resistant lines continue to segregate (Singh and Munoz, 1999). The cause of instability is not known; the CBB resistant gene(s) may be unstable; or, because three or more genes or QTLs are involved in controlling CBB resistance (Singh and Munoz, 1999). Thus, to maintain high levels of CBB resistance, evaluations should be made under replicated trials and single plant selections must be made under high disease pressure in each generation (Fourie, 2002).

CBB resistance QTL has been identified from common bean, and its related species, tepary bean (*Phaseolus acutifolius* L.) and Scarlet runner bean (*Phaseolus coccineus*

L.), (Singh and Munoz, 1999). In *P. acutifolius,* three linked dominant genes were identified as controlling CBB resistance, (Singh and Munoz, 1999).

Hybridization between *P. vulgaris* and *P. acutifolius,* by embryo rescue, was initiated at CIAT, Palmira, Colombia, in 1989. More than 10, 000 advanced-generation progenies were obtained from recurrent and congruity backcrosses (i.e., backcrossing alternately to either species) of the interspecific F_1 hybrids and gene pyramiding (i.e. combining different sources of CBB resistance genes) (Singh and Munoz, 1999). Congruity backcrossing is used to overcome hybridization barriers such as genotype incompatibility, early embryo abortion, hybrid sterility and lower frequencies of hybridization (Fourie, 2002).

Near-immune lines (XAN 159, XAN 160, XAN 161 and OAC 88-1) were derived from crosses between *P. acutifolius* and *P.vulgaris,* although resistance instabilities were reported in XAN 159 and its progeny, it is still widely used in resistance breeding programmes (Beebe and Corrales, 1991).

CIAT developed six CBB-resistant lines from interspecific hybridization of *P. vulgaris* and *P. acutifolius* and gene pyramiding (VAX1, VAX 2, VAX 3, VAX 4, VAX 5, and VAX 6). The level of resistance possessed in VAX 3, VAX 4, and VAX 6 are as high as those found in *P. acutifolius* accessions. These lines also possess much better tropical adaptation, plant type, and seed color (Singh and Munoz, 1999).

More recently, CIAT and collaborator transferred CBB resistance to red mottled seed class (RMX lines) of Andean origin through gamete selection (Project IP-1, 2001; personal communication).

2.2.6 Assessment of resistance in breeding lines

Breeding of *Phaseolus* emphasize identification of germplasm as a source of resistance, tolerant varieties to *Xap* have been developed. For this reason, different plant pathogenic bacteria inoculations techniques in crops are required (Izquierdo *et al.*, 2001). Certainly, more valuable than the pathogen itself has been the methods to assess resistance of bean lines and the pathogenicity of the pathogen (Gilbertson *et al*., 1991). Different inoculation techniques are used to evaluate CBB resistance, these

include; aspersion (inoculation sprayed under pressure on leaves) and wounding of leaves using scissors, razor blades, needles, surgical blades etc (Fourie, 2002). It is difficult to standardize the inoculation methods because of wide range of environments in which such evaluations are conducted and the varying availability of laboratory and field facilities (Gilbertson *et al*., 1991).

Factors that influence disease expression include; the method used to inoculate the plants, the inoculum concentration and whether the test is conducted in the field or the green house (Izquierdo *et al.*, 2001). For field evaluations, some of the characteristics that are important in developing and/or selecting inoculation methods are that they can be easily applied to large numbers of plants, they emulate to some extent the natural mode of infection of the pathogen in the field, and that they provide reproducible results (Coyne and Schuster, 1983).

Efficiency of inoculation techniques depends on environmental conditions, plant (growth habit, phenological stage, handling after inoculation etc) and pathogen (concentration, inoculum age etc) (Coyne and Schuster, 1983). Multiple interactions among these factors complicate genotype evaluations, mainly due to pathogenicity loss or variation when handling the bacteria in the laboratory (Fourie, 2002).

Inoculum concentration is another important factor when inoculating plants with blight bacteria. Inoculum concentrations that are too low may indicate that a susceptible line is moderately resistant where as an inoculum concentration that is too high may indicate that a moderately resistant line is susceptible (Gilbertson *et al*., 1990). Evaluation of large numbers of genotypes in the field or controlled environment requires fast and optimum production of inoculum concentrations of 10^6 to 10^{10} CFU (colony forming units mL^{-1}), and inoculation must be carried out with young bacteria (48 h maximum age) (Izquierdo *et al.*, 2001).

In general, isolation, production, and identification of bacterial colony occurs in solid yeast-extract-dextrose-calcium-carbonate agar (YDC) culture media prepared from yeast extract (10g), dextrose (20g), calcium carbonate (20g) and agar (15g) in 1 litre of distilled water. This medium is selective for *Xanthomonas* (Izquierdo *et al.*, 2001). The bacteria is aerobic, Gram negative (0.4 to 0.9µ diameter; 0.4 to 2.6µ length) and

mobile, with polar flagella (Gilbertson *et al*., 1990). Bacteria colonies are bright yellow, convex shaped, with lypolitic activity, and they hydrolise gelatin, casein and starch (Schaad and Stall, 1980).

Various scales have been used for evaluating disease reaction. Rating scales should be standardized and utilized uniformly when comparing lines with CBB resistance (Fourie, 2002). A rating scale commonly used in describing rating of leaf disease is adapted from van Schoonhoven and Pastor-Corrales, (1987) (Table 5).

Table 5: Scale to evaluate the reaction of bean germplasm to fungal and bacterial pathogens

Rating	Description	Comments
1	No visible symptoms	Germplasm useful as parents or
2,3	Very light symptoms resulting in little or no economic damage	commercial varieties
4,5,6	Visible and conspicuous symptoms resulting in only limited economic damage	Germplasm can be used as commercial varieties or as sources of resistance to certain diseases
7,8,9	Severe to very severe symptoms causing considerable yield loss or plant death	Germplasm in most cases is not useful as parents or commercial varieties

Source: Bean breeding scales. Beaver (2007)

2.2.7 Marker assisted selection

Evaluation of field reactions is costly in terms of time and space (Fourie, 2002). In addition, resistance to common bacterial blight is difficult to evaluate and select in common bean-breeding populations because of complex inheritance and low heritability. Several authors have suggested the possibility of using molecular marker based selection as a way to improve the efficiency of breeding common bean cultivars with bacterial blight resistance (Jung *et al.,* 1999).

The molecular markers mostly used to identify QTL conditioning resistance to CBB are SCARs. SCARs are longer primers developed from RAPD fragments (Paran and Michelmore, 1993). SCAR markers were developed to overcome the problems associated with RAPD markers (Anuniwa *et al.,* 2009). The RAPD technique is sensitive to reaction conditions, which results in poor reproducibility (Ardiel *et al*., 2002). To improve the utility of RAPDs in marker-associated selection, longer

primers have been developed from RAPD fragments (Paran and Michelmore, 1993). These longer primers generate a Sequence-Characterized Amplified Region (SCAR), which can particularly be useful to follow the inheritance of the marked region of the genome (Anuniwat *et al.,* 2009). The SCAR markers are preferred over RAPD markers as they detect only a single locus and their amplification is less sensitive to reaction conditions (Anuniwa *et al.,* 2009).

SCAR markers are robust and reliable, they detect a single locus and may be codominant (Collard *et al.*, 2005). SCAR markers improve selection because of multiplex polymerase chain reaction (PCR) and relative ease of scoring for complex inherited CBB resistance (Mutlu *et al.,* 2005). Yu *et al*., (2000) reported that using SCAR marker $BC420_{900}$, a single reaction was sufficient to identify the presence or absence of the band in a plant which is not so with other markers e.g RAPDs, and that this not only improved the marker´s reproducibility and accuracy but also reduced chemical costs for marker analysis by 2.5 fold.

SCAR markers BC420, SU91, and SAP6 are being used for MAS of CBB resistance and to validate QTL present in resistant lines selected by phenotypic selection (Milklas *et al.*, 2005). Unfortunately, all the three markers are dominant. Dominant markers can identify plants that have at least one copy of the gene of interest, but cannot easily be used to distinguish plants with one copy of gene (heterozygous) from plants with two copies of a gene (homozygous) (Vandemark *et al.,* 2008).

Both BC420 and SU91 located on linkage group B6 and B8 respectively, are derived from the common bean breeding line XAN 159, SAP6 on linkage group B10 is derived from greet northern landrace cultivar Montana No.5 (Vandemark *et al.,* 2008). The different chromosome positions of these three SCAR markers make them attractive sources for introgressing independent QTL conditioning resistance to CBB into susceptible bean cultivars (Vandemark *et al.,* 2008). In fact, all the three markers can be multiplexed in a single PCR reaction to expedite MAS for combined resistance to CBB (Miklas *et al.*, 2005). Effective MAS evidence of these QTLs (SU91, SAP 6 and BC420) outside the original mapping populations has been repeatedly demonstrated by other researchers (Kelly *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Sites

The development of the crosses was done in the bean screen house at CIAT headquarters, Cali, Colombia. Initial screening and advancement of the materials was done at Chitedze Agricultural Research Station, Kasinthula Sub research Station and Bunda College of Agriculture in Malawi. The inoculation and disease evaluation of the populations was carried out in the bean screen house and in the field at ARC-Potchefstroom in South Africa. MAS was conducted in the ARC biotechnology laboratory.

3.2 Genetic materials.

Bean seeds used in the study were sourced from CIAT. Simple crosses were made from CBB resistant sources (Plate 3); VAX 3, VAX 6 of Meso-American origin, RMX 2, RMX 19 RMX 20 of Andean origin and susceptible Andean lines (SEQ, RMA, RAA, DRK, CMB, SAB) (Plate 4). VAX 3 and VAX 6 (together with VAX 1, VAX2 and VAX5) were developed at CIAT from interspecific hybridization of *P. vulgaris* and *P. acutifolius* and gene pyramiding. VAX3 and VAX6 possess high levels of CBB resistance (scores of 1-2 on 1-9 scale) which are as high as those found in *P. acutifolius* accessions. RMX 2, RMX 19 and RMX20 are all large seeded with red and cream mottled seeds. RMX 2 was developed from cross of (A483/MONTCALM) X ((MAM48/A486) X (VAX2/KABOON)) (Table 6 and Fig 1). The other RMX donors were also developed using a similar crossing scheme to that of RMX2. RMX 19; CAL143 X ((A483/G6416) X (VAX3/AFR298)) and RMX20; CAL143 X ((A483/G6416) X (VAX3/AFR298)).

Table 6: Pedigree for CBB parent RMX2

Parent	Pedigree
P1	MAM48 x A486
P2	VAX2 x KABOON
P3	P1 x P2
P4	A483 x MONTCALM

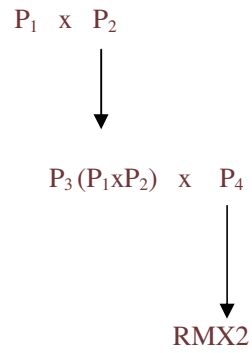


Figure.1. Diagrammatical presentation for the crossing scheme for RMX2

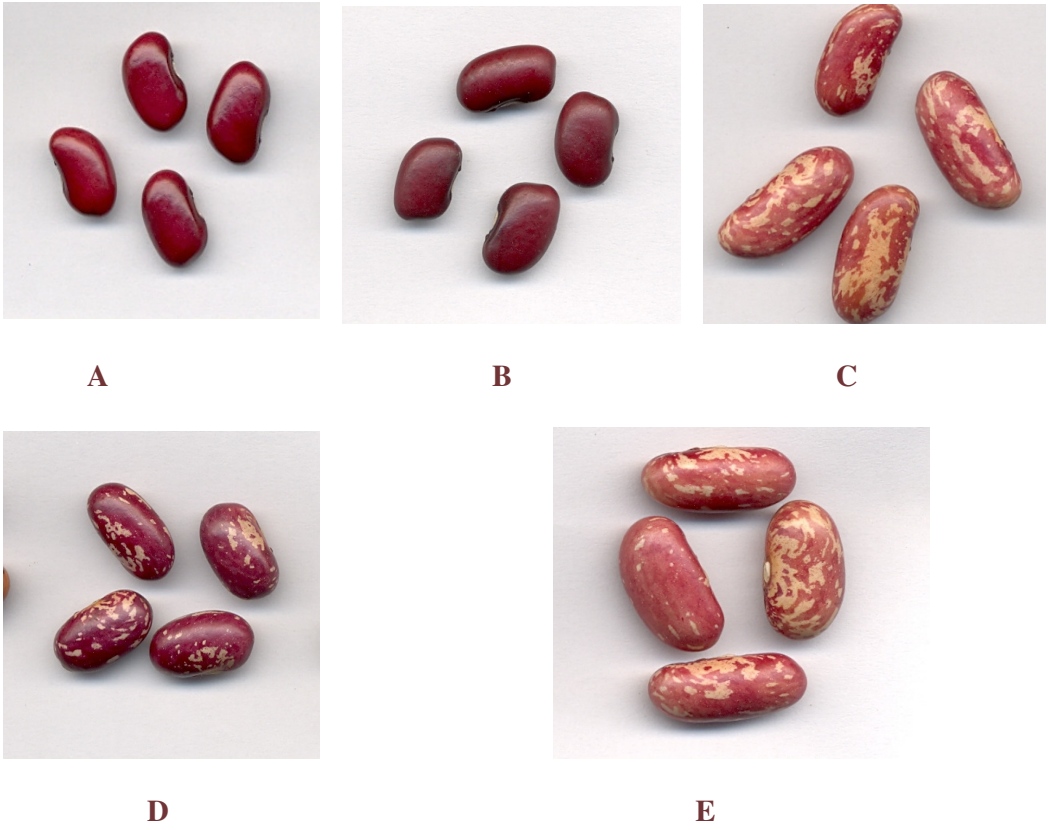


Plate 3. Phenotype of donor parental lines used in the development of the population, (A=VAX3, B=VAX6, C=RMX20, D= RMX2, E= RMX19)

The recipient parents that were used are of Andean origin (Plate 4) and they possess different traits (Blair, personal communication). The red mottled RMA lines are large seeded, resistant to angular leaf spot (ALS), BRB lines are resistant to BCMV and are red mottled, cream mottled or red. CMB lines are cream mottled in colour, resistant to BCMV. SEQ lines are tolerant to drought whilst RAA are large red lines also tolerant to drought. DRK lines are dark red kidney beans with high levels of drought

tolerance. SAB lines are large seeded, drought tolerant and are found in different colours. Appendix 1 shows the seed types for all the parents that were used in the crosses.

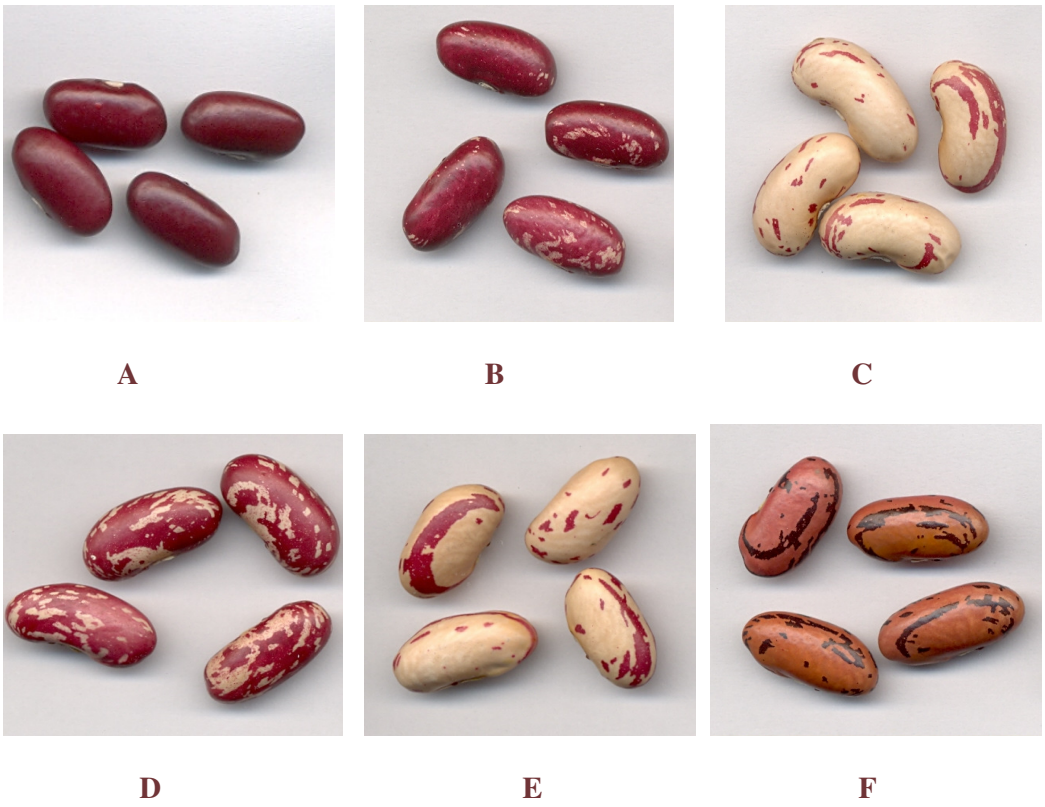


Plate 4. Phenotype of selected recipient parents used in the crosses (A= BRB267, B=BRB215, C=CMB107, D=RMA71, E=SAB568, G=SEQ 1004)

3.2.1 Crosses used in the study

A total of 15 simple crosses were made at CIAT-CALI in 2008A and 17 crosses in 2008B. The donor parents were VAX3, VAX6, RMX19, RMX2 and RMX19 (Table 7). There were a total of 27 Andean recipient parents, 2 of the donors were Meso-American and 3 were Andean. In total there were 32 different parents.

Table 7: Pedigrees for simple crosses that were evaluated for CBB resistance

Donor	Recipient	Population	Donor	Recipient	Population
VAX 3	BRB 211	BRB 211 / VAX 3	RMX 2	RMA 71	RMA 71/RMX2
VAX 3	BRB 214	BRB 214 / VAX 3	VAX6	BRB264	BRB264 /VAX6
VAX 6	BRB 215	BRB 215 / VAX 6	VAX3	BRB 265	VAX3 / BRB 265
VAX 3	BRB 264	BRB 264 / VAX 3	RMX 2	SEQ 1027	SEQ 1027/RMX2
VAX 6	BRB 265	BRB 265 / VAX 6	VAX3	BRB 267	BRB 267/VAX3
RMX 19	RBB 268	BRB 268 / RMX19	RMX2	BRB266	BRB266/RMX2
VAX 3	CMB 106	CMB 106 / VAX 3	RMX19	SEQ 11	SEQ 11/ RMX19
RMX 2	CMB 107	CMB 107 / RMX 2	VAX 3	SEQ 1003	SEQ1003/VAX 3
VAX 3	RAA 21	RAA 21 / VAX 3	RMX 2	DRK 149	DRK 149/RMX 2
RMX 20	RMA 70	RMA 70 / RMX 20	VAX6	SEQ1006	SEQ1006/VAX6
RMX 19	RMA 72	RMA 72 / RMX 19	VAX6	RAA21	RAA21/VAX6
VAX 6	RMA 72	RMA 72 / VAX 6	VAX6	CMB106	CMB106/VAX6
RMX 20	SEQ1036	SEQ 1036 RMX20	VAX6	SAB568	SAB568/VAX6
RMX 19	SAB 516	SAB 516 RMX 19	RMX2	SAB514	SAB514/RMX2
RMX 19	BRB 266	BRB 266 / RMX 19	RMX2	SAB576	SAB576/RMX2
RMX 19	RMA70	RMA 70/RMX 19	VAX6	SAB575	SAB575/VAX6

3.2.2 Single plant selections

Several generation advancements of the crosses were made in Malawi. Single plant selections based on phenotypic traits were made at F_3 and F_4 for the 17 and 15 populations respectively. Number of selections varied among the populations. Segregation to CBB resistance was observed within and among populations. The criteria used in the selection included resistance to CBB, BCMV, pod load, maturity period, yield and seed colour. The major criterion was resistance to CBB.

Most populations were severely affected by virus (Plate 5) and this affected the selection process. Some plants were infected at the early seedling stage and ended up being stunted and distorted. The plants that resisted both BCMV and CBB were tagged for selection (Plate 5).

Plate 5. CBB and BCMV susceptible plant (a) and resistant plant (b)

A total of 167 single plant selections were made from the F_3 populations and 142 were made from the F_4 populations. Seed increase of each selected plant was done at Bunda College of Agriculture farm. Planting was done on single 4 m row (unreplicated). All the necessary agronomic practices were followed to ensure good plant growth and development i.e. standard agronomic practices for tillage, fertilization, insect control, and weed controls were applied. At harvest, seeds from each row were bulked and advanced to the next generation without further selection.

3.3 Phenotypic screening of parents and progenies

3.3.1 Determining agronomic traits and CBB reaction in parental lines

A total of 32 different parents that were used in the development of the study populations were planted at Bunda College farm during 2009 winter season. Phenological and morphological data collected on the parental materials included; date of planting, days to flowering-measured in days-after-planting when 50% of the plants have one or more flowers, CBB leaf ratings using a scale of 1 to 9 as described by van Schoonhoven and Pastor-Corrales (1987), seed size expressed as weight in grams of 100 randomly chosen seeds (Small: Less than 25g; Medium: 25g to 40g; Large: more than 40g) and seed yield in Kg/ha.

3.3.2 Green house screening for CBB resistance

Four seeds of each of the parental line and the progenies were planted on 19th January 2010 in 20-liter plastic bags in sterile soil and maintained in a greenhouse at 18^{o}C night/28^{o}C day. Data collected on the parents included; date of planting, days to flowering-measured in days-after-planting when 50% of the plants have one or more flowers, flower colour, growth habit, CBB leaf disease using a scale of 1 to 9 as

described by van Schoonhoven and Pastor-Corrales, (1987), days to maturity measured as days-after-planting and collected at physiological maturity (PM), number of pods per plant at physiological maturity, pod length (cm), number of seeds per pod and seed yield.

The Bacterial pathogens (isolates *Xf*260 and *Xf*410) used in this research were obtained from ARC. *Xf*260 and *Xf*410 were isolated from field grown beans and have been previously used to examine the interaction of QTLs conferring resistance to CBB (vandemark *et al*, 2009). Inoculum was prepared from 48-hour old cultures grown on yeast-extract-dextrose-calcium-carbonate agar (YDC) medium. The bacteria cells were suspended in sterile distilled water, which was adjusted to 10^8 CFU/ml using a Shimadzu UV-260 spectrophotometer. Fourteen to twenty day old plants with fully expanded first trifoliate leaves were inoculated using the multiple needle inoculation method (Plate 6a and 6b). A leaf was placed on a sponge saturated with a bacterial suspension in a petri dish and perforated with a multiple needle. Bacterial inoculum was drawn into the wounds upon removal of the multiple needle device.

Inoculated plants were maintained in a greenhouse at 18°C night/28°C day and scored for CBB infection at 14 days after inoculation using a 1–9 scale (van Schoonhoven and Pastor-Corrales, 1987) with 1 being resistant and 9 susceptible.

Plate 6. (a)Multiple needle inoculation method. (b) Reaction of leaves few days after the inoculation

3.3.3 Field and greenhouse screening for CBB resistance in the $F_{3.5}$ and $F_{4.6}$ genotypes

In the field, 167 $F_{3.5}$ progenies and 142 $F_{4.6}$ progenies were evaluated in two separate experimental fields, arranged in 13*13 and 12*12 simple triple lattice respectively. Two controls used in the experiment were CBB resistant parent RMX20 and CBB susceptible parent SEQ11. Each progeny was planted on a single 5m row plot replicated three times. The plots were machine planted at a density of 35seeds/row. All the necessary agronomic practices were followed to ensure adequate plant growth and development. Plants were first inoculated at 2-3 trifoliate stage.

During the first inoculation, each trifoliate from every plant within the row was inoculated using multiple needle method, soon after the needles, the trial was re-inoculated using a motorized backpack sprayer to enhance disease development. The second inoculation was done seven days after the first inoculation using a motorized backpack sprayer. Inoculum was prepared similar to that of the greenhouse with the exception that non-sterile water was used. Reaction to *Xcp* evaluated per row was first recorded 14 days after first inoculation on a 1-9 scale (van Schoonhoven and Pastor-Corrales, 1987), with 1 being resistant and 9 susceptible. Scoring for *Xcp* was repeated at 7 and 14 days after the first evaluation.

3.4 Genotypic screening for CBB resistance

3.4.1 Leaf sampling from the greenhouse

Young leaves from each parent and their progenies were harvested and put into two separate tubes, the first tube had leaf material from a single plant (pure sample) and a second tube consisted of leaf materials from 3 plants (bulk sample) from the same pot.

3.4.2 Isolation of genomic plant DNA

The protocol used in the DNA isolation was a modified version of the method described by Graham *et al.,* (1994). Dry leaf samples from a Freeze-drying machine (Lyophilizer) were ground to fine powder, about 5 grams of the powdered was transferred to a 1.5ml eppindorf tube. A total of 900 µl of pre-heated CTAB extraction buffer [100 mM Tris (tris (hydroxymethyl) aminomethane, pH 8.0]; 20 mM EDTA (ethylenediaminetetraacetate, pH 8.0); 1.4 M NaCl; 2% (w/v) CTAB (hexadecyltrimethylammonium bromide); 0.2% (v/v) β-mercaptho-ethanol] was

added and thoroughly mixed with the powdered tissue. The suspension was then incubated in a water bath at 65^{o}C for one hour with occasional mixing once after every 15 minutes.

A volume of 800 µl Chloroform: isoamyl alcohol-IAA (24:1) was added and the suspension was mixed by gentle inversion. The tubes were then centrifuged at 12 000 rpm for 10 minutes at 4^{o}C to resolve the phases. After centrifugation, the upper aqueous phase (supernatant) was transferred to a fresh tube where 400 µl ice-cold isopropanol was added and mixed by gentle inversion and incubated at room temperature for 30 minutes. The suspension was centrifuged at 4,000 rpm at 4^{o}C for 30 minutes to collect precipitate. The supernatant was then discarded into a chemical waste jar and the pellet washed with 500 µl ice-cold ethanol 70% and incubated at room temperature for 20 minutes. DNA was precipitated at 4000 rpm at 4^{o}C for 5 minutes; the sap was then carefully discarded to avoid dislodging of the pellet. The pellet was then air-dried in a fume hood for 2-3 hours, and resuspended in 200 µl TE buffer (10Mm Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0). DNA concentrations were estimated by measuring absorbance using NanoDrop ND-1000 (NanoDrop technologies, USA) 10487 Spectrophotometer. DNA samples were diluted to a working solution of 15 ng µl^{-1} and stored at -20^{o}C before polymerase chain reactions (PCR).

3.4.3 PCR optimization and reactions for SCAR markers

SCAR primers, BC420, SAP6 and SU91 (Table 8) were synthesized by Invitrogen (Life technologies, Glasgow, United Kingdom) based on primer sequences obtained from Miklas *et al*. (2005). Primers were suspended in TE buffer to a concentration of 30ng/µl**.** SCAR markers were used for the polymerase chain reaction based on the protocol of Williams *et al.*, (1990) with minor modifications.

Table 8: SCAR markers used in genotypic screening for CBB resistance

SCAR	Sequence (5'-3')	Size bp*	Tagged locus	Resistant source	Linkage Group
SU91-5`	CCACATCGGTTAACATGAGT		major	XAN 159	B8
SU91-3`	CCACATCGGTGTCAACGTGA	700	QTL		
SAP6-5`	GTCACGTCTCCTTAATAGTA		major	GN#1 sel	B10
SAP6-3`	GTCACGTCTCAATAGGCAAA	820	QTL	27	
BC4205`	GCAGGGTTCGAAGACACACTGG		major	XAN 159	B6
BC4203`	GCAGGGTTCGCCCAATAACG	900	QTL		

*Expected band size

Adapted from Milkals *et al*., 2005.

Multiplex PCR, PCR technique where several products are amplified in a single reaction requires extensive optimization because primer-dimers (the bright fuzzy bands at the bottom of the gel) and other non-specific products may interfere with amplification of specific products. In this experiment, several steps were carried out using positive parents in order to obtain expected yield. Amplification reactions were performed in a 20 µl reaction volume containing 1X of Promega 5X (Promega Corporation, Madison, Wisconsin) reaction buffer and 2 mM $MgCl_2$. Multiplex PCR was optimized on type of enzyme, DNA concentration and dNTP concentrations.

Once conditions for amplifying individual products were determined, multiplex PCR was first carried out on parents. Reactions were performed using a PCR Sprint Thermal Cycler, multi block system (Hybaid Limited, UK) programmed for 5 min at 92^{o}C, 35 cycles of 1 min at 92 ^{o}C, 58 ^{o}C and 72^{o}C at 1:30 minutes. Followed by 1 cycle at 72^{o}C for 5 min and 4^{o}C on hold. PCR results were analyzed using a 2% agarose gel stained with ethidium bromide using UNTAN buffer (0.4 M trisbase, 0.02 M EDTA, pH 7.4). Since multiplex PCR was used, SU91 (700 bp) SAP6 (820bp) and BC420 (900 bp) were distinguishable by size using ultraviolet light fluorescence. Gels were photographed under UV light with (Gel Doc 1000 BIO-RAD Laboratories, Inc. USA) gel documentation system. The size of bands were compared with molecular weight marker (Fermentas, Middle Range 5000bp DNA ladder).

3.4.4 Marker assisted selection

Parents were first screened for presence of SCAR markers SAP6, SU91 and BC420. Populations from parental combinations that were polymorphic for the markers were used in MAS**.**

The $F_{3.5}$ and $F_{4.6}$ progenies were evaluated for the presence of CBB resistance QTL in MAS. Presence of marker was scored as 1 and 0 for marker absence. The marker scores (genotypic) were compared with the phenotypic scores from the greenhouse. The CBB scores were coded as 1 for resistance (1-3), 2 for intermediate reaction (4-6) and 5 for susceptible (7-9). Relationships between genotypic and phenotypic scores for each population were determined.

3.5 Data analysis

General analysis of variance

Grain yield analysis for parents was done using GenStat Discovery Edition (Lawes Agricultural Trust, Rothamsted, U.K). The following statistical model was used:

$$Y_{ij} = \mu + ßj + \acute{\alpha}_i + e_{ij}$$

Where μ is overall mean

ß*j* is the effect of j^{th} block

$\acute{\alpha}_i$ is the effect of i^{th} treatment

e_{ij} is random error term

Multiple comparison procedure (Duncan's Multiple Range) was used to determine means which are significantly different from others (Gomez and Gomez, 1984).

Correlation coefficient (r) analysis (Gomez and Gomez, 1984).

This was used to determine the relationship between yield, yield components and CBB in the parental lines. Correlation analysis was also done to establish association between marker and phenotypic scores for the $F_{3.5}$ and $F_{4.6}$ populations.

CHAPTER FOUR

4.0 RESULTS

4.1 Performance of parental lines during 2009 winter/dry season

Evaluation of the parental lines was conducted at Bunda during 2009 off season. Analysis of variance indicated highly significant ($P \leq 0.001$) differences for yield among the 32 parents (Table 9).

Table 9: Analysis of Variance for parental yield (kg/ha)

Source of variation	d.f.	m.s.	F pr.
Replication	2	128859	
Parents (genotypes)	31	669853	≤ 0.001
Residual	62	193364	
Total	95		

d.f: Degrees of freedom m.s: mean sum of square

Mean yield indicated that VAX 6, a small red seeded line of Meso-American origin had the lowest yield (1107 kg/ha) amongst the donor parents (VAX3, RMX19, RMX2 and RMX20) followed by another Meso-American line VAX 3 (1417 kg/ha). The Andean donor parents (large seeded RMX lines) had their mean yield above 1500 kg/ha (Table 10).

SEQ1004 and SEQ1006 the CBB susceptible but drought tolerant parents, had the highest mean yield of 2637 kg/ha and 2624 kg/ha respectively (Table 10). CMB106, had mean yield of 2127 kg/ha which was not significantly different from the mean yield of RMX 20 (2497 kg/ha) a CBB donor of Andean origin. SEQ 1003, a drought donor parent had the lowest mean yield (1049 kg/ha) which was not significantly different from a red mottled line resistant to angular leaf spot (ALS) RMA 70 (1105 kg/ha).

Table 10: Mean yield (kg/ha) of 32 parental lines of beans evaluated at Bunda Farm in the 2009 dry season

No	Parent (genotype) Identity	Mean yield (Kg ha-1)	
1	CMB 106	2548	jk*
2	CMB 107	2127	f-k
3	SAB 514	2483	ijk
4	SAB 516	2278	hijk
5	SAB 568	1332	a-e
6	SAB 575	2054	e-k
7	SAB 576	1713	a-i
8	SAB 581	1891	d-k
9	RAA 21	1482	a-h
10	BRB 267	1945	d-k
11	SEQ 11	2072	f-k
12	BRB 268	2152	g-k
13	SEQ 1004	2637	k
14	SEQ1006	2624	k
15	SEQ 1036	1650	a-i
16	RMA 71	1930	d-k
17	BRB 215	1513	a-h
18	BRB 264	1909	e-k
19	BRB 265	1819	b-j
20	BRB 266	1725	a-j
21	RMA 72	1828	c-j
22	BRB 214	2257	hijk
23	RMX 20	2497	jk
24	RMX 19	1652	a-i
25	RMX 2	1674	a-i
26	BRB 211	1356	a-f
27	VAX 6	1107	ab
28	DRK 149	1131	abc
29	RMA 70	1105	a
30	VAX 3	1417	a-g
31	SEQ 1027	1186	abcd
32	SEQ 1003	1049	a
Grand mean:		**1817**	
CV%		**24.2**	

*Means followed by different letters differ significantly according to Duncan's multiple range ($P \leq 0.05$)

CBB reactions for parental lines during the 2009 off season indicated no significant differences $P > 0.05$ among the resistant and susceptible parents (Table 11).

Table 11: Analysis of Variance for CBB reaction during 2009 dry season

Source of variation	d.f.	m.s.	F pr.
Replicate	2	0.073	0.413
Treatment	31	4.666	
Residual	62	4.406	
Total	95		

CBB grand mean score for the parents was 5 with most of the susceptible parents having intermediate scores of 4-6 (Table 12).

Table 12: Mean CBB scores for the parental genotypes evaluated at Bunda

Parent Identity	**Mean CBB**
CMB 106	6
CMB 107	4
SAB 514	5
SAB 516	5
SAB 568	7
SAB 575	6
SAB 576	6
SAB 581	4
RAA 21	5
BRB 267	6
SEQ 11	5
BRB 268	5
SEQ 1004	6
SEQ1006	5
SEQ 1036	6
RMA 71	3
BRB 215	6
BRB 264	4
BRB 265	5
BRB 266	6
RMA 72	4
BRB 214	7
RMX 20	3
RMX 19	3
RMX 2	3
BRB 211	7
VAX 6	2
DRK 149	6
RMA 70	5
VAX 3	2
SEQ 1027	6
SEQ 1003	6
Grand mean	**5**
L.s.d.	**3.426**
CV%	**38.1**

The donor parents VAX3 and VAX6 had resistant scores of 2; RMX lines had a resistant score of 3 (Table 12). Regression analysis showed that there was no significant relationship (P> 0.05) between seed yield and CBB (Table 13).

Table 13: Regression analysis for seed yield and CBB

	d.f.	m.s.	F pr.
Regression	1	219042	0.430
Residual	94	348858	
Total	95	347492	
Correlation Coefficient r= 0.0814572			
R^2 = 0.663527			

The correlation coefficient (r = 0.0814572), indicated a relatively weak relationship between seed yield and CBB. The coefficient of determination (R^2) value for the two variables was low, signifying that CBB only accounted for 0.66% of the variability in seed yield. Correlation analysis between seed yield and seed size showed a negative association (r =-0.981) between the two variables.

4.2 Reaction of the parental lines to Xf260 and Xf410 isolates (ARC-Potchefstroom greenhouse)

Reaction of the parental lines to the South African isolates was assessed in the ARC greenhouse. The parental genotypes differed significantly (p<0.001) for their foliar disease symptoms following inoculations with *Xf*260 and *Xf*410 isolates (Table 14).

Table 14: Analysis of Variance for CBB reaction of the parents evaluated in the green house-ARC Potchefstroom in 2010

Source of variation	d.f.	m.s.	F pr.
Replication	3	0.9772	
Parent (genotype)	28	19.5043	<.001
Residual	79	0.6583	
Total	110		

Mean scores for the parental lines was 6 with a coefficient of variation of 13.8% (Table 15).

Table 15: Parental reaction to *Xf*260 and *Xf*410 CBB isolates

Parental line	Mean CBB (1-9 scale)	
CMB106	8	fg*
CMB107	6	cd
SAB 514	7	ef
SAB 516	8	fg
SAB 568	7	ef
SAB 575	8	fg
SAB 576	6	cd
SAB 581	6	cd
RAA 21	6	cd
BRB267	6	cd
SEQ 11	7	ef
BRB 268	8	fg
SEQ1004	8	fg
SEQ 1006	6	cd
SEQ 1036	7	ef
RMA 71	4	b
BRB 215	6	c d
BRB 264	6.7	e
BRB 265	8	fg
BRB 266	9	g
RMA 72	7	ef
BRB 214	6	cd
RMX 20	4	b
RMX 19	5	bc
RMX 2	5	bc
BRB 211	7	ef
VAX 6	2	a
DRK 149	6	cd
RMA 70	6	cd
VAX 3	1	a
SEQ 1027	5	bc
SEQ 1003	6	cd
BBSS 130	1	a
BBSS 5	1	a
Grand mean		**6**
CV%		**13.8**

*Means followed by different letters differ significantly according to Duncan's Multiple Range Test (p=0.05)

South African parental lines BBSS5 and BBSS130 had highest resistance (score of 1) together with small seeded lines VAX 3 and VAX 6 (score of 1 and 2 respectively). Large seeded Andean donor parent RMX 20 had similar score (rating of 4) with Andean line RMA 71; a CBB susceptible parent but resistant to ALS. RMX 2 and 19

had mean scores of 5 together with drought resistant parent SEQ 1027. CBB susceptible but BCMV resistant parent, (BRB 266) was the most affected by the isolates with mean score of 9.

The associations between CBB infestation and most measured/derived parental agronomic traits were expect for flower colour and pod length (Table 16). The correlation between CBB rating and seed yield (r=-0.917) was significant (P<0.001) just as the one between CBB rating and days to maturity (p<0.001).

Table 16: Association between *Xap* inoculation and agronomic traits

Trait	Correlation coefficient (r)	Coeffiecient of determination ($R^{2)}$	P value
Flower color	0.323	0.1	0.225
No. pods per plant	-0.426	0.18	0.062
Pod length	0.073	0.0036	0.835
No of seeds per pod	-0.133	0.02	0.556
Days to maturity	-0.944	0.89	<0.001
Seed yield	-0.917	0.84	<0.001

4.3 Optimisation of multiplex PCR

To optimize the multiplex PCR conditions, the effect of polymerase enzyme type, template DNA and dNTP concentration were assessed. Template DNA from 3 CBB donors (VAX4, VAX5 and VAX6) of Meso-American background was used to determine the appropriate enzyme to be used in the multiplex reaction.

Appropriately-sized products were obtained in reactions using 1 unit of Go-Taq flex (Promega Corporation, Madison, Wisconsin) opposed to use to 1 unit of supertherm enzyme (Promega Corporation, Madison, Wisconsin) (Plate 7).

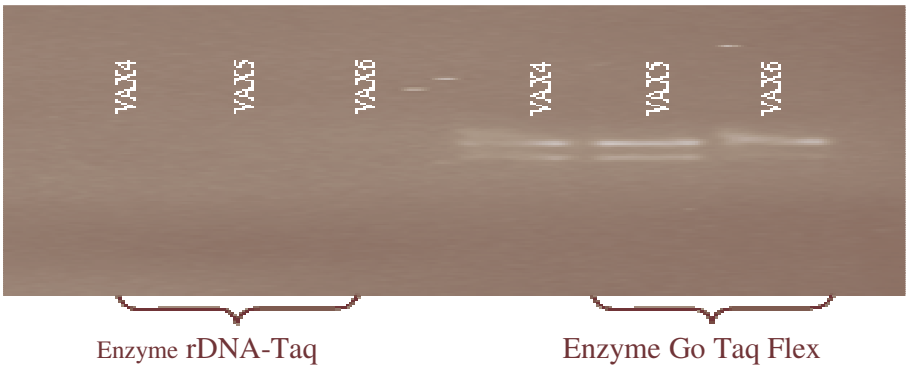
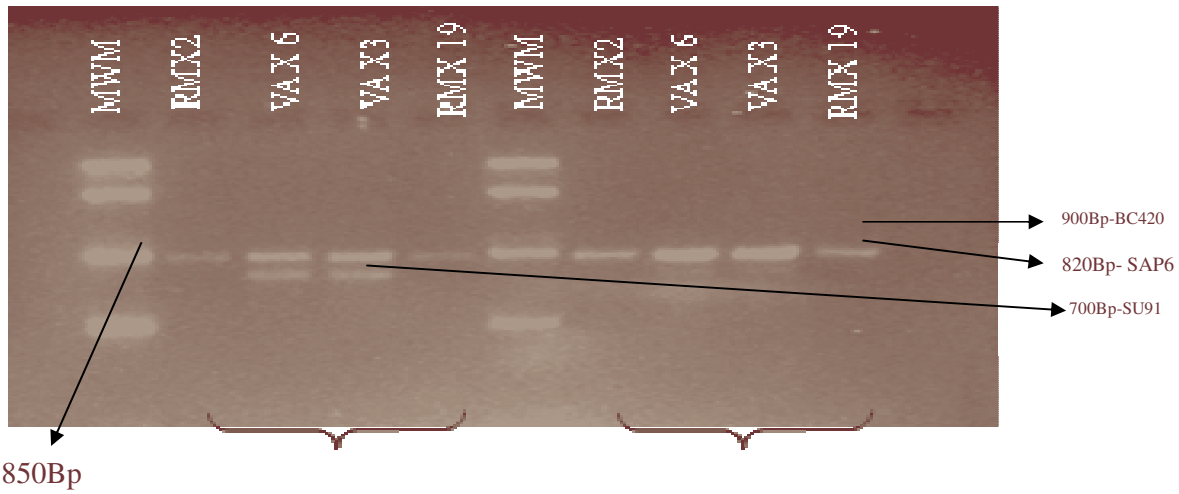


Plate7. Multiplex PCR profiles using different enzymes

Presence of bands on VAX4, VAX5 and VAX6 PCR product with one unit of Go Taq flex enzyme showed that Go Taq is the right enzyme to use in the multiplex reaction. Absence of bands on rDNA-Taq signified that the enzyme was not the appropriate one to use in the multiplex reaction.

An experiment on DNA concentration was carried out using two Andean donor parents (RMX2 and RMX19) and two Meso-American donors (VAX3 and VAX6). A gel photograph of two reactions prepared using 50ng and 60ng DNA concentrations and 1 unit of promega Go-Taq enzyme showed that 50ng DNA gave the double bands for VAX lines (Plate 8) same as those observed in the enzyme experiment (Plate 7).



DNA	50 ng	60 ng
Enzyme	1U	1U

Plate 8. PCR Profile for SCAR markers SU91 and SAP6 at two DNA template concentrations

Presence of both SU91 and SAP6 band in the VAX3 and VAX6, and SAP6 band in the RMX lines signified that 50ng was suitable for a reaction that combines different primers. Absence of SU91 band in the VAX lines performed using 60ng of DNA showed that higher concentration of DNA affected the SU91 primer.

Changes in dNTPs concentration using 50ng DNA (extracted from RMX2, RMX19, VAX3 and VAX6) with 1 unit enzyme yielded different products that gave an idea of the appropriate concentration that can be used in PCR multiplex reaction for SCAR markers SU91, SAP6 and BC420 (Plate 9).

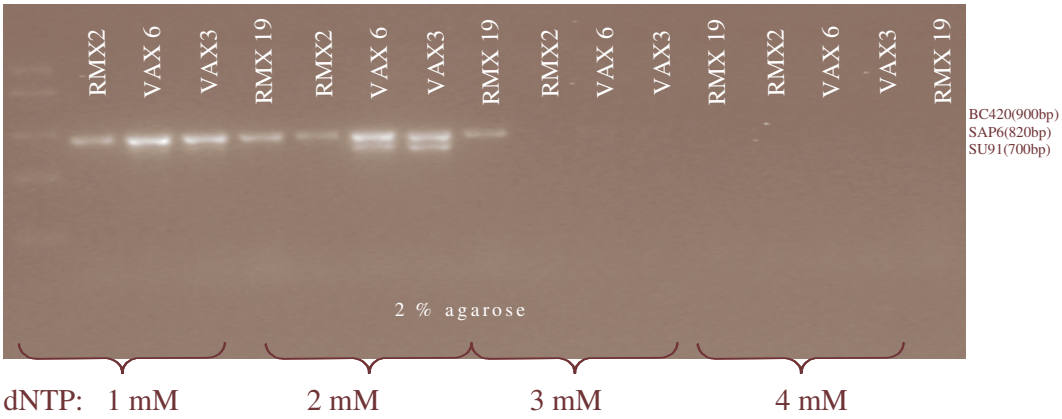


Plate 9: PCR profile for selected CBB parents at different dNTP rates

Absence of bands on 3 mM and 4 mM dNTP concentrations signified the negative effect of higher concentrations of dNTP on the multiplex reaction. Absence of SU91 band on the VAX lines performed using 1 mM indicated that the lower concentration did not work positively with the primers. Concentration of 2 mM yielded the expected bands for both VAX and RMX lines. Subsequent PCR experiments were conducted using the optimised parameters as described above.

4.4 Presence of SCAR markers among the parental lines

Assessment of the parental lines for their presence of SCAR markers was conducted in the ARC Biotechnology laboratory. The gel results showed that all the donor parents (RMX2, RMX19, RMX, VAX3 and VAX6) used in the crosses had SAP6 marker (Plate 10). In addition to SAP6, the VAX donor parents also had the band for

SU91 but lacked BC420, a major QTL present in some of the South African CBB breeding lines.

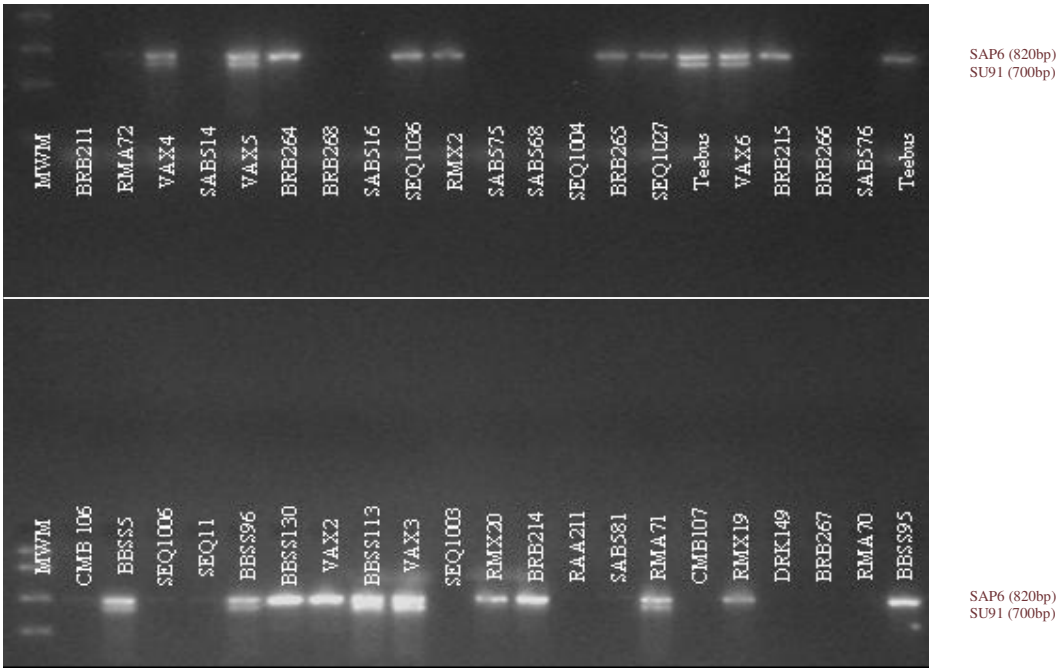


Plate 10-Parental gel of duplex markers-SAP6 and SU91

The results showed that CBB susceptible lines that are tolerant to drought (SEQ1036 and SEQ1027) possess the SAP6 marker though their CBB scores showed that they were susceptible and intermediate to the *Xf*260 and *Xf*410 isolates (Table 17). The BCMV resistant parents (BRB265 and BRB211) that are susceptible to CBB showed presence of SAP6 band.

Comparison of CBB scores from the greenhouse and marker presence showed that the parental lines with 2-3 markers were those that had significantly high resistance (scores of 1-2) (Table 17). The ALS resistant parent, RMA 71, had both SAP6 and SU91 with a CBB score of 4. Parents that had either one marker (SAP6) or none had intermediate reaction to CBB (scores of 4-6) indicating that the combination of markers SAP6 and/ or SU91 and BC420 resulted in significant reduction of the severity.

Table 17: Reaction to CBB and presence of SCAR markers in parental lines

Parental line	CBB score	Reaction	Markers and their resistant source SU91 (XAN 159)	SAP6 (GN#1 sel27)	SAP6 (XAN 159
CMB 106	8 fg*	susceptible	-*	-	-
CMB 107	6 cd	susceptible	-	-	-
SAB 514	7 ef	susceptible	-	-	-
SAB 516	8 fg	susceptible	-	-	-
SAB 568	7 ef	susceptible	-	-	-
SAB 575	8 fg	susceptible	-	-	-
SAB 576	6 cd	susceptible	-	-	-
SAB 581	6 cd	susceptible	-	-	-
RAA 21	6 cd	susceptible	-	-	-
BRB 267	6 cd	susceptible	-	-	-
SEQ 11	7 ef	susceptible	-	-	-
BRB 268	8 fg	susceptible	-	-	-
SEQ 1004	8 fg	susceptible	-	-	-
SEQ1006	6 cd	susceptible	-	-	-
SEQ 1036	7 ef	susceptible	-	+	-
RMA 71	4 b	intermediate	+	+	-
BRB 215	6 cd	susceptible	-	-	-
BRB 264	7 e	susceptible	-	-	-
BRB 265	8 fg	susceptible	-	+	-
BRB 266	9 g	susceptible	-	-	-
RMA 72	7 ef	susceptible	-	-	-
BRB 214	6 cd	susceptible	-	-	-
RMX 20	4 b	intermediate	-	+	-
RMX 19	5 bc	intermediate	-	+	-
RMX 2	5 bc	intermediate	-	+	-
BRB 211	7 ef	susceptible	-	+	-
VAX 6	2 a	resistant	+	+	-
DRK 149	6 cd	susceptible	-	-	-
RMA70	6 cd	susceptible	-	-	-
VAX 3	1 a	resistant	+	+	-
SEQ 1027	5 bc	intermediate	-	+	-
SEQ 1003	6 cd	susceptible	-	-	-
BBSS 5	1 a	resistant	+	+	+
BBSS 96	1 a	resistant	+	+	+

*+ Presence of marker, or - absence of marker

* Means followed by different letters differ significantly according to Duncan's multiple range test

4.5. Reaction of $F_{3.5}$ and $F_{4.6}$ lines to South African CBB isolates Xf260 and Xf410

4.5.1: Field and greenhouse evaluations of $F_{3.5}$ progenies

Evaluations for CBB reaction in the $F_{3.5}$ progenies were conducted at ARC Potchefstroom field during 2010 season. Statistical analysis showed presence of highly significant ($p<.001$) differences among the $F_{3.5}$ progenies to *Xf*260 and *Xf*410 isolates (Table 18).

Table 18: Analysis of Variance for CBB reaction of $F_{3.5}$ progenies to *Xf*260 and *Xf*410 isolates evaluated in the field during 2010 season

Source of variation	d.f	m.s.	F pr.
Replication	2	2.2191	
Blocks/replicate	36	2.1096	
Treatments	168	1.3327	<.001
Residual$^{\pi}$	261	0.4862	
Total	467		

$^{\pi}$Corrected for a single block for each replication

The resistant control (RMX20) showed segregation reaction towards susceptibility with average score of 6 (Table 19). The susceptible control SEQ11 had a score of 6, similar to the resistant control (RMX20). Most of the genotypes showed intermediate reaction with scores of 4-6 signifying that the populations in this generation are still segregating for CBB resistance. The most resistant progenies, SEQ1006/VAX 6-1 and SEQ1006/VAX 6-2, had scores of 3 while progenies from RMX2/SAB576 series were susceptible with a score of 7 (Table 19).

Table 19: Mean CBB scores for most resistant and the most susceptible progenies from the field

Cross Identity	CBB rating
SEQ 1006 / VAX 6-1	3
SEQ 1006 / VAX 6-2	3
SAB568-2/VAX6	4
SAB575-3/VAX6	4
SEQ 1003 / VAX 3-6	4
SEQ 1003 / VAX 3-10	4
SEQ 1006 / VAX 6-5	4
SAB576-2/RMX2	7
SAB576-5/RMX2	7
SAB576-6/RMX2	7
SAB576-8/RMX2	7
SAB576-11/RMX2	7
Control RMX20	6
Control SEQ11	6
Grand mean	**5**
L.s.d (0.05)	**1.2**
CV%	**12.1**

Out of 169 progenies evaluated in the field; 61 had their scores above grand mean (5), accounting for 37% of the total progenies, 63% showed susceptibility with scores of 6 and 7 (Appendix 2). From the 61 progenies with scores above average of 5, only 7 progenies had scores of 3-4 (SEQ1006/VAX6-1, SEQ1006/VAX6-2, SAB568/ VAX6-2, SAB575/VAX6-3, SEQ1003/VAX3-6, SEQ1003/VAX 3-10, SEQ 1006/VAX 6-5) and all these came from Andean/Meso-American parents (Appendix 2). From the 54 average progenies (score of 5), a total of 26 came from Andean/Andean parentage and 28 from Andean/Meso-American parents. Of the susceptible progenies 26 came from Andean/Meso-American parentage and the rest came from Andean/Andean parents (Appendix 2).

CBB evaluations for the $F_{3.5}$ progenies were also carried out in the ARC greenhouse. Using the *Xf*260 and *Xf*410 isolates, statistical analysis showed significant differences ($p < .001$) among the progenies (Table 20).

Table 20: Analysis of Variance for CBB reaction of $F_{3.5}$ progenies to *Xf*260 and *Xf*410 isolates evaluated in the greenhouse

Source of variation	d.f	m.s.	F pr.
Replication	3	0.626	
Treatment (crosses)	167	8.553	<.001
Residual	428	1.024	
Total	598	1697.594	

The $F_{3.5}$ generation had an average greenhouse score of 5 (Table 20); the resistant control RMX 20 had a score of 4 and 7 for susceptible control SEQ11. There were 8 progenies that had their scores above the susceptible control (Appendix 3). Three of these came from Andean/Meso-American parentage while the remainder was from Andean/Andean parents. The highest resistant progeny came from SAB575/VAX6 population with a score of 1 while the most susceptible were BRB 267/VAX3 progenies with score of 9 (Table 21).

Table 21: Mean CBB scores for most resistant and the most susceptible progenies evaluated in the greenhouse

Cross Identity	**CBB Score**
SAB575-6/VAX6	1
SAB 575-5/YAX6	1
SAB575-1/VAX6	2
SAB575-3/VAX6	2
SEQ1003/VAX 3-15	2
BRB266-1/RMX2	8
BRB266-11/RMX2	8
SEQ 1003 / VAX 3-11	8
SEQ 1004 / RMX 8-4	8
SEQ 1006 / VAX 6-7	8
BRB 267-1/VAX3	9
Control RMX20	4
Control SEQ11	7
Grand mean	**5**
L.s.d (0.05)	**1.4**
CV%	**21.6**

Seventy-eight progenies had scores of 1-4 (above average) accounting for 46% resistance above average (Appendix 3). Most of the evaluated progenies showed intermediate resistance/susceptibility (scores of 4-6) signifying that the populations in this generation are still segregating for CBB resistance. A total of eighteen progenies were susceptible in the greenhouse with scores of 7-9 (Appendix 3). Amongst the susceptibles, 12 came from Andean/Andean parents and 6 were Andean/Mesoamerican progenies (i.e 67% Andean/Andean against 33% Andean/Mesoamerican). Resistant progenies in the greenhouse came from both Andean/Andean and Andean/Mesoamerican parentage (Appendix 3). Out of 25 resistant progenies, 12 were from Andean/Mesoamerican parentage and 13 from Andean/Andean parents.

Correlation analysis for field and greenhouse scores showed that there was no statistically significant relationship (r = 0.0925) between field and greenhouse experiments.

4.5.2: Field and greenhouse evaluations of $F_{4.6}$ progenies

Assessment of the $F_{4.6}$ progenies for their reaction CBB was carried out in the field and greenhouse during 2010 season**.** Statistical analysis for the field evaluations showed that there were highly significant differences (p<.001) among the 144 $F_{4.6}$ progenies for their reaction to *Xf*260 and *Xf*410 isolates (Table 22).

Table 22: Analysis of Variance for CBB reaction of $F_{4.6}$ crosses to *Xf*260 and *Xf*410 isolates

Source of variation	d.f	m.s.	F pr.
Replication	2	1.6266	
Block/Replicate	33	1.2119	
Treatment	142	0.7441	<.001
Residual$^{\pi}$	199	0.3850	
Total	376		

$^{\pi}$Corrected for a single block for each replication

The mean score for the field evaluation was 5 (Table 23). Both positive (RMX 20) and negative (SEQ11) controls had average score of 5 indicating intermediate

reaction. Most progenies had mean score of 5 signifying segregation for resistance/susceptibility. The highly resistant progenies had scores of 3 (Table 21). Two of the 4 resistant progenies came from Andean/Meso-American parents (BRB265/VAX6-9, BRB265/VAX6-13) and the other two from Andean/Andean parants (BRB268/RMX19-1, SEQ1036/RMX20-12).

Table 23: Mean CBB scores for most resistant and the most susceptible crosses from the field

Cross Identity	Mean CBB
BRB265/VAX6-9	3
SEQ1036/RMX20-12	3
BRB265/VAX6-13	3
BRB268/RMX19-1	3
BRB211/VAX 3-15	4
RMA72/VAX6-21	4
RMA72/RMX19-33	4
BRB215/VAX6-5	4
BRB215/VAX6-3	4
BRB268/RMX19-13	4
RMA72/VAX6-14	4
RMA72/VAX6-12	6
CMB106/VAX3-3	6
RMA72/VAX6-2	6
RMA72/VAX6-35	6
RMA72/RMX19-16	6
Grand mean	**5**
L.s.d (0.05)	**1.1**
CV%	**13.3**

Out of 144 progenies evaluated in the field, a total of 71 (Appendix 4) had their average scores above the mean. A total of 6 progenies were susceptible in the field with scores of 6 (Appendix 4). Five of the susceptible progenies came from Andean/Meso-American parentage and only one from Andean/Andean cross.

Greenhouse evaluations for the $F_{4.6}$ progenies showed that there were significant CBB differences ($p<.001$) among the $F_{4.6}$ progenies for their reaction to *Xf*260 and *Xf*410 isolates (Table 24).

Table 24: Analysis of variance for CBB reaction of $F_{4.6}$ progenies to *Xf*260 and *Xf*410 isolates

Source of variation	d.f.	m.s.	F pr
Replicate	3	4.465	
Treatment(genotypes)	142	5.635	<.001
Residual	317	1.846	
Total	462	1205.136	

The greenhouse average score for the $F_{4.6}$ was 6; the resistant control (RMX20) had average score of 4 signifying intermediate resistance towards resistance. The suspetible control (SEQ11) had a score of 6. There were 55 progenies with CBB scores above the susceptible control. Amongst these susceptible progenies, 33 were from Andean/Andean crosses and 22 from Andean/Meso-American parentage (Appendix 5). The most resistant progeny (BRB215/VAX6-2) had average score of 2 and came from Meso-American/Andean parents (Table 25).

Table 25: Mean CBB scores for most resistant and the most susceptible crosses from the greenhouse

Cross Identity	**Mean CBB**
BRB215/VAX6-2	2
BRB214/VAX3-17	3
BRB265/VAX6-11	3
RMA72/VAX6-21	3
BRB214/VAX3-10	3
BRB265/VAX6-8	3
RMA72/VAX6-22	3
BRB264/VAX3-13	3
RMA72/VAX6-26	3
BRB268/RMX19-12	8
RMA72/RMX19-37	8
BRB214/VAX3-16	8
BRB264/VAX3-9	8
CMB106/VAX3-4	8
RMA72/RMX19-20	9
CMB106/VAX3-1	9
RMA72/VAX6-5	9
Grand mean	**6**
L.s.d (0.05)	**1.9**
CV%	**22.7**

A total of 9 out of 144 progenies had scores of 2 and 3 signifying resistance. All the 9 resistant progenies came from crosses that had either VAX3 or VAX6 as donor parent (Appendix 5). None of the Andean donors had resistant progeny in this generation. A total of 107 progenies were susceptible to the isolates with scores of 6-9 (Appendix 5). This showed that 74% of the progenies were susceptible to the isolates, only 0.06% resisted and 25.94% had intermediate reaction. From the susceptible progenies, 50% came from Andean/Andean crosses and 50% from Andean/Mesoamerican cross.

Correlation analysis for field and greenhouse scores showed that there was no statistically significant relationship (r=0.094) between field and greenhouse scores.

4.6 Marker assisted selection

MAS was conducted in Potchefstroom-ARC Biotechnology laboratory for the $F_{4.6}$ and $F_{3.5}$ progenies from parental combinations (parent 1 and 2) that were polymorphic for the markers (Table 26). Parents for both $F_{3.5}$ and $F_{4.6}$ progenies were screened for presence of the SCAR markers. Using PCR products for the parents (Plate 10), the markers present in the parents were detected.

Table 26: Parental combinations applicable for MAS

Parent 1	Marker	Parent 2	Marker
VAX 3	SAP6, SU91$^{\alpha}$	BRB 211	
VAX 3	SAP6, SU91*	BRB 214	SAP6
VAX 6	SAP6, SU91*	BRB 215	SAP6
VAX 3	SAP6, SU91*	BRB 264	SAP6
VAX 6	SAP6, SU91*	BRB 265	SAP6
RMAX 19	SAP6$^{\surd}$	BRB 268	
VAX 3	SAP6, SU91$^{\alpha}$	CMB 106	
RMX 2	SAP6$^{\surd}$	CMB 107	
VAX 3	SAP6, SU91$^{\alpha}$	RAA 21	
RMX 20	SAP6$^{\surd}$	RMA 70	
VAX 6	SAP6, SU91*	RMA 72	SAP6
RMX 19	SAP6$^{\surd}$	SAB 516	
BRB 266		RMX 19	SAP6$^{\surd}$
RMX 2	SAP6*	RMA 71	SAP6, SU91
SAB 514		RMX 8	SAP6$^{\surd}$
BRB 265	SAP6	VAX 3	SAP6, SU91$^{\alpha}$
BRB 267		VAX 3	SAP6, SU91$^{\alpha}$
BRB 266		RMX 2	SAP6$^{\surd}$
RMX 19	SAP6$^{\surd}$	SEQ 11	
VAX 3	SAP6, SU91$^{\alpha}$	SEQ 1003	
RMX 2	SAP6$^{\surd-+}$	DRK 149	
RMX 2	SAP6$^{\surd}$	SEQ 1004	
VAX 6	SAP6, SU91$^{\alpha}$	SEQ 1006	
VAX 6	SAP6, SU91$^{\alpha}$	RAA 21	
CMB 106		VAX 6	SAP6, SU91$^{\alpha}$
SAB 568		VAX 6	SAP6, SU91$^{\alpha}$
SAB 514		RMX 2	SAP6$^{\surd}$
SAB 576		RMX 2	SAP6$^{\surd}$
SAB 575		VAX 6	SAP6, SU91$^{\alpha}$
RMX 19	SAP6$^{\surd}$	RMA 70	
BRB 264	SAP6*	VAX 6	SAP6, SU91

$^{\alpha}$-Polymorphic for SAP6 and SU91. *Polymorphic for SU91. $^{\surd}$Polymorphic for SAP6

4.6.1 Marker Assisted Selection for the $F_{3.5}$ progenies

A total of 11 $F_{3.5}$ populations were evaluated in MAS. These populations came from the parental combinations that were polymorphic for the markers. PRC products were run on agarose gels and are presented in series of plates below.

Progenies from BRB265/VAX3 (Plate 11) population were selected for possessing SU91 since SAP6 was monomorphic for both parents. Using the phenotypic scores, 8

progenies were resistant, 16 intermediate and 2 susceptible (Appendix 6). The resistant plants had a mean CBB of 3, with a range of 2-3. Using genotypic scores, 50% of the resistant progenies had SU91 marker confirming successful transfer of resistance from VAX3, and 50% lacked the marker.

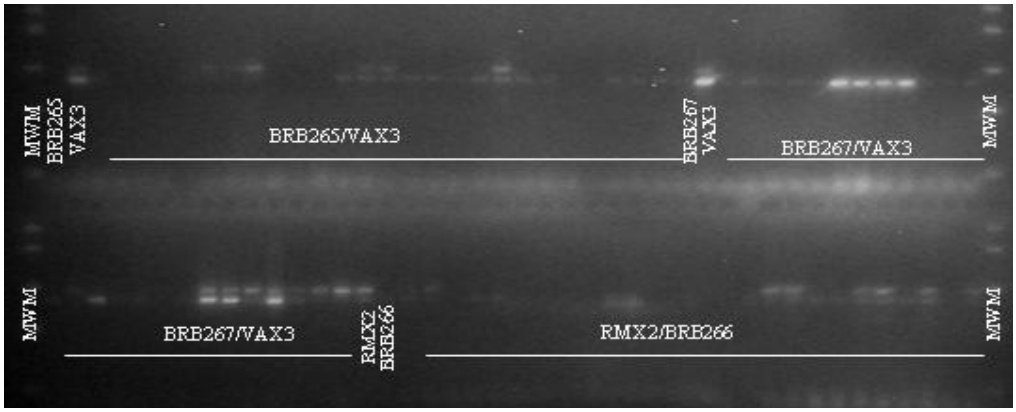


Plate 11: BRB265/VAX3, BRB267/VAX3 and BRB266/RMX2 populations (Evaluated for presence of SU91700$_{bp}$-lower band and SAP6820$_{bp}$-upper band)

A cross of BRB267/VAX3 was polymorphic for both SU91 and SAP6 (Plate 11). Out of 27 progenies in this population, 7 were resistant with CBB scores of 2 to 3 (Appendix 6). Six of the resistant progenies had SU91 band but lacked SAP6. One progeny (BRB 267-8/VAX3) had SAP6 with a score of 2. Progenies from a cross of BRB266/RMX2 (Plate 11) were scored for SAP6, a marker present in the donor RMX2. Out of 24 progenies from BRB266/RMX2 population, 4 were resistant with scores of 3, only one of the resistant progenies had both SAP6 and SU91 (Appendix 6). The other 3 progenies were resistant but without the marker.

A cross of Andean and Mesoamerican parent (SEQ1003/VAX3) had a total of 30 progenies and was scored for SU91 (Plate 12). Ten of the progenies had resistant phenotypic scores ranging from 1-3 (Appendix 6). Nine of the resistant progenies had SU91 marker, only one of the lacked both SU91 and SAP6 marker.

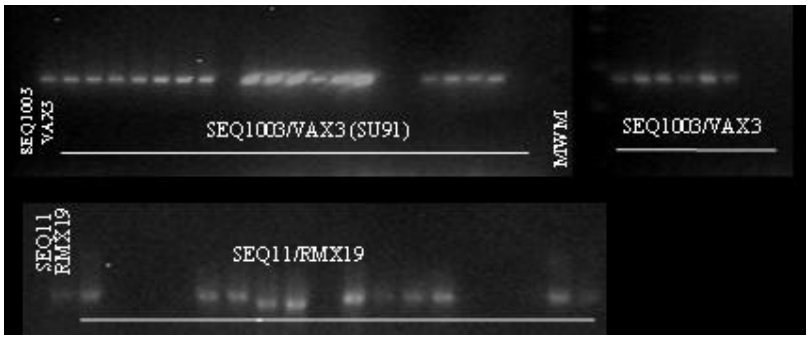


Plate 12: SEQ1003/VAX3 and SEQ11/RMX19 populations (screened for presence of SU91700$_{bp}$-lower band and SAP6820$_{bp}$-upper band)

Two of the progenies from the Andean/Andean (SEQ11/RMX19) population were scored as resistant with scores of 2 and 3 (Appendix 6), they both possessed SU91 marker though their donor parent only possessed SAP6 marker (Plate 12).

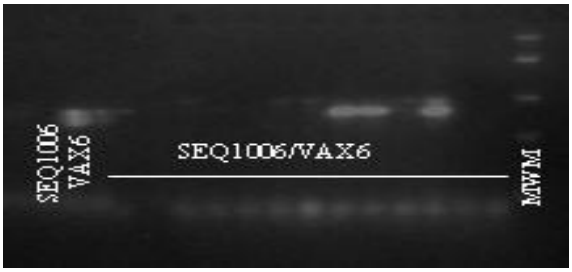


Plate 13: SEQ1006/VAX6 population (Evaluated for presence of SU91700$_{bp}$-lower band)

Progenies from SEQ1006/VAX6 (Plate 13) were scored for SU91 marker. The population had 12 progenies (Plate 13) four of them were phenotypically resistant (Appendix 6). Three of the resistant progenies had SU91 marker, one progeny lacked the marker (Appendix 6).

A cross of BRB264/VAX6 was scored for SU91, the marker that was polymorphic in the parental lines (Plate 14). The population had 10 progenies. One out of ten progenies was resistant with phenotypic score of 3 but did not have the marker (Appendix 6).

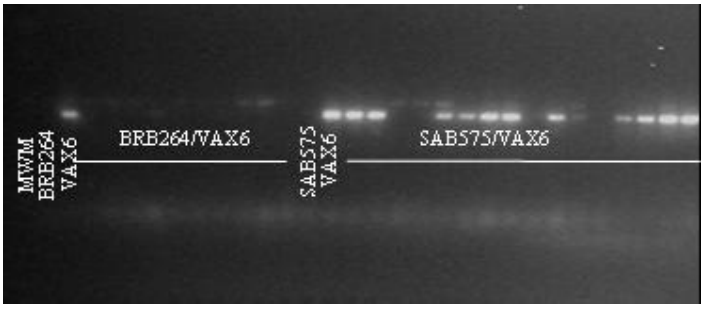


Plate 14: BRB264/VAX6 and SAB575/VAX6 populations (Screened for presence of SU91700$_{bp}$-lower band and SAP6820$_{bp}$-upper band)

Progenies from SAB575/VAX6 population were scored for both SAP6 and SU91 marker. A total of 9 out of 16 progenies in this population had resistant phenotypic scores (1-3), six of the resistant genotypes had SU91 marker (Plate 14), one genotype with a score of 3 had SAP6 marker and two progenies with average phenotypic scores of 2 possessed both SAP6 and SU91 marker (Appendix 6).

A cross of two Andean parents; RMA70/RMX19 (Plate 15) had average score of 4 (Appendix 6). A total of 16 progenies were tested for presence of the SAP6 marker, five out of six phenotypically resistant progenies had SAP6 marker and one progeny lacked the marker. Crosses of SAB568/VAX6 and RAA211/VAX6 did not have any progenies that had resistant scores (Appendix 6). Genotypic screening showed that all the progenies from these populations (SAB568/VAX6 and RAA211/VAX6) had SAP6 marker (Plate 15).

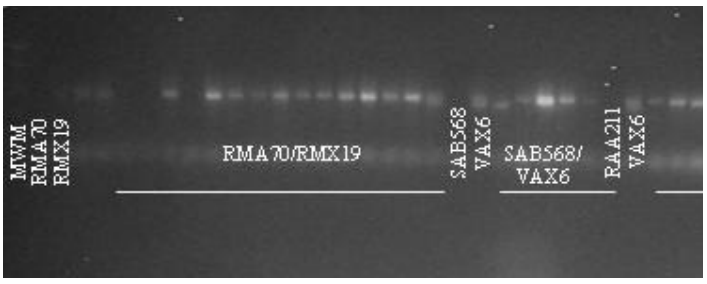


Plate 15: RMA70/RMX19, SAB568/VAX6 and RAA21/VAX6 populations (screened for presence of SU91700$_{bp}$-lower band and SAP6820$_{bp}$-upper band)

All the populations in this $F_{3.5}$ generation had their p value from regression analysis greater than 0.05 expect SEQ1006/VAX6 population which was significant for a

combination of SAP6/SU91 (p=0.004) but not significant for the either SAP6 or SU91 alone. The regression indicated that there were no significant differences in the progenies carrying either one marker (SAP6 or SU91) or both (SAP6/SU91) when compared with the phenotypic scores. Although the markers (SU91 and SAP6) did not have significant correlation (p>0.005) with the phenotypic scores, there were evidence of successful transfer resistance in some progenies (Table 27).

Table 27: $F_{3.5}$ CBB reaction per population and their presence of SCAR markers

Population	CBB reaction *	SAP6+/ SU91+	SU91-/ SAP6-	SAP6+	SAP6-	SU91+	SU91-
BRB265/VAX3	Resistant					4	4
	Intermediate					9	7
	Susceptible						2
BRB267/VAX3	Resistant			1		6	
	Intermediate	3	4			5	
	Susceptible	2	1	2		3	
BRB266/RMX2	Resistant	1	3				
	Intermediate	2	7			4	
	Susceptible	3				4	
SEQ11/RMX19	Resistant					2	
	Intermediate		6	8			
	Susceptible		2	1			
SEQ1003/VAX3	Resistant		1			9	
	Intermediate		6			12	
	Susceptible					2	
SEQ1006/VAX6	Resistant					3	1
	Intermediate					1	4
	Susceptible						3
BRB264/VAX6	Resistant						1
	Intermediate					1	2
	Susceptible						6
SAB575/VAX6	Resistant	2		1		6	
	Intermediate	1	2	1		2	
	Susceptible	1					
RMA70/RMX19	Resistant			5	1		
	Intermediate			8	2		
	Susceptible						
SAB568/VAX6	Resistant						
	Intermediate			2			
	Susceptible			4			
RAA211/VAX6	Resistant						
	Intermediate			3			
	Susceptible						

* Resistant = 1-3, Intermediate = 4-6, susceptible = 7-9

The summary of the gel results reported in the Plates 11-15 indicate that there were resistant progenies in all the populations except two Andean/Meso-American populations of SAB568/VAX6 and RAA211/VAX6 (Table 27). However, the intermediate progenies in the RAA211/VAX6 population had SAP6 marker. In the SAB568/VAX6 population both susceptible and intermediate progenies possessed the SAP6 marker. Most of the progenies in this generation had intermediate reaction to CBB. Amongst the 51 resistant progenies, 30 had SU91 and 7 had SAP6 marker, the remaining 14 progenies possessed either a combination of the two markers or had no marker (Table 27). Most of the resistant progenies came from Andean/Meso-American parents. There were resistant genotypes in the Andaean/Andean populations of RMA70/RMX19, SEQ11/RMX19 and BRB266/RMX2.

4.6.2 Marker Assisted Selection for the $F_{4.6}$ progenies

A total of 9 populations in the $F_{4.6}$ populations were polymorphic for SU91, SAP6 or a combination of SAP6/SU91 markers and were evaluated in MAS. Gel photographs were used to score for the presence of the marker.

Population of BRB211/VAX3 had a total of 27 progenies (Plate 16) and a population score of 5 (Appendix 7). The progenies were screened for SU91 marker. Three progenies had resistant CBB reaction and two of them had SU91 marker (Appendix 7).

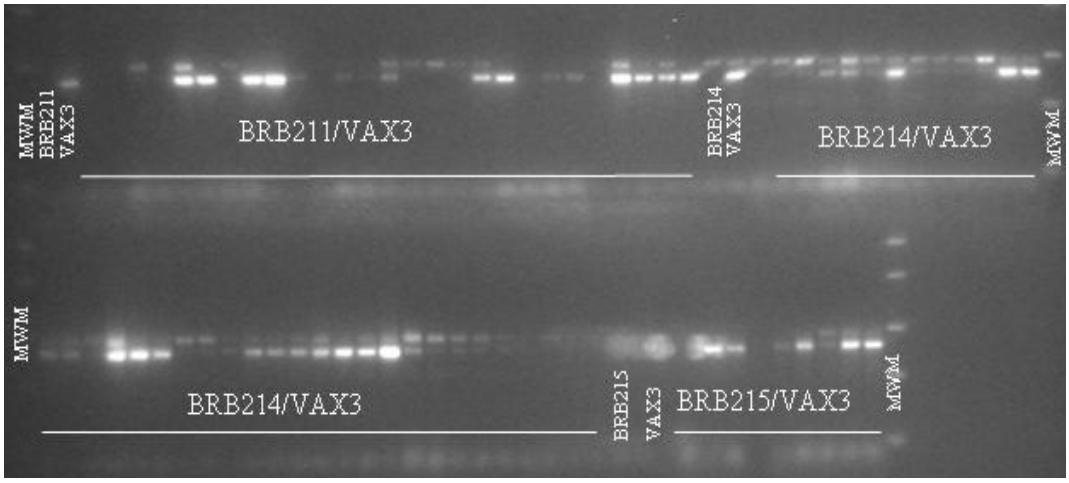


Plate 16: BRB211/VAX3, BRB214/VAX3 and BRB215/VAX3 populations (Evaluated for presence of $SU91700_{bp}$-lower band and $SAP6820_{bp}$-upper band)

A cross of BRB214/VAX3 was polymorphic for SU91 marker (Plate 16). The population had 37 progenies, 10 of them had resistant scores and they all possessed the SU91 marker (Appendix 7). A cross of BRB215/VAX3 had 10 genotypes that had intermediate reaction to CBB (Appendix 7). Although they had intermediate reaction, nine of the 10 progenies carried the SU91 marker (Plate 16).

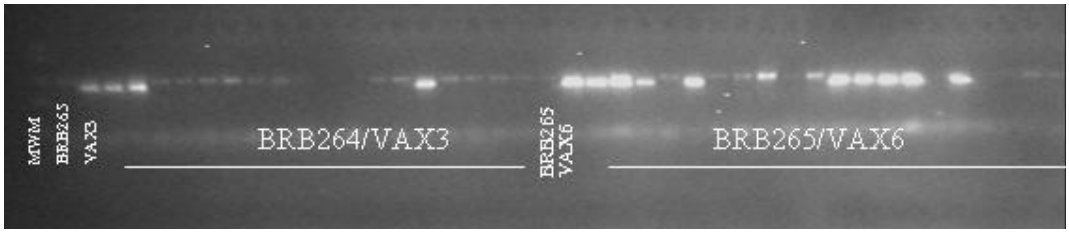


Plate 17: BRB264/VAX3 and BRB265/VAX6 populations-(evaluated for presence of SU91700$_{bp}$-lower band and SAP6820$_{bp}$-upper band)

A total of 18 progenies from BRB264/VAX3 were evaluated for their CBB resistance and screened for their presence of SU91 marker (Plate 17). Three out of 18 progenies had scores of 3 from the greenhouse (Appendix 7), two of these resistant progenies possessed SU91 marker and one lacked the marker. Population of BRB265/VAX6 had an average CBB score of 5 (Appendix 7). Scoring for SU91 marker was done on the 20 progenies (Plate 17). Six of the phenotypically resistant genotypes possessed the SU91 marker.

Cross of two Andean parents (CMB107/RMX2) had a population score of 6 (Appendix 7). There were no resistant progenies from this population though most of them carried the SAP6 marker (Plate 18).

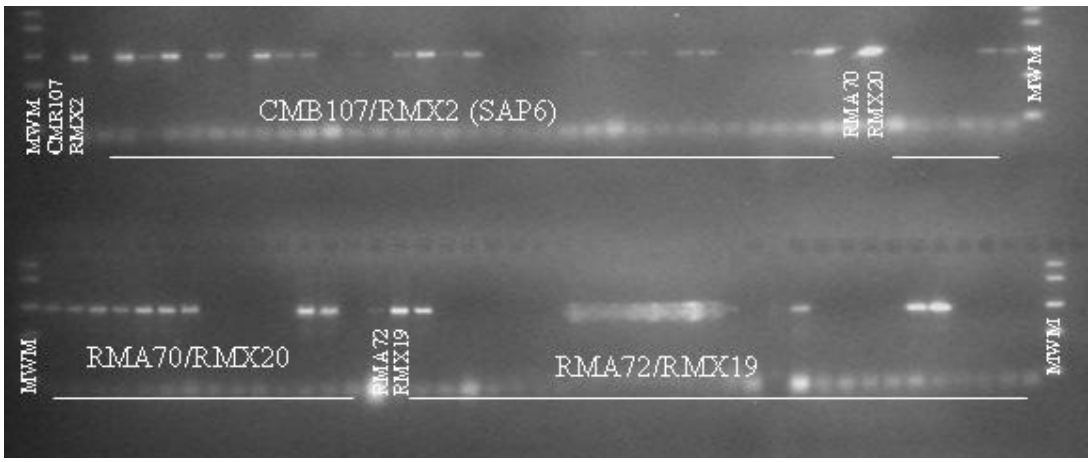


Plate 18: CMB107/RMX2, RMA70/RMX20 and RMA72/RMX19 populations (screened for presence of and SAP6 820$_{bp}$)

Population of RMA70/RMX20 was screened for SAP6 marker (Plate 18). None of the 19 progenies had a resistant score though most of the progenies with intermediate reaction had SAP6 (Appendix 7). Another cross between large seeded lines; RMA72/RMX19, did not yield resistant progenies but SAP marker was present in both intermediate and susceptible progenies.

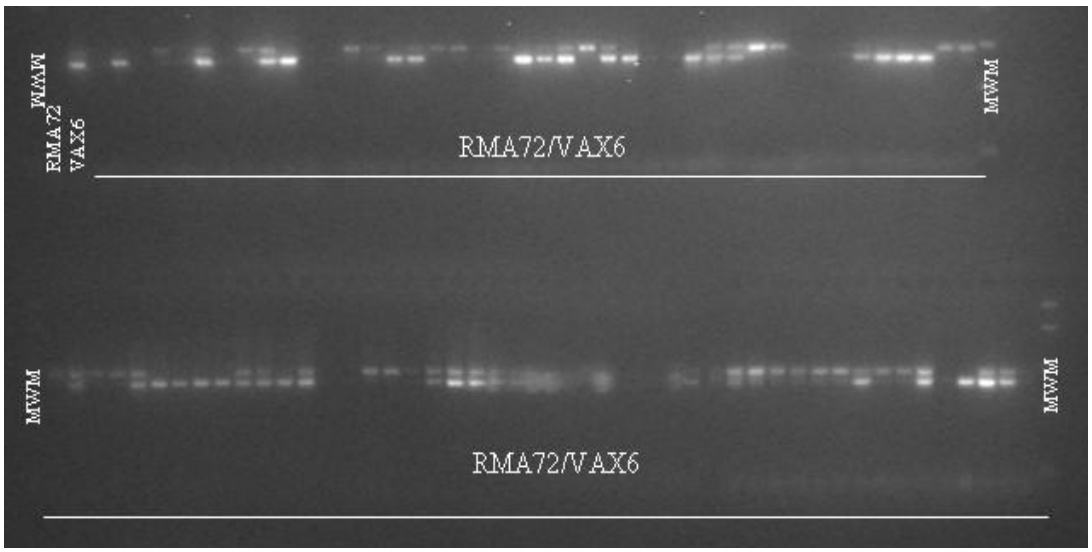


Plate 19: RMA72/VAX6 population (screened for presence of SU91700$_{bp}$-lower band and SAP6820$_{bp}$-upper band)

A cross of RMA72/VAX6 had 88 progenies and was screened for both SAP6 and SU91 markers (Appendix 7). A total of 21 progenies were resistant and they had either SU91 marker or a combination of SAP6/SU91 (Plate 19). None of the resistant progenies possessed SAP6; but the marker was present in both susceptible and intermediate genotypes. Some susceptible lines also possessed both SU91 and SAP6 markers.

Regression analysis showed highly significant relationship ($p<0.001$) between phenotypic scores and marker scores for SU91 in BRB265/VAX6 population. The relationship was also significant ($p<0.001$) for CBB scores and presence of SU91 in the RMA 72/VAX6 population. In the same population (RMA 72/VAX6) there was statistically significant relationship ($p<0.001$) between phenotypic scores and genotypic scores for progenies that had both SAP6/SU91.

Evaluation of individual populations indicated that genotypes from Andean/Andean parents did not resist the isolates (Table 28). Though the Andean/Andean crosses (CMB107/RMX2, RMA70/RMX20 and RMA72/RMX19) did not have any resistant progenies, most of the intermediate and susceptible genotypes possessed the SAP6 marker (Table 28).

Table 28: F4.6 CBB reaction (phenotyped in ARC-Potchefstroom greenhouse-2010) and for the presence of SCAR markers SU91 and SAP6

Population	CBB reaction	SAP6+/ SU91+	SU91-/ SAP6-	SAP6+	SAP6-	SU91+	SU91-
BRB211/VAX3	Resistant					2	1
	Intermediate					14	6
	Susceptible						4
BRB214/VAX3	Resistant					10	
	Intermediate					13	3
	Susceptible					8	2
BRB215/VAX3	Resistant						
	Intermediate					6	1
	Susceptible					2	
BRB264/VAX3	Resistant					2	1
	Intermediate						5
	Susceptible					1	9
BRB265/VAX6	Resistant					6	
	Intermediate					3	5
	Susceptible						6
CMB107/RMX2	Resistant						
	Intermediate			12	12		
	Susceptible			6	2		
RMA70/RMX20	Resistant						
	Intermediate			8	6		
	Susceptible			3	2		
RMA72/RMX19	Resistant						
	Intermediate			4	6		
	Susceptible			9	8		
RMA72/VAX6	Resistant	15				9	
	Intermediate	14	12	15		4	
	Susceptible	6	7	7			

Andean/Mesoamerican crosses had more of the intermediates than the resistant progenies (Table 28). The intermediate genotypes from both Andean/Andean and Andean /Mesoamerican parents possessed either SAP6 or SU91 marker. None of the

progenies in this generation possessed SAP6 and resisted the isolates, most of the resistant progenies had SU91 marker, and the other resistant progenies had a combination of SAP6/SU91 while others were resistant without the marker.

Reaction of the two generations ($F_{3.5}$ and $F_{4.6}$) to the South African *Xf*260 and *Xf*410 isolates indicated that both generations are still segregating for their resistance to CBB. The mean score for $F_{3.5}$ was 5 for both greenhouse and field experiments (Table 19 and 21). The $F_{4.6}$ generation had mean score of 5 from the field and 6 from the greenhouse (Table 23 and 25 respectively). Both generations had most of their progenies with scores of 4-6 (Appendix 2-4) signifying segregation of the progenies to their CBB resistance. There were evidence of successful transfer of resistance in both generations; this was evidenced by presence of resistant progenies (Table 27 and 28). Most resistant progenies possessed SU91 marker and some a combination of the two markers (SAP6/SU91), SAP6 was also detected in some resistant progenies. The intermediate and resistant progenies in both generations had either SAP6 or SU91; however, most of them possessed the SAP6 marker.

CHAPTER FIVE

5.0 DISCUSSION:

The main objective of the current study was to select CBB resistant genotypes to be used as parental lines for production of disease free seed in order to increase bean productivity. Specifically, the study aimed at identifying parental traits that support conventional breeding and MAS for CBB tolerance. Secondly, the study was to detect the presence of SCAR markers associated with CBB resistance in bean breeding populations and lastly, asses the efficiency/effectiveness of the SCAR markers in MAS. Each specific objective had activities that lead to its achievement.

5.1 Evaluation of parental lines to CBB resistance and identifying traits that support conventional breeding and MAS

5.1.1 Assesment of parental lines for agronomic traits and resistance to CBB

High yields were recorded for the parental lines with grand mean of 1816.99 kg/ha. The highest yielding lines had large seed size (>40g/100g) i.e are of Andean center of origin and they had intermediate CBB scores (4-6). Improving CBB resistance in the high yielding Andean parentals through inter-gene pool hybridization lines is potential for the development of cultivars with both improved resistance and high yielding. The limiting factor in the inter gene pool is the incompatibilities that exist in the progenies from the hybridization of the two gene pools. Johnson and Gepts, (1999) found that crosses between two gene pools potentially provide a source of additional genetic diversity but their progenies have been characterized by phenotypic abnormalities and reduced productivity. Genetic variation is potential for the development of improved cultivars, but sometimes, incompatibilities between the nuclear and cytoplasmic genomes may be detected in the progenies between the two gene pools of common bean (Yu, *et al.,* 1998; Gallegos *et al.*, 2007).

Despite the high yields in the Andean large seeded lines, there was negative correlation (r-0.981) between seed yield and seed size. These results indicate that the two variables are not related though the coefficient of determination (r^2 =0.962361) showed that 96% of yield was explained by variation in seed size. The negative correlation results are consistent with Sexton *et al.,* (1998) findings that seed yield is negatively associated with cultivar seed size in common bean (*Phaseolus vulgaris* L.).

Furthermore, Johnson and Gepts, (1999) found association between seed weight in common bean to be negatively correlated with seed yield.

CBB was negatively correlated with the agronomic traits measured in the greenhouse during 2010. The negative relationship provides evidence of the damaging effects of this disease on bean production. The findings on the significant ($P<0.001$) negative correlation (-0.917) between seed yield and CBB corresponds with previous reports that high disease score and low seed yield as well as other seed related traits are significantly negative related (Tar'an *et al.*, 2001). There were also significant ($P<0.001$) negative correlation (-0.944) between CBB and days to maturity of the parental lines. Delayed maturity and other agronomic traits, including growth habit, days to flowering, canopy height and width, branching pattern, lodging, and days to maturity were found previously to be significantly associated with disease severity index (Kolkman and Kelly, 2002**).** For selected traits related to seed yield components the R^2 values indicate that CBB explained most of the variation in seed yield per se ($R^2= 0.84$) and days to maturity ($R^2= 89$).

There was a low positive correlation coefficient r = 0.08 between disease rating and yields obtained during 2009 off season. These results indicated that CBB did not have any significant effect on seed yield during the off season because of low pressure of the disease. CBB infection occurs most readily during warm, wet weather, especially hard, wind-driven rains (Schwartz *et al.,* 2007). The evaluation of the parental lines was carried out during the off season when temperatures are usually low and dry, hence low CBB incidence and severity. On the other had, the trial was irrigated using fallow/flooding method which is not a good dispersal mechanism for the disease. Previous reports indicate that bacteria are disseminated within and among fields by splashing water especially when using overhead irrigation (Schwartz *et al.,* 2007).

5.1.2 Assessment of parental lines for CBB resistance to *Xf*260 and *Xf*410 South African Isolates

Reaction of parental lines to *Xf*260 and *Xf*410 isolates indicated that RMX donor parents were segregating for their resistance. VAX 3 and VAX6 had low scores (1 and 2) signifying resistance. The behaviour of the VAX lines was expected since they

were specifically bred for CBB resistance and that level of resistance possessed in VAX 3, VAX 4, and VAX 6 are as high as those found in *P. acutifolius* accessions. (Singh and Munoz, 1999). Unexpected results were for RMA 71, a donor parent for ALS which had lower CBB score (4) than donor parents RMX 2 (5) and RMX 19 (5), its score was not significantly different from RMX 20 which had a score of 4. Disease severity of the RMX lines may be attributed to virulence of the isolates used in the study. Isolate virulence are evident though physiological specialisation on *P. vulgaris* is unknown (Fourie, 2002). These results are also supported by previous results that the role of specific QTL and interaction between QTL on the expression of resistance to CBB can be influenced by the isolates of *Xap* used to infect the plants (Vandemark, *et al*, 2009).

The other factor that may have contributed to the resistance of VAX lines and susceptibility of RMX lines is that RMX lines are of Andean origin and VAX lines are Meso- American origin, although they were both bred for CBB resistance, reports indicate that susceptibility in large seeded bean types (Andean origin) is more problematic than in small seeded (Beebe and Pastor-Corrales, 1991). On the other hand, both donor parents (RMX and VAX lines) were developed in Colombia and this current study tested them using African isolates, the findings that the RMX lines of Andean origin were intermediate and susceptible to the African isolates, correspond with findings by Marquez *et al.,* (2007) that African *Xcp* isolates were highly pathogenic on large-seeded Andean common bean and significantly less pathogenic on small-seeded. In addition, Singh and Munoz (1999) documented that, a *Phaseolus* genotype may show resistance to some strains of bacterium but susceptible to others and Fourie, (2002) reported that, since CBB is controlled by QTL, its expression may differ over environments or population.

Susceptibility of the RMX donor parents may also mean that their resistance is not yet stable. Such instabilities in CBB donor parents were previously documented. Resistance instabilities have also been reported in XAN159 and its progenies; however, it is still used widely in resistance breeding programmes (Fourie, 2002).

5.1.2 Parental traits that can support MAS

The parental lines were screened for presence of marker BC420, SAP6 and SU91. All the three markers were amplified in a single PCR reaction as reported by Miklas *et al*., (2005) that all the three markers can be multiplexed in a single PCR reaction to expedite MAS for combined resistance to CBB. Although multiplexing is possible for these CBB markers, extensive optimization is a necessity because primer-dimers and other non-specific products may interfere with amplification of specific products. Results in Plate 7-9 indicate that type of enzyme, DNA concentrations and dNTP concentrations are some of the optimization steps that are crucial in multiplex reactions.

Marker screening in the parental lines showed that none of the parental lines used in the development of the populations had BC420 (Plate 10). SCAR marker BC420 was only present in BBSS5 and BBSS 113 parental lines from South Africa (data not shown). The RMX donor parents only had SAP6 and the VAX parents had both SAP6 and SU91. Presence of one marker (SAP6) in the RMX parental lines may have affected their level of resistance. Resistance to CBB in common bean is a quantitative trait, and, as a result, the level of resistance conferred by a single QTL is partial, developing 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; Nodari *et al.,* 1993). Results from O'Boyle *et al*., (2007) indicated that; the combination of multiple QTL for CBB resistance provides a higher level of resistance than that conferred by single QTL.

The two VAX lines (VAX3 and VAX6) had SAP6 and SU91 marker but lacked BC420, they however exhibited lower CBB scores (Table 15) same as those in the South African donors which posses three makers (SAP6, SU91, BC420). There were no significant statistical differences between the VAX lines with SAP6 and SU91 and South African lines with additional marker BC420 (Table 17). These findings are consistent with, Singh and Munoz, (1999) who reported that some lines with two of three QTL alleles, such as VAX 3 and VAX6 can have very high levels of resistance as those found in *P. acutifolius* accessions. Other lines with only two QTL alleles, however, only have intermediate resistance, which indicates minor QTL alleles are also important when breeding for durable resistance to CBB (Liu *et al.*, 2007).

The use of BC420 marker is not practical in certain market classes as it is linked to the V locus that results in an undesirable darker seed coat colour (Miklas *et al.*, 2006; Mutlu *et al.*, 2005). Absence of marker BC420 in the VAX lines could also indicate that the linkage between the V-locus and resistance gene has been broken and that although resistant gene may be present, the marker is absent (Fourie, 2002). The possibility also exist that other untagged genes could contribute to the high levels of resistance present in the VAX lines, as genes from different sources have been pyramided into these lines (Fourie, 2002).

Surprising results were for ALS donor parent RMA71 which had both SU91 and SAP6 markers. In addition, SAP6 marker was present in most of the recipient parents (Plate 10 and Table 17). Presence of SAP6 in both resistant and susceptible parents was not a surprise, these findings corresponds with Duncan *et al.,* (2008) who reported that there were no significant differences in CBB severity for plants with or without SAP6, and that SAP6 marker was found in both resistant and susceptible cultivars. Assessment of the parental lines for their genotypic markers helped in determining the populations that were polymorphic for the markers and the applicable for MAS.

5.2 Detecting presence of CBB SCAR markers (SU91, SAP6 and BC420) in breeding populations and effectiveness of the SCAR markers in MAS.

5.2.1 Phenotypic selection for $F_{3.5}$ and $F_{4.6}$ progenies

A combined screening (MAS and phenotypic) was applied to the $F_{3.5}$ and $F_{4.6}$ progenies for their resistance to CBB. The combined strategy has been reported by many others as the most effective in developing lines with improved CBB resistance and that phenotypic selection is needed to retain minor effect QTL and select for epistatic interactions that contribute to improved resistance (Miklas *et al.*, 2005). Fourie, (2002) reported that use of molecular marker alone to select for resistance does not always result in lines with superior resistance, some minor genes contributing to CBB resistance are lost when relying on markers only. The combined use of both phenotypic screening and molecular markers is, therefore important in developing CBB resistance lines.

There were high statistic significant differences among the $F_{3.5}$ ($p<0.001$) and $F_{4.6}$ ($p<0.001$) progenies in their reaction to South African isolates (*Xf*260 and *Xf*410) adjusted to 10^8 CFU/ml concentration. Bean researchers have used *Xcp* and *Xcpf* concentrations of 10^7 to 10^8 cfu mL^{-1} for germplasm screening, genetics, and breeding purposes (Asensio-Manzanera *et al*., 2006; Navarrete and Acosta, 2005; Miklas *et al*., 1996). Marquez *et al.,* 2007; reported that a density of 10^7 cfu mL^{-1} or less may not differentiate among CBB susceptible, intermediate, and resistant common bean genotypes in some greenhouse and filed environments

The correlation for CBB scores between the greenhouse and field experiment was 0.0925. for $F_{3.5}$ and 0.094 for $F_{4.6}$. The correlation coefficients ($r = 0.0925$ and 0.094) indicated a weak relationship between CBB scores from the two experiments. The weak relationship signified that the progenies evaluated in the greenhouse differed with the field genotypes to their CBB reaction though the isolates were the same. The CBB differences may be due to differences between the two experiments screened under different environments (greenhouse vs. field) and also because of the differences in inoculation and evaluation methods. These results are consistent with Liu, *et al.,* (2007) who reported CBB rating differences between two experiments screening under different environments (greenhouse vs. field). Contrary to Seattler, (1991), who reported that inoculum concentrations ranging from 3-6x10^7 cfu/ml gave greenhouse reactions that correlated with reactions observed in the field, the concentration of inoculum used in this experiment was adjusted to 10^8 CFU/ml, hence no correlation between the two experiments. In addition, Fourie, (2002), indicated that climate and environmental conditions can influence more disease development in the field and possibilities of escapes also exist.

The performance of the two generations did not differ though the two are in different generations. They both had RMX2, RMX19, RMX20, VAX3, and VAX6 as donors, all the recipients were of Andean origin hence some similarities. The mean CBB scores for $F_{3.5}$ in the greenhouse and field was 5, $F_{4.6}$ had mean of 5 from the field and 6 from the greenhouse. The intermediate mean scores for both generations indicated segregating reaction and instability of CBB resistance though the populations have been selfed for half a dozen times. Fourie, (2002); Singh and Munoz, (1999), reported that the most serious problem in CBB resistance breeding has been the instability of

resistance because segregation has been recorded in populations after more than twelve generations of selfing.

The highest resistant progeny for field and greenhouse in the $F_{3.5}$ and $F_{4.6}$ generation came from a cross between Mesoamerican donor (VAX lines) and an Andean recipient this indicated successful transfer of resistance, also signified the presence of high resistance in the Mesoameriacn donor lines hence need for broadening the genetic base of cultivars and inter-gene pool hybridization (Singh *et al*., 2002). Mesoameriacans possess more favorable alleles and QTL imparting higher level of resistance to both biotic and abiotic stress than their large-seeded Andean counterparts (Asensio *et al.,* 2005).

Surprisingly, two of the progenies from Andean/Andean cross in the $F_{4.6}$ generation had high resistance (scores of 3). Presence of high resistance in Andean/Andean progenies (SEQ1036/RMX20-12 and BRB268/RMX19-1) shows that improving resistance in the Andean lines may yield lines that have high resistance same as progenies that have Mesoamerican background. These findings also indicate that there are gains from the interracial hybridization. Interracial hybridization broadens the genetic base of cultivars and gains from selection (Singh *et al*., 2002). On the other hand, a cross between a Mesoameraican and Andean had the highest susceptibility (score of 9) in both $F_{3.5}$ and $F_{4.6}$ generations (Tables 21 and 25) this may mean unsuccessful transfer of the resistance QTL but may also be an indication of incompatibility between the two races. Previous reports indicate that barriers such as genotype incompatibility, early embryo abortion, hybrid sterility and lower frequencies of hybridization occur in the crosses from inter-gene pool (Fourie, 2002). Yu *et al*., (1998), reported of incompatibilities between the nuclear and cytoplasmic genomes of Andean and Meso-Ameriacan gene pools.

5.2.2 Marker assisted selection in the $F_{3.5}$ and $F_{4.6}$ progenies

Successful applications of marker-assisted selection (MAS) in breeding for quantitatively inherited disease resistance in various crop plants have been reported (Mutlu *et al*., 2004). MAS results for this study indicated that, SU91 marker was associated with those progenies that showed higher resistance in both $F_{3.5}$ and $F_{4.6}$

generations. Such results were previously obtained by O'Boyle *et al*., (2007), who reported that the presence of the SCAR marker SU91 was clearly associated with high levels of CBB leaf resistance.On the contrary, SAP6 was found in most of the intermediate and susceptible progenies. This indicated that the isolates X*f*260 and X*f*410 used in the artificial inoculation were more aggressive on the progenies such that SAP6 was suppressed and could not be present in the resistant genotypes. It is also possible that the severe disease environment masked any effect SAP6 might have on resistance to CBB in the different populations tested in this study. Vendemark *et al*., (2009) reported that screening of populations using artificial inoculation of plants in the greenhouse with African isolates *Xap* (*Xf*260 and *Xf*410) resulted in more severe disease than was observed during initial screening with *Xap* isolates from Dominican republic. This clearly indicates that *Xf*260 and *Xf*410 isolates are aggressive.

There were some progenies from BRB266/RMX19 and SEQ11/RMX19 populations that possessed SU91 marker (Table 27). Presence of SU91 in the progenies was a surprise since none of the parents carried SU91 marker. However, there are high chances that this marker SU91 could have been inherited from parents used to develop the donor parent RMX 2 ((A483 X MONTCALM) X ((MAM 48 X A 486) X (VAX 2 X KABOON)) and RMX19 (CAL143 x ((A 483 x G 6416) x (VAX3 x AFR298))). Perez and Kelly, (2008), documented findings where presence of SU91 marker in PTD99099 was a surprise as the marker is derived from a tepary resistance source but pedigree PDT99099 did not indicate such connection.

5.2.3 Effectiveness of the SCAR markers in MAS

Using direct selection, 36 of the $F_{3.5}$ and 82 of the $F_{4.6}$ genotypes were susceptible (Tables 27 and 28). In fact, 61% of the $F_{3.5}$ susceptible plants, 51% of the $F_{4.6}$ and hundreds of the intermediates from both generations had the SCAR markers (either SAP6, SU91 or combination of the two). These results strongly agree with the need to combine phenotypic and MAS selection. All the plants which had markers present could have been scored as resistant because they possessed the markers yet they are phenotypically susceptible. Selection of such plants (susceptible but with the marker

present) would increase the time and the cost of the development of a resistant line (Duncan *et al*., 2008).

Some progenies possessed both markers but were intermediate and susceptible to the isolates. The failure of the combination of both markers to provide elevated levels of resistance may suggest some type of episitatic interaction of these QTL in common bean disease-resistance pathway or uneven distribution of marker genotypes in the study (O'Boyle *et al.*, 2007).

A total of 51 $F_{3.5}$ plants were phenotypically resistant to X*f*260 and X*f*410 South African isolates. On comparing marker results with inoculation test data, 40 of 51 resistant plants had marker band indicating an accuracy of 78%. Conversely 22% of the resistant plants did not contain any of the markers. Using direct selection, 46 plants in the $F_{4.6}$ generation were resistant. Based on marker information, 44 of the resistant plants had the marker band indicating 96% accuracy. Only 4% of the phenotypically resistant plants did not have the marker band. Absence of the marker in the resistant plant may mean that these plants have the resistant QTL but lack the marker, resistant QTL may be present in some plants that have no marker i.e the marker may be deleted during crossover and may not segregate together with the QTL (Fourie, 2002). Duncan *et al.,* (2008), found some percentage of CBB resistant plants which did not contain any of the SCAR markers, demonstrating the quantitative nature of CBB resistance and reported on the need for the development of additional molecular markers linked with these QTLs. These findings also emphasize on the need for phenotypic selection besides MAS. Phenotypic selection is needed to retain minor effect QTL and select for epistatic interactions that contribute to improved resistance (Miklas *et al*., 2005).

Out of the 40 plants with markers in the $F_{3.5}$, 30 had SU91 marker, 7 had SAP6 and 3 had a combination of both markers. A total of 29 plants out of 44 in the $F_{4.6}$ had SU91 marker present, 15 plants had a combination of SU91/SAP6 and none of the resistant plants had SAP6. SCAR marker SAP6 was present in most plants with segregating and susceptible reaction. Some susceptible plants possessed a combination of both markers (Table 31 and 32). This signifies that there may be some epistatic interaction between the two QTLs. O'Boyle *et al*, (2007) reported that, failure of combination of

both markers to provide elevated levels of resistance may suggest some type of epistatic interaction of these QTL in a common disease-resistance pathway.

The marker results show that SAP6 did not contribute to resistance in most of the populations. Vandemark *et al.,* (2009), reported that SAP6 has been associated with resistance to CBB in other populations but it has also been previously detected in bean cultivars susceptible to CBB reflecting specificity of SAP6 towards distinct races of *Xap* isolates. Furthermore, previous reports indicate that the lack of effect of SAP6 towards resistance to CBB could be due to recombination between the SAP6 marker and the QTL conditioning resistance (Vandemark *et al.,* 2009).

Use of the combination of isolates (X*f*260 and X*f*410) examined that SU91 was more influential in reaction to CBB than SAP6. However, previous results suggest that the role of specific QTL and interaction between QTL on the expression of resistance to CBB can be influenced by the population examined and the isolates of *Xap* used to infect the plants (Vandemark *et al*., 2009).

In general, there was no correspondence between the CBB resistant plants and the presence of the SCAR markers. These findings indicate that not all resistant plants had the marker band and not all plants that possessed the marker were resistant. Similar results were also reported by Yu, (1999), that the number of lines resistant to CBB and the number of lines with the SCAR marker was much lower than the expected and that there might be either a gametic or a zygotic selection pressure against CBB resistance or an unknown genetic factor tightly linked to the QTL for CBB resistance that was under negative selection pressure.

CHAPTER SIX

6.0: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The overall objective of the study was to select CBB resistant genotypes and from the findings of this study, it is concluded that, 51 of the $F_{3.5}$ and 46 of the $F_{4.6}$ progenies were resistant with phenotypic scores of 1-3. These progenies will be advanced and later used in breeding programmes as donor parental lines or as breeders' seed.

The first specific objective of the study aimed at identifying traits that can support MAS and conventional breeding. Results obtained pointed to seed yield and days to maturity as the traits of importance and are affected by CBB. Susceptibility to CBB is manifested through reduction of seed yield while resistance is associated with delayed maturity and relatively higher seed yield. Traits like flower colour, pods length and number of pods per plant, seed size are not affected by CBB and may not be selected for as phenotypic markers for CBB in conventional breeding. Parental survey for genotypic markers and determining their polymorphism is an essential initial step in MAS. Information on marker present in both donor and recipient parents helps in choosing populations that are applicable for MAS.

Secondly; the study aimed at detecting the presence of SCAR markers associated with CBB resistance in bean breeding populations. None of the parental lines possessed BC420 and it was not tested for in the progenies. SAP6 was present in most of the progenies evaluated but it was not associated with phenotypic resistance. Presence of SU91 marker was associated with phenotypic resistance though some progenies possessed the marker but were intermediate and susceptible to CBB.

The last objective was to assess the effectiveness of the SCAR markers SAP6, SU91 and BC420 in MAS. From the findings, SCAR marker SU91 proved to be more effective than SAP6 because SU91 was associated with resistance in most of the progenies. SU91 was present in many of the progenies that had phenotypic resistance signifying the effectiveness of this marker in MAS. Furthermore, effectiveness of SU91 was also confirmed by highly significant relationship ($p<0.001$) between phenotypic scores and marker scores for SU91 marker in some of the populations.

The populations studied proved that the use of SAP6 in MAS should be closely monitored as it may not reflect phenotypic resistance and that resistance to CBB cannot be assumed to be present simply based on detection of SAP6 in a plant genome.

6.2 Recommendation

I recommend that, selected disease resistant breeding progenies from this study be evaluated for other diseases and agronomic characters including yield and maturity across contrasting environments.

On the other hand, the resistant progenies may be crossed to some of South African CBB donor parent that have three markers (SU91, SAP6 and BC420) to increase their resistance. There are some progenies that were resistant but without the marker, these need to be backcrossed to their donor parents so that they posses both the QTLs and the marker.

Resistance from Andean parental lines (RMX2, RMX19 and RMX20) needs to be improved by further crossing them with Mesoamerican lines that have high resistance. Parental line RMA71 (susceptible to CBB) had both SAP6 and SU91 markers and had phenotypic score of 4; this line should be considered for CBB resistance.

Future research plans

Although CBB has been extensively studied, resistance in the Andean genotypes continues to be a major constraint in dry bean production. Andean genotypes are large seeded and have market seed colours. Continued efforts in finding new sources of resistance and improvement of current levels of resistance in the Andean cultivars are needed. Pyramiding of QTL for CBB resistance in the Andean genotypes is possible and can solve the low resistance problem. Given opportunity, gene pyramiding in the Andean genotypes will be the next step. Marker technology allows the possibility of combining multiple QTL for a trait-of-interest in a single genotype. Therefore, apart from CBB, other biotic constraints of economic importance may be pyramided in the same CBB resistant materials.

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APPENDICES

Appendix 1. Seed types for both CBB donor and recipient parents.

BRB267

BRB 211

BRB 214

BRB 215

BRB 268

BRB 266

BRB 265

BRB 264

RAA 21

DRK 149

CMB 107

CMB 106

RMA 71

RMA 70

RMA 69

RMA 68

RMX 19

RMX 8

RMX 2

RMA 72

RMX 20

SAB 516

SAB 514

SAB 581

SAB 576

SAB 575

SAB 568

SEQ 1036

SEQ 1004

SEQ 1003

SEQ 11

SEQ 1006

SEQ 1027

VAX 6

VAX 3

Appendix 2. $F_{3.5}$ mean CBB scores from the field in their ascending order

Trt	Identity/Cross	CBB	Trt	Identity/Cross	CBB
155	SEQ 1006 / VAX 6-1	3	126	SEQ 11 / RMX 19-8	5
156	SEQ 1006 / VAX 6-2	3	127	SEQ 11 / RMX 19-9	5
102	SAB568-2/ VAX6	4	128	SAB 575-5/VAX6	5
125	SAB575-3/VAX6	4	130	SEQ 11 / RMX 19-12	5
135	SEQ 1003 / VAX 3-6	4	136	SEQ 1003 / VAX 3-7	5
139	SEQ 1003 / VAX 3-10	4	138	SEQ 1003 / VAX 3-9	5
157	SEQ 1006 / VAX 6-5	4	140	SEQ 1003 / VAX 3-11	5
6	BRB 265-19/VAX3	5	141	SEQ 1003 / VAX 3-14	5
7	BRB 265-22/ VAX3	5	142	SEQ 1003 / VAX 3-15	5
8	BRB 265-24 /VAX3	5	148	DRK 149 / RMX 2-9	5
26	BRB 266 / RMX 8-1	5	151	SEQ 1004 / RMX 8-2	5
29	BRB 266 / RMX 8-6	5	152	SEQ 1004 / RMX 8-4	5
43	BRB 267-5/VAX3	5	154	SEQ 1004 / RMX 8-7	5
45	BRB 267-8/VAX3	5	158	SEQ 1006 / VAX 6-6	5
49	BRB 267-12/VAX3	5	159	SEQ 1006 / VAX 6-7	5
51	RMA 70 / RMX 19-6	5	161	SEQ 1027 / RMX 2-2	5
56	RMA 70 / RMX 19-21	5	163	SEQ 1027 / RMX 2-4	5
57	RMA 70 / RMX 19-22	5	164	SEQ 1027 / RMX 2-5	5
64	RMA 71 / RMX 2-10	5	165	SEQ 1027 / RMX 2-6	5
65	RMA 71 / RMX 2-13	5	166	SEQ 1027 / RMX 2-7	5
71	RMA 71 / RMX 2-21	5	167	RAA 21 / VAX 6-14	5
72	RMA 71 / RMX 2-22	5	2	BRD 265-3 /VAX3	6
73	RMA 71 / RMX 2-23	5	4	BRB 265-13 /VAX3	6
74	RMA 71 / RMX 2-24	5	5	VAX3 / BRB 265-15	6
75	RMA 71 / RMX 2-25	5	9	VAX3 / BRB 265-25	6
76	RMA 71 / RMX 2-26	5	10	VAX3 / BRB 265-26	6
78	RMA 71 / RMX 2-28	5	11	BRB264-1 /VAX6	6
79	RMA 71 / RMX 2-29	5	12	BRB264-5 /VAX6	6
80	RMA 71 / RMX 2-30	5	14	BRB266-1 /RMX2	6
81	RMA 71 / RMX 2-31	5	16	BRB266-3/RMX2	6
82	RMA 71 / RMX 2-32	5	17	BRB266-4/RMX2	6
83	RMA 71 / RMX 2-33	5	18	BRB266-5/RMX2	6
86	SAB514-4/RMX2	5	19	BRB266-6/RMX2	6
87	SAB514-1/RMX8	5	20	BRB266-7/RMX2	6
101	SAB514-19/RMX8	5	21	BRB266-8/RMX2	6
104	SAB575-1/VAX6	5	22	BRB266-9/RMX2	6
107	SAB575-6/VAX6	5	23	BRB266-10/RMX2	6
109	SAB575-8/VAX6	5	24	/BRB266-11/RMX2	6
122	SEQ 11 / RMX 19-4	5	27	BRB 266 / RMX 8-4	6
123	SEQ 11 / RMX 19-5	5	28	BRB 266 / RMX 8-5	6

Trt	Identity/Cross	CBB	Trt	Identity/Cross	CBB
36	BRB 266 / RMX 8-20	6	117	SAB576-10 /RMX2	6
39	BRB 266 / RMX 8-23	6	119	SEQ 11 / RMX 19-1	6
40	BRB 266 / RMX 8-26	6	120	SEQ 11 / RMX 19-2	6
41	BRB 267-1/VAX3	6	121	SEQ 11 / RMX 19-3	6
44	BRB 267-6/VAX3	6	124	SEQ 11 / RMX 19-6	6
47	BRB 267-10/VAX3	6	129	SEQ 11 / RMX 19-11	6
48	BRB 267-11/VAX3	6	131	SEQ 1003 / VAX 3-1	6
50	RMA 70 / RMX 19-1	6	132	SEQ 1003 / VAX 3-2	6
52	RMA 70 / RMX 19-8	6	133	SEQ 1003 / VAX 3-4	6
53	RMA 70 / RMX 19-14	6	134	SEQ 1004 / RMX 8-3	6
54	RMA 70 / RMX 19-18	6	137	SEQ 1003 / VAX 3-8	6
55	RMA 70 / RMX 19-19	6	143	SEQ 1003 / VAX 3-16	6
58	RMA 71 / RMX 2-1	6	144	DRK 149 / RMX 2-2	6
59	RMA 71 / RMX 2-3	6	145	DRK 149 / RMX 2-3	6
62	RMA 71 / RMX 2-8	6	146	DRK 149 / RMX 2-4	6
63	RMA 71 / RMX 2-9	6	147	DRK 149 / RMX 2-8	6
66	RMA 71 / RMX 2-15	6	149	DRK 149 / RMX 2-10	6
67	RMA 71 / RMX 2-16	6	150	SEQ 1004 / RMX 8-1	6
68	RMA 71 / RMX 2-17	6	153	SEQ 1004 / RMX 8-5	6
69	RMA 71 / RMX 2-18	6	160	SEQ 1027 / RMX 2-1	6
70	RMA 71 / RMX 2-19	6	162	SEQ 1027 / RMX 2-3	6
77	RMA 71 / RMX 2-27	6	168	CONTROL SEQ 11	6
84	VAX6 / CMB106-2	6	169	CONTROL RMX 20	6
85	RMX2 / SAB514-3	6	3	VAX3 / BRD 265-10	6.4
88	RMX8 / SAB514-2	6	1	VAX3 / BRD 265-1	6.6
89	RMX8 / SAB514-3	6	13	VAX6 / BRB264-14	7
90	RMX8/SAB514-4	6	15	RMX2 /BRB266-2	7
91	RMX8 / SAB514-6	6	25	RMX2 /BRB266-12	7
92	RMX8 / SAB514-7	6	31	BRB 266 / RMX 8-10	7
93	RMX8 / SAB514-8	6	32	BRB 266 / RMX 8-14	7
94	RMX8 / SAB514-9	6	33	BRB 266 / RMX 8-16	7
95	RMX8 / SAB514-11	6	34	BRB 266 / RMX 8-18	7
96	RMX8 / SAB514-14	6	35	BRB 266 / RMX 8-19	7
97	RMX8 / SAB514-15	6	37	BRB 266 / RMX 8-21	7
98	RMX8 / SAB514-16	6	38	BRB 266 / RMX 8-22	7
99	RMX8 / SAB514-17	6	42	VAX3 / BRB 267-4	7
103	VAX6 / SAB568-3	6	46	VAX3 / BRB 267-9	7
105	VAX6 / SAB575-2	6	60	RMA 71 / RMX 2-4	7
106	VAX6 / SAB575-4	6	61	RMA 71 / RMX 2-5	7
108	VAX6 / SAB575-7	6	100	RMX8 / SAB514-18	7
111	RMX2 / SAB576-3	6	110	RMX2 / SAB576-2	7
112	RMX2 / SAB576-4	6	113	RMX2 / SAB576-5	7
116	RMX2 / SAB576-9	6	114	RMX2 / SAB576-6	7
118	RMX2 / SAB576-11	7	115	RMX2 / SAB576-8	7

Appendix 3. $F_{3.5}$ mean CBB scores from the green house in their ascending order

Trt	Identity/Cross	CBB	Trt	Identity/Cross	CBB
107	VAX6 / SAB575-6	1	69	RMA 71 / RMX 2-18	4
128	VAX 6/SAB 575-5	1	79	RMA 71 / RMX 2-29	4
101	RMX8 / SAB514-19	2	81	RMA 71 / RMX 2-31	4
104	VAX6 / SAB575-1	2	82	RMA 71 / RMX 2-32	4
125	VAX6/SAB575-3	2	86	RMX2 / SAB514-4	4
142	SEQ 1003 / VAX 3-15	2	94	RMX8 / SAB514-9	4
3	VAX3 / BRD 265-10	3	98	RMX8 / SAB514-16	4
4	VAX3 / BRD 265-13	3	99	RMX8 / SAB514-17	4
43	VAX3 / BRB 267-5	3	100	RMX8 / SAB514-18	4
45	VAX3 / BRB 267-8	3	105	VAX6 / SAB575-2	4
55	RMA 70 / RMX 19-19	3	108	VAX6 / SAB575-7	4
67	RMA 71 / RMX 2-16	3	112	RMX2 / SAB576-4	4
68	RMA 71 / RMX 2-17	3	113	RMX2 / SAB576-5	4
70	RMA 71 / RMX 2-19	3	115	RMX2 / SAB576-8	4
71	RMA 71 / RMX 2-21	3	117	RMX2 / SAB576-10	4
74	RMA 71 / RMX 2-24	3	123	SEQ 11 / RMX 19-5	4
89	RMX8 / SAB514-3	3	124	SEQ 11 / RMX 19-6	4
95	RMX8 / SAB514-11	3	130	SEQ 11 / RMX 19-12	4
97	RMX8 / SAB514-15	3	134	SEQ 1004 / RMX 8-3	4
111	RMX2 / SAB576-3	3	135	SEQ 1003 / VAX 3-6	4
122	SEQ 11 / RMX 19-4	3	136	SEQ 1003 / VAX 3-7	4
131	SEQ 1003 / VAX 3-1	3	138	SEQ 1003 / VAX 3-9	4
157	SEQ 1006 / VAX 6-5	3	139	SEQ 1003 / VAX 3-10	4
158	SEQ 1006 / VAX 6-6	3	141	SEQ 1003 / VAX 3-14	4
7	VAX3 / BRD 265-22	4	144	DRK 149 / RMX 2-2	4
13	VAX6 / BRB264-14	4	145	DRK 149 / RMX 2-3	4
17	RMX2 /BRB266-4	4	146	DRK 149 / RMX 2-4	4
19	RMX2 /BRB266-6	4	147	DRK 149 / RMX 2-8	4
20	RMX2 /BRB266-7	4	150	SEQ 1004 / RMX 8-1	4
25	RMX2 /BRB266-12	4	151	SEQ 1004 / RMX 8-2	4
29	BRB 266 / RMX 8-6	4	160	SEQ 1027 / RMX 2-1	4
31	BRB 266 / RMX 8-10	4	165	SEQ 1027 / RMX 2-6	4
34	BRB 266 / RMX 8-18	4	169	CONTROL RMX 20	4
36	BRB 266 / RMX 8-20	4	2	VAX3 / BRD 265-3	5
38	BRB 266 / RMX 8-22	4	9	VAX3 / BRB 265-25	5
40	BRB 266 / RMX 8-26	4	11	VAX6 / BRB264-1	5
44	VAX3 / BRB 267-6	4	16	RMX2 /BRB266-3	5
51	RMA 70 / RMX 19-6	4	21	RMX2 /BRB266-8	5
53	RMA 70 / RMX 19-14	4	23	RMX2 /BRB266-10	5
54	RMA 70 / RMX 19-18	4	30	BRB 266 / RMX 8-8	5
56	RMA 70 / RMX 19-21	4	32	BRB 266 / RMX 8-14	5
57	RMA 70 / RMX 19-22	4	42	VAX3 / BRB 267-4	5
60	RMA 71 / RMX 2-4	4	47	VAX3 / BRB 267-10	5
66	RMA 71 / RMX 2-15	4	52	RMA 70 / RMX 19-8	5

Trt	Identity/Cross	CBB	Trt	Identity/Cross	CBB
58	RMA 71 / RMX 2-1	5	73	RMA 71 / RMX 2-23	6
72	RMA 71 / RMX 2-22	5	76	RMA 71 / RMX 2-26	6
75	RMA 71 / RMX 2-25	5	77	RMA 71 / RMX 2-27	6
83	RMA 71 / RMX 2-33	5	78	RMA 71 / RMX 2-28	6
85	RMX2 / SAB514-3	5	80	RMA 71 / RMX 2-30	6
88	RMX8 / SAB514-2	5	84	VAX6 / CMB106-2	6
90	RMX8/SAB514-4	5	87	RMX8 / SAB514-1	6
93	RMX8 / SAB514-8	5	91	RMX8 / SAB514-6	6
110	RMX2 / SAB576-2	5	92	RMX8 / SAB514-7	6
114	RMX2 / SAB576-6	5	96	RMX8 / SAB514-14	6
116	RMX2 / SAB576-9	5	102	VAX6 / SAB568-2	6
118	RMX2 / SAB576-11	5	109	VAX6 / SAB575-8	6
127	SEQ 11 / RMX 19-9	5	119	SEQ 11 / RMX 19-1	6
132	SEQ 1003 / VAX 3-2	5	120	SEQ 11 / RMX 19-2	6
137	SEQ 1003 / VAX 3-8	5	121	SEQ 11 / RMX 19-3	6
148	DRK 149 / RMX 2-9	5	126	SEQ 11 / RMX 19-8	6
149	DRK 149 / RMX 2-10	5	133	SEQ 1003 / VAX 3-4	6
154	SEQ 1004 / RMX 8-7	5	143	SEQ 1003 / VAX 3-16	6
155	SEQ 1006 / VAX 6-1	5	153	SEQ 1004 / RMX 8-5	6
162	SEQ 1027 / RMX 2-3	5	156	SEQ 1006 / VAX 6-2	6
1	VAX3 / BRD 265-1	6	161	SEQ 1027 / RMX 2-2	6
5	VAX3 / BRD 265-15	6	164	SEQ 1027 / RMX 2-5	6
6	VAX3 / BRD 265-19	6	167	RAA 21 / VAX 6-14	6
8	VAX3 / BRD 265-24	6	12	VAX6 / BRB264-5	7
15	RMX2 /BRB266-2	6	22	RMX2 /BRB266-9	7
18	RMX2 /BRB266-5	6	27	BRB 266 / RMX 8-4	7
26	BRB 266 / RMX 8-1	6	48	VAX3 / BRB 267-11	7
28	BRB 266 / RMX 8-5	6	106	VAX6 / SAB575-4	7
33	BRB 266 / RMX 8-16	6	129	SEQ 11 / RMX 19-11	7
37	BRB 266 / RMX 8-21	6	163	SEQ 1027 / RMX 2-4	7
39	BRB 266 / RMX 8-23	6	166	SEQ 1027 / RMX 2-7	7
46	VAX3 / BRB 267-9	6	168	CONTROL SEQ 11	7
49	VAX3 / BRB 267-12	6	14	RMX2 /BRB266-1	8
50	RMA 70 / RMX 19-1	6	24	RMX2 /BRB266-11	8
59	RMA 71 / RMX 2-3	6	35	BRB 266 / RMX 8-19	8
61	RMA 71 / RMX 2-5	6	103	VAX6 / SAB568-3	8
62	RMA 71 / RMX 2-8	6	140	SEQ 1003 / VAX 3-11	8
63	RMA 71 / RMX 2-9	6	152	SEQ 1004 / RMX 8-4	8
64	RMA 71 / RMX 2-10	6	159	SEQ 1006 / VAX 6-7	8
65	RMA 71 / RMX 2-13	6	41	VAX3 / BRB 267-1	9

Appendix 4. $F_{4.6}$ mean CBB scores from the field in their ascending order

Trt	Cross/Identity	CBB	Trt	Cross/Identity	CBB
35	BRB265/VAX6-9	3	126	RMA72/VAX6-33	4
134	SEQ1036/RMX20-12	3	57	CMB106/VAX3-9	4
38	BRB265/VAX6-13	3	43	BRB268/RMX19-6	4
39	BRB268/RMX19-1	3	47	BRB268/RMX19-12	4
8	BRB211/VAX 3-15	4	82	RMA70/RMX20-17	4
115	RMA72/VAX6-21	4	18	BRB214/VAX3-15	4
94	RMA72/RMX19-33	4	41	BRB268/RMX19-4	4
25	BRB215/VAX6-5	4	33	BRB265/VAX6-7	4
23	BRB215/VAX6-3	4	26	BRB264/VAX3-1	5
48	BRB268/RMX19-13	4	89	RMA72/RMX19-24	5
110	RMA72/VAX6-14	4	19	BRB214/VAX3-16	5
36	BRB265/VAX6-11	4	58	CMB107/RMX2-1	5
135	SEQ1036/RMX20-17	4	98	RMA72/RMX19-37	5
31	BRB264/VAX3-18	4	62	CMB107/RMX2-5	5
44	BRB268/RMX19-9	4	59	CMB107/RMX2-2	5
65	CMB107/RMX2-8	4	80	RMA70/RMX20-13	5
30	BRB264/VAX3-13	4	54	CMB106/VAX3-6	5
46	BRB268/RMX19-11	4	5	BRB211/VAX 3-10	5
105	RMA72/VAX6-8	4	140	BRB266/RMX19-3	5
132	SEQ1036/RMX20-10	4	32	BRB265/VAX6-6	5
122	RMA72/VAX6-29	4	24	BRB215/VAX6-4	5
63	CMB107/RMX2-6	4	90	RMA72/RMX19-25	5
53	CMB106/VAX3-5	4	99	RMA72/VAX6-1	5
141	RMA69/VAX3-3	4	29	BRB264/VAX3-12	5
72	RAA21/VAX3-9	4	97	RMA72/RMX19-36	5
71	CMB107/RMX2-17	4	144	CONTROL-RMX2	5
14	BRB214/VAX3-7	4	1	BRB211/VAX 3-1	5
74	RMA70/RMX20-1	4	77	RMA70/RMX20-4	5
45	BRB268/RMX19-10	4	9	BRB211/VAX 3-16	5
3	BRB211/VAX 3-6	4	78	RMA70/RMX20-5	5
83	RMA70/RMX20-18	4	92	RMA72/RMX19-31	5
49	CMB106/VAX3-1	4	10	BRB214/VAX3-1	5
107	RMA72/VAX6-11	4	119	RMA72/VAX6-26	5
76	RMA70/RMX20-3	4	123	RMA72/VAX6-30	5
73	RAA21/VAX3-10	4	103	RMA72/VAX6-5	5
116	RMA72/VAX6-22	4	81	RMA70/RMX20-14	5
133	SEQ1036/RMX20-11	4	17	BRB214/VAX3-14	5
117	RMA72/VAX6-23	4	96	RMA72/RMX19-35	5
4	BRB211/VAX 3-7	4	114	RMA72/VAX6-20	5
37	BRB265/VAX6-12	4	13	BRB214/VAX3-6	5
2	BRB211/VAX 3-3	4	42	BRB268/RMX19-5	5
127	RMA72/VAX6-34	4	129	SEQ1036/RMX20-6	5
113	RMA72/VAX6-18	4	137	SAB516/RMX19-4	5
118	RMA72/VAX6-25	4	101	RMA72/VAX6-3	5

Trt	Cross/Identity	CBB	Trt	Cross/Identity	CBB
66	CMB107/RMX2-9	5	136	SAB516/RMX19-1	5
125	RMA72/VAX6-32	5	104	RMA72/VAX6-7	5
143	CONTROL-SEQ1003	5	21	BRB215/VAX6-1	5
20	BRB214/VAX3-17	5	131	SEQ1036/RMX20-9	5
142	RMA69/VAX3-5	5	95	RMA72/RMX19-34	5
61	CMB107/RMX2-4	5	139	BRB266/RMX19-2	5
16	BRB214/VAX3-12	5	50	CMB106/VAX3-2	5
84	RMA72/RMX19-12	5	64	CMB107/RMX2-7	5
138	SAB516/RMX19-5	5	27	BRB264/VAX3-3	5
120	RMA72/VAX6-27	5	112	RMA72/VAX6-17	5
67	CMB107/RMX2-11	5	121	RMA72/VAX6-28	5
85	RMA72/RMX19-13	5	79	RMA70/RMX20-6	5
93	RMA72/RMX19-26	5	22	BRB215/VAX6-2	5
60	CMB107/RMX2-3	5	69	CMB107/RMX2-13	5
40	BRB268/RMX19-2	5	109	RMA72/VAX6-13	5
111	RMA72/VAX6-15	5	88	RMA72/RMX19-20	5
106	RMA72/VAX6-9	5	102	RMA72/VAX6-4	5
86	RMA72/RMX19-14	5	75	RMA70/RMX20-2	5
6	BRB211/VAX 3-13	5	15	BRB214/VAX3-10	5
70	CMB107/RMX2-14	5	52	CMB106/VAX3-4	5
7	BRB211/VAX 3-14	5	56	CMB106/VAX3-8	5
34	BRB265/VAX6-8	5	28	BRB264/VAX3-9	5
91	RMA72/RMX19-26	5	11	BRB214/VAX3-2	6
68	CMB107/RMX2-12	5	108	RMA72/VAX6-12	6
124	RMA72/VAX6-31	5	51	CMB106/VAX3-3	6
55	CMB106/VAX3-7	5	100	RMA72/VAX6-2	6
130	SEQ1036/RMX20-7	5	128	RMA72/VAX6-35	6
12	BRB214/VAX3-4	5	87	RMA72/RMX19-16	6

Appendix 5. $F_{4.6}$ mean CBB scores from the green house in their ascending order

Trt	Cross/Identity	CBB	Trt	Cross/Identity	CBB
22	BRB215/VAX6-2	2	84	RMA72/RMX19-12	6
20	BRB214/VAX3-17	3	101	RMA72/VAX6-3	6
36	BRB265/VAX6-11	3	122	RMA72/VAX6-29	6
115	RMA72/VAX6-21	3	127	RMA72/VAX6-34	6
15	BRB214/VAX3-10	3	134	SEQ1036/RMX20-12	6
34	BRB265/VAX6-8	3	48	BRB268/RMX19-13	6
116	RMA72/VAX6-22	3	53	CMB106/VAX3-5	6
30	BRB264/VAX3-13	3	140	BRB266/RMX19-3	6
119	RMA72/VAX6-26	3	6	BRB211/VAX 3-13	6
35	BRB265/VAX6-9	4	8	BRB211/VAX 3-15	6
118	RMA72/VAX6-25	4	14	BRB214/VAX3-7	6
109	RMA72/VAX6-13	4	16	BRB214/VAX3-12	6
106	RMA72/VAX6-9	4	17	BRB214/VAX3-14	6
121	RMA72/VAX6-28	4	25	BRB215/VAX6-5	6
105	RMA72/VAX6-8	4	39	BRB268/RMX19-1	6
107	RMA72/VAX6-11	4	55	CMB106/VAX3-7	6
126	RMA72/VAX6-33	4	62	CMB107/RMX2-5	6
131	SEQ1036/RMX20-9	4	64	CMB107/RMX2-7	6
144	CONTROL-RMX2	4	67	CMB107/RMX2-11	6
7	BRB211/VAX 3-14	4	73	RAA21/VAX3-10	6
1	BRB211/VAX 3-1	4	79	RMA70/RMX20-6	6
5	BRB211/VAX 3-10	4	81	RMA70/RMX20-14	6
26	BRB264/VAX3-1	5	92	RMA72/RMX19-31	6
108	RMA72/VAX6-12	5	97	RMA72/RMX19-36	6
124	RMA72/VAX6-31	5	99	RMA72/VAX6-1	6
12	BRB214/VAX3-4	5	100	RMA72/VAX6-2	6
113	RMA72/VAX6-18	5	117	RMA72/VAX6-23	6
45	BRB268/RMX19-10	5	120	RMA72/VAX6-27	6
112	RMA72/VAX6-17	5	125	RMA72/VAX6-32	6
4	BRB211/VAX 3-7	5	129	SEQ1036/RMX20-6	6
29	BRB264/VAX3-12	5	137	SAB516/RMX19-4	6
59	CMB107/RMX2-2	5	141	RMA69/VAX3-3	6
63	CMB107/RMX2-6	5	135	SEQ1036/RMX20-17	6
143	CONTROL-SEQ1003	5	2	BRB211/VAX 3-3	6
83	RMA70/RMX20-18	5	51	CMB106/VAX3-3	6
23	BRB215/VAX6-3	5	57	CMB106/VAX3-9	6
69	CMB107/RMX2-13	5	61	CMB107/RMX2-4	6
31	BRB264/VAX3-18	6	65	CMB107/RMX2-8	6
68	CMB107/RMX2-12	6	66	CMB107/RMX2-9	6
89	RMA72/RMX19-24	6	128	RMA72/VAX6-35	6
95	RMA72/RMX19-34	6	58	CMB107/RMX2-1	7
133	SEQ1036/RMX20-11	6	76	RMA70/RMX20-3	7
3	BRB211/VAX 3-6	6	78	RMA70/RMX20-5	7
9	BRB211/VAX 3-16	6	85	RMA72/RMX19-13	7
11	BRB214/VAX3-2	6	114	RMA72/VAX6-20	7
33	BRB265/VAX6-7	6	24	BRB215/VAX6-4	7

Trt	Cross/Identity	CBB	Trt	Cross/Identity	CBB
38	BRB265/VAX6-13	7	75	RMA70/RMX20-2	7
43	BRB268/RMX19-6	7	13	BRB214/VAX3-6	7
93	RMA72/RMX19-26	7	42	BRB268/RMX19-5	7
104	RMA72/VAX6-7	7	54	CMB106/VAX3-6	7
139	BRB266/RMX19-2	7	74	RMA70/RMX20-1	7
10	BRB214/VAX3-1	7	123	RMA72/VAX6-30	7
21	BRB215/VAX6-1	7	40	BRB268/RMX19-2	8
37	BRB265/VAX6-12	7	44	BRB268/RMX19-9	8
80	RMA70/RMX20-13	7	46	BRB268/RMX19-11	8
90	RMA72/RMX19-25	7	102	RMA72/VAX6-4	8
138	SAB516/RMX19-5	7	136	SAB516/RMX19-1	8
18	BRB214/VAX3-15	7	77	RMA70/RMX20-4	8
27	BRB264/VAX3-3	7	86	RMA72/RMX19-14	8
32	BRB265/VAX6-6	7	87	RMA72/RMX19-16	8
41	BRB268/RMX19-4	7	91	RMA72/RMX19-26	8
56	CMB106/VAX3-8	7	94	RMA72/RMX19-33	8
60	CMB107/RMX2-3	7	132	SEQ1036/RMX20-10	8
70	CMB107/RMX2-14	7	47	BRB268/RMX19-12	8
72	RAA21/VAX3-9	7	98	RMA72/RMX19-37	8
82	RMA70/RMX20-17	7	19	BRB214/VAX3-16	8
96	RMA72/RMX19-35	7	28	BRB264/VAX3-9	8
110	RMA72/VAX6-14	7	52	CMB106/VAX3-4	8
111	RMA72/VAX6-15	7	88	RMA72/RMX19-20	9
130	SEQ1036/RMX20-7	7	49	CMB106/VAX3-1	9
71	CMB107/RMX2-17	7	103	RMA72/VAX6-5	9

Appendix 6: $F_{3.5}$ CBB from the greenhouse and their marker scores

No	IDENTITY		CBB	SAP6	SU91
P1	**BRB265**	**5***	8	1	0
P2	**VAX3**		1	1	1
1	VAX3 / BRB 265-1		5	0	0
2	VAX3 / BRB 265-1		4	0	0
3	VAX3 / BRB 265-1		7	0	0
4	VAX3 / BRB 265-1		6	0	0
5	VAX3 / BRB 265-3		5	0	0
6	VAX3 / BRB 265-3		5	1	0
7	VAX3 / BRB 265-10		2	1	0
8	VAX3 / BRB 265-10		3	1	0
9	VAX3 / BRB 265-13		4	0	0
10	VAX3 / BRB 265-13		3	0	0
11	VAX3 / BRB 265-13		3	0	0
12	VAX3 / BRB 265-13		3	0	1
13	VAX3 / BRB 265-15		4	1	1
14	VAX3 / BRB 265-15		6	1	1
15	VAX3 / BRB 265-22		4	0	1
16	VAX3 / BRB 265-22		3	0	1
17	VAX3 / BRB 265-22		5	0	1
18	VAX3 / BRB 265-22		5	0	1
19	VAX3 / BRB 265-24		6	1	1
20	VAX3 / BRB 265-24		5	0	1
21	VAX3 / BRB 265-24		6	0	1
22	VAX3 / BRB 265-24		6	0	0
23	VAX3 / BRB 265-25		7	0	0
24	VAX3 / BRB 265-25		4	0	1
25	VAX3 / BRB 265-26		3	0	1
26	VAX3 / BRB 265-26		3	0	1
	BRB266	**5***	8	1	0
	RMX2		5	1	0
1	RMX2 /BRB266-1		8	0	1
2	RMX2 /BRB266-1		7	1	1
3	RMX2 /BRB266-2		6	0	0
4	RMX2 /BRB266-2		6	0	1
5	RMX2 /BRB266-3		7	0	1
6	RMX2 /BRB266-3		5	0	0
7	RMX2 /BRB266-4		4	0	0
8	RMX2 /BRB266-4		4	0	0
9	RMX2 /BRB266-5		5	0	0
10	RMX2 /BRB266-5		6	0	1
11	RMX2 /BRB266-6		4	0	1
12	RMX2 /BRB266-6		3	0	0

No	IDENTITY		CBB	SAP6	SU91
P1	**BRB267**	**5***	6	0	0
P2	**VAX3**		1	1	1
1	VAX3 / BRB 267-1		9	0	1
2	VAX3 / BRB 267-1		9	0	1
3	VAX3 / BRB 267-1		9	0	1
4	VAX3 / BRB 267-4		6	0	1
5	VAX3 / BRB 267-4		5	0	1
6	VAX3 / BRB 267-5		2	0	1
7	VAX3 / BRB 267-5		3	0	1
8	VAX3 / BRB 267-5		2	0	1
9	VAX3 / BRB 267-5		3	0	1
10	VAX3 / BRB 267-6		3	0	1
11	VAX3 / BRB 267-6		5	0	1
12	VAX3 / BRB 267-6		5	0	1
13	VAX3 / BRB 267-6		4	0	1
14	VAX3 / BRB 267-8		2	1	0
15	VAX3 / BRB 267-8		3	0	1
16	VAX3 / BRB 267-9		5	0	0
17	VAX3 / BRB 267-9		6	0	0
18	VAX3 / BRB 267-10		5	0	0
19	VAX3 / BRB 267-10		5	0	0
20	VAX3 / BRB 267-11		8	0	0
21	VAX3 / BRB 267-11		8	1	1
22	VAX3 / BRB 267-11		4	1	1
23	VAX3 / BRB 267-11		7	1	0
24	VAX3 / BRB 267-12		7	1	1
25	VAX3 / BRB 267-12		6	1	1
26	VAX3 / BRB 267-12		5	1	1
27	VAX3 / BRB 267-12		7	1	0
	SEQ11	**5***	7	0	0
	RMX19		5	1	0
1	SEQ 11 / RMX 19-1		6	1	0
2	SEQ 11 / RMX 19-1		6	0	0
3	SEQ 11 / RMX 19-2		5	0	0
4	SEQ 11 / RMX 19-2		7	0	0
5	SEQ 11 / RMX 19-3		6	1	0
6	SEQ 11 / RMX 19-3		6	1	0
7	SEQ 11 / RMX 19-4		3	0	1
8	SEQ 11 / RMX 19-4		3	0	1
9	SEQ 11 / RMX 19-5		4	0	0
10	SEQ 11 / RMX 19-5		5	1	0
11	SEQ 11 / RMX 19-6		5	1	0
12	SEQ 11 / RMX 19-6		4	1	0

13	RMX2 /BRB266-7	5	0	0
14	RMX2 /BRB266-7	3	0	0
15	RMX2 /BRB266-8	5	0	0
16	RMX2 /BRB266-8	5	1	1
17	RMX2 /BRB266-9	7	1	1
18	RMX2 /BRB266-9	8	0	1
19	RMX2 /BRB266-10	4	0	1
20	RMX2 /BRB266-10	6	1	1
21	RMX2 /BRB266-11	7	1	1
22	RMX2 /BRB266-11	7	0	1
23	RMX2 /BRB266-12	3	1	1
24	RMX2 /BRB266-12	4	0	0
	SEQ1006 5*	6	1	0
	VAX6	2	1	1
1	SEQ 1006 / VAX 6-1	6	0	1
2	SEQ 1006 / VAX 6-1	5	0	0
3	SEQ 1006 / VAX 6-2	8	0	0
4	SEQ 1006 / VAX 6-2	5	0	0
5	SEQ 1006 / VAX 6-2	6	1	0
6	SEQ 1006 / VAX 6-2	4	1	0
7	SEQ 1006 / VAX 6-5	3	0	1
8	SEQ 1006 / VAX 6-5	4	0	1
9	SEQ 1006 / VAX 6-6	2	1	0
10	SEQ 1006 / VAX 6-6	2	1	1
11	SEQ 1006 / VAX 6-7	8	0	0
12	SEQ 1006 / VAX 6-7	8	0	0
	BRB264 6*	7	1	0
	VAX6	2	1	1
1	VAX6 / BRB264-1	6	1	0
2	VAX6 / BRB264-1	6	1	0
3	VAX6 / BRB264-1	4	1	0
4	VAX6 / BRB264-1	5	0	1
5	VAX6 / BRB264-5	7	1	0
6	VAX6 / BRB264-5	7	0	0
7	VAX6 / BRB264-5	7	1	0
8	VAX6 / BRB264-5	6	1	0
9	VAX6 / BRB264-14	3	1	0
10	VAX6 / BRB264-14	5	0	0
	SAB575 3*	8	0	0
	VAX6	2	1	1
1	VAX6 / SAB575-1	1	0	1
2	VAX6 / SAB575-1	1	0	1
3	VAX6 / SAB575-2	5	1	0
4	VAX6 / SAB575-2	3	1	0
5	VAX6 / SAB575-4	7	1	1
6	VAX6 / SAB575-4	6	0	1
7	VAX6 / SAB575-6	1	1	1
8	VAX6 / SAB575-6	1	1	1
9	VAX6 / SAB575-7	4	0	0

13	SEQ 11 / RMX 19-8	6	1	0
14	SEQ 11 / RMX 19-8	4	0	0
15	SEQ 11 / RMX 19-9	6	0	0
16	SEQ 11 / RMX 19-9	5	0	0
17	SEQ 11 / RMX 19-11	6	1	0
18	SEQ 11 / RMX 19-11	7	1	0
	SEQ1003 4*	6	0	0
	VAX3	1	1	1
1	SEQ 1003 / VAX 3-1	4	0	1
2	SEQ 1003 / VAX 3-1	2	0	1
3	SEQ 1003 / VAX 3-1	1	0	1
4	SEQ 1003 / VAX 3-1	4	0	1
5	SEQ 1003 / VAX 3-2	5	0	1
6	SEQ 1003 / VAX 3-2	4	0	1
7	SEQ 1003 / VAX 3-2	4	0	1
8	SEQ 1003 / VAX 3-2	6	0	0
9	SEQ 1003 / VAX 3-4	7	0	1
10	SEQ 1003 / VAX 3-4	5	0	1
11	SEQ 1003 / VAX 3-6	2	0	1
12	SEQ 1003 / VAX 3-6	3	0	1
13	SEQ 1003 / VAX 3-7	5	0	1
14	SEQ 1003 / VAX 3-7	3	0	1
15	SEQ 1003 / VAX 3-8	4	0	0
16	SEQ 1003 / VAX 3-8	5	0	0
17	SEQ 1003 / VAX 3-9	3	0	1
18	SEQ 1003 / VAX 3-9	4	0	1
19	SEQ 1003 / VAX 3-9	4	0	1
20	SEQ 1003 / VAX 3-9	4	0	1
21	SEQ 1003 / VAX 3-10	4	0	0
22	SEQ 1003 / VAX 3-10	4	0	0
23	SEQ 1003 / VAX 3-11	6	0	1
24	SEQ 1003 / VAX 3-11	8	0	1
25	SEQ 1003 / VAX 3-14	3	0	1
26	SEQ 1003 / VAX 3-14	4	0	1
27	SEQ 1003 / VAX 3-15	1	0	1
28	SEQ 1003 / VAX 3-15	3	0	1
29	SEQ 1003 / VAX 3-16	4	0	0
30	SEQ 1003 / VAX 3-16	5	0	0
	RAM 70 4*	6	0	0
	RMX19	5	1	0
1	RMA 70 / RMX 19-1	6	1	0
2	RMA 70 / RMX 19-1	6	1	0
3	RMA 70 / RMX 19-6	4	0	0
4	RMA 70 / RMX 19-6	3	0	0
5	RMA 70 / RMX 19-8	5	1	0
6	RMA 70 / RMX 19-8	5	0	0
7	RMA 70 / RMX 19-14	4	1	0
8	RMA 70 / RMX 19-14	3	1	0
9	RMA 70 / RMX 19-18	4	1	0

10	VAX6 / SAB575-7	4	0	1	10	RMA 70 / RMX 19-18	4	1	0
11	VAX6 / SAB575-8	5	1	1	11	RMA 70 / RMX 19-19	3	1	0
12	VAX6 / SAB575-8	6	0	0	12	RMA 70 / RMX 19-19	5	1	0
13	VAX6/SAB575-3	2	0	1	13	RMA 70 / RMX 19-21	3	1	0
14	VAX6/SAB575-3	2	0	1	14	RMA 70 / RMX 19-21	4	1	0
15	VAX 6/SAB 575-5	1	0	1	15	RMA 70 / RMX 19-22	3	1	0
16	VAX 6/SAB 575-5	1	0	1	16	RMA 70 / RMX 19-22	4	1	0
	SAB568 8*	7	0	0		**RAA21 6***	6	0	0
	VAX6	2	1	1		**VAX6**	2	1	1
1	VAX6 / SAB568-2	7	0	1	1	RAA 21 / VAX 6-14	6	1	0
2	VAX6 / SAB568-2	7	1	0	2	RAA 21 / VAX 6-14	6	1	0
3	VAX6 / SAB568-3	9	1	0	3	RAA 21 / VAX 6-14	6	1	0
4	VAX6 / SAB568-3	9	1	0					
5	VAX6 / SAB568-3	6	1	0					
6	VAX6 / SAB568-3	8	1	0					

***Population CBB mean score**

Appendix 7. $F_{4.6}$ CBB from the greenhouse and their marker scores

No	Identity	CBB	SAP6	SU91	No	Identity	CBB	SAP6	SU91
	BRB 211 5*	**7**	**1**	**0**		**BRB 214 5***	6	1	0
	VAX 3	**1**	1	1		**VAX 3**	1	1	1
1	BRB211/VAX 3-13	5	0	0	1	BRB214/VAX3-2	7	1	0
2	BRB211/VAX 3-13	6	0	0	2	BRB214/VAX3-2	6	1	1
3	BRB211/VAX 3-16	7	1	0	3	BRB214/VAX3-2	4	1	1
4	BRB211/VAX 3-16		0	0	4	BRB214/VAX3-14	7	1	1
5	BRB211/VAX 3-16	5	1	1	5	BRB214/VAX3-14	7	1	1
6	BRB211/VAX 3-16	5	0	1	6	BRB214/VAX3-17	6	1	1
7	BRB211/VAX 3-3	7	1	0	7	BRB214/VAX3-17	7	1	1
8	BRB211/VAX 3-3	5	0	1	8	BRB214/VAX3-7	6	1	1
9	BRB211/VAX 3-3	4	0	1	9	BRB214/VAX3-7	6	1	1
10	BRB211/VAX 3-15	6	0	1	10	BRB214/VAX3-7		1	1
11	BRB211/VAX 3-15	7	0	0	11	BRB214/VAX3-7		1	1
12	BRB211/VAX 3-15	6	0	1	12	BRB214/VAX3-4	5	1	1
13	BRB211/VAX 3-15	5	0	1	13	BRB214/VAX3-4	5	1	1
14	BRB211/VAX 3-14	3	1	1	14	BRB214/VAX3-4	4	1	1
15	BRB211/VAX 3-14	6	1	0	15	BRB214/VAX3-4	4	1	1
16	BRB211/VAX 3-14	5	1	0	16	BRB214/VAX3-16	3	1	1
17	BRB211/VAX 3-14	3	1	0	17	BRB214/VAX3-16	2	1	1
18	BRB211/VAX 3-6	4	1	1	18	BRB214/VAX3-16	3	0	1
19	BRB211/VAX 3-6	7	0	1	19	BRB214/VAX3-16	2	0	1
20	BRB211/VAX 3-1	4	0	0	20	BRB214/VAX3-15	9	1	0
21	BRB211/VAX 3-1	5	0	1	21	BRB214/VAX3-15	8	1	1
22	BRB211/VAX 3-7	5	0	1	22	BRB214/VAX3-1	7	1	1
23	BRB211/VAX 3-7	5	0	0	23	BRB214/VAX3-1	6	1	1
24	BRB211/VAX 3-10	6	1	1	24	BRB214/VAX3-17	2	1	1
25	BRB211/VAX 3-10	2	1	1	25	BRB214/VAX3-17	2	1	1
26	BRB211/VAX 3-10	5	1	1	26	BRB214/VAX3-17	2	1	1

27	BRB211/VAX 3-10	4	0	1	27	BRB214/VAX3-17	2	0	1
	BRB 215 6*	6	1	0	28	BRB214/VAX3-8	2	1	1
	VAX 3	1	1	1	29	BRB214/VAX3-8	4	1	1
1	BRB215/VAX6-2	4	0	1	30	BRB214/VAX3-6	8	1	1
2	BRB215/VAX6-2	5	0	0	31	BRB214/VAX3-6	7	1	1
3	BRB215/VAX6-2	7	0	1	32	BRB214/VAX3-12	6	1	1
4	BRB215/VAX6-5	4	0	1	33	BRB214/VAX3-12	6	1	1
5	BRB215/VAX6-5	5	0	1	34	BRB214/VAX3-12	5	1	1
6	BRB215/VAX6-3	8	0	1	35	BRB214/VAX3-12	6	0	0
7	BRB215/VAX6-3	4	0	1	36	BRB214/VAX3-10	6	1	0
8	BRB215/VAX6-3	8	1	1	37	BRB214/VAX3-10	6	0	0
9	BRB215/VAX6-4	5	1	1		**BRB 264 6***	7	1	0
10	BRB215/VAX6-4	6	1	1		**VAX 3**	1	1	1
	BRB 265 5*	8	1	0	1	BRB264/VAX3-12	3	0	1
	VAX 6	2	1	1	2	BRB264/VAX3-12	3	1	1
1	BRB265/VAX6-8	3	1	1	3	BRB264/VAX3-18	8	1	0
2	BRB265/VAX6-8	3	1	1	4	BRB264/VAX3-18	6	1	0
3	BRB265/VAX6-6	5	0	1	5	BRB264/VAX3-18	7	1	0
4	BRB265/VAX6-6	7	1	0	6	BRB264/VAX3-18	7	1	0
5	BRB265/VAX6-6	4	1	1	7	BRB264/VAX3-13	5	1	0
6	BRB265/VAX6-6	6	1	0	8	BRB264/VAX3-13	6	1	0
7	BRB265/VAX6-12	7	1	0	9	BRB264/VAX3-1	6	1	0
8	BRB265/VAX6-12	7	1	0	10	BRB264/VAX3-1	7	0	0
9	BRB265/VAX6-12	6	0	0	11	BRB264/VAX3-1	8	0	0
10	BRB265/VAX6-12	7	1	0	12	BRB264/VAX3-1	7	1	0
11	BRB265/VAX6-9	3	1	1	13	BRB264/VAX3-9	3	1	0
12	BRB265/VAX6-9	3	1	1	14	BRB264/VAX3-9	7	1	1
13	BRB265/VAX6-7	3	1	1	15	BRB264/VAX3-9	5	1	0
14	BRB265/VAX6-7	3	1	1	16	BRB264/VAX3-3	9	1	0
15	BRB265/VAX6-13	6	0	0	17	BRB264/VAX3-3	8	1	0
16	BRB265/VAX6-13	6	1	1	18	BRB264/VAX3-3	8	1	0
17	BRB265/VAX6-11	5	0	0		**CMB107 6***	6	0	0
18	BRB265/VAX6-11	6	0	0		**RMX2**	5	1	0
19	BRB265/VAX6-11	8	1	0	1	CMB107/RMX2-14	6	0	0
20	BRB265/VAX6-11	8	1	0	2	CMB107/RMX2-14	8	1	0
	RMA70 6*	6	0	0	3	CMB107/RMX2-8	6	1	0
	RMX20	4	1	0	4	CMB107/RMX2-8	7	1	0
1	RMA70/RMX20-17	4	0	0	5	CMB107/RMX2-12	4	0	0
2	RMA70/RMX20-17	5	0	0	6	CMB107/RMX2-12	6	1	0
3	RMA70/RMX20-4	6	0	0	7	CMB107/RMX2-12	6	0	0
4	RMA70/RMX20-4	7	0	0	8	CMB107/RMX2-4	6	1	0
5	RMA70/RMX20-13	6	1	0	9	CMB107/RMX2-4	7	1	0
6	RMA70/RMX20-18	6	1	0	10	CMB107/RMX2-4	6	1	0
7	RMA70/RMX20-18	5	1	0	11	CMB107/RMX2-4	5	0	0
8	RMA70/RMX20-6	6	1	0	12	CMB107/RMX2-2	8	1	0
9	RMA70/RMX20-6	7	1	0	13	CMB107/RMX2-2	7	0	0
10	RMA70/RMX20-5	6	1	0	14	CMB107/RMX2-17	7	1	0
11	RMA70/RMX20-5	6	1	0	15	CMB107/RMX2-17	7	1	0
12	RMA70/RMX20-14	6	1	0	16	CMB107/RMX2-11	5	1	0

13	RMA70/RMX20-14	7	1	0	17	CMB107/RMX2-11	6	1	0
14	RMA70/RMX20-1	7	0	0	18	CMB107/RMX2-9	6	0	0
15	RMA70/RMX20-1	7	0	0	19	CMB107/RMX2-9	6	0	0
16	RMA70/RMX20-3	5	0	0	20	CMB107/RMX2-13	6	0	0
17	RMA70/RMX20-3	6	0	0	21	CMB107/RMX2-13	7	0	0
18	RMA70/RMX20-2	7	1	0	22	CMB107/RMX2-5	4	1	0
19	RMA70/RMX20-2	6	1	0	23	CMB107/RMX2-5	5	0	0
	RMA72 7*	7	0	0	24	CMB107/RMX2-3	6	1	0
	RMX19	5	1	0	25	CMB107/RMX2-3	6	0	0
1	RMA72/RMX19-34	5	1	0	26	CMB107/RMX2-7	6	1	0
2	RMA72/RMX19-34	6	0	0	27	CMB107/RMX2-7	6	1	0
3	RMA72/RMX19-24	8	0	0	28	CMB107/RMX2-6	7	0	0
4	RMA72/RMX19-24	6	0	0	29	CMB107/RMX2-6	6	0	0
5	RMA72/RMX19-31	7	0	0	30	CMB107/RMX2-6	5	0	0
6	RMA72/RMX19-31	7	0	0	31	CMB107/RMX2-1	6	1	0
7	RMA72/RMX19-37	8	1	0	32	CMB107/RMX2-1	7	1	0
8	RMA72/RMX19-13	8	1	0		**P1 RMA72 5***	7	0	0
9	RMA72/RMX19-13	8	1	0		**P2 VAX6**	2	1	1
10	RMA72/RMX19-35	7	1	0	1	RMA72/VAX6-26	6	0	0
11	RMA72/RMX19-35	7	1	0	2	RMA72/VAX6-26	4	1	1
12	RMA72/RMX19-26	7	1	0	3	RMA72/VAX6-20	7	0	0
13	RMA72/RMX19-26	6	1	0	4	RMA72/VAX6-20	6	1	0
14	RMA72/RMX19-26	9	1	0	5	RMA72/VAX6-20	7	1	0
15	RMA72/RMX19-26	7	1	0	6	RMA72/VAX6-20	8	1	1
16	RMA72/RMX19-14	7	0	0	7	RMA72/VAX6-33	5	0	0
17	RMA72/RMX19-14	8	1	0	8	RMA72/VAX6-33	6	1	0
18	RMA72/RMX19-12	7	0	0	9	RMA72/VAX6-33	3	1	1
19	RMA72/RMX19-12	6	0	0	10	RMA72/VAX6-33	2	0	1
20	RMA72/RMX19-36	6	0	0	11	RMA72/VAX6-4	7	0	0
21	RMA72/RMX19-36	6	0	0	12	RMA72/VAX6-4	8	0	0
22	RMA72/RMX19-20	6	1	0	13	RMA72/VAX6-23	5	1	0
23	RMA72/RMX19-20	5	1	0	14	RMA72/VAX6-23	6	1	0
24	RMA72/RMX19-16	8	0	0	15	RMA72/VAX6-8	4	1	1
25	RMA72/RMX19-16	8	0	0	16	RMA72/VAX6-8	4	1	1
26	RMA72/RMX19-25	8	0	0	17	RMA72/VAX6-14	7	1	0
27	RMA72/RMX19-25	8	0	0	18	RMA72/VAX6-14	7	1	0
	P1 RMA72 5*	7	0	0	19	RMA72/VAX6-29	8	0	0
	P2 VAX6	2	1	1	20	RMA72/VAX6-29	6	1	0
21	RMA72/VAX6-29	3	0	1	47	RMA72/VAX6-5	9	1	1
22	RMA72/VAX6-27		1	1	48	RMA72/VAX6-25	3	0	1
23	RMA72/VAX6-27	3	1	1	49	RMA72/VAX6-25	4	0	1
24	RMA72/VAX6-27	7	1	0	50	RMA72/VAX6-25	4	0	1
25	RMA72/VAX6-27	8	1	1	51	RMA72/VAX6-25	3	0	1
26	RMA72/VAX6-12	2	0	1	52	RMA72/VAX6-9	5	1	1
27	RMA72/VAX6-12	6	0	0	53	RMA72/VAX6-9	3	1	1
28	RMA72/VAX6-12	6	0	0	54	RMA72/VAX6-9	5	0	1
29	RMA72/VAX6-12		0	1	55	RMA72/VAX6-9	2	1	1
30	RMA72/VAX6-22	2	1	1	56	RMA72/VAX6-15	8	0	0
31	RMA72/VAX6-22	3	1	1	57	RMA72/VAX6-15	7	0	0

32	RMA72/VAX6-35	6	1	0	58	RMA72/VAX6-1	5	1	0
33	RMA72/VAX6-35	7	1	0	59	RMA72/VAX6-1	6	1	0
34	RMA72/VAX6-7	8	0	0	60	RMA72/VAX6-30	7	1	1
35	RMA72/VAX6-7	7	0	0	61	RMA72/VAX6-30	8	1	1
36	RMA72/VAX6-7	5	0	0	62	RMA72/VAX6-13	2	1	1
37	RMA72/VAX6-21	2	1	1	63	RMA72/VAX6-13	2	1	1
38	RMA72/VAX6-21	3	0	1	64	RMA72/VAX6-13	4	1	1
39	RMA72/VAX6-21	3	0	1	65	RMA72/VAX6-13	3	1	1
40	RMA72/VAX6-21	3	0	1	66	RMA72/VAX6-28	2	1	1
41	RMA72/VAX6-34	7	1	0	67	RMA72/VAX6-28	2	1	1
42	RMA72/VAX6-34	5	1	1	68	RMA72/VAX6-28	5	0	0
43	RMA72/VAX6-34	5	1	1	69	RMA72/VAX6-28	6	1	1
44	RMA72/VAX6-5	9	1	1	70	RMA72/VAX6-3	5	0	0
45	RMA72/VAX6-5	9	1	0	71	RMA72/VAX6-3	6	0	0
46	RMA72/VAX6-5	9	1	0	72	RMA72/VAX6-32	6	1	1
81	RMA72/VAX6-17	4	1	0	73	RMA72/VAX6-32	6	1	1
82	RMA72/VAX6-31	5	1	1	74	RMA72/VAX6-2		1	1
83	RMA72/VAX6-31	4	0	0	75	RMA72/VAX6-2	6	1	1
84	RMA72/VAX6-11	4	0	0	76	RMA72/VAX6-2	6	1	0
85	RMA72/VAX6-11	4	0	1	77	RMA72/VAX6-2	6	1	0
86	RMA72/VAX6-18	6	1	1	78	RMA72/VAX6-17	6	1	0
87	RMA72/VAX6-18	2	1	1	79	RMA72/VAX6-17	5	1	0
88	RMA72/VAX6-18	6	0	0	80	RMA72/VAX6-17	4	1	1

***CBB mean score per population**