STUDY OF YIELD STABILITY AND BREEDING FOR COMMON BACTERIAL BLIGHT RESISTANCE IN SOUTH AFRICAN DRY BEAN GERMPLASM

By

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DISSERTATION ABSTRACT

Dry bean is an important legume crop worldwide. In South Africa it is a major source of plant protein and income among growers. The crop is mainly produced in the Free State, Mpumalanga, Gauteng, KwaZulu-Natal, North-West, Limpopo and Eastern Cape provinces, all exhibiting different agro- ecological conditions such as temperature, humidity, soil fertility and rainfall. Crop productivity is low such that national demand outstrips production. The main attributing factors to low production include abiotic and biotic stresses. Yield instability due to environmental changes and common bacterial blight disease (CBB) caused by *Xanthomonas axonopodis* pv *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans* (Xff) are considered to be major attributing factors to low productivity. The overall goal of the research was to contribute to the improvement dry bean production in South Africa through identifying stable and high yielding cultivars from the released cultivars, identifying mega environments for dry bean production and conducting pre-breeding experiments that will results in the generation of information relevant to common bacterial resistance breeding in South Africa.

Yield stability and adaptation was studied using data of 30 released cultivars from the national cultivar trials planted in 21 locations of which 17 are in South Africa, two in Swaziland and two in Lesotho. Additive main effect multiplicative interaction (AMMI) statistical model was used to investigate yield stability and adaptation in the study. The results revealed that variances due to environment, genotype and genotype by environment interaction were all significant ($P<0.01$). The AMMI analysis also showed that interaction principal components (IPCA1, IPCA2 and IPCA3) were all significant ($P<0.01$). Based on the AMMI stability value, cultivars G6 and G14 were both identified as stable and high yielding. Their yield exceeded the grand mean yield of 1.3 t ha^{-1}. Cultivars G20, G26 and G29 were high yielding with mean yield exceeding the grand mean, but exhibiting specific adaptation.

A total of 60 genotypes were evaluated for both CBB resistance and grain yield. Eight of the evaluated genotypes were from the local dry bean breeding programmes. These local cultivars were considered as checks in the experiment. The evaluation study was conducted at two localities in South Africa (Cedara and Potchefstroom) under artificial inoculation using a mixture of two local isolates (Xf260 and Xf410). Relative area under disease progress curve (RAUDPC) was used to determine reaction to CBB. The evaluated germplasm exhibited different reactions to CBB. Approximately 20% of genotypes exhibited resistance across the two locations, 43% and 37% showed moderate and susceptible reaction to CBB, respectively.

Mean grain yield was 1.29 t ha^{-1}. The majority of susceptible genotypes yielded below the mean. However, there was a weak negative correlation (r = -0.48, P = 0.05) between disease reaction and yield. Genotypes ADP-0041, ADP-0790, M-125, ADP-0096, ADP-0544 and M-191 were selected as both high yielding and resistant. These genotypes exhibited good levels of resistance and yielded above 1.7 t ha^{-1}. Genotypes ADP- 0055, ADP-0099 and ADP-0103 were selected on the basis of yield. These genotypes yielded above 1.7 t ha^{-1} but showed susceptible reaction to CBB.

A study was initiated using two crosses from South African market class cultivars (Teebus-RCR 2 x Teebus-RR 1 and RS 7 x Tygerberg) to investigate the mode of gene action governing inheritance, estimate heritability, establish the significance of maternal effects in CBB resistance and determine the efficacy of marker assisted selection (MAS) in CBB resistance breeding using two SCAR markers BC420 and SU91. Beside additive-dominant gene effects, epistatic gene effects were also detected. Dominant gene effect was of more significance than additive gene effects in both crosses. Duplicate epistasis was detected in the Teebus-RCR 2 x Teebus-RR 1 cross. Gene dispersion was detected in both crosses. Heritability of CBB resistance was moderate in both crosses, maternal effect was of significance in the two crosses and lastly, resistance was found to be linked to two QTL SCAR markers (BC420 and SU91) in Teebus- RCR2. Both markers were absent in RS 7. SU91 was found to be the only marker that could be effectively utilized in MAS. The implication of these findings for CBB resistance breeding is that it will affect the selection strategy to be deployed and also the choice of a female parent in resistance breeding programme. Backcross breeding, recombinant breeding, delayed selection, choosing a resistant parent as a female parent and using MAS especially in crosses involving Teebus-RCR 2, could yield positive result in CBB resistance breeding programme if these parents are to be used.

In general, the study confirmed the existence of genotype by environment interaction in South African dry bean growing environment, and valuable common blight resistant and high yielding dry bean lines, which could be used in dry bean breeding, were identified. Lastly, the inheritance mechanism of common blight resistance and the efficacy of marker assisted breeding for the trait were established.

DECLARATION

I, Wilson Nkhata, declare that,

The research reported in this dissertation, except where otherwise indicated, is my original work.

This dissertation has not been submitted for any degree or examination at any university.

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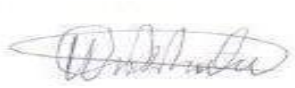
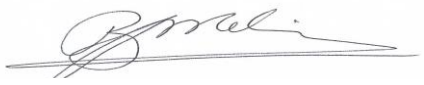
Signature  Date: 14th March 2017

Wilson Nkhata

As the candidate's supervisors, we agree the submission of this dissertation:

Signature  Date: 14th March 2017

Prof Rob Melis (Main Supervisor)

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Dr Deidré Fourie (Co-Supervisor)

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DEDICATION

The dissertation is dedicated to the work and contribution of Prof Rob Melis and Dr Deidré Fourie to research of dry bean in Africa

TABLE OF CONTENTS

ABBREVIATIONS

ADP	Andean Diversity Panel
AFLP	Amplified Fragment Length Polymorphism
AMMI	Additive Main Effect Multiplicative Interaction
ANOVA	Analysis of Variance
ARC-GCI	Agricultural Research Council-Grain Crop Institute
ASV	AMMI Stability Value
CBB	Common bacterial blight
CIAT	Centre for International Tropical Agriculture
CV	Coefficient of Variation
DAFF	Department of Agriculture, Forestry and Fisheries
DDAP	Days to each disease assessment after planting
DF	Degrees of freedom
DNA	Deoxyribonucleic acid
DPM	Days to physiological maturity
FD	Days to flowering
GEI	Genotype by environment interaction
GGE	Genotype main effects and genotype by environment interaction
IMCDA	Improvement Masters in Cultivar Development for Africa
IPCA	Interaction principal components Axis
LSD	Least significance difference
Masl	Metres above sea level

MAS	Marker assisted selection
MS	Mean sum of squares
NCT	National Dry Bean Cultivar Trials
PCR	Polymerase chain reaction
PIC	Phaseolus improvement cooperative
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RAUDPC	Relative area under disease progress curve
REML	Residual maximum likelihood
RLT	Relative lifetime
SCAR	Sequence conserved amplified repeats
SS	Sum of squares

INTRODUCTION TO DISSERTATION

1 Background

Dry bean (*Phaseolus vulgaris L.*) (2n =22) is the third most important grain legume crop in the world and represents 50% of grain legumes for direct consumption (Zhang et al., 2008; Blair et al., 2009). The crop is grown on all continents between 52 ^{o} N and 32 ^{o} S, from sea level to as high as 3000 m above sea level. Production in Africa is mainly concentrated in East and Southern Africa where a total of 3.7 million ha of arable land is devoted to the crop annually (Kimani et al., 2001). Mean annual production in South Africa is 56,411 metric tons (2006-2015) on an area of 47,677 ha which does not meet the annual demand of 120,000 metric tons (Kleingeld, 2015). In South Africa, the crop is mainly produced in the Free State, Mpumalanga, Gauteng, KwaZulu-Natal, North West, Limpopo and Eastern Cape provinces.

Table 1. Dry bean production from 2006-2015 in South Africa

Year	Harvested area (ha)	Quantity (MT)	Average yield (t ha^{-1})
2006	60,000	61,500	1.0
2007	51,000	35,290	0.7
2008	40,000	52,223	1.3
2009	42,000	62,520	1.5
2010	41,600	47,899	1.2
2011	41,900	40,992	1.0
2012	37,050	47,689	1.3
2013	43,550	60,600	1.4
2014	55,670	82,129	1.5
2015	64,000	73,330	1.1

Source; Kleingeld, C. (2015)

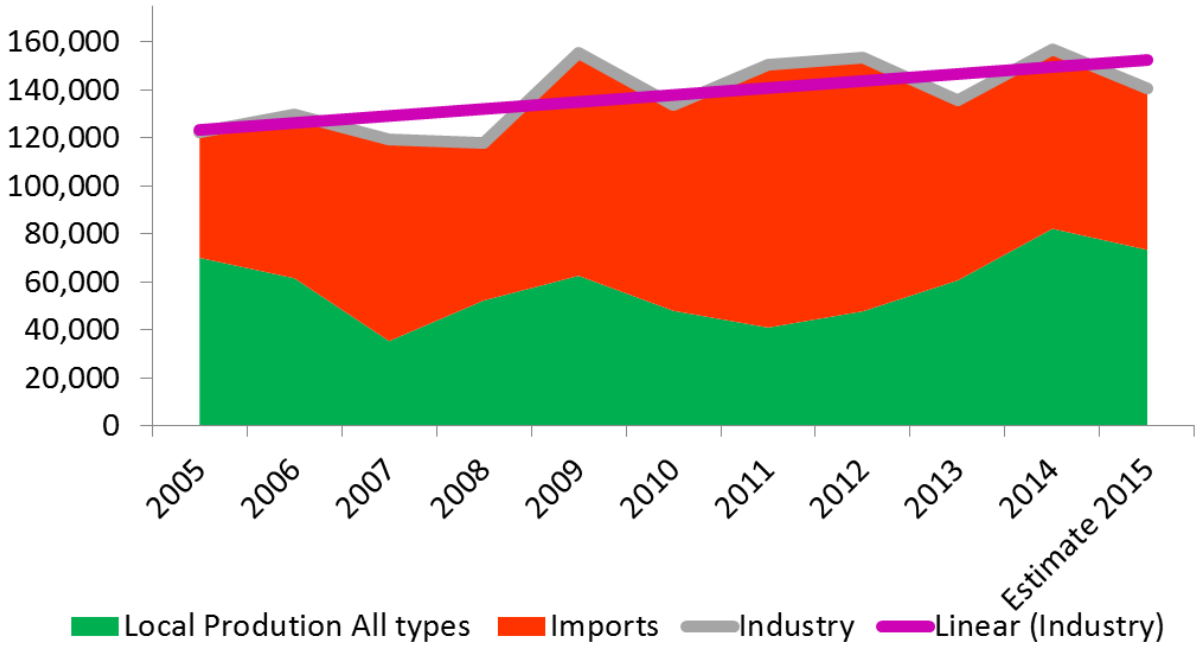

Figure 1. National dry bean consumption for South Africa 2005-2015

Source; Kleingeld, C. (2015)

2 Importance of dry bean

Dry bean is rich in proteins, carbohydrates and vitamins. It is also rich in minerals such as phosphorus, copper, manganese and magnesium. The crop is ideal in enhancing food security and in combating nutritional deficiencies. Due to its high protein content, dry bean has been described as "*the meat for the poor*" as it is the best substitute of animal protein, which is expensive (Kimani et al., 2001). In sub-Saharan Africa, dry bean is mainly used as relish (sauce). The leaves, green pods, fresh seeds and dry grains of this crop can be consumed. Dry bean improves soil fertility by fixing nitrogen in the soil, an intrinsic feature of legumes (Rondon et al., 2007). This is an added advantage, especially when intercropped with maize because it reduces amounts of inorganic fertilizers to be applied, as these are expensive, especially for the resource limited farmers. Apart from being a source of food and improving soil fertility, dry beans also provide a supplementary source of income to farmers, especially rural women.

3 Dry bean production constraints in South Africa

The mean yield (1.2 t ha^{-1}) of dry bean in South Africa is much less as compared to achievable yields in North America (3 t ha^{-1}) (Graham and Ranalli, 1997). Apart from low yield per ha, dry bean production in South Africa is further characterised by yield instability. The causes of low

yield and yield instability are abiotic stresses (unreliable rainfall, adverse temperatures and low soil fertility) and biotic stresses (diseases and insects). Among the diseases, common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans* (Xff) is considered as one of the most important bacterial diseases in South Africa. The disease occurs in almost all dry bean producing areas in the country (Fourie, 2002) and may result in yield loss of up to 60% under severe conditions (Marquez et al., 2007).

4 Problem statement and justification

The demand for dry beans in South Africa outstrips the production. Yield instability and CBB are major causes of low production. Thus the need of identifying cultivars that are stable, high yielding and resistant to CBB. This study was, therefore, undertaken to evaluate local cultivars for yield stability and adaptation and to screen new introductions for yield factors and the reaction to CBB. In addition, the study investigated the efficacy of marker assisted selection in breeding for CBB resistance in South Africa.

5 Overall research goal

The overall goal of the research was to contribute to the improvement of dry bean production in South Africa through identifying stable and high yielding cultivars and conducting pre-breeding experiments that will result in generation of information relevant to common bacterial resistance breeding in South Africa.

The specific objectives for the research were as follows:

- To determine the grain yield stability and adaptability of 30 South African dry bean cultivars grown in 21 environments.
- To evaluate newly introduced dry bean germplasm for yield and resistance to common bacterial blight.
- To investigate the heritability of common blight resistance and the efficacy of marker assisted selection for the trait in breeding for common bacterial blight resistance.

6 Dissertation outline

The dissertation is in the form of discrete research chapters, each following the format of a stand-alone research paper. This is the dominant dissertation format adopted by the University of KwaZulu-Natal. As such, there is some non-avoidable repetition of references and some introductory information between the chapters. The advantage of this format is that it facilitate publication of research papers since each paper stands as a research article. The referencing system used in this dissertation is based on the “Crop Science Society of America (CSSA)”, referencing style and follows the specific style in the “Crop Science Journal”.

Table 2. The outline of the dissertation is as follows

Chapter	Title
	Dissertation introduction
1	Literature review
2	Yield stability and adaptation of analysis of South African dry bean cultivars
3	Evaluation of newly introduced dry bean germplasm for resistance to common bacterial blight and for yield
4	Investigating heritability and efficacy of marker assisted selection in breeding for common bacterial blight resistance in South African dry beans
5	General overview of the study and implications for breeding

7 References

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CHAPTER 1
LITERATURE REVIEW

1.1. Introduction

This chapter is a review of the origin, domestication, botany and diversity of dry beans. It also highlights constraints associated with dry bean production in brief and specifically of the common bacterial blight, its epidemiology and the taxonomy of the pathogen causing the disease. Efforts on breeding resistance to common bacterial blight and achievements are also reviewed, which includes the genetics of the common bacterial blight disease resistance and defence mechanisms to the disease in a plant. The chapter further discusses the mating designs and their importance in genetic studies in brief and application of generation mean analysis and how it has been exploited by breeders in genetic studies. The effect of genotype by environment interaction in plant breeding programme and its analytical techniques used in quantifying it, is also highlighted.

1.2. Botany and genetic diversity of dry bean

Dry bean (*Phaseolus vulgaris* .L) is native of America and two gene pools of wild dry beans exist, namely, Mesoamerican and Andean gene pools (Singh and Schwartz, 2010). The Andean dry beans are large seeded, while the Mesoamerican are small seeded (Singh, 2001). It is believed that domestication of the two gene pools, started almost at the same time, between 7000 to 8000 years back around the Andean and Mexican highlands, respectively (Gepts and Debouck, 1991). The domesticated dry bean evolved over time from wild dry bean through continuous selection, mutation and migration. Growth habit, seed size, seed retention, and maturity are some of the distinct features that distinguish the modern cultivated dry bean from their ancestral wild form (Gentry, 1969). In terms of distribution from the two centres of origin to the rest of the world, the Andean beans are presently predominantly grown in Africa, Europe and north-eastern United States, while Mesoamerican beans are predominately grown in South America, an indication that the distribution of the two gene pools followed two different routes (Gepts and Bliss, 1985). A comparison of the genetic diversity of the two gene pools based on molecular analysis showed that Mesoamerican dry beans have a wider genetic diversity than Andean dry beans, although the latter have a remarkable morphological diversity (Santalla et al., 2004). Another prominent and interesting feature of these two gene pools is that hybridization between them can result in weak F1 plants which is reported to be an attribute of independent evolution (Gepts and Bliss, 1985; Gepts and Debouck, 1991).

1.3. Taxonomy of dry bean

The dry bean (*Phaseolus vulgaris* L.*)* belongs to the family Fabaceae (Leguminosae) sub-family Papilionoideae and genus Phaseolus. The genus is found in the Mexican mountains, containing 50 species of which five are being cultivated, namely, dry bean (*Phaseolus vulgaris* L.*)*, lima bean (*Phaseolus lunatus* L.), scarlet runner bean (*Phaseolus coccineus* L.), year-bean (*Phaseolus polyanthus* Greenman) and tepary bean (*Phaseolus acutifolius* A.Gray). Among these five, dry bean (*Phaseolus vulgaris* L.*)* is the most cultivated and widely distributed. It is cultivated both in the tropical and subtropical environment and approximately 90% of the total global land for the production of the Phaseolus genus is allocated to dry bean (*Phaseolus vulgaris* L.*)* (Singh, 1989). The crop is a diploid and has uniform chromosomal number, 2n=2x=22. Diploid is a common feature among all species of Phaseolus genus (Mercado-Ruaro and Delgado-Salinas, 2000). Dry bean has a complete flower and it is a self-pollinated crop.

1.4. Morphology of dry bean

Dry bean is a typical herbaceous dicot plant having primary tap roots where secondary roots emerge. The other distinct feature of dry bean roots are root hairs which emerge later on the secondary and primary root covering an area of 10 cm. It has a shallow tap root, which does not go beyond 20 cm (Debouck, 1991; Graham and Ranalli, 1997). Like any other member of the sub-family Papilionoideae, dry bean has nodules which are distributed on the lateral roots of the upper and middle parts of the root system. The nodules are usually 2 mm to 5 mm in diameter and are colonized by Rhizobium bacteria which fix atmospheric nitrogen to the soil (Debouck et al., 1986). The main stem is cylindrical or slightly angular in cross section. The stem is larger in diameter than any branch. It can be either erect, semi-prostrate or prostrate, but it tends to be vertical, either when the bean is growing alone or with support. Stem colour and hairiness varies from one variety to the other and also to some extent with the age of the plant. The primary leaves of dry bean are unifoliate and secondary leaves are trifoliate. These are inserted at the nodes of the stem and branches. Flower initiation is within 28-42 days from the day of planting, though it may take longer than this in climbing beans grown at higher altitude. Flowers rise from the axially bud complexes (triad) located in the axil of the leaves. As typical example of the Papilionoideae flower, the flower is initially enveloped by the oval or round bracteoles which open at a later stage. Flowers have different colours; white, pink and purple. Flower colour is reported to be genetically independent to seed colour. Flowers are zygomorphic with ten stamen and a single multi-ovuled ovary. This floral morphology favors self-pollination. The ovaries, which are future seeds, arise alternately on both sides of the placenta suture and the seed that arises is non- endospermic and its nutritional reserves are stored in the two cotyledons. About 3 to 10 seeds can be achieved

from one pod. Dry bean seeds are of various shapes and colour with seed size ranging from 50 mg/seed in wild species to 2000 mg/seed in large Colombian cultivars (Debouck, 1991; Graham and Ranalli, 1997).

1.5. Dry bean growth habit

Dry bean cultivars may be classified based on their growth habit. Essentially dry bean has two growth habits, namely determinate and indeterminate. Determinate type growth ceases when an inflorescence appears and usually the bean plant has few nodes and internodes. With the indeterminate type, the stem remains vegetative and it continues to develop, forming nodes and internodes even during the reproductive phase. Studies on dry bean growth habit at CIAT, resulted in classification of the growth habit into four groups namely; Type I which has determinate growth habit characterized with well-developed stem and lateral branches. Growth ceases once inflorescence is formed and the plants are usually short with few branches. Type II indeterminate which has an erect stem with more nodes and internodes than type I though it has inability to climb and continue to grow during flowering. Type III has indeterminate prostrate growth habit. The stem is prostrate or semi-prostrate with well-developed branches and if provided with support, it can climb, hence are also referred to as semi-climbers. Type IV indeterminate which has a climbing growth habit with a long main stem. The stem develops double twisting capacity from the first trifoliate leaves and it is poorly branched with a prolonged flowering period (Graham and Ranalli, 1997).

1.6. Genetic diversity of dry bean

The initial parallel domestication of the two dry bean gene pools, intensive selection and dispersal during domestication, have resulted in a large genetic diversity of the crop (Acosta-Gallegos et al., 2007; Blair et al., 2009; Kwak and Gepts, 2009). Studies on genetic diversity has utilized morphological markers such as seed size, geographical and ecological distribution, biochemical markers for example isozymes, allozymes and phaesolin seed proteins and molecular markers such as microsatellites, random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) (Singh et al., 1991b; Galvan et al., 2003; Blair et al., 2009). Blair et al. (2009) used microsatellite markers and seed characteristics such as seed length, width and thickness in assessing the genetic diversity of 604 genotypes from the CIAT germplasm and core collections. A similar method was used to establish the genetic diversity of dry bean in China (Zhang et al., 2008). It was found that French dry beans have lower genetic diversity than those from Argentina using ISSR and also that ISSR markers were more reliable than RAPD in identifying beans by gene pool. These ISSR markers were reported to be limited in revealing variations between

genotypes (Galvan et al., 2003). In Italy two types of DNA markers, namely ISSRs and SSRs, were used to establish the diversity of local genotypes and the results from all markers revealed that 71% of genotypes were from the Andean gene pool (Sicard et al., 2005).

Genetic diversity studies have also been used in classifying the crop on the basis of 100 seed mass into three categories namely; large seeded (>40 g 100-seed weight^{-1}) medium seeded (25–40 g 100-seed weight^{-1}) and small-seeded (<25 g 100-seed weight^{-1}) (Evans, 1980). The large seeded belonged to the Andean pool, while the medium and small seeded were Mesoamerican (Zhang et al., 2008). Similar studies have led to further classification of these primary gene pools into seven races. Races are a group of genotypes which are clustered together and members of each race have distinct features which include, morphology traits and agro-ecology adaptation (Singh et al., 1991a; Acosta-Gallegos et al., 2007). The races in the Andean gene pool are Nueva Granada, Peru and Chile, while for Mesoamerican are Mesoamerica, Durango, Jalisco and Guatemalan (Beebe et al., 2000; Zhang et al., 2008). Acosta-Gallegos et al. (2007) reported that in the Mesoamerican gene pool, Mesoamerica and Durango races are the most widely grown dry beans and on the other hand Jalisco and Guatemalan have received little attention by researchers in terms of improvement. Knowledge and access on genetic diversity have been reported to be useful in the breeding programme as it broadens the genetic base thus assuring continued crop improvement (Acosta-Gallegos et al., 2007; Zhang et al., 2008).

1.7. Dry bean production

Dry bean is the third most important grain legume crop in the world and it represents 50% of grain legume for direct consumption (Zhang et al., 2008; Blair et al., 2009). It is the main source of dietary protein in Latin America and East Africa (Graham and Ranalli, 1997). Wortmann (1998) reported that annual consumption of dry bean exceeds 50 kg per person in the African Great Lakes Region. The crop is grown on all continents between 52 ^{o}N and 32 ^{o}S, from sea level to as high as 3000 m above sea level. However, production is largely concentrated in Latin America and Eastern and Southern Africa (Pachico, 1989; Graham and Ranalli, 1997). The total area under production worldwide is estimated to be over 18 million ha (Graham and Ranalli, 1997; FAO, 2015). In developing countries small farmers, especially women, are primary producers of the crop and it is cultivated both as a sole and secondary crop in association with staple crops such as maize, sorghum, millet, banana, root and tubers (Wortmann, 1998). Production among small holder farmers is low per unit area and reported to be less than 0.5 t ha^{-1} in Latin America and Africa when compared to yield achieved in highly mechanized commercial farms in North America (3 t ha^{-1}) and also in experimental field (5 t ha^{-1}) (Graham and Ranalli, 1997).

1.8. Dry bean yield limiting factors

There is a great variation in terms of factors that limit production in large commercial farms of dry bean in the USA and Europe compared to those of small scale farmers of Latin America and Africa (Graham and Ranalli, 1997). Production constraints of dry bean in Latin America and Africa which limit yields have been reported by several authors (Saettler, 1989; Wortmann, 1998; Kimani et al., 2001). These include abiotic and biotic stresses.

Abiotic factors that limit dry bean production include a range of physical environmental factors and these are either climatic or soil related. The most predominant climatic related factor is inadequate rainfall which result in moisture deficit and under severe condition the problem causes complete crop loss (Wortmann, 1998). Soil related production limiting problems includes essential nutrient deficiency (low nitrogen, phosphorus and potassium), poor exchangeable bases and aluminum and manganese toxicity (Kimani et al., 2001).

Diseases and insect pests, are major biotic factors that limit dry bean production. Diseases are reported to impact more in the tropics than in cool temperate climate. Farming systems in the tropics allow two to three planting cycles per year, which results in a continuing presence of pathogen inoculum. Similarly, under field conditions, insect pests cause more damage in Africa and Latin America than in USA and Europe (Graham and Ranalli, 1997). The important dry bean diseases include angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), bacterial brown spot (*Pseudomonas syringae* pv. *syringae*), bean common mosaic virus (BCMV), bean common mosaic necrosis virus (BCMNV), beet curly top virus (BCTV), bean golden mosaic virus (BGMV), bean golden yellow mosaic virus (BGYMV), common bacterial blight (*Xanthomonas axonopodis* pv *phaseoli* (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans*), halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*), root rots (*Aphanomyces, Fusarium, Pythium, Rhizoctonia, Thielaviopsis species*), rust (*Uromyces appendiculatus*), web blight (*Thanatephorus cucumeris*) and white mold (*Sclerotinia sclerotiorum*) (Beebe et al., 1991; Miklas et al., 2006; Singh and Schwartz, 2010). Insect pests that are of economic importance in dry bean production include, bean pod weevil (*Apion godmani* Wagner), bruchids (*Coleoptera: Bruchidae*), leafhoppers (*Empoasca* spp.*; Homoptera cicadellidae*), thrips palmi Karny (*Thysanoptera thripidae*), stem maggot (*Ophiomyia phaseoli O. spencerella*, and *O. centrosematis*) and aphid (*Aphis fabae*, and *A. craccivora*) (Graham and Ranalli, 1997; Wortmann, 1998; Miklas et al., 2006). Apart from abiotic and biotic factors, agronomic factors such as late planting, poor weed management, continuous cropping and use of unimproved seed have been reported to limit dry bean production (Kimani et al., 2001).

Table 1.1. Losses to dry bean production in Africa, ranked in descending order of importance, by constraint

Constraint	Eastern and central Africa	Southern Africa	Sub-Saharan Africa
	Losses in tons/year		
Biotic			
Angular leaf spot	281,300	93,500	384,200
Anthracnose	247,400	69,800	328,000
Bean stem maggot	194,400	96,400	297,100
Bruchids	163,000	77,600	245,600
Root rots	179,800	31,000	221,100
Common bacterial blight	145,900	69,800	220,400
Aphids	136,300	58,900	196,900
Rust	118,700	72,400	191,400
Bean common mosaic	144,600	29,900	184,200
Abiotic			
Drought	291,200	94,600	396,000
N deficiency	263,600	125,200	389,900
P deficiency	234,200	120,400	355,900
Exchangeable bases	152,700	65,800	220,000
Al/Mn toxicity	97,500	60,300	163,900

Source : Kimani et al. (2001)

1.9. Common bacterial blight of dry bean

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans* (Xff), is an important disease of dry bean (*Phaseolus vulgaris* L.) worldwide. The disease was first described in the United States of America in the 1800s (Zaumeyer and Thomas, 1957; López et al., 2006). Presently, CBB is prevalent in tropical and sub-tropical production regions worldwide (Singh and Schwartz, 2010; Viteri and Singh, 2014). The range of yield losses due to CBB is poorly documented, although some researchers have reported that it ranges from 20 to 60% (Marquez et al., 2007; Fourie et al., 2011). The extend of yield loss is dependent on favorable weather conditions, disease pressure and susceptibility of the cultivars (Zaumeyer and Thomas, 1957; e Silva et al., 1989). The disease is of world economic importance due to its epidemiology and yield losses. Apart from dry bean, CBB also occurs in scarlet runner beans (*Phaseolus coccineus* L.), mung bean (*Vigna radiate* L.), tepary bean (*Phaseolus acutifolius* A.), soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L. Walp) (Zaumeyer and Thomas, 1957; Saettler, 1989).

1.9.1. Epidemiology of common bacterial blight

The common bacterial blight pathogen is a warm temperature pathogen, with an optimum temperature of 28 ^{o}C (Saettler, 1989). Growth stops at temperatures below 16 ^{o}C and above 32 ^{o}C. High relative humidity also favors condition for the pathogen growth (e Silva et al., 1989; Saettler, 1989). The severity of common bacterial blight is partially controlled in the tropics by maize-bean intercrop farming systems. Maize offers a physical barrier and thereby hinders the spread of the pathogen in the field (Saettler, 1989; Fininsa, 2003).

Common bacterial blight is a seed borne disease of dry beans and the pathogen survives in the seed as long as the seed is viable (Fininsa and Tefera, 2001; Karavina et al., 2008). The mode of transmission of the pathogen is primarily seed and the pathogen can be present internally or externally. Infested debris and soil are also important as sources of primary inoculum due to the fact the pathogen has the ability of overwintering in these for long periods (Arnaud-Santana et al., 1991; Fininsa and Tefera, 2001). Several factors have been reported to affect the concentration of inoculum, including initial inoculum present, favorable temperatures, relative humidity and rain during overwintering. Fininsa and Tefera (2001) reported that soil factors, such as rate of decomposition and activities of microorganism, influence the growth rate of the pathogen. Volunteer plants present in the field may also harbor the pathogen and is a threat to the new crop. Saettler (1989) reported that spread of CBB is affected by rain, windblown soils, irrigation water, humans, animals and insects such as whitefly and beetles. Implements and humans operating in the field injure plants and thereby create passages for the entry of the pathogen.

Factors influencing the quantity of inoculum from primary sources include, concentration of inoculum initially present, favorable temperatures, relative humidity and rain during overwintering, location of debris and effectiveness of seed treatment (Fininsa and Tefera, 2001). The stage of the epidemic is mainly determined by the initial source of inoculum. Fininsa and Tefera (2001) reported that primary inoculum from infested seed and infested debris causes earlier epidemics resulting in larger yield losses than inoculum from the soil which causes later epidemics.

Symptoms caused by CBB usually appear on leaves, pods, stem and seeds. On leaves, initial symptoms appear as water-soaked spots which eventually enlarge and coalesce with adjacent lesions (Saettler, 1989). Infected tissues appear flaccid and lesions are often encircled by a narrow zone of lemon yellow tissue. These lesions, which are irregular in shape, develop either on the edges of the leaf or on the leaf surface. Premature defoliation of heavily infected leaves is a typical symptom of the disease. Pod lesions appear as water-soaked which may enlarge and become dark, red and slightly sunken (Saettler, 1989). In stems the predominant symptom is hypertrophy and darkening of the stem at an early stage of vegetative growth. In seed the

predominant symptoms are buttered yellow spots on white or light coloured seeds. These are difficult to observe on dark coloured seeds. The seed shape can be deformed and damaged, resulting in poor germination and reduced vigour (Zaumeyer and Thomas, 1957; Saettler, 1989).

Common bacterial blight can be controlled chemically or by good agricultural practices. The use of certified pathogen free seed, crop rotation, deep ploughing and practising field hygiene, have been recommended for control of CBB. However, these measures have failed to register success in Africa because the majority of dry bean farmers are poorly resourced and usually use on-farm saved seed or seed from fellow farmers, which is mostly infested by the pathogen. Additionally, farmers have small arable lands making it hard for them to practise crop rotation and in general they use simple implements in land preparation restricting deep ploughing. Use of various chemicals such as copper hydroxide and potassium methyldithiocarbamate, particularly when applied early, have been reported to control foliage infection. However, spraying chemicals does not significantly reduce pod infection and increase grain yield but increases the cost of production (Saettler, 1989; Mkandawire et al., 2004). The use of resistance cultivars, and integrating cultural and chemical control methods, is the most effective way of controlling the disease.

1.9.2. Taxonomy of common bacterial blight pathogen

Xanthomonas axonopodis pv *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans* (Xff) (Smith) Dye belongs to the kingdom bacterial; phylum proteobacteria; class gammaproteobacteria; order Xanthomonadales; Family: Xanthomonadaceae; genus *Xanthomonas.* The genus was classified in 1939 (Dowson, 1939) and has been subjected to many taxonomic studies as it causes many plant diseases which are of economic importance (Vauterin et al., 1990). Genetic studies have established that the genus has a separate rRNA branch in the *Proteobacteria* gamma taxon (De Vos and De Ley, 1983). Morphologically, Xap is a non-spore forming, motile, aerobic, gram-negative rod, 0.4-0.9 x 0.6-2.6 µm, with a single polar flagellum. Agar colonies are convex, yellow and wet-shining. In culture, on complex media or media containing tyrosine, a brown, diffusible pigment is produced by so-called *fuscans* strains (Xff). Several methods have been developed over the years to test its presence in the seeds and other parts of the plants (Ednie and Needham, 1973).

1.9.3. Modes of penetrations

The CBB pathogen enters leaves via stomata or wounds created by mechanical damage due to wind-blown soil particles, leaf feeding insects and humans. Subsequently, the pathogen invades the intercellular spaces, causing a gradual dissolution of the middle lamella. Cell disintegration occurs due to formation of bacterial pockets (Zaumeyer and Thomas, 1957; Kaiser and Vakili,

1978; Saettler, 1989; Akhavan et al., 2013). Apart from entering into the leaves, the pathogen can also enter the stem directly through the stomata of the hypocotyl and epicotyl for the leaf vascular structures and infected cotyledons (Kaiser and Vakili, 1978; Akhavan et al., 2013). The pathogen can invade the pod through the pedicel into funiculus through the raphe leading into the seed coat, where it either remains in the seed coat or in the cells of the seed and from there spread to the cotyledon region. The pathogen found in the seed is the source of infection to the germinating young plants (Zaumeyer and Thomas, 1957; Akhavan et al., 2013). The pathogen can remain viable in the seed coat for several years and a single inoculum can contaminate an area of more than 8 m around it, translating to one diseased plant in 10 000 plants being sufficient to cause a severe epidemic under favorable conditions (Akhavan et al., 2013). Direct penetration of the pathogen into the seed coat has not been observed (Zaumeyer and Thomas, 1957; Saettler, 1989).

1.9.4. Genetic variability of common bacterial blight pathogen

Extensive research on the genetic diversity of the common bacterial blight pathogen has been conducted in different dry bean growing regions and it has offered a prerequisite for developing CBB resistant cultivars adapted to a specified area (Mkandawire et al., 2004; López et al., 2006; Alavi et al., 2008; Zamani et al., 2011). Studies have reported that the common bacterial blight pathogen exhibits genetic diversity and that there is correlation between the gene pool predominantly grown in the area and the pathogen strain due to co-evolution. Mkandawire et al. (2004) reported that Xap and Xff dominate in Malawi, whereas Xff dominate in Tanzania. In Tanzania Xff strains were associated with Andean and Mesoamerican dry beans (Mkandawire et al., 2004). The findings were in line with what was reported in a similar study, where it was found that 60% of CBB was caused by Xff and the remaining was due to Xap in East Africa (Opio et al., 1996). Though very aggressive, Xff has been reported to exhibit lower genetic diversity than Xap (Mkandawire et al., 2004).

Host pathogen co-evolution has similarly been reported in the fungal dry bean diseases i.e. *Colletotrichum lindemuthianum* (causal agent of anthracnose), *Phaeoisariopsis griseola* (causal agent of angular leafspot) and *Uromyces appendiculatus* (causal agent of bean rust),in which genetically distinct pathogens tend to be more pathogenic to genotypes of one gene pool than the other (Guzmán et al., 1995; Sicard et al., 1997; López et al., 2006). Contrary to these results, López et al. (2006) conducted a study to determine the diversity of CBB bacteria in the north-central part of Spain in order to establish whether Spanish CBB strains were similar with those from other geographical regions. They found that there was no genetic diversity and that strains were similar to those from other geographical regions, an indication that there was no co-evolution

of bacterial strain in Spain as was the case in Eastern Africa. Different techniques have been used to study diversity of the Xanthomonas genus. These include biochemical, physiological, pathogenicity, polymerase chain reaction (PCR) and repetitive polymerase chain reaction (rep-PCR). However, the inability of biochemical and physiological techniques to classify the bacteria beyond specie level, has resulted in less exploitation of the techniques in classifying Xanthomonas bacteria. Pigmentation has been used to distinguish Xap from Xff. Several studies on genetic diversity of pathogens have utilized molecular markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), rep-PCR and restriction fragment length polymorphism (RFLP) (Mkandawire et al., 2004; López et al., 2006; Alavi et al., 2008; Zamani et al., 2011). In Iran, rep- PCR and RFLP were used (Zamani et al., 2011) and in Spain, rep-PCR and RAPD were used in pathogen genetic diversity studies (López et al., 2006). The rep PCR technique is useful in studying genetic diversity of bacterial pathogens due to its ability in fingerprinting gram negative bacteria such as Xanthomonas, Pseudomonas, Ralstonia and Agrobacterium (De Bruijn, 1992)

1.9.5. Host plant resistance

Plant diseases are caused by malfunction of host cells and tissues from an injurious physiological process, resulting from continued irritation by a pathogenic agent or environmental factor, leading to development of pathological condition called symptoms (Agrios, 2005). Two outcomes can be expected when pathogens invade a host plant, depending mainly on whether the plant is able to hinder the growth, development and spread of the pathogens (Agrios, 2005). Plants may be susceptible due to their inability to recognize the pathogen or to offer a desired defense mechanism that could restrict development and spread of the pathogen. On the other hand, plants may have the ability to hinder the growth, development and spread of the pathogen and these are classified as resistant (Dempsey and Klessig, 1995; Parlevliet, 2002). Host plants have developed several defense mechanisms that enables them to restrict the development of infections. These include the presence of physical barriers that inhibit the pathogen to penetrate the plant, i.e. thick cuticle layer, size and location of stomata, among others. Host plants can also release chemical compounds into its environment that inhibits pathogen development, for example fungitoxic exudates in certain plant species inhibits fungi development. Compounds such as phenols, tannins and avenacin present in cells offers a good chemical defense mechanism in plants (Heath, 1981; Agrios, 2004). Heath (1981) reported a non-host resistance, a form of resistance where plants are not considered to be host of the pathogen in question. The other form of resistance is referred to as true resistant, which is genetically controlled through incompatibility between itself (host) and the pathogen (Agrios, 2004). According to Agrios (2004), true resistance

can either be horizontal resistance or vertical resistance. Horizontal resistance is the form of resistance that is non-specific but rather quantitative and controlled by many genes, hence referred to as polygenic resistance (Agrios, 2004).

Common bacterial blight resistance is an example of polygenic resistance since it is controlled by more than one gene (Saettler, 1989; Chataika et al., 2011). In this form of resistance, a single gene may not play a role in resistance alone, but in combination with other genes. Horizontal resistance does not protect plants from being infected, but rather slows the development of the disease, hence slowing the spread of the disease in the field (Agrios, 2004). Vertical resistance is a race-specific form of resistance usually controlled by one major gene (monogenic resistance) or very few genes. In this form of resistance, a cultivar may be resistant to some races of the pathogen and susceptible to other races of the same pathogen (Agrios, 2004). Vertical resistance is characterized by incompatibility between the host and pathogen and when attacked, the host responds by hypersensitive reaction. Hypersensitive reaction is the rapid localized death of host cells in response to infection. Horizontal resistance is more durable and difficult to overcome, while vertical resistance is easy to overcome due to continuous pathogen co-evolution resulting in the existing of new races. In order to overcome polygenic resistance, a pathogen race needs to possess virulence genes that can overcome all resistance genes present (Agrios, 2004).

1.9.6. Sources of resistance

The availability of good sources of resistance is a prerequisite for a successful resistance breeding programme. Exploration of resistant sources has not been limited to *P. vulgaris* but across Phaseolus species. The first source of CBB resistance was transferred from *P. acutifolius to P. vulgaris* using embryo rescue technique (Honma, 1956). To date sources of resistance have been identified from the primary, secondary and tertiary gene pools. These sources have different levels of resistance (Singh and Munoz, 1999; Singh and Miklas, 2015). Some of the resistant dry bean germplasm, with pyramided CBB resistant genes, include Calima 9, Montana No 5, ICB3, ICB6, XAN 159, XAN 160, VAX-3, -4, -5, and -6, Badillo, Chase and Coyne (Beebe and Corrales, 1991; Singh and Miklas, 2015). The primary gene pool possesses low level of resistance, while the secondary and tertiary gene pools possess intermediate and high level of resistance respectively (Beebe and Corrales 1991; Singh et al., 2001; Marquez et al., 2007; Singh and Miklas, 2015). The above sources have been extensively used in common bacterial blight breeding programmes (Fourie and Herselman, 2002; Mutlu et al., 2005; O'Boyle et al., 2007; Tryphone et al., 2012).

1.9.7. Inheritance mechanism

The mode of inheritance to CBB has been extensively studied by several researchers (Valladares-Sanchez et al., 1979; e Silva et al., 1989; Arnaud-Santana et al., 1994; Chataika et al., 2011; Zapata et al., 2011). In most of these genetic studies, it has been established that inheritance to CBB resistance is quantitative and the mode of gene action is mainly additive, often with dominance and epistasis effects (Saettler, 1989). Eskridge and Coyne (1996) reported that up to five genes were responsible in controlling CBB. In a similar study two major genes were reported to control CBB in parental genotypes, used as sources of resistance in Malawi dry bean breeding programme (Chataika et al., 2011). Apart from major genes, CBB resistance has been associated with minor genes (e Silva et al., 1989; Chataika et al., 2011). Contrary to reports that CBB resistance is controlled by many genes, Zapata et al. (2011) reported that a single dominant gene was responsible in controlling CBB after crossing PR0313-58 9 x Rosada Nativa. Similar results were also reported in Tanzania after crossing Kablangenti, a local adapted variety with VAX 4 a resistant breeding line (Tryphone et al., 2012). Heritability of CBB resistance ranges from low to high (Tryphone et al., 2012).

Genetic studies on inheritance to CBB resistance have been extended to secondary and tertiary gene pools. Urrea et al. (1999) reported that in some tepary genotypes such as in Neb-T-6-s and PI 321637-s have resistance to CBB that is governed by one dominant gene, while in Neb T-8a-s resistance is governed by two dominant genes with complementary effects. In other related studies within *P. acutifolius,* resistance was governed by a single dominant gene (Drijfhout and Blok, 1987). Due to the inconsistency of the results on inheritance mechanism, the genetics of CBB resistance seems to be complex and remains unclear, especially in crosses with the secondary and tertiary gene pools. This justifies the importance of studying the mode of inheritance of genotypes that are used as sources of resistance for CBB in a breeding programme.

1.9.8. Marker assisted selection in common bacterial blight resistance

Marker assisted breeding has advantages over classical breeding and these includes, ability to screen resistance at seedling stage, screening for resistance even when there are disease escapes and high efficiency in screening for environment dependent traits (O'Boyle et al., 2007). There has been an increase in application of markers in breeding for CBB resistance over the past two decades. This indirect selection of CBB resistance has been possible due to great progress registered in development of markers used to identify quantitative traits loci (QTL)

conditioning resistance (Fourie, 2002). The QTLs conditioning resistances to CBB are from primary, secondary and tertiary gene pools of Phaseolus (Vandemark et al., 2009).

At present four QTL markers linked to CBB resistance are available and these are SU19, BC420, SAP6 and X11.4 (Viteri et al., 2014). All these are sequenced characterized amplified region (SCAR) markers. SU91, BC420, SAP6 and X11.4 are located on linkage groups B6, B8, B10 and B11, respectively (Viteri et al., 2014; Singh and Miklas, 2015). These QTLs have been associated with undesirable traits. For example SU 91 with low yields (O'Boyle et al., 2007) and BC40 with undesirable seed coat colour (Mutlu et al., 2005). Epistatic interaction among these QTL has been observed (O'Boyle et al., 2007; Vandemark et al., 2009). Genotypes pyramided with both SU91 and BC420 have shown to exhibit lower resistance than genotypes that possess either of the two (O'Boyle et al., 2007). Expression of resistance of these has been linked with environment, dry bean population and bacterium race (Park et al., 1999). SAP 6 was found to be susceptible in other populations (Singh and Miklas, 2015). To date, markers have been used to develop and test populations, pyramiding and confirming resistance in developed progenies (Fourie, 2002; Mutlu et al., 2005; O'Boyle et al., 2007). Confirming the efficacy of markers prior to their use in a breeding programme is of importance (Park et al., 1999; Fourie, 2002).

1.9.9. Screening for resistance

Inoculation is the method used to bring the plants in contact with the pathogen. The pathogen itself is referred to as inoculum. For a disease to occur the inoculum has to initiate infection (Agrios, 2004). The reaction of common bacterial blight in dry beans depends on the host genotype, the bacterial strains, inoculum concentration and the environment (e Silva et al., 1989). Several inoculation techniques have been developed that are being used in screening for CBB resistance (Saettler, 1989). Some of the techniques have been reported to be effective in green house screening trials, while others are effective in field screening experiments (Saettler, 1989). Most of the techniques involve injuring the plants in order to introduce the inoculum in the plant through the wounds. Instruments such as scissors, needles and razors are used to injure plant organs such as cotyledon, leaves, stems and pods. Techniques that do not injure plants include soaking leaves in a bacterial solution and vacuum infiltration. All these techniques are used for both greenhouse and field evaluation, though the techniques with artificial injury are primarily used in greenhouse evaluations as they are labor intensive and ideal for small population. Manual or motorized sprays can be used to spray the inoculum to the plant at a distance of approximately 30 to 60 cm (Zapata 1985).

Reports indicate that optimal inoculation requires a highly concentrated inoculum of between 10 million to 100 million cells/ml from an aggressive isolate must be used (Zapata et al., 1985). In order to get good infection, isolates from infected leaves can be cultured on a yeast-extract-dextrose-calcium-carbonate (YDC) nutrient agar at 27 ^{o}C for 48 to 72 h (Zapata et al., 1985; Fourie, 2002). In most studies, inoculation is done at 21 to 28 days after planting (Mutlu et al., 2008). Apart from artificial inoculation some field evaluation studies have relied on natural inoculum (Chataika et al., 2011). The disadvantage of relying on natural inoculum is that in the absence of favorable conditions for the pathogen, disease development will be slow or insignificant.

1.10. Mating designs and genetic analysis and generation mean analysis

Several mating designs can be used in developing improved varieties including bi-parental, North Carolina (I, II, III), line X tester, polycross, topcross and diallel designs. A reliable choice of the mating design and good parent selections are important factors in a successful CBB breeding programme (Khan et al., 2009). Apart from being tools for developing improved cultivars, mating designs are used in generating genetic information on mode of gene action. This encompasses general combining ability and specific combining ability associated with the trait and also determines the genetic gain in the breeding programme (Acquaah, 2009). Progenies developed from these mating design have a defined genetic relationship with their parents and preceding generation, which is easily established once studied (Kearsey and Pooni, 1998). A bi-parental mating design, which simply involves mating of two parents selected from large population, is the simplest design (Acquaah, 2009; Nduwumuremyi et al., 2013).

Quantitative traits are governed by several genes and it becomes difficult to estimate the effect of a single gene on a trait, but rather all genes associated with the inheritance of that particular trait (Acquaah, 2009; Shashikumar et al., 2010). The traits can be governed by various gene actions namely additive, dominance, epistasis and over dominance. It is important to test the significance of the gene action model to establish relationship between generations and to estimate gene effect (Ajay et al., 2012).

Generation mean analysis (GMA), first proposed in 1949, is one of the methods used to estimate the type of gene action associated with the inheritance of the trait by establishing the relationship between generations (Mather, 1949). According to Mather and Jinks (1982) the principle of GMA is that parents must be unrelated and homozygous. The model assumes that when the data does not fit the simple additive-dominance model then epistasis is present (Mather and Jinks, 1982). Several researchers have used the model in studies of inheritance mechanism of different traits

in different crops (van Ginkel and Scharen, 1987; e Silva et al., 1989; Ajay et al., 2012; Bitaraf and Hoshmand, 2012). Extensive exploitation of the model in studies of gene effects is due to its ability to partition the effects into additive, dominance and epistasis (Ajay et al., 2012).

Scaling test have been developed that establishes generation relationships between means and variances (Mather, 1949; Cavalli, 1952). The first scaling test was proposed in 1949 and was referred to as the A,B,C and D or Mather scaling test (Mather, 1949). The scale was limited to six generations only and used the six parameter model: P1, P2, F1, F2, BCP1 and BCP2. Though the scaling test is limited to six generations, it accounts for additive, dominance and epistasis gene actions. To address the weakness of Mather scaling test, Cavalli (1952) developed the joint scaling test, a test that is not limited to a specified number of generations (Cavalli, 1952). The notations used in the joint scaling test are [m] which is the mean of F2, [d] additive gene effect and [h] dominance gene effect (Hayman and Mather, 1955). The Cavalli's scaling test involves two steps. Firstly, the gene effect is estimated from the available families using weighted square method to get expected values. Thereafter, using statistical tools, the expected values are compared with the observed and significance indicates presence of epistasis.

1.11. Genotype by environment interaction

Genotype by environment interaction (GEI) is defined as differential genotypic expression across environments. Genotype by environment interaction is problematic when it is significant and larger than the genotype main effect, which is a common scenario in yield trials (Romagosa and Fox, 1993). It reduces association between phenotypic and genotypic values and may cause selections from one environment to perform poorly in another, forcing plant breeders to combine selection and stability in one criterion (Kang, 1993; Romagosa and Fox, 1993; Fox et al., 1997). Regardless of complicating the process of selection, it is regarded as a major element in determining many key aspects of a breeding programme which includes; whether to aim at wide adaptation or specific adaptation selection programme to be deployed and choice of evaluation sites (Fox et al., 1997). The most important GEI is the crossing over type which result in changes in the ranking of genotypes across environments (Fox et al., 1997). Becker (1981) proposed the concept of genotype stability and distinguished stability into biological stability, in which a genotype maintains a constant yield across environments, and agronomic stability, which is defined as genotype's ability to respond to improved environmental conditions. The latter is related to ranking. A stable genotype is the one that is consistently well ranked. In trials, which the same location and genotypes are included over years, the GEI term from the analysis of variance is partitioned into genotype x location (G.L), genotype x years (G.Y) and genotype x location x years (G.L.Y) (Fox et al., 1997).

1.12. Methods for analysing yield stability in multi-environment trials

Several statistical methods have been exploited to quantity GEI and the available techniques for assessing yield stability have been reviewed by several authors. The focus has been on their advantages and disadvantages in comparison to each other (Romagosa and Fox, 1993; Fox et al., 1997; Farshadfar, 2008; Yang et al., 2009). These techniques include analysis of variance, regression analysis and multivariate techniques among others. In analysing the yield stability of cultivars the use of two or more statistical techniques must be employed for a reliable decision in a breeding programme. An example is the recommendation that 50% of GEI should be explained before linear regression is used (Seif and Pederson, 1978; Romagosa and Fox, 1993).

In analysis of variance, the magnitude of sum of squares of relevant terms as well as the variance components are used to quantify sources of variation. The majority of plant breeders do a pairwise analysis of variance between test genotypes and standard check varieties to detect which genotypes show the same adaptation pattern with checks in multi-environment trials (Fox et al., 1997). However, this method is labor demanding when dealing with many genotypes. Failure of showing a detailed exploration of the patterns of variance by GEI variance is reported as a major shortfall of the technique (Romagosa and Fox, 1993; Fox et al., 1997).

In linear regression (Finlay and Wilkinson's 1963), the observed values of all genotypes are regressed on the environmental indices. The slope of the regression of an individual genotype value against an environment index, estimates genotypic stability. The method partitions GEI term from analysis of variance into heterogeneity of regressions and deviations from regressions (Crossa, 1990). Linear regression, though widely used, has received some criticism (Romagosa and Fox, 1993).

Additive main effect multiplicative interaction (AMMI) is one of the powerful multivariate techniques for multi-environment trials (Romagosa and Fox, 1993). The technique is a very useful tool in GEI trials as it gives a quick visualization and exploration of patterns (Yang et al., 2009). In addition, being user friendly, with the readily availability software, it has contributed to extensive exploitation by breeders, as reported in several publications (Crossa et al., 1991; Gauch Jr, 1992; Annicchiarico, 1997; Worku and Zelleke, 2007; Abuali et al., 2014). It has the ability to extract genotype and environment effect and uses the principal component analysis (PCA) to explain the interaction (Zobel et al., 1988). AMMI is categorized into different families on the basis of whether it has PCA or not, for example AMMI without PCA is AMMI0 and only measures GEI effects, while an AMMI with PCA1 is AMMI1 (Fox et al., 1997). AMMI is a daughter technique of PCA. Farshadfar (2008) pointed out that the analysis is effective where the assumption of linearity of responses of genotype to a change environment is not fulfilled. Similarly, Yang et al. (2009), in

their review paper, highlighted the limitation of AMMI and concluded that it has to be used with caution. Regardless of the limitations, AMMI has the ability to reveal adequately about the complex relationships among locations or among genotypes (Farshadfar, 2008) and reveals more information of GEI than the regression methods (Romagosa and Fox, 1993). The failure of the AMMI model to make provision for a quantitative stability measure, which is essential in quantifying and ranking genotypes in terms of yield stability, resulted in the development of AMMI stability value (ASV) (Purchase et al., 2000). Low ASV indicates that the genotype has a wider adaptation and high ASV indicates specific adaptation (Farshadfar, 2008).

1.13. Conclusion

The reviewed literature provides evidence that common bacterial blight is one of the major production constraint in dry bean production globally. The disease has been well researched compared with other dry bean diseases from breeding standpoint, and reliable sources of resistance have been identified. However, due to continuous evolution of the pathogen there is a need of continuous research in breeding for common bacterial blight resistance.

1.14. References

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CHAPTER 2

YIELD STABILITY AND ADAPTATION ANALYSIS OF SOUTH AFRICAN DRY BEAN CULTIVARS

ABSTRACT

Genotype by environment interaction is a common phenomenon in plant breeding and usually contributes to selections from one environment to perform poorly in another environment. The study was initiated to determine grain yield stability and adaptability of South African dry bean cultivars using additive main effect multiplicative model (AMMI) technique. A field experiment that constituted 30 dry bean cultivars was conducted in 21 locations of which 17 were in South Africa, two in Swaziland and two in Lesotho. A row by column design with three replications was used to lay out the experiment. The results revealed that variances due to environment, genotype and genotype by environment interaction were all significant ($P<0.01$). Additive main effect multiplicative model (AMMI) analysis also revealed that interaction principal components (IPCA1, IPCA2 and IPCA3) were significant ($P<0.01$). Based on the AMMI stability value, cultivars G6 and G14 were both stable and high yielding and recorded yield of 1.46 t ha^{-1} and 1.45 t ha^{-1} respectively. Cultivars G20, G26 and G29 were high yielding and recorded yield of 1.46 t ha^{-1}, 1.48 t ha^{-1} and 1.54 t ha^{-1} respectively. However, these cultivars exhibited specific adaptation to selected environments.

Key words: Additive multiplicative main effect interaction, AMMI stability value, Dry bean, Genotype by environment interaction and Cultivars

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2.1. Introduction

Dry bean (*Phaseolus vulgaris* L.) (2n =22) is the third most important grain legume crop in the world and represents 50% of grain legumes for direct consumption (Zhang et al., 2008; Blair et al., 2009). The crop is grown on all continents between 52 ^{o} N and 32 ^{o} S, from sea level to as high as 3000 m above sea level. Production in Africa is mainly concentrated in East and Southern Africa where a total of 3.7 million ha of arable land is devoted to the crop annually (Kimani et al., 2001). Mean annual production in South Africa is 56,411 metric tons (2006-2015) on an area of 47,677 ha which does not meet the annual demand of 120,000 metric tons (Kleingeld, 2015). Dry bean production in South Africa is further characterised by yield instability due to changes in environmental conditions. The crop is mainly produced in the Free State, Mpumalanga, Gauteng, KwaZulu-Natal, North West, Limpopo and Eastern Cape provinces, all exhibiting different agro-ecological conditions such as temperature, soil fertility and rainfall. The Grain Crops Institute of the Agricultural Research Council (ARC-GCI), in collaboration with the Department of Agriculture, Forestry and Fisheries (DAFF) and seed companies, annually conducts multi-location trials in the most important dry bean producing provinces to evaluate the stability and adaptation of all the released dry bean cultivars. Information from these trials enables the identification of superior cultivars that are widely or specific adapted.

Genotype by environment interaction (GEI) is the differential genotypic expression across environments. Genotype by environment interaction is problematic when it is significant and larger than the genotype main effect, which is a common scenario in yield trials (Romagosa and Fox, 1993). It reduces association between phenotypic and genotypic values and may cause selections from one environment to perform poorly in another, forcing plant breeders to combine selection and stability in one criterion (Romagosa and Fox, 1993; Fox et al., 1997). The most important GEI is the crossover type, which results in changes in the ranking of genotypes across environments (Fox et al., 1997). Becker (1981) proposed the concept of genotype stability and distinguished stability into biological stability (a genotype maintains a constant yield across environments), and agronomic stability (a genotype's ability to respond to improved environmental conditions). The latter, relates to ranking, whereas a stable genotype consistently has a high ranking. With trials that have been conducted on the same locations and genotypes over years, the GEI term from the analysis of variance can be partitioned into genotype x location (G.L), genotype x years (G.Y) and genotype x location x years (G.L.Y) (Fox et al., 1997).

Genotype by environment interaction and stability analysis are very important in dry bean breeding programmes (Kang et al., 2006). In dry bean, GEI has been associated with plant growth habit, seed size and plant architecture (Kelly et al., 1987). Apart from identifying cultivars that are

both high yielding and stable, multi-location trials have also played a role in identifying redundant or non-informative locations, which has resulted in efficient utilization of the limited resources by excluding these environments from the dry bean evaluation trials (Kang et al., 2006). Several statistical methods are exploited in quantify GEI. These techniques include analysis of variance, regression analysis and multivariate techniques. The additive main effect multiplicative interaction model (AMMI) is a very powerful multivariate technique for quantifying GEI. The technique has the advantage of estimating GEI interaction of a genotype and partitioning it into interaction effects due to the environment (Abuali et al., 2014). It is a recommendation that when analyzing data for yield stability, two or more statistical techniques are required to make a reliable decision in a breeding programme, due to some shortfalls these techniques have individually. For example it is recommended that 50% of GEI should be explained before linear regression is used (Seif and Pederson, 1978; Romagosa and Fox, 1993). The study was, therefore, initiated to determine grain yield stability and adaptability of thirty South African dry bean cultivars grown in 21 environments.

2.2. Materials and methods

Data used in the analysis is from the National Dry Bean Cultivar Trials (NCT) conducted during the 2014/2015 growing season on 21 locations in three countries namely: South Africa, Swaziland and Lesotho. The dry bean genotypes used in the study and their agronomic traits are shown in Table 2.1. Detailed geographical information of each location is shown in Table 2.2. Cultivars were planted in a 5 X 6 latinized row by column design with three replications. Plots consisted of four rows of 5 m in length with inter- and intra-row spacing of 75 cm and 7.5 cm respectively. Inorganic fertilizer was applied based on the recommendations from the soil analysis results in all locations. Both chemical and manual weeding were conducted to control weeds. At crop maturity, the middle two rows of each plot were harvested and the yield recorded. The combined ANOVA model (Equation 2.1) for multi-environment trials was used in the analysis using GenStat 17th edition (Payne et al., 2014). The model includes additive terms for main effects of genotype and environment collectively as well as extra additive terms that accounts for interaction.

Equation 2.1. Combined ANOVA model for multi-environment trials

$$Y_{ij} = \mu + g_i + e_j + (ge)_{ij} + \in_{ij}$$

Where Y_{ij} is the yield of the genotype i in environment j, μ is overall yield mean, g_i and e_j are genotypic and environmental effect, $(ge)_{ij}$ is the effect of interaction between the i^{th} genotype and j^{th} environment, $\in_{ij}$ is the mean random error of the i^{th} genotype and e_j environment.

Yield stability and adaptation was determined by AMMI. Both AMMI ANOVA table and AMMI biplot were generated. The AMMI analysis was based on the model as described by Crossa et al., (1991) (Equation 2.2). The analysis was performed in GenStat 17th edition (Payne et al., 2014).

Equation 2.2. Additive multiplicative main effect interaction model

$$Y_{ij} = \mu + g_i + e_j + \Sigma_{k=1}\lambda_k \alpha_{ik}\, \gamma_{jk} + \epsilon_{ij}$$

Where, Y_{ij} is the yield of genotype i^{th} in the j^{th} environment, μ is the grand mean, g_i is the mean of i^{th} genotype minus the grand mean, e_j is the mean of j^{th} environment minus the grand mean. λ_k is the square root of the eigen value of the principal component analysis (PCA) axis, α_{ik} and γ_{jk} are the principal component scores for PCA_k of the i^{th} genotype and j^{th} environment respectively and ϵ_{ij} is the residual error. AMMI stability value (ASV) (Equation 2.3) (Purchase et al., 2000) was used to determine which cultivars showed specific or general adaptation.

Equation 2. 3. Additive main effect multiplicative stability value

$$ASV = \sqrt{\left[\frac{SSPCA1}{SSPCA2}(IPCA1\ score)\right]^2 + (IPCA2\ scores)^2}$$

Where, $\frac{SSPCA1}{SSPCA2}$ is the weight given to IPCA 1 (interaction principal component axis 1) value by dividing IPCA 1 and sum of squares with IPCA2 (interaction principal component axis 2) sum of squares. Larger IPCA scores regardless of signage indicated specific adaptation and lower IPCA scores regardless of signage indicated general adaptation. Different signage of IPCA scores indicate crossover GEI (Romagosa and Fox, 1993).

2.3. Agronomic characteristics of the cultivars

The evaluated cultivars (Table 2.1) had different agronomic characteristics. Out of 30 genotypes only four were small white (SW) canning beans. The rest were red speckled sugar dry beans (RSS). All RSS dry beans, except G9, were of type II indeterminate growth habit. The SW were all of type I determinate growth habit. Number of seed per 100 g ranged from 178 to 512.

Table 2.1. List of cultivars evaluated and their agronomic characterisics

Genotype Code	Type	Growth habit	Seed /100 g
G1	RSS	II	239
G2	RSS	II	250
G3	RSS	II	224
G4	RSS	II	233
G5	RSS	II	205
G6	RSS	II	204
G7	RSS	II	212
G8	RSS	II	239
G9	RSS	I	223
G10	RSS	II	237
G11	SW	I	512
G12	RSS	II	246
G13	RSS	II	220
G14	RSS	II	209
G15	RSS	II	245
G16	RSS	II	241
G17	RSS	II	202
G18	RSS	II	202
G19	RSS	II	220
G20	RSS	II	229
G21	RSS	II	245
G22	RSS	II	316
G23	RSS	II	178
G24	RSS	II	217
G25	RSS	II	226
G26	SW	I	450
G27	SW	I	398
G28	SW	I	401
G29	RSS	II	204
G30	RSS	II	232

RSS = Red speckled beans, SW = Small white beans, I = Determinate type I beans and II = Indeterminate type II beans.

Table 2.2. List of trial locations and their geographical information

Province/Country	Location Name	Location code	Altitude (Masl)	Latitude (^{0}S)	Longitude (^{0}E)
North West					
	Biesiesvlei	BI	1532	26.37	26.00
	Grootpan	GN	1557	22.77	28.68
	Lichtenburg	LG	1504	26.65	26.17
	Potchefstroom	PM	1340	26.70	27.10
Mpumalanga					
	Middleburg	MG	1476	25.77	29.47
	Delmas	DS	1550	26.15	28.68
	Ermelo	EO	1788	26.53	29.98
	Loskop	LP	1489	30.50	24.10
Free State					
	Clarens	CS	1942	28.51	27.88
	Harrismith	HH	1661	28.28	29.13
	Ficksburg	FG	1590	28.87	27.88
	Kransfontein	KN	1666	30.28	26.03
	Petrussteyn	PN	1715	27.65	28.13
KwaZulu-Natal					
	Grey town	GU	1021	29.10	30.60
	Nqutu-Zizameleni	NQ	1207	28.30	30.74
	Kosktad	KD	1229	30.55	29.24
Swaziland					
	Hebron	HB	1243	26.19	31.07
	Mangcongo	MO	1400	31.00	26.35
Lesotho	Maseru	MU	1673	29.32	27.48
	Leribe	LE	1642	28.88	28.05

Masl =Metres above sea level.

2.4. Results

2.4.1. Combined analysis of variance for grain yield

The analysis of variance for grain yield (Table 2.3) revealed significant differences (P<0.01) for environment, genotype and genotype x environment interaction. The combined ANOVA partitioned the treatment as follows; sources of variance due to environment, genotype, and genotype by environment interaction were; 77.1%, 1.1% and 9.3% of total sum of squares respectively. The grand mean yield was 1.33 t ha^{-1}. The combined coefficient of variation (CV) for all trials across the 21 testing environments was 29.9%.

Table 2.3 Combined analysis of variance for grain yield over different environment

Source of Variation	DF	SS	MS
Genotype	29	17.45	0.6**
Environment	20	1229.12	61.46**
GEI	580	147.50	0.25**
Residual	1260	200.31	0.16
Total	1889	1594.38	
Yield mean (t ha^{-1})	1.33	CV	29.9%

** Significant at P<0.01. DF = Degrees of freedom, CV = Coefficient of variation, SS = Sum of squares, MS = Mean of squares, GEI = Genotype x Environment interaction.

2.4.2. Additive multiplicative main effect analysis of variance for grain yield

The results from additive multiplicative main effect interaction (AMMI) analysis of variance (Table 2.4) was significantly different (P<0.01) for all cultivars and for all environments. The partitioning of variance components by AMMI ANOVA revealed that 1.1% was due to genotype, 77.1% due to environment main effect, 9.3% was due to genotype x environment interactions of the total sum of squares. Furthermore, the three interaction principal components (IPCA1, IPCA2 and IPCA3) accounted for 29%, 15.6% and 11.8%, respectively of the total of genotype by environment interaction sum of squares and were significantly different (P<0.01). The interaction principal components IPCA1, IPCA2 and IPCA3 cumulatively accounted for 56.4% of the total genotype by environment interaction sum of squares.

Table 2.4. AMMI analysis of grain yield in dry bean cultivars over environments

Source of variation	DF	SS	MS
Total	1889	1594.40	0.84
Treatments	629	1394.10	2.22**
Genotypes	29	17.50	0.60**
Environments	20	1229.10	61.46**
Block	42	39.40	0.94**
GEI	580	147.50	0.25**
IPCA 1	48	42.80	0.89**
IPCA 2	46	23.00	0.50**
IPCA 3	44	17.40	0.40**
Residuals	442	64.20	0.16
Error	1218	160.90	0.13

** Significant at P<0.01, DF = Degrees of freedom, SS = Sum of squares, MS = Mean sum of squares, GEI = Genotype x Environment interaction, IPCA 1 = Interaction principal component axis 1, IPCA 2 = Interaction principal components axis 2, IPCA 3 = Interaction principal component 3.

2.4.3. Mean yield and AMMI stability values for cultivars

Mean yield for the 30 cultivars ranged between 1.07 t ha^{-1} and 1.54 t ha^{-1} (Table 2.5). Cultivar G24 had the lowest yield and cultivar G29 the highest. Forty percent of the cultivars (G10, G14, G15, G18, G20, G21, G28, G29, G30, G6 and G8) yielded above the grand mean of 1.33 t ha^{-1}. Additive main effect multiplicative interaction stability value (ASV) for cultivars ranged from 1.55 to 0.20 (Table 2.5). Cultivar G13 had the highest ASV, while G14 had the lowest.

Table 2.5. Mean yield, first, second and third IPCA scores and ASV of cultivars evaluated

Cultivar code	Mean GY (t ha^{-1})	IPCAg[1]	IPCAg[2]	IPCAg[3]	ASV
G1	1.26	-0.23	-0.61	0.19	0.74
G10	1.35	0.10	0.08	-0.14	0.21
G11	1.27	0.43	0.18	-0.56	0.82
G12	1.28	-0.34	-0.28	-0.49	0.70
G13	1.32	0.83	0.15	0.35	1.55
G14	1.45	0.10	0.04	0.08	0.20
G15	1.38	0.27	-0.08	0.23	0.51
G16	1.30	0.07	-0.23	-0.04	0.26
G17	1.26	0.23	-0.06	0.25	0.43
G18	1.43	0.21	0.13	0.12	0.41
G19	1.27	0.37	-0.64	0.50	0.93
G2	1.27	-0.30	0.07	-0.29	0.55
G20	1.46	-0.44	0.39	0.20	0.90
G21	1.43	-0.19	0.48	0.28	0.60
G22	1.30	0.05	-0.24	-0.11	0.26
G23	1.18	0.32	-0.17	-0.22	0.63
G24	1.07	-0.29	-0.37	-0.09	0.66
G25	1.33	-0.14	0.30	0.41	0.40
G26	1.48	0.50	0.31	0.11	0.98
G27	1.30	0.49	0.35	-0.36	0.98
G28	1.35	0.24	0.21	-0.45	0.50
G29	1.54	-0.43	0.48	-0.31	0.93
G3	1.30	-0.40	0.02	0.17	0.74
G30	1.42	0.39	-0.11	-0.22	0.73
G4	1.32	0.01	-0.47	-0.22	0.47
G5	1.31	-0.27	-0.19	0.19	0.53
G6	1.46	-0.14	0.06	0.35	0.27
G7	1.32	-0.59	0.34	0.06	1.15
G8	1.34	-0.32	0.24	0.24	0.64
G9	1.23	-0.54	-0.37	-0.24	1.07

IPCAg [1] = Interaction principal component axis for genotype scores 1, IPCAg [2] = Interaction principal component axis for genotype scores 2, IPCAg [3] = Interaction principal component for genotype scores 3, ASV = AMMI stability value, Mean GY = Mean grain yield.

2.4.4. Mean yield and AMMI stability values for environments

Yield across environments ranged from 0.23 t ha^{-1} to 3.49 t ha^{-1} (Table 2.6). Thirty-eight percent of the environments (LE, LP, GU, CS, BA, GN, KD, and DS) recorded mean yield above the grand mean of 1.33 t ha^{-1}. KN recorded the lowest yield, while DS was the highest yielding environment. Additive main effect multiplicative interaction stability value (ASV) for environments ranged from 2.83 to 0.15 (Table 2.6). Environment DS had the highest ASV, while MG had the lowest.

Table 2.6. Mean yield, first, second and third IPCA scores and ASV of environments

Environment	Mean GY (t ha^{-1})	IPCAe[1]	IPCAe[2]	IPCAe[3]	ASV
BA	1.82	0.96	0.55	0.67	1.87
BI	0.95	-0.09	-0.12	-0.06	0.21
CS	1.71	0.05	0.80	0.10	0.81
DS	3.49	-1.50	0.39	0.19	2.83
EO	1.04	0.25	0.03	-0.09	0.47
FG	0.95	-0.08	-0.24	-0.28	0.28
GN	2.07	-0.25	0.50	0.39	0.69
GU	1.71	-0.05	-0.26	0.09	0.28
HH	1.20	0.27	-0.02	-0.53	0.49
HN	0.23	-0.08	-0.15	0.03	0.21
KD	3.24	-0.06	-0.83	0.77	0.83
KN	0.23	0.19	-0.04	-0.23	0.35
LE	1.46	-0.33	-0.02	-0.19	0.62
LG	0.86	0.10	-0.01	-0.19	0.19
LP	1.51	0.38	0.00	-0.22	0.71
MG	1.28	0.08	0.03	-0.25	0.15
MO	0.79	0.05	-0.23	0.42	0.25
MU	0.77	-0.07	-0.46	0.12	0.48
NQ	0.81	0.02	-0.42	-0.34	0.43
PM	1.03	-0.02	0.32	-0.49	0.32
PN	0.85	0.20	0.14	0.10	0.41

IPCAe[1] = Interaction principal component axis for environment scores 1, IPCAe[2] = Interaction principal component for environment scores 2, IPCAe[3] = Interaction principal component for environment scores 3, ASV = AMMI stability value, Mean GY = Mean grain yield.

2.4.5. Interaction principal components scores for cultivars and environments

The results of IPCA scores for cultivars and environments had both positive and negative scores for the cultivars and environments respectively (Tables 2.5 and 2.6). The IPCAs scores for both cultivars and environment varied. Some cultivars recorded higher IPCAs scores than others, similarly, some environments had higher IPCAs scores than others.

2.4.6. First four selections per environment

The AMMI analysis identified the best four cultivars per location (Table 2.7). In terms of 'which won where' cultivar G29 was the highest and won in seven environments followed by G26 and G19, which won in four environments each. Cultivars G30, G20, G13, G11, G7, G6 and G7 won in one environment each. Differential ranking of cultivars across the environments indicated the presence of crossover type of GEI.

Table 2.7. First four selections per enviroment

Environment	Mean GY (t ha^{-1})	Score	Ranking per environment			
			1st	2nd	3rd	4th
BA	1.82	0.9619	G13	G26	G18	G15
LP	1.51	0.383	G26	G30	G27	G13
HH	1.20	0.2651	G11	G28	G30	G27
EO	1.04	0.2534	G26	G30	G13	G18
PN	0.85	0.2048	G26	G13	G18	G29
KN	0.23	0.1866	G30	G26	G29	G28
LG	0.86	0.101	G29	G30	G26	G28
MG	1.28	0.077	G29	G30	G26	G28
MO	0.79	0.0524	G19	G6	G15	G26
CS	1.71	0.0456	G29	G21	G20	G26
NQ	0.81	0.0182	G4	G12	G30	G9
PM	1.03	-0.0221	G29	G28	G11	G27
GU	1.71	-0.0501	G6	G19	G1	G14
KD	3.24	-0.0648	G19	G1	G6	G5
MU	0.77	-0.0745	G19	G1	G4	G6
FG	0.95	-0.0767	G29	G12	G4	G30
HN	0.23	-0.0789	G29	G6	G20	G14
BI	0.95	-0.0909	G29	G6	G20	G14
GN	2.07	-0.2525	G20	G21	G29	G25
LE	1.46	-0.3336	G29	G20	G7	G12
DS	3.49	-1.5049	G7	G20	G29	G8

Mean GY =Mean grain yield.

2.4.7. Additive multiplicative main effect interaction bi-plot

The AMMI bi-plot analysis (Figure 2.1) revealed that environment DS, KD, BA and CS had the greatest effect on GEI interaction. Cultivar G19 had specific adaptation with high yielding environments. Cultivars G7, G8, G20, G21 and G29 had a positive interaction with environment DS, hence were specifically adapted to DS. Cultivar G29 showed better specific adaptation to DS than cultivars G7, G8, G20 and G21, because it has the longer vector to DS than that of the latter. Cultivar G13 interacted positively with environment BA and was the only cultivar that showed better specific adaptation to BA among the cultivars that were adapted to BA, namely, G11, G18, G28 and G27. All cultivars in the fourth quadrant of bi-plot displayed specific adaptation to KD. The following cultivars; G6, G10, G14, G16, G18 and G22 were all close to the centre of the bi-plot revealing general adaptation to the testing environments. Cultivar G2 had an angle of less than 90^0 with environment GN. Other cultivars showed similar relationship with other

environments. Environments that are close to each other in a bi-plot have similar response. Environments (BI, HN, FG, GU and MO; MG, LP, EO KN and PN) exhibited this relationship in the bi-plot. The bi-plot analysis of GEI based on the AMMI2 model for the first two interaction principal component scores, namely IPCA1 and IPCA2, revealed that the two IPCAs cumulatively contributed 44.6% of the GEI.

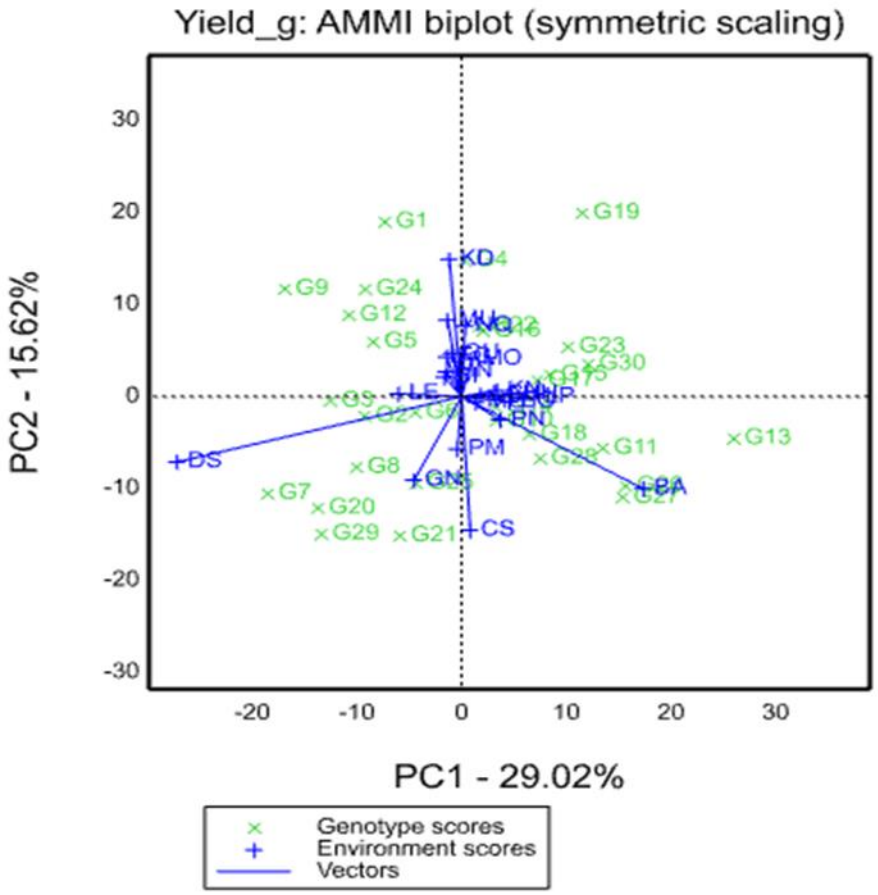

Figure 2.1. Bi-plot analysis of GEI based on AMMI2 for the first two interaction principal component scores

2.5. Discussion

The combined analysis of variance revealed that the highest source of variation was due to environment. This clearly indicates that the testing environments were diverse, which suggest that the 21 locations had different biotic and abiotic production limiting factors. Similar results were also reported in previous studies focused on yield stability for other crops (Annicchiarico, 1997; Worku and Zelleke, 2007; Farshadfar, 2008; da Silveira et al., 2013). The magnitude of the source of variation of GEI was 8.5 larger than that of genotype. This reveals that there was

significant genotypic response across testing environments and that this response was the main contributor of inconsistency in ranking of tested dry bean cultivars across the environments. Fox et al. (1997) reported that GEI results in selections from one environment performing badly in another environment due to crossover. These results are in line with the results which previous investigators found in similar studies (Kang et al., 2006; Farshadfar, 2008; Mehari et al., 2014). Farshadar (2008) found that the GEI was 2.5 larger than that of genotypes in a yield study for bread wheat grain in Iran. In another similar study conducted on malt barley, the magnitude of GEI was 4.7 larger than that of genotypes (Mehari et al., 2014).

The AMMI ANOVA table revealed that GEI was significant and it contributed less than 50% to the total sum of squares justifying that the AMMI model was sufficient in determining stability. Romagosa and Fox (1993) recommended that regression analysis should be performed on stability studies when GEI account for more than 50%. The first three principal component analysis (IPCAs) were significant and sufficient in explaining the additive main effects and that the data fitted the model. Zobel et al. (1988) found that IPCA1, IPCA2, and IPCA3 accounted 76% of the variation in yield stability of soybean cultivars evaluated and argued that such a large value, though statistically sufficient, was undesirable in describing the additive main effect. The IPCA1 contribution was larger than the subsequent IPCAs, though all of them being significant means that genotypic variation was more important among dry bean cultivars than the remaining variations, which are associated with other IPCAs. IPCA1 account for genotypic variation and the subsequent IPCAs account for the remaining variation (Crossa, 1990).

The study reveals that among the tested dry bean cultivars, some were specifically adapted to selected environments and some showed general adaptation. These results are in line with previous similar studies (Kang et al., 2006; Pereira et al., 2009). Cultivars that had large IPCA1 scores were specifically adapted to environments with IPCA1 score of the same sign. For example G11, which had a positive IPCA1 score of 0.43, was specifically adapted to LP with a positive IPCA1 score of 0.38. Similarly, G2, with a negative IPCA1 score of -0.30, was adapted to GN with a negative IPCA1 score of -0.25. The majority of the tested cultivars demonstrated this relationship. Romagosa and Fox (1993) reported that a large genotypic PCA1 score reflects more specific adaptation to environments with PCA1 scores of the same sign. Cultivar G2 showed specific adaptation to environment GN so was G11 to environment LP. These two cultivars had angles of less than 90^0 to the respective environments as displayed on the bi-plot. Fox et al. (1997) reported that a genotype with an angle less than 90^0, has specific adaptation and positive interaction with that particular environment. Environment MG is the largest contributor to yield stability among tested environments as it has the lowest ASV. Environments with the largest

contribution to phenotypic stability are ideal for conducting preliminary tests in the selection scheme (da Silveira et al., 2013).

Stable cultivars have low ASV and are close to the centre in a bi-plot (Fox et al., 1997; Purchase et al., 2000). Cultivars G6, G10, G14, G18 and G22 had low ASV, exhibiting general adaptation to the testing environments and therefore were regarded as stable cultivars. The AMMI bi-plot groups environments with similar response together (Fox et al., 1997). KN and NP were among environments that had similar responses. Environments with similar responses could be clustered together into mega-environments and replaced with other testing environments that are representative of the region where the cultivar will be grown or could be excluded as testing environments (Hongyu et al., 2014). Kang et al. (2006) reported similar results independently, using genotype main effects and genotype by environment interaction (GGE) bi-plot and recommended that it is possible to drop some of these environments and non-informative ones as a measure of controlling the scarce resources in breeding programme that goes with trial management. It was observed that all the type I cultivars were unstable. Kelly et al. (1987) reported that stability in dry bean is associated with growth habit.

2.6. Conclusion

The study reveals the presence of GEI in South African dry bean cultivars, which justify that dry bean breeding programmes should consider breeding for both wide adaptation and specific adaptation. Furthermore, the breeding programmes can reduce the number of testing environments in order to save resources incurred in trial management. Cultivars G6 (1.46 t ha^{-1}) and G14 (1.45 t ha^{-1}) were both stable and high yielding and can be recommended for general adaptation. On the other hand, cultivars G20 (1.46 t ha^{-1}), G26 (1.48 t ha^{-1}) and G29 (1.54 t ha^{-1}) can be recommended for high potential dry bean growing environments as they demonstrated specific adaptation to such environments.

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CHAPTER 3

EVALUATION OF NEWLY INTRODUCED DRY BEAN GERMPLASM FOR YIELD AND COMMON BACTERIAL RESISTANCE

ABSTRACT

Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans* (Xff) is a disease of economic importance in South Africa. Identifying genotypes that are both high yielding and resistant to the disease, will contribute to the improvement of national dry bean production. The study aimed at identifying resistant, high yielding genotypes from introduced germplasm. Sixty cultivars and lines were evaluated at two localities in South Africa (Cedara, KZN and Potchefstroom, North West) under artificial inoculation, using a mixture of two local isolates (Xf260 and Xf410). Eight local cultivars were included as checks. Relative area under disease progress curve (RAUDPC) was used to determine the reaction of these genotypes to CBB. The evaluated germplasm exhibited different reactions to CBB. Approximately 20% of genotypes exhibited resistance across the two locations, 43% and 37% showed moderate and susceptible reaction to CBB, respectively. The mean grain yield was 1.29 t ha^{-1}, with the majority of susceptible genotypes yielding below the mean. There was, however, a weak negative correlation ($r = - 0.49$, $P<0.001$) between disease reaction and yield. Genotypes ADP-0041, ADP-0790, M-125, ADP-0096, ADP-0544 and M-191 were selected as both high yielding and resistant. These genotypes exhibited good levels of resistance and yielded above 1.7 t ha^{-1}. Genotypes ADP-0055, ADP-0099 and ADP-0103 were selected on the basis of their high yield. These genotypes yielded above 1.7 t ha^{-1}, but showed susceptible reaction to CBB. Disease onset on genotypes was detected using relative life time (RLT). The onset of disease varied significantly among genotypes. Disease was first detected in RS 7. Strong positive correlation ($r = 0.54$, $P = 0.001$) between RAUDPC and RLT was noted. In general, genotypes with white flowers and those with indeterminate growth habit showed a high level of resistance reaction to CBB.

Key words: Common bacterial blight (CBB), Dry bean, Relative area under disease progress curve (RAUDPC), Relative life time (RLT)

3.1. Introduction

Dry bean (*Phaseolus vulgaris*. L) is a crop of significance worldwide. In Africa it is considered to be the main legume crop, especially among smallholders. It is more important in East and Southern Africa, where it is a major source of dietary proteins among the rural people. In South Africa, dry bean is consumed both by the poor and rich people. Despite its importance, production is still low due to a number of production constraints associated with the crop. Production constraints include both abiotic and biotic stresses. Among these constraints, common bacterial blight (CBB), a seed borne disease caused by *Xanthomonas axonopodis* pv *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *Fuscans* is considered to be of significant economic importance across the dry bean production environment worldwide (Arnaud-Santana et al., 1994). It is more severe in areas of high temperature and humidity (Saettler, 1989; O'Boyle et al., 2007). The disease reduces yield up to 60% and also lowers the quality of the grain (Jung et al., 1996; Marquez et al., 2007). All aerial parts of the bean plant are affected by the CBB pathogen.

It has been established that planting infected seeds, continuous cropping, infected volunteer plants and plant debris are primary sources of inoculum for CBB in dry bean (Saettler, 1989; Fininsa and Tefera, 2001). The majority of dry bean farmers in Africa are poorly resourced smallholder farmers who plant farm saved seeds contaminated with seed borne diseases such as CBB. They have limited land to practice crop rotation in order to break the life cycle of the pathogen and little capital to invest in practices that maintains high field hygiene or purchase bactericides that can control the disease in the field (Mkandawire et al., 2004). Chemical control using agro-chemicals has not proved to be highly effective and just raises the production cost (Mkandawire et al., 2004; O'Boyle et al., 2007; Fourie et al., 2011). Planting resistant dry bean varieties is the only viable solution to control CBB in dry bean production, especially in smallholder agriculture (Mkandawire et al., 2004).

Evaluation of the reaction to common bacterial blight in dry bean germplasm is very important since it allows identification of genotypes that are resistant and also establishes their level of resistance (Singh and Munoz 1999; Duncan 2010). Genotypes can be grouped into three categories based on their reaction to CBB namely susceptible, moderate resistant (intermediate) and resistant. Such information has great value in dry bean breeding research as it informs

researchers about which genotypes should be either used as parental materials in the breeding programme, or released to farmers. In summary, CBB screening studies are a prerequisite for a successful resistance breeding programme. The aim of the study was, therefore, to evaluate newly introduced dry bean germplasm in order to identify genotypes that are both high yielding and resistant to CBB.

3.2. Materials and methods

3.2.1. Plant materials

The reaction to CBB was evaluated in the field using 60 dry bean genotypes of diverse genetic background, including 39 lines from the Andean Diversity Panel (ADP) and 13 from the Phaseolus Improvement Cooperative (PIC) lines. Five cultivars from the Agricultural Research Council-Grain Crop Improvement (ARC-GCI) dry bean breeding programme and three from Pro-Seed, a seed company based in KwaZulu-Natal South Africa were used as checks in the study.

3.2.2. Experimental sites

The germplasm accessions were screened for resistance to CBB under artificial infection at Potchefstroom and Cedara during the 2015/2016 growing season. Potchefstroom is located in North West province of South Africa at an altitude of 1321 metres above sea level (masl), while Cedara is located in KwaZulu-Natal province and is at an altitude of 1053 masl.

3.2.3. Experimental design and management

The experiment was laid using 10 x 6 alpha lattice design with three replications. The number of rows per plot at both locations was two, which were spaced at 75cm apart. The length of a plot at Potchefstroom was 5 m and 4 m at Cedara. The intra-row spacing for Potchefstroom trial was 7 cm and 5 cm for the Cedara trial. At both locations spreader rows of the highly susceptible cultivar, Teebus, were planted around the trial. Both fields were weeded twice manually and no fertilizer was applied.

3.2.4. Field inoculation

A mixture of two local isolates (Xf260 and Xf410) was used for inoculation. Inoculum was prepared by suspending 48 to 72 h old cultures in tap water. The suspension was adjusted to 10^8 CFU/ml. Culture plates used at Cedara were transported at room temperature and suspensions were made on site, while at Potchefstroom due to closeness to the field, suspensions were made in the laboratory. Inoculation was done immediately after preparing the suspension. The Potchefstroom trial was irrigated after inoculation in order to increase humidity, while the Cedara trial was rain

fed. Both trials were inoculated at 28, 35 and 42 days after planting using a Stihl SR 430 mist blower.

3.2.5. Data collection

Disease severity was based on the percentage of leaf area infected for the whole plot using a standardised CIAT scale of 1 to 9 (Corrales and van Schoonhoven, 1987). The evaluations were conducted three times at an interval of 14 days after first inoculation. The scores were transformed into percentages 1 = 5%, 2 =15%, 3 = 25%, 4 = 35%, 5 = 45%, 6 = 55%, 7 = 65%, 8 = 75% and 9 = 85%. The percentage values were used to calculate the relative area under disease progress curve (RAUDPC). Both days to each disease assessment from planting (DDAP) and days to physiological maturity (DPM) were recorded and used to quantify relative life time (RLT) for each genotype (Tschanz, 1984) (Equation 3.1). The function of RLT was to standardise the growth stage of genotypes. Relative area under disease progress curve was calculated according to Campbell and Madden (1990) (Equation 3.2) using RLT as the independent variable and percentage severity scores as dependant variable. Grain yield (Appendix 3.4) and other non-yield traits (growth habit, days to 50% flowering, flower colour, bean type) were recorded (Appendix 3.1).

Equation 3.1. Formular for calculating relative life time

$$RLT = [(DDAP/DPM) * 100]$$

Where RLT is relative life time, DDAP is days to each disease assessment from planting and DPM is days to physiological maturity.

Equation 3.2. Formular for calculating area under disease progress curve

$$RAUDPC = \sum_{i}^{n-1} \left(\frac{y_1 + y_{i+1}}{2} \right) (t_{i+1} + t_i)$$

Where RAUDPC is relative area under disease progress curve, "n" is the number of evaluations, "y" is the disease percentage for each assessment and "t" is the relative life time (RLT) of each assessment

3.2.6. Data analysis

Data were analysed using analysis of variance in REML (Residual maximum likelihood) in GenStat 17th edition (Payne et al., 2014). Means were separated by least significant difference

(LSD) at P = 0.05. Phenotypic correlation analysis was performed in Genstat 17th edition (Payne et al., 2014) using two way model at P = 0.05. Relationship between CBB reaction and growth habit, CBB reaction and flower colour were also performed using t-statistic assuming unequal variance at P = 0.05 in excel, Microsoft office 2013.

3.3. Results

3.3.1. Weather data

The mean seasonal temperature (Table 3.1) for the two environments varied. Potchefstroom recorded higher mean temperature than Cedara. Potchefstroom recorded the best optimum favourable mean monthly temperatures for common bacterial blight disease development compared with Cedara. Cedara, however, recorded higher rainfall (Table 3.1) during the growing season than Potchefstroom.

Table 3.1. Rainfall and mean temperature of Cedara and Potchestroom during growing season

Month	Cedara		Potchefstroom	
	Mean Temp(ºC)	Total Rain (mm)	Mean Temp (ºC)	Total Rain(mm)
Nov-15	24.8	54.1	30.5	36.6
Dec-15	27.9	84.6	33.4	64.7
Jan-16	26.5	158.3	30.8	94.7
Feb-16	27.5	115.3	31.6	80.0
Mar-16	26.8	95.5	28.7	60.2
Apr-16	25.4	20.1	26.5	77.0
May-16	22.5	18.5	22.1	42.4
Average	25.9	-	29.1	-
Total	-	546.4	-	455.6

Source; Agricultural research council.

3.3.2. Analysis of variance

A summary of analysis of variance (ANOVA) for the two environments (Cedara and Potchefstroom) and combined ANOVA for relative life time (RLT), relative area under disease progress curve (RAUDPC) and grain yield (GY at t ha^{-1}) is presented in Table 3.2.There were large significant differences (P<0.001) between genotypes for RLT and RAUDPC at both location and in the combined analysis. Highly significant differences (P<0.001) between genotypes for grain yield in Potchefstroom were observed.

Table 3.2. Analysis of variance for relative life time, relative area under disease progress curve and grain yield for the genotypes evaluated at two locations in South Africa

Source of variation	DF	RLT		RAUDPC		GY	
		MS	F.pr	MS	F.pr	MS	F.pr
Cedara							
Replication	2	3.427	0.232	803005	0.496		
Block	5	13.020	<0.001	3351143	0.016		
Rep*Block	10	11.068	<0.001	6176693	<0.001		
Genotype	59	12.829	<0.001	5926578	<0.001		
Residual	103	2.311		1138868			
Total	179	6.579		3056425			
Potchefstroom							
Replication	2	8.590	0.506	6035509	<0.001	0.2654	0.467
Block	5	22.680	0.117	2146813	0.008	0.5778	0.149
Rep*Block	10	55.060	<0.001	2577149	<0.001	0.3991	0.332
Genotype	59	41.750	<0.001	9599908	<0.001	1.1288	<0.001
Residual	103	12.530		645268		0.3463	
Total	179	24.770		3806892		0.6127	
Combined							
Environment	1	1350.540	<0.001	71254584	<0.001		
Replication	2	2.049	0.806	5437572	0.025		
Env*Rep	2	9.971	0.352	1400942	0.384		
Genotype	59	47.177	<0.001	13303378	<0.001		
Residual	295	9.507		1457483			
Total	359	19.395		3620581			

DF = Degrees of freedom, RLT = Relative lifetime RAUDPC = Relative area under disease progress curve, GY= Grain yield in t ha^{-1}, MS = Mean of squares.

3.3.3. Correlations among traits

Table 3.3 is a summary of correlations of relative area under disease progress curve (RAUDPC), relative life time (RLT), grain yield (t ha^{-1}) and other traits among the 60 evaluated dry bean genotypes. Both significant positive and negative correlations between traits were observed. RAUDPC was significantly positively correlated with RLT (P<0.001). Days to flowering was highly significantly (P<0.001) positively correlated with DPM and GH. Grain yield was highly significantly (P<0.001) positively correlated with GH. RAUDPC was highly significantly (P<0.001) negatively correlated with DPM and grain yield. RLT was highly significantly (P<0.001) negatively correlated with FD and DPM.

Table 3.3. Correlations among traits

Traits	FD	DPM	RAUDPC	RLT	GY
FD	-				
DPM	0.76***	-			
RAUDPC	-0.58	-0.53***	-		
RLT	-0.76***	-0.99***	0.54***	-	
GY	0.23	0.21	-0.49***	-0.22	-

Significant at: * P<0.05; ** P<0.01; *** P<0.001. FD = Days to flowering, DPM = Days to physiological maturity, RAUDPC = Relative area under disease progress curve, RLT = Relative life time, GY = Grain yield.

3.3.4. Relative life time

The RLT pooled mean (Appendix 3.2) for the two locations was 65.2%. The RLT means for Cedara and Potchefstroom were 63.3 and 67.2% respectively. Pooled RLT ranged from as 71.6% to 56.2%. Pooled data indicated that genotype ADP-0601 recorded the highest RLT, while Tygerberg recorded the lowest with the majority of genotypes in the range of 63% to 67%. At Cedara RS 7 recorded the lowest RLT of 51.2% and ADP- 0376 recorded the highest 66.4%, while at Potchefstroom M-24 had the lowest RLT of 58.4% and ADP-0601 recorded the highest RLT of 76.7%. Using the RLT scale the disease was first detected at Cedara. At Cedara the disease was first detected on genotype RS 7 and latest detection was found on ADP-0376. At Potchefstroom it was detected first in M-24 and last on ADP-0601.

3.3.5. Relative area under disease progress curve

The combined mean (Appendix 3.2) for RAUDPC of the two environment was 3531. Relative area under disease progress curve ranged from 6442 in ADP-0601 to 869 in RS 7 with 37% of genotypes having RAUDPC of above 4001, 43% between 2001 and 4000 and 20% with less than 2000. Mean RAUDPC was lower at Cedara than at Potchefstroom with a difference of 890. Gadra recorded the highest RAUDPC at Cedara and Werna the lowest. Genotypes, ADP-0611 and RS 7 had the highest and lowest RAUDPC of 7123 and 774 at Potchefstroom, respectively. Approximately 30% and 27% of genotypes recorded RAUDPC of less than 2000 at Cedara and Potchefstroom, respectively. In addition, there was a strong positive significant correlation (r = 0.54, P<0.001) between RAUDPC and RLT (Table 3.3).

3.3.6. Genotypes reaction to common bacterial blight disease

Genotypes with RAUDPC of less than 2000 were considered resistant to CBB, genotypes with RAUDPC values between 2001 and 4000 were considered to exhibit moderate resistance and

genotypes with RAUDPC of greater than 4001 were considered susceptible to CBB. Based on pooled RAUDPC data (Appendix 3.3), approximately 20% of genotypes exhibited resistance across the two locations, while 43% and 37% showed moderate and susceptible reaction to CBB respectively. Five of eight South African dry bean genotypes showed resistance reaction to CBB (Werna, RS 7, Teebus-RCR 2, Caledon and Ukulinga). Four ADP lines showed resistant reaction across the two locations (ADP-0531, ADP-0041, ADP-0211 and ADP-0790). Only three PIC lines, M-139, M-145 and M-197 showed resistant reaction to CBB at both locations. Teebus-RR 1 and Gadra were the two local genotypes that exhibit susceptibility to CBB among the local dry bean genotypes across two locations. Tygerberg, also a local genotype, exhibited moderate resistance at both locations. Pooled data revealed that 19 ADP genotypes were susceptible at both locations and only one PIC line exhibited the same reaction to CBB. The majority of genotypes exhibited susceptible reaction at Potchefstroom than at Cedara. The proportional distribution to CBB reaction among genotypes at Cedara was 30% resistant, 42% moderate and 28% susceptible while at Potchefstroom the distribution was 27, 15 and 58% respectively. Genotypes M-126 and ADP 0540 showed resistance to CBB at Cedara only.

3.3.7. Relationship between disease reaction and grain yield

Grain yield was recorded at Potchefstroom only (Appendix 3.4). Grain yield at Cedara was not recorded due to a hailstorm that occurred during the flowering stage and also high severity of Ascochyta blight disease. Almost all genotypes showed susceptibility to the disease. The most susceptible genotypes showed a high level of leaf defoliation. The mean grain yield at Potchefstroom was 1.29 t ha^{-1}. Yield ranged from 2.91 t ha^{-1} to 0.08 t ha^{-1}. Genotypes ADP-0041 and ADP-0611 recorded the highest and lowest yield, respectively. Five of the eight local dry bean genotypes were among the top ten high yielding genotypes and these were Caledon (2.4 t ha^{-1}), Teebus-RCR 2 (2.3 t ha^{-1}), Teebus-RR 1 (2.29 t ha^{-1}), Tygerberg (2.17 t ha^{-1}) and Werna (1.79 t ha^{-1}). ADP-0790 and Teebus RR 1 were the only two susceptible genotypes among the top ten high yielding genotypes. Gadra and Ukulinga were the two local cultivars yielding below the mean yield. Only five genotypes that exhibit resistance to CBB yielded below the mean. A negative correlation was noted (Table 3.3) ($r = - 0.49$, $P<0.001$) between RAUDPC and grain yield.

3.3.8. Relationship between selected morphological traits and reaction to common bacterial blight

The analysis output for t-statistic for two sample assuming unequal variance (Table 3.4) showed that the RAUDPC mean for genotypes with white flowers was lower than that of genotypes with purple flowers. Dry bean genotypes with a determinate growth habit had a higher mean of

RAUDPC than indeterminate genotypes. This is therefore, an indication that both white flower and indeterminate genotypes exhibited higher level of resistance as compared to purple flower and determinate genotypes among the evaluated genotypes.

Table 3.4. Relationship between flower and growth habit with reaction to common bacterial blight

Trait	Type	Number of genotypes	RAUDPC Mean
Flower colour	White	28	2816.321
	Purple	32	4155.813
Growth habit	Determinate	24	3815.615
	Indeterminate	36	3312.853

P<0.05 t-statistic; Two sample assuming unequal variance

3.4. Discussion

High significant variation of RAUDPC between genotypes is an indication that these genotypes had different levels of resistance to CBB. A great variation in terms of resistance levels within dry bean genotypes has been reported (Singh and Munoz, 1999; Duncan et al., 2011). Difference in RAUDPC values of genotypes between two environments is an indication that the two testing environments exhibited different favourable climatic conditions for CBB development. Environmental conditions have been reported to influence CBB development (Singh and Munoz, 1999; Mutlu et al., 2005). The RAUDPC values for the majority of genotypes were higher at Potchefstroom than at Cedara. Potchefstroom had optimum environmental conditions for CBB development compared to Cedara, which included high temperatures and humidity (Saettler, 1989; Singh and Munoz, 1999; Fourie, 2002) . The optimum temperature for CBB development ranges from 28 ^{o}C to 32 ^{o}C (Saettler, 1989). Irrigation at Potchefstroom increased humidity, favouring CBB development. High significant differences of RLT between genotypes is an indication that onset of the disease varied among genotypes despite that all genotypes were inoculated at the same time. The explanation of this phenomena is that the evaluated genotypes reached the critical susceptible stage differently (Hartman et al., 1991). Plant age is reported to be among the major factors that influence reaction to CBB in dry beans (Singh, 1991). Dry bean plants are reported to be more susceptible at flowering stage (Fourie D. Personal communication). High significant difference between the environments for RLT is an indication that the onset of CBB disease varied between the two environments. The disease appeared earlier in Cedara than in Potchefstroom. Favourable temporal conditions for CBB development might be the main attributing factors to the phenomena. Yang et al. (1991) reported that disease onset varies between environment.

In addition, strong positive correlation between RAUDPC and RLT is an indication that disease development was directly linked with plant growth stage. These results are similar to previous studies (Beebe and Corrales, 1991; Singh and Munoz, 1999). Fei et al. (1997) Indicated that temporal changes in plant physiology are associated with changes in host reaction to infection and colonisation. Using RLT in calculating RAUDPC could be the best strategy in selection for disease resistant genotypes in plant breeding programmes as it factors in the time the disease was first detected. Earlier CBB infection has greater impact on yield loss than late infection (Singh and Munoz, 1999). Using this selection criteria, Teebus-RCR 2 was resistant, which confirms findings of previous studies (Fourie and Herselman, 2002). Werna, and RS 7 exhibited resistance to CBB which confirms multiple season unpublished data (Fourie D. Personal Communication). However, Tygerberg, known to be susceptible genotype in previous investigations using other selection criteria, was moderately resistant using the present selection criteria and yielded above the mean yield. The possible reason for high yield in Tygerberg is that the disease was detected late, supporting the concept that late epidemic causes less yield loss (Singh and Munoz, 1999). Flower colour has been associated with CBB resistance (Park et al., 1999). In this study genotypes with white flowers exhibited a higher level of resistance than those with purple flowers. These results contradict reports from previous investigations, which reported positive association of purple flower and CBB resistance (Mutlu et al., 2005; Vandemark et al., 2008). A good example to cite is Teebus-RCR 2, a white flowered genotype bred for resistance to CBB through backcross breeding with XAN 159 (Fourie and Herselman, 2002). Park et al. (1999) emphasized the importance of investigating the association of flower colour and resistance to CBB in specific populations. Indeterminate genotypes were observed to exhibit more resistance than determinate genotypes. The suggested reason is that extended vegetative growth in indeterminate genotypes improves plant immunity. These results are similar to a previous report (Singh, 1991)

There were highly significant differences between genotypes for yield, an indication that these genotypes had varying yield potentials. The reason for the variation in yield was due to divergence of the genotypes in terms of agronomic characteristics. All small seeded genotypes were among the high yielding genotypes regardless of being susceptible or not to CBB, which confirms previous report that small seeded genotypes generally tend to yield more than large seeded genotypes (Beaver, 1999). The other attributing factor to the high yields obtained from these small seeded genotypes, is that they are well adapted to local growing environments, since they were all from local breeding programmes. In this study, a weak negative correlation between yield and severity to CBB was noted. Negative correlations have been reported between yield and disease severity in similar studies (Fourie, 2002; Scott and Michaels, 1992;). Correlations values of -0.48 and -0.72 between CBB severity and yield were reported in independent studies (Fourie, 2002;

Scott and Michaels, 1992). The suggested attributing factors for such findings are; presence of tolerant genotypes, different agronomic characteristics, variation in adaptability to the growing conditions, different yield potential and inaccuracies associated with visual rating of diseases. Agrios (2005) indicated that tolerant genotypes may exhibit disease symptoms but still yield well. An example of such genotypes is ADP-0055. Small seeded genotypes usually yield higher than large seeded genotypes (Beaver, 1999). O'Brien and Van Bruggen (1992) pointed out that low level of precision associated with foliar disease rating contributes to lack of reliability of yield-disease correlation results and concluded that mostly there is no relationship between the two variables. Other investigators also reported that measurement of disease severity based on leaf area usually relate less to yield (Waggoner and Berger, 1987; Filho et al., 1997).

3.5. Conclusion

The use of RAUDPC with consideration of RLT proved to be a good strategy in measuring disease reaction, which enabled the identification of resistant, moderate resistant and susceptible genotypes in the study. Approximately 20% of the genotypes exhibited resistance reaction while 43 and 37% showed moderate and susceptible reaction respectively across both locations. Genotypes ADP-0041, ADP-0790, M-125, ADP-0096, ADP-0544 and M-191 were selected as both high yielding and resistant. These genotypes had RAUDPC values of less than 2000 and yielded above 1.7 t ha^{-1}. Genotypes; ADP- 0055, ADP-0099 and ADP-0103 were selected on the basis of yield. These genotypes yielded above 1.7 t ha^{-1}, but showed susceptible reaction to CBB. Selected genotypes, either on the basis of reaction to CBB or yield, need to be evaluated further to confirm the results. Once confirmed they can be used in the breeding programme to improve resistance or yield in already available market class cultivars or released as new cultivars.

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3.8. Appendices

Appendix 3.1. Characteristics of 60 dry bean genotypes evaluated for resistance to common bacterial blight and yield

Genotype	GH	FD	FLCOR	Bean type	DPM
Werna	I	44	White	Red Speckled Sugar	89
ADP-0560	D	37	Purple	Red mottled	83
ADP-0457	I	45	White	Red mottled	90
ADP-0553	D	36	White	Red mottled	89
Teebus-RCR 2	I	48	White	Small White Canning	89
ADP-0023	I	36	Purple	Red mottled	85
Teebus RR 1	I	42	White	Small White Canning	85
ADP-0544	D	44	White	Red mottled	89
Tygerberg	I	46	White	Red Speckled Sugar	101
M-24/PIC-005	I	46	Purple	Red mottled	93
ADP-0099	D	39	Purple	Red mottled	84
ADP-0754	D	37	Purple	Red mottled	84
ADP-0022	D	34	Purple	Red mottled	80
ADP-0036	D	43	White	Red mottled	86
ADP-0601	I	34	White	Red mottled	78
ADP-0055	I	41	Purple	Red mottled	85
ADP-0577	D	44	Purple	Red mottled	86
ADP-0186	I	42	Purple	Red mottled	85
ADP-0096	I	37	Purple	Red mottled	81
M-191/PIC-098A	I	41	Purple	Red mottled	88
ADP-0053	I	40	Purple	Red mottled	85
ADP-0540	D	45	Purple	Red mottled	88
ADP-0612	D	40	White	Red mottled	83
ADP-0435	I	39	White	Red mottled	85
ADP-0103	I	36	White	Red mottled	82
ADP-0751	D	43	Purple	Red mottled	87
Caledon	I	44	White	Small White Canning	89
ADP-0437	D	39	Purple	Red mottled	81
M-126/PIC-029	I	43	White	Red mottled	89
Gadra	D	36	Purple	Red Speckled Sugar	79
ADP-0211	I	43	White	Red mottled	86
M-125/PIC-029	I	45	White	Red mottled	87
M-139/PIC-029	D	41	White	Red mottled	87
ADP-0013	D	38	Purple	Red mottled	84
ADP-0041	I	42	Purple	Red mottled	91
ADP-0758	I	41	Purple	Red mottled	86
ADP-0390	D	40	Purple	Red mottled	83
ADP-0324	I	37	Purple	Red mottled	83
M-128/PIC-029	I	43	White	Red mottled	87
M-124/PIC-029	I	44	White	Red mottled	88
ADP-0376	D	38	Purple	Red mottled	85
M-12/PIC-001	I	40	Purple	Red mottled	90

Genotype	GH	FD	FLCOR	Bean type	DPM
ADP-0531	D	46	White	Red mottled	92
ADP-0750	I	39	Purple	Red mottled	84
ADP-0765	D	39	White	Red mottled	88
M-190/PIC-098A	I	41	Purple	Red mottled	87
ADP-0790	I	47	White	Red mottled	93
M-23	D	46	White	Red mottled	87
ADP-0208	D	39	Purple	Red mottled	86
ADP-0447	I	42	White	Red mottled	88
ADP-0611	D	38	Purple	Red mottled	82
ADP-0752	I	40	Purple	Red mottled	83
M-127/PIC-029	I	44	White	Red mottled	87
Ukulinga	D	48	White	Red Speckled Sugar	91
M-145/PIC-029	I	45	White	Red mottled	89
RS 7	I	45	White	Red mottled	92
ADP-0427	I	42	Purple	Red mottled	84
ADP-0561	D	36	Purple	Red mottled	81
M-197/PIC-098	I	43	Purple	Red mottled	86
ADP-0006	I	36	Purple	Red mottled	82

GH = Growth habit, FD = 50% days to flowering, FLCOR = Prominent flower colour, DPM = Days to physiological maturity.

Appendix 3.2. Common bacterial blight disease relative life time (RLT) and relative area under disease progress curve (RAUDPC) of 60 dry bean genotypes evaluated in artificially inoculated field trials at Cedara and Potchefstroom

Genotype	RLT			RAUDPC		
	Cedara	Potch	Mean	Cedara	Potch	Mean
Werna	62.69	63.48	63.08	781	961	871
ADP-0560	64.65	70.91	67.78	5879	4507	5193
ADP-0457	62.82	63.16	62.99	1381	5475	3428
ADP-0553	63.64	63.02	63.33	1830	4334	3082
Teebus-RCR 2	61.09	64.99	63.04	813	1237	1025
ADP-0023	64.12	68.67	66.4	3819	5406	4612
Teebus RR 1	63.64	66.95	65.29	4852	5577	5215
ADP-0544	60.08	65.5	62.79	1142	3261	2201
RS 7	51.38	60.97	56.17	963	774	869
M-24	61.72	58.44	60.08	2283	4698	3490
ADP-0099	64.13	69.49	66.81	3484	4197	3841
ADP-0754	64.12	69.83	66.98	3925	5050	4488
ADP-0022	65.64	74.42	70.03	4600	5628	5114
ADP-0036	64.39	65.24	64.81	1296	3066	2181
ADP-0601	65.88	76.71	71.3	5875	7010	6442
ADP-0055	64.39	67.48	65.93	4762	4940	4851
ADP-0577	65.4	63.4	64.4	3223	5413	4318
ADP-0186	63.88	68.43	66.16	4343	5991	5167
ADP-0096	64.39	73.08	68.73	5367	2110	3738
M-191	61.77	66.41	64.09	4543	1446	2994
ADP-0053	65.4	65.65	65.52	1922	4998	3460
ADP-0540	61.6	65.96	63.78	1141	4807	2974
ADP-0612	65.4	68.38	66.89	2571	5423	3997
ADP-0435	64.39	66.31	65.35	2896	4328	3612

Genotype	RLT			RAUDPC		
	Cedara	Potch	Mean	Cedara	Potch	Mean
ADP-0103	63.88	72.2	68.04	4475	5144	4809
ADP-0751	62.01	66.24	64.13	2506	4648	3577
Caledon	62.04	63.75	62.89	1012	1475	1244
ADP-0437	64.37	73.74	69.06	2935	5242	4089
M-126	61.15	64.9	63.03	3825	1620	2722
Gadra	66.41	75.06	70.73	5921	6791	6356
ADP-0211	62.69	68.31	65.50	1181	1560	1371
M-125	62.22	66.48	64.35	3319	1749	2534
M-139	62.5	67.49	64.99	1066	1743	1404
ADP-0013	65.38	69.83	67.61	3774	5253	4514
ADP-0041	62.61	61.72	62.16	1174	1481	1328
ADP-0758	62.71	66.82	64.77	2517	4857	3687
ADP-0390	66.14	69.78	67.96	4491	5710	5100
ADP-0324	63.16	72.8	67.98	3727	5802	4764
M-128	62.24	65.42	63.83	4357	3442	3900
M-124	62.69	64.18	63.43	2941	3533	3237
ADP-0376	66.41	65.69	66.05	5005	6423	5714
M-12	61.32	63.95	62.63	2632	3869	3250
ADP-0531	60.9	60.58	60.74	1131	916	1024
ADP-0750	65.38	69.14	67.26	3142	5983	4563
ADP-0765	64.87	64.65	64.76	2564	5106	3835
M-190	62.24	68.69	65.46	4107	2711	3409
ADP-0790	60.08	60.58	60.33	1536	1461	1499
M-23	65.14	66.05	65.59	4750	5197	4974
ADP-0208	63.64	69.15	66.39	3712	4414	4063
ADP-0447	63.64	65.49	64.56	2519	4481	3500
ADP-0611	64.89	72.86	68.87	3766	7123	5445

Genotype	RLT			RAUDPC		
	Cedara	Potch	Mean	Cedara	Potch	Mean
ADP-0752	62.07	73.71	67.89	2120	5687	3903
M-127	63.16	66.93	65.05	3478	3143	3311
Ukulinga	61.9	62	61.95	1427	959	1193
M-145	62.94	63.95	63.44	1816	1707	1761
Tygerberg	62.94	60.21	61.57	3680	3552	3616
ADP-0427	63.16	70.98	67.07	2277	1850	2063
ADP-0561	65.4	73.84	69.62	5646	5669	5658
M-197	63.88	66.2	65.04	1516	1771	1643
ADP-0006	65.63	70.59	68.11	5414	5827	5620
Mean	63.31	67.18	65.24	3086	3976	3531

Potch = Potchefstroom.

Appendix 3.3. Common bacterial blight reaction at Cedara and Potchestroom field trials and overall reaction averaged over the two locations

Genotype	Cedara	Potch	Pooled reaction
Werna	R	R	R
RS 7	R	R	R
ADP-0531	R	R	R
Teebus-RCR 2	R	R	R
Ukulinga	R	R	R
Caledon	R	R	R
ADP-0041	R	R	R
ADP-0211	R	R	R
M-139	R	R	R
ADP-0790	R	R	R
M-197	R	R	R
M-145	R	R	R
ADP-0427	R	R	R
ADP-0036	R	R	R
ADP-0544	R	R	R
M-125	R	R	R
M-126	R	M	M
ADP-0540	R	M	M
M-191	M	M	M
ADP-0553	M	M	M
M-124	M	M	M
M-12	M	M	M
M-127	M	M	M
M-190	M	M	M
ADP-0457	M	M	M
ADP-0053	M	S	M
M-24	M	S	M
ADP-0447	M	S	M
ADP-0751	M	S	M
ADP-0435	M	S	M
Tygerberg	M	S	M
ADP-0758	M	S	M
ADP-0096	M	S	M
ADP-0765	M	S	M
ADP-0099	M	S	M
M-128	M	S	M
ADP-0752	M	S	M
ADP-0612	M	S	M
ADP-0208	M	S	S

Genotype		Cedara	Potch	Pooled reaction
ADP-0437		M	S	S
ADP-0577		M	S	S
ADP-0754		M	S	S
ADP-0013		M	S	S
ADP-0750		S	S	S
ADP-0023		S	S	S
ADP-0324		S	S	S
ADP-0103		S	S	S
ADP-0055		S	S	S
M-23		S	S	S
ADP-0390		S	S	S
ADP-0022		S	S	S
ADP-0186		S	S	S
ADP-0560		S	S	S
Teebus RR 1		S	S	S
ADP-0611		S	S	S
ADP-0006		S	S	S
ADP-0561		S	S	S
ADP-0376		S	S	S
Gadra		S	S	S
ADP-0601		S	S	S
Reaction Summary (%)	R	30	27	20
	M	42	15	43
	S	28	58	37

Potch = Potchefstroom.

Appendix 3.4. Grain yield performance and reaction to common bacterial blight of the 60 evaluated genotypes using artificial inoculation at Potchestroom trial

Yield Ranking	Genotype	Grain yield (t ha^{-1})	RAUDPC	Reaction
1	ADP-0041	2.91	1481	R
2	ADP-0790	2.62	1461	R
3	ADP-0055	2.51	4940	S
4	Caledon	2.36	1475	R
5	Teebus-RCR 2	2.30	1237	R
6	Teebus RR 1	2.29	5577	S
7	Tygerberg	2.17	3552	M
8	M-125	2.00	1749	R
9	ADP-0096	1.84	2110	M
10	Werna	1.79	961	R
11	ADP-0544	1.76	3261	M
12	ADP-0099	1.76	4197	S
13	ADP-0103	1.75	5144	S
14	M-191	1.73	1446	R
15	ADP-0211	1.68	1560	R
16	M-128	1.65	3442	M
17	M-12	1.64	3869	M
18	ADP-0560	1.62	4507	S
19	ADP-0013	1.58	5253	S
20	ADP-0437	1.56	5242	S
21	M-197	1.56	1771	R
22	ADP-0758	1.45	4857	S
23	M-190	1.44	2711	M
24	ADP-0752	1.42	5687	S
25	M-124	1.37	3533	M
26	M-126	1.34	1620	R
27	RS 7	1.34	774	R
28	ADP-0053	1.25	4998	S
29	M-127	1.24	3143	M
30	ADP-0006	1.23	5827	S
31	ADP-0427	1.20	1850	R
32	ADP-0036	1.20	3066	M
33	ADP-0531	1.19	916	R
34	ADP-0447	1.18	4481	S
35	M-145	1.18	1707	R
36	ADP-0754	1.17	5050	S
37	ADP-0561	1.09	5669	S
38	ADP-0324	1.08	5802	S
39	ADP-0023	1.05	5406	S
40	ADP-0750	1.01	5983	S

Yield Ranking	Genotype	Grain yield (t ha^{-1})	RAUDPC	Reaction
41	ADP-0612	0.98	5423	S
42	ADP-0751	0.97	4648	S
43	ADP-0540	0.95	4807	S
44	ADP-0457	0.94	5475	S
45	ADP-0022	0.93	5628	S
46	M-24	0.83	4698	S
47	ADP-0765	0.81	5106	S
48	ADP-0186	0.77	5991	S
49	Ukulinga	0.70	959	R
50	ADP-0208	0.70	4414	S
51	Gadra	0.68	6791	S
52	ADP-0601	0.65	7010	S
53	ADP-0435	0.62	4328	S
54	ADP-0553	0.59	4334	S
55	M-23	0.51	5197	S
56	ADP-0577	0.48	5413	S
57	M-139	0.42	1743	R
58	ADP-0390	0.28	5710	S
59	ADP-0376	0.16	6423	S
60	ADP-0611	0.084	7123	S
Mean	Mean	1293	3976	

CHAPTER 4

HERITABILITY AND EFFICACY OF MARKER ASSISTED SELECTION IN BREEDING FOR COMMON BACTERIAL BLIGHT RESISTANCE IN SOUTH AFRICAN DRY BEAN GERMPLASM

ABSTRACT

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans* (Xff) is an important disease of dry beans (*Phaseolus vugalis* L.). Although several sources of resistance to CBB have been identified, the disease remains a major challenge in dry bean production worldwide. The study was initiated using two crosses between South African market class cultivars (Teebus-RCR 2 x Teebus-RR 1, and RS 7 x Tygerberg) to: investigate the mode of gene action governing inheritance, estimate heritability, establish the role of maternal effects in CBB resistance and determine the efficacy of marker assisted selection (MAS) in CBB resistance breeding using two SCAR markers BC420 and SU91. Both additive-dominant and epistatic gene effects were detected. Dominant gene effects were of more significance than additive gene effects in both crosses. Duplicate epistasis was detected in Teebus-RCR 2 x Teebus-RR 1 cross. Gene dispersion was detected in both crosses. Heritability of CBB resistance was moderate in both crosses, maternal effect were of significant in the two crosses and lastly resistance was found to be linked to two QTL SCAR markers (BC420 and SU91) in Teebus-RCR 2. Both markers were absent in RS 7. SU91 was found to be the only marker that could be effectively utilized in MAS. The implications of these findings for CBB resistance breeding is that it will affect the selection strategy to be deployed and also the choice of a female parent in resistance breeding programmes. Backcross breeding, recombinant breeding, delayed selection, choosing a resistant parent as a female parent and using MAS, especially in crosses involving Teebus-RCR 2, could yield positive results in CBB resistance breeding programmes if these parents are to be used.

Key words: Common bacterial blight (CBB), dry bean, Gene effects, Heritability, Maternal effects, Variance components, Marker assisted selection (MAS)

4.1. Introduction

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis pv phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans* (Xff) is an important disease of dry beans (*Phaseolus vugalis* L.). The disease occurs in all dry bean growing environments worldwide (Singh and Schwartz, 2010; Viteri et al., 2014). The common bacterial blight pathogen is a motile gram negative bacteria which attacks dry bean stems, leaves, pods and seeds. The symptoms of the disease to some extent depends on the plant organ attacked. Though not well documented, CBB results in yield loss that can exceed 60% (Marquez et al., 2007). The extent of yield loss depend mainly on weather, disease pressure, susceptibility of the cultivars, and time of the epidemic (Arnaud-Santana et al., 1994; Singh and Munoz, 1999).

Common bacterial blight is a seed borne disease of dry bean and the pathogen survives as long as the seed is viable. Its mode of transmission is mainly through infected seed. Both internally or externally infested seed are a good inoculum source of the disease. Other sources of inoculum is plant debris. In Africa, CBB control through planting disease free seeds and high field hygiene has failed to register success due to limited resources (Mkandawire et al., 2004). Planting resistant varieties is reported to be the most viable method of control as it is cheap and effective (Fourie, 2002b; Vandemark et al., 2009). Efforts in breeding for CBB resistance has resulted in identification of resistant sources both in the primary and other gene pools (Singh and Miklas, 2015).

Common bacterial blight in dry bean has been reported to be quantitatively inherited (Chataika et al., 2011; Tryphone et al., 2012). The inheritance is governed by additive gene action with dominance and epistatic effects (Rava et al., 1987; e Silva et al., 1989; O'Boyle et al., 2007). Heritability is dependent on dry bean population, bacterial isolates, inoculation method and plant organs (e Silva et al., 1989; Arnaud-Santana et al., 1994). Heritability values ranging from low to high, have been reported in independent studies (Arnaud-Santana et al., 1994; Tryphone et al., 2012). High complexity of the genetics of CBB resistance has been reported in dry bean (Park et al., 1999). At present there are no reports that CBB resistance is under influence of maternal effect.

Molecular markers linked to quantitative trait loci conditioning resistance to CBB have been identified through genetic studies of CBB resistance. The identified markers offer a promising alternative to disease screening for identifying resistant genotypes (Vandemark et al., 2008). At present four characterized sequence repeats (SCAR) markers, SU19, BC420, SAP6 and X11.4, are available (Viteri et al., 2014). These markers are being utilized in CBB resistance breeding programmes to speed up selection through marker assisted selection (MAS) (Fourie, 2002a;

Mutlu et al., 2005). Success of these markers has been associated with environment, dry bean population and bacterium race, hence, testing their efficacy prior to utilization has been recommended (Park et al., 1999; Fourie, 2002a).

Generation mean analysis has been utilized before to study the genetics of common bacterial blight (Rava et al., 1987; e Silva et al., 1989). The objectives of this study were to determine the mode of gene action governing common bacterial blight resistance, establish the significance of maternal effects in CBB resistance, estimate heritability and determine the efficacy of MAS using two SCAR markers BC420 and SU91 linked to QTL derived from XAN 159 in South African dry bean cultivars.

4.2. Materials and methods

The inheritance of resistance to common bacterial blight was studied using two susceptible cultivars, Teebus-RR 1 and Tygerberg, and two CBB resistant cultivars, Teebus-RCR 2 and RS 7, selected from the local breeding programme. The selected genotypes are used as parents in dry bean improvement programme in South Africa. Teebus-RCR 2 and Teebus-RR 1 are small white canning dry bean cultivars and RS 7 and Tygerberg large red speckled sugar bean cultivars. The four parents were crossed using a simple bi-parental mating design. Teebus-RCR 2 was crossed to Teebus-RR 1 and RS 7 to Tygerberg, with reciprocals. In total eight generations were generated for each cross. The generations were as follows: P1, P2, F1, RF1, F2, RF2, BCP1 and BCP2. RF1 and RF2 were reciprocals of F1 and F2 respectively. BCP1 and BCP2 were backcrosses to P1 and P2 respectively. In the study P1 were the resistant parents and P2 were susceptible parents. Crosses were conducted in the greenhouse at ARC-GCI in Potchefstroom from July 2015 to February 2016.

4.2.1 Greenhouse inoculation

Reaction to CBB was evaluated in a greenhouse at ARC-GCI, Potchefstroom during the 2015/2016 growing season. Mean day- and night temperatures of the greenhouse were 24 ^{0}C and 18 ^{0}C, respectively. The trial was planted on 30th March 2016 and laid out in a randomised complete block design (RCBD) with three replications. The eight generations of each cross were raised in polythene plastic pots of 30 cm in diameter. The pots were filled with sterilized pot mix as a growth media. The number of plants varied depending on generation. A mixture of two aggressive local isolates (Xf260 and Xf410) were used for inoculation. Inoculum was prepared by suspending 48 to 72-h-old cultures in distilled water. The colony density was determined with a spectrometer. The inoculum density was 10^8 CFU/ml (Colony Forming Units/ml). The multiple needle technique was used to inoculate fully first expanded trifoliate leaves (Andrus, 1948).

4.2.2 Leaf sample preparation and deoxyribonucleic acid (DNA) extraction

Young leaves from parents and progenies were harvested and washed with sterile distilled water. Washed leaves were freeze dried separately for each plant and kept at -20 ^{0}C for further use. Deoxyribonucleic acid (DNA) was isolated using a modified version of the method described by Graham et al. (1994). Freeze dried leaves were ground to fine powder for DNA extraction and a volume of 750 µl CTAB buffer (100 mM Tris [tris (hydroxymethyl) aminomethane], pH 8.0; 20 mM EDTA (ethylenediaminetetraacetate), pH 8.0; 1.4 mM NaCl; 2% (w/v) CTAB (hexadecyltrimethylammonium bromide); 0.2% (v/v) ß-mercaptho-ethanol added to approximately 250 µl of the fine leaf powder in a 1.5 ml microfuge tube. The suspension was thoroughly mixed and the tube incubated at 65°C for one hour. A 500 µl volume of chloroform: isoamyl alcohol (24:1) was added and the suspension mixed by gentle inversion. After centrifugation at 14 000 rpm for 3 min, the upper aqueous layer was transferred to a fresh tube containing 500 µl isopropanol, mixed by gentle inversion and incubated at room temperature for 20 min. The suspension was centrifuged at 14 000 rpm for 5 min, 500 µl 70% (v/v) ethanol added and incubated at room temperature for 20 min. DNA was precipitated at 14 000 rpm for 5 min, the pellet air-dried for 1 hr, and resuspended in TE buffer (10 mM Tris-Cl, pH 8.0; 1 mM EDTA, pH 8.0). Resuspended DNA was extracted with 1/10 volume 7.5 M ammonium acetate and an equal volume of chloroform: isoamyl alcohol (24:1). The aqueous layer was transferred to a fresh tube containing two volumes of cold absolute ethanol. Precipitated DNA was washed three times in cold 70% (v/v) ethanol, the pellet air-dried, and resuspended in TE buffer. DNA was treated with RNase for 2 hr at 37°C, after which concentration and purity were estimated by measuring absorbances at A_{260} and A_{280}. DNA samples were diluted to a working solution of 200 ng/µl.

4.2.3 SCAR markers and PCR reactions

SCAR primers, SU91 and BC420 (Table 4.1) were synthesized by GibcoBRL (Life Technologies, Glasgow, United Kingdom), based on primer sequences obtained from Miklas et al. (2000). Primers were suspended in TE buffer to a concentration of 200 pmol/µl. A work solution of 10 pmol/µl was prepared. SCAR markers were used for the polymerase chain reaction (PCR) based on the protocol of Williams et al. (1990) with minor modifications. Amplification reactions were performed in a 25 µl reaction volume containing Promega (Promega Corporation, Madison, Wisconsin) reaction buffer (500 mM KCl; 100 mM Tris-HCl, pH 9.0; 1% (v/v) Triton X-100), 2 mM $MgCl_2$, 100 µM of each dNTP (dATP, dCTP, dGTP, dTTP), 10 pmol primer, 1 unit *Taq* DNA polymerase (Promega) and 15 ng template DNA. Reactions were performed using a PCR Sprint Thermal Cycler (Hybaid Limited, UK) programmed for 5 min at 94^{o}C, 30 cycles of 1 min at 94^{o}C,

1 min at 58ºC for SU91 and BC420 primers, and 1.5 min at 72ºC, followed by one cycle of 5 min at 72ºC.

Amplification products were analysed by electrophoresis in 1.5% (w/v) agarose gels (Seakem LE) at 80V for 2 h using UNTAN buffer (0.4 M Trisbase, 0.02 M EDTA, pH 7.4) and detected by staining with 1 µg/ml ethidium bromide. Gels were photographed under UV light with Polaroid 667 film.

Table 4.1. SCAR markers used to screen population

Primer	Sequence(5'-3')	PCR product size	Resistance source	Linkage group
SU91-1	CCACATCGGTTAACATGAGT	700 bp	XAN159	B8
SU91-2	CCACATCGGTGTCAACGTGA			
BC420-1	GCAGGGTTCGAAGACACATGG	900 bp	XAN159	B6
BC420-2	GCAGGTTCGCCCAATAACG			

4.2.4 Data collection

Common bacterial blight severity was rated once at fourteen days after inoculation using the 1 - 9 scale (Aggour and Coyne, 1989; Vandemark et al., 2009) to describe disease symptoms: 1 = no necrotic lesions and/or chlorosis; 2 - 3 = 1-25.5% leaf area affected; 4 - 6 = 26-64.5% leaf area infected and 7- 9 = 65 -100% leaf area infected.

Resistant reaction Susceptible reaction

Figure 4.1. Common bacterial blight reaction in the greenhouse

4.2.5 Data analysis

Data for the generations was analysed using unbalanced analysis of variance in GenStat 17th edition (Payne et al., 2011). Means were separated by least significant difference (LSD) test at P = 0.05.

4.2.6 Gene effect estimation

A generation mean analysis was conducted on each of the two crosses separately to estimate additive, dominance and epistasis gene effects using the joint scaling test (Cavalli, 1952). Gene effects were defined by Gamble (1962) notations as follows; [m] = mean of homozygotes parents, [a] = pooled additive gene effect, [d] = pooled dominance gene effect, [aa] = pooled additive x additive epistatic gene effects, [ad] = pooled additive x dominance epistatic gene effects, [dd] = pooled dominance x dominance epistatic effects. Means of various generation were unequal due to large differences in family sizes and as such they were adjusted through weighting as described by Kearsey and Pooni (1998). Coefficients that determine the degree of relationship of various generations was used to calculate gene effects variables for individual generations. Regression analysis in GenStat 17th edition (Payne et al., 2011) for sets of variables was performed to test for significance at P = 0.05.

Table 4.2. Generalised genetic and interaction model of the generation mean

	Genetic effects coefficients					
Generation	**[m]**	**[a]**	**[d]**	**[aa]**	**[ad]**	**[dd]**
P1	1	1	0	1	0	0
P2	1	-1	0	1	0	0
F1	1	0	1	0	0	1
F2	1	0	0.5	0	0	0.25
BCP1	1	0.5	0.5	0.25	0.25	0.25
BCP2	1	-0.5	0.5	0.25	0.25	0.25

Source; Kearsey and Pooni (1998)

Maternal effect was determined using generation mean analysis following similar procedure described earlier on generation mean analysis. Maternal gene effects were defined using Kearsey and Pooni (1998) notations as follows; [c] = cytoplasmic effect, [a_m] = additive maternal gene effect, [d_m] = dominance maternal gene effect, [aa_m] = additive x additive maternal gene effect, [ad_m] = additive x dominance maternal gene effects, [da_m] =dominance x additive maternal gene effects and [dd_m] = dominance x dominance maternal gene effects.

Table 4.3. The generalized genetic, maternal and interaction effect model of the generation means

	Progeny genotype			**Maternal genotype**			**Genotype/maternal interaction**			
Generation	**[m]**	**[a]**	**[d]**	**[a_m]**	**[d_m]**	**[c]**	**[aa_m]**	**[ad_m]**	**[da_m]**	**[dd_m]**
P1	1	1	0	1	0	1	1	0	0	0
P2	1	-1	0	-1	0	-1	1	0	0	0
F1(P1 x P2)	1	0	1	1	0	1	0	0	1	0
RF1(P2 x P1)	1	0	1	-1	0	-1	0	0	-1	0
F2(F1 x F1)	1	0	0.5	0	1	1	0	0	0	0.5
RF2(RF1x RF1)	1	0	0.5	0	1	-1	0	0	0	0.5

Source; Kearsey and Pooni (1998)

4.2.7 Heritability estimation

Broad sense heritability (h^2_b) was calculated as a ratio of genetic variance to phenotypic variance, while narrow sense heritability (h^2_n) was calculated as a ratio of additive variance to phenotypic variance. Variance components (s^2) were calculated using equations 4.1, 4.2, 4.3. and 4.4. (Kearsey and Pooni, 1998) Both broad and narrow sense heritability equations 4.5 and 4.6, respectively, were expressed in percentage. Heritability was classified as suggested by Robinson et al. (1949). Where 0 - 30% low, 30.1 - 60% moderate and greater than 60.1% high.

Equation 4.1. Formula for calculating environmental variance

$$VE = \frac{1}{3}\left[s^2 P1 + s^2 P2 + s^2 F1\right]$$

VE = Environment variance, $s^2 P1$ = P1 population variance, $s^2 P2$ = P2 population variance, $s^2 F1$ = F1 population variance.

Equation 4.2. Formula for calculating additive variance

$$VA = 2s^2 F2 - s^2 BCP1 - s^2 BCP2$$

VA = Additive variance, $s^2 F2$ = F2 population variance, $s^2 BCP1$ = BCP1 population variance, $s^2 BCP2$ = BCP2 population variance.

Equation 4.3. Formula for calculating dominance variance

$$VD = s^2 BCP1 + s^2 BCP2 - s^2 F2 - VE$$

VD = Dominance variance

Equation 4.4. Formula for calculating additive x dominance variance

$$VAD = \frac{1}{2}\left[s^2 BCP1 - s^2 BCP2\right]$$

VAD = Additive x dominance variance

Equation 4.5. Formula for calculating broad sense heritability

$$Broad\ sense\ heritability\ (h^2{}_b) = [(VA + VD)/(VA + VD + VE)] \times 100\%$$

Equation 4.6. Formula for calculating narrow sense heritability

$$Narrow\ sense\ heritability\ (h^2{}_n) = [(VA)/(VA + VD + VE)] \times 100\%$$

4.3. Results

4.1.1. Generation mean analysis of variances for reaction to common bacterial blight

The analysis of variance (ANOVA) for severity to CBB among generations (Table 4.4) of Teebus-RCR 2 x Teebus-RR 1 revealed a high significant difference (P<0.001). The generation grand mean score was 4.79. Among the generations, P1 had the lowest mean score followed by BCP1 with mean scores of 1.57±0.64 and 2.45±0.64, respectively. P2 and BCP2 had the highest mean scores of 7.08± 0.59 and 6.38 ±0.64 respectively. Both F1 and F2 progenies had means higher than the mid parent value.

The ANOVA (Table 4.4) for severity to CBB among generations of RS 7 x Tygerberg revealed highly significant differences (P = 0.001). The generation grand mean score was 3.64. Among generations, P1 had the lowest score followed by BCP1 with mean scores of 2.37±0.49 and 2.62±0.37 respectively. P2 and F2 had the highest mean scores of 6.19±0.51 and 4 ±0.24 respectively. The mean scores of backcross progenies were close to their recurrent parents for Teebus-RCR 2 x Teebus-RR 1 and RS 7 x Tygerberg. In both crosses large coefficient of variation was detected an indication that there was great divergence between generations.

Table 4.4. Generation means and ANOVA of severity to CBB in two dry bean crosses

Generation	Teebus RCR 2 x Teebus-RR 1	RS 7 x TYGERBERG
	CBB mean severity score±SE	CBB mean severity score±SE
P1	1.57±0.63	2.37±0.49
P2	7.08±0.60	6.19±0.51
F1	5.51±0.44	3.25±0.29
F2	5.74±0.28	4.00 ±0.24
BCP1	2.45±0.52	2.62±0.37
BCP2	6.38±0.60	3.39±0.57
Grand mean	4.79	3.64
Generation MS	111.76	45.57
Error MS	7.11	5.18
P-Value	<.001	<.001
CV (%)	51.91	63.28
Repeatability	0.83	0.90
$LSD_{0.05}$	1.45	1.17

CV = Coefficient of variation, Error MS = Error mean of squares, Generation MS = Generation mean of squares, LSD = Least significant difference, SE = Standard error, CBB = Common bacterial blight.

4.1.2. Maternal effects generation analysis of variance for reaction to common bacterial blight

Analysis of variance for maternal effects of severity to CBB (Table 4.5) for a cross between Teebus-RCR 2 and Teebus-RR 1 revealed high significant differences (P<0.001) among generations. The generation grand mean score was 4.5. The mean scores of CBB in F1 and F2 progenies were closer to the mean score of the susceptible parent (P2) and higher than their reciprocals. Common bacterial blight disease mean scores of RF1 and RF2 were 3.07±0.60 and 4.09±0.32, respectively. The mean scores of generations ranged from 7.05±0.61 to 1.55±0.64. P2 having the highest mean score and P1 having the lowest.

Analysis of variance for maternal effects of severity to CBB (Table 4.5) for a cross between RS 7 and Tygerberg revealed high significant differences (P<0.001) among generations. The generation grand mean score was 3.66. P1 had the lowest CBB mean score of 2.36±0.49 followed by RF1 with mean scores of 2.90±0.30. P2 had the highest mean score of 6.20±0.51. The mean scores of F1 and F2 were higher than their reciprocals.

Table 4.5. Generation means and ANOVA of maternal effect for severity to CBB of two dry bean crosses

Generation	Teebus-RCR 2 x Teebus-RR 1	RS 7 x TYGERBERG
	CBB mean severity score±SE	CBB mean Severity score±SE
P1	1.55±0.64	2.36±0.49
P2	7.05±0.61	6.20±0.51
F1	5.48±0.45	3.25±0.30
F2	5.73±0.29	4.00±0.24
RF1	3.07±0.60	2.90±0.30
RF2	4.09±0.32	3.29±0.26
Grand mean	4.50	3.66
Generation MS	97.49	45.37
Error MS	7.42	5.24
P-Value	<.001	<.001
CV (%)	56.38	64.96
Repeatability	0.80	0.72
$LSD_{0.05}$	1.38	1.00

CV = Coefficient of variation, Error MS = Error mean of squares, Generation MS = Generation mean of squares, LSD = Least significant difference, SE = Standard error, CBB = Common bacterial blight.

4.1.3. Gene action

Data for reaction to CBB (Table 4.6) of Teebus-RCR 2 x Teebus-RR 1 did not fit a simple additive-dominance model, but the six parameter model which include interactions (i.e. additive,

dominance, additive by additive, additive by dominance and dominance by dominance). The high significant differences (P<0.001) for all variables of the six parameter model, indicated that data was sufficient and fitted the di-genic model well. Dominance and dominance x dominance gene action had different signs indicating the presence of duplicate type of epistasis. Since dominance was positive and highly significant it indicates that the type of dominance present is directional. In addition, negative signs associated with gene effects were also observed, revealing significance in the direction of susceptible parent. Data for reaction to CBB (Table 4.6) in a cross between RS x Tygerberg did not fit both simple additive dominant model and the di-genic six parameter model. Additive gene effects were non-significant. Both dominance and additive x additive gene effects were significant (P<0.05). However, additive x additive gene effects predominate dominance gene effect. Additive x dominance gene effects were significant (P<0.01) in the direction of susceptible parent. In both crosses dominance gene effect predominate additive gene effects indicating the presence of gene dispersion.

Table 4.6. Estimates of gene effects of reaction to CBB for the two crosses of dry bean

Gene action	**Teebus-RCR 2 x Teebus-RR 1**	**RS 7 x TYGERBERG**
	Estimates ±SE	**Estimates±SE**
[m]	-35.76±6.25***	5.371±0.407***
[a]	8.73±2.43***	2.31±2.32
[d]	21.69±1.04***	3.19±1.57*
[aa]	28.6±4.69***	9.65±4.78*
[ad]	-31.08±5.75***	-15.01±5.49**
[dd]	-9.252±0.822***	-
Epistasis type	Duplicate	-

Significant at: * P<0.05; ** P<0.01; *** P<0.001. SE = Standard error, [m] = Mid parent, [a] = Additive gene action, [d] = Dominance gene action, [aa] = Additive x additive gene action, [ad] = Additive x dominant gene action, [dd] = Dominant x dominant gene action.

Cytoplasmic effects were significant (P<0.01) for Teebus-RCR 2 x Teebus-RR 1 and for RS x Tygerberg (P<0.001) in both crosses (Table 4.7). Data for both crosses could not fit both the simple and the six parameter model for maternal effects. For Teebus-RCR 2 x Teebus-RR 1 additive and additive x additive maternal effects were significant (P<0.001). Only additive maternal gene effects were significant (P<0.001) in RS 7 x Tygerberg. The absence of dominance and its epistasis gene effects indicated that maternal inheritance of common bacterial blight resistance is governed by fixable gene effects only.

Table 4.7. Estimates of maternal gene effects of reaction to CBB for the two crosses of dry bean

Maternal gene effects	Teebus -RCR 2 x Teebus-RR 1	RS 7 x TYGERBERG
	Estimates ±SE	Estimates ±SE
[m]	-58.22± 3.98***	5.76±0.17***
[a]	1.62±0.58**	-4.02±0.25***
[d]	20.44±0.85***	-0.94±0.18***
[c]	0.27±0.11**	0.79±0.06***
[a_m]	-3.53±0.32***	0.41±0.10***
[aa_m]	27.19±16.73***	-

Significant at: * P<0.05; ** P<0.01; *** P<0.001. SE = Standard error, [m] = Mid parent, [a] = Additive gene action, [d] = Dominance gene action, [c] = Cytoplasmic effect, [a_m] = Additive maternal gene action, [aa_m] = Additive x additive maternal gene action.

4.1.4. Heritability estimate

Heritability estimate using variance components for both broad and narrow sense heritability of common bacterial blight resistance in two crosses is presented in Table 4.8. Broad sense heritability was high in both Teebus-RCR 2 x Teebus-RR 1 (74%) and RS 7 x Tygerberg (78%) crosses. Narrow sense heritability was moderate in both crosses (36% for Teebus-RCR 2 x Teebus-RR 1 and 59% for RS 7 x Tygerberg). Due to significant epistatic interaction (Table 4.6), the effective number of genes governing resistance was not calculated. Since narrow sense heritability was moderate, CBB resistance in both crosses was shown to be a quantitative inherited trait.

Table 4.8. Genetic variance components, broad sense heritability and narrow sense heritability for reaction to CBB

Parameter	Teebus-RCR 2 x Teebus-RR 1	RS 7 x Tygerberg
VA	6.43	2.98
VD	6.77	7.85
VE	4.63	2.49
VAD	3.18	0.68
h^2_b (%)	74	78
h^2_n(%)	36	59

VA = Additive variance, VD = Dominance variance, VE = Environment variance, VAD = Additive x Dominance variance, h^2_b = Broad sense heritability, h^2_n = Narrow sense heritability.

4.1.5. Confirming the presence of markers in parental genotypes

Polymerase chain reaction (PCR) results revealed both the presence and absence the two QTL linked SCAR markers (SU91 and BC420) associated with CBB resistance (Table 4.9). Both markers were present in the Teebus-RCR 2 dry bean cultivar. The markers were absent in Teebus-RR 1, RS 7 and Tygerberg dry bean cultivars. The presences of the two QTL markers in Teebus-RCR 2 indicates their effect on resistance to CBB. Teebus-RCR 2 exhibited good resistance to CBB in the greenhouse experiment (phenotypic data). The absence of markers in Teebus-RR 1 and Tygerberg confirms greenhouse evaluation results. These two cultivars were susceptible to CBB and recorded higher mean score of CBB (Table 4.4). Though the two QTL linked markers were absent in RS 7, the cultivar exhibited good resistance to CBB in the greenhouse (Table 4.4).

Table 4.9. Presence and absence of molecular markers in genotypes based on PCR results

Genotype	Marker	
	SU91	**BC420**
Teebus-RCR 2	+	+
Teebus-RR 1	-	-
RS 7	-	-
Tygerberg	-	-

4.1.6. Proportionality of individual plants with markers in progenies of Teebus-RCR 2 and Teebus-RR 1

Table 4.10 is a summary of results of the agarose gel for the PCR (Figures 4.2, 4.3 and 4.4) for individual segregating plants of respective generation of a cross between Teebus-RCR 2 x Teebus-RR 1. SU91 was present in all generations, while BC420 was only present in F2 and RF2. The percentage of SU91 markers in individual plants for respective generation was as follows; BC1 (96%), BC (50%), F1 (72%), RF1 (100%), F2 (60%) and RF1 (91%). The percentage distribution of BC420 in F2 and RF2 was 1 and 9% respectively.

Table 4.10. Number of individual plants with markers in segregating generation of Teebus-RCR 2 x Teebus-RR 1

		Marker			
		SU91		BC420	
Generation	Total number of plant	Present	Percentage	Present	Percentage
BC1	26	25	96	-	-
BC2	16	8	50	-	-
F1	29	21	72	-	-
RF1	23	23	100	-	-
F2	72	43	60	1	1
RF2	65	59	91	6	9

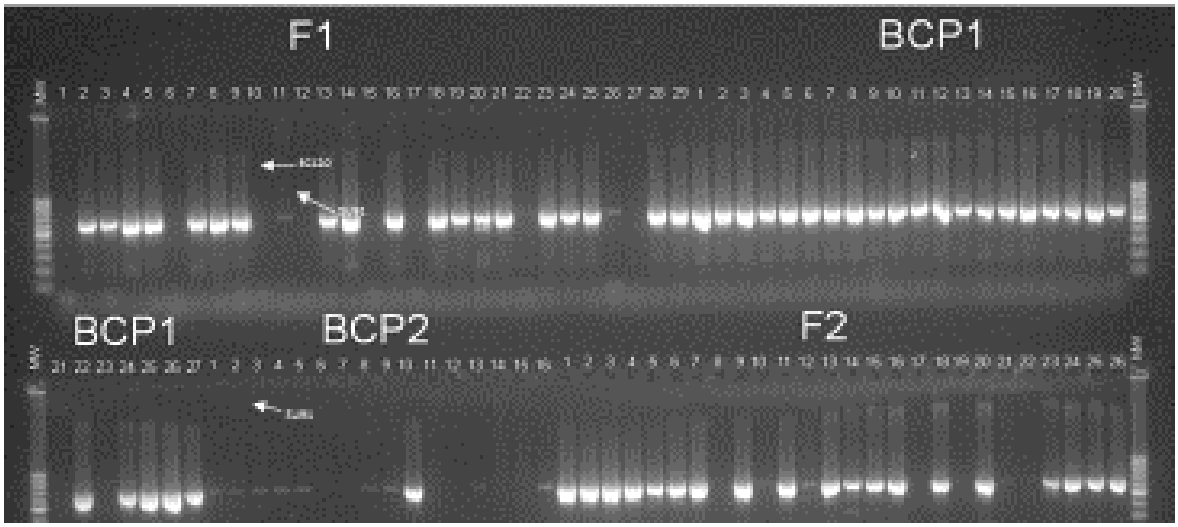

Figure 4.2: Agarose gel showing SCAR markers of primer BC420 and SU91 in F1, BCP1, BCP2 and F2 of Teebus-RCR 2 and Teebus-RR 1

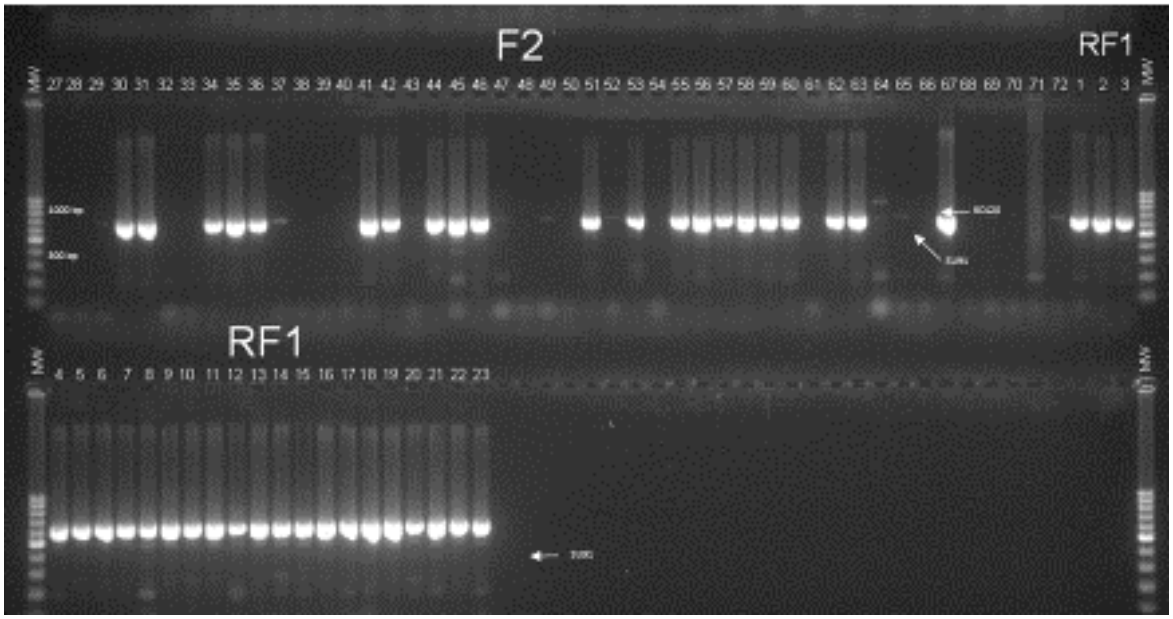

Figure 4.3: Agarose gel showing SCAR markers of primer BC420 and SU91 in RF2 and RF1 of Teebus-RCR 2 and Teebus-RR 1

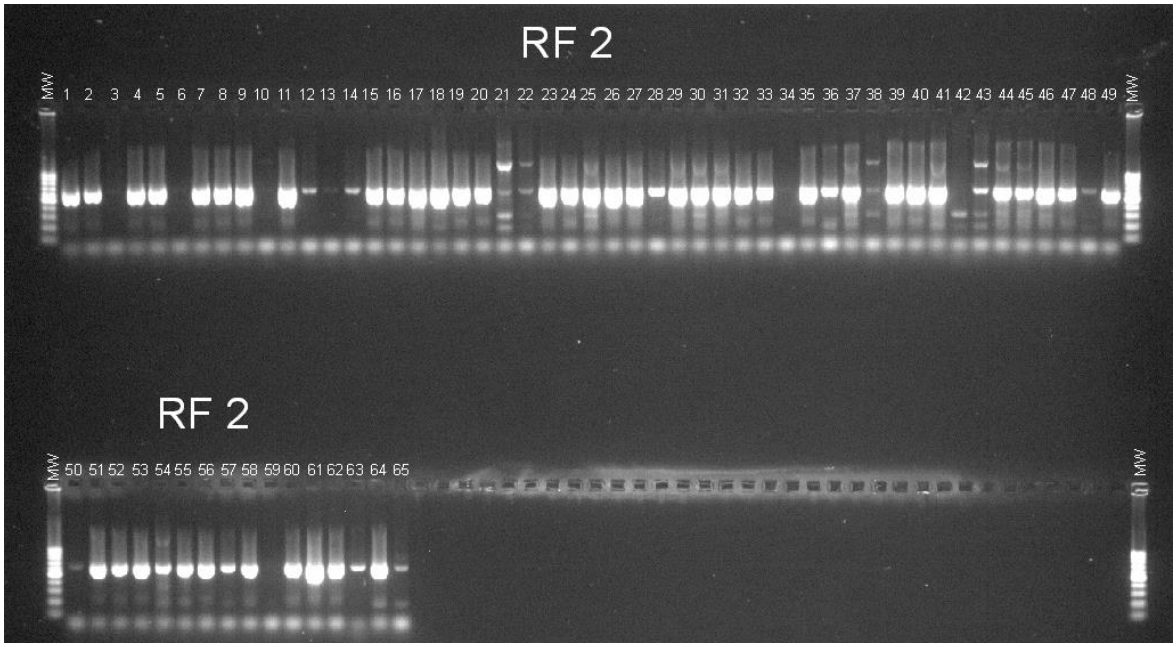

Figure 4.4: Agarose gel showing SCAR markers of primer BC420 and SU91 in RF2 of Teebus-RCR 2 and Teebus-RR 1

4.1.7. Confirming the efficiency of marker assisted selection using F2 plants of Teebus-RCR 2 x Teebus-RR 1

There was a high significant difference (P<0.001) in reaction to CBB using Chi square between the expected and the observed confirming that inheritance to CBB in Teebus-RCR 2 and Teebus-

RR 1 is governed by many genes with epistatic gene effects (Table 4.11). Phenotypic data against SCAR marker SU91 indicated that there was no significant difference between the observed and expected plants with SU91 marker. All plants that were phenotypically resistant had SU91 SCAR marker. However, there was a high significant difference (P<0.001) between the phenotype data and the SCAR marker BC420. BC420 was absent in the majority of plants, but the plants were phenotypically resistant.

Table 4.11. Inheritance of common bacterial blight and the SCAR markers SU91 and BC420 in F2 population of Teebus-RCR 2 x Teebus-RR 1

	Phenotype	Observed	Expected	Chi-Square	DF	FPr	Significant
Reaction	Resistant	35	54	26.74	1	7.879	***
	Susceptible	37	18				
Marker							
SU91	Present	43	35	3.56	1	3.851	NS
	Absent	29	37				
BC420	Present	1	35	34.75	1	7.879	***
	Absent	29	37				

Significant at: *** P<0.001, NS = Non significant, DF = Degrees of freedom, FPr = F-probability value.

4.4. Discussion

Highly significant differences between generations for reaction to CBB in both crosses is an indication that the generations were different from each other. The large difference in mean score for CBB reaction between the parents P1 and P2 used in both crosses is an indication that parents used in the study were divergent in the trait investigated, which is desirable for a generation mean analysis (Jinks and Mather, 1982). The mean score for reaction to CBB was lower in F1 than F2. This was due to an increase in proportion of susceptible plants to resistant plants in F2. Backcross generations had their means close to their recurrent parent because backcrossing increases the frequency of recurrent parent alleles in the genome of the progeny (Xu, 2010). The higher mean scores of F1 and F2 than their reciprocals indicate the role of maternal effects. Plants generated from reciprocal crossing exhibited high level of resistance. This implies that the choice of a female parent will be of paramount importance in common bacterial blight resistance breeding. Maternal effects have been reported in foliar diseases in other crops. Derera et al. (2007) reported that grey leaf spot in maize was under the influence of maternal effects. The frequency of susceptible plants increases from RF1 to RF2 evident by higher mean score in RF2 than RF1.

A clear understanding of mode of gene action conditioning expression of traits of interest is fundamental in breeding programmes. It helps in developing breeding strategies for crop improvement (Zalapa et al., 2006). The joint scaling test (Cavalli, 1952), which was used to

determine gene action for reaction to CBB in this study, was significant for both crosses. For Teebus-RCR 2 x Teebus-RR 1, data adequately fitted the six parameter model. e Silva et al. (1989) reported similar results in a cross between Rio Doce and XAN 112. For the cross RS 7 x Tygerberg, data failed to fit both the six parameter model and the simple additive model, but epistatic gene effects was present. Rava et al. (1987) reported similar results in two crosses CNF 0010 x Cornell and Jules x CNF 0010. The failure of the model to fit the simple additive dominance model indicated that resistance to CBB is governed by many genes. Despite that, both additive and dominant gene actions were significant in Teebus-RCR 2 x Teebus-RR 1. Dominant gene action was predominant over additive.

In RS 7 x Tygerberg dominant gene effects were significant, while additive were not. These results do not agree with earlier reports where additive gene action was found to be more of significant in CBB resistance (Rava et al., 1987; e Silva et al., 1989). This implies that selection should be delayed in both crosses until homozygosity has increased in the population (Ajay et al., 2012). All epistatic interactions were significant, however, additive x additive epistatic effect was more significant in Teebus-RCR 2 x Teebus-RR 1 than the RS 7 x Tygerberg cross. This is consistent with the previous studies by Rava et al. (1987). This suggest that transgressive segregants of higher resistance than parents can be achieved in advanced generations. Additive x additive interactions are fixable, hence can be utilised in selecting a pure line (Rava et al., 1987). Likewise, the significance of additive by additive gene effects in RS 7 x Tygerberg indicates an opportunity that resistance can be fixed and exploited. The presence of duplicate epistasis in Teebus-RCR 2 x Teebus-RR 1 is an indication that variability in segregating generations will be reduced, making the selection process difficult and rigorous, justifying the need of incorporating marker assisted selection (Kumar and Patra, 2010; Ajay et al., 2012). High total significant magnitude of non-fixable epistatic effects for both crosses also indicates that selection is likely to be very ineffective and rigorous. Dominance gene effect predominated additive gene effect in both crosses, supporting the presence of gene dispersion. This implies that transgressive segregants can be developed easily if these dispersed genes are brought together (Kearsey and Pooni, 1998).

Maternal effects have not been reported before in CBB resistance studies and generation mean analysis has not been exploited in investigating maternal effects. In the study, maternal effects were found to be of importance in both crosses. Maternal effects on foliar diseases in other crops have been reported. Derera et al. (2007) found that resistance to Phaeosphaeria leaf spot in maize disease was conditioned by maternal genes. Data could not fit both the simple maternal additive-dominance and the six parameter model for Teebus-RCR 2 x Teebus-RR 1. In RS 7 x Tygerberg data fitted the simple additive-dominant model. Additive maternal effects were

significant for both crosses, however, in Teebus-RCR 2 x Teebus-RR 1 it was to the direction of susceptible parent. Kearsey and Pooni (1998) indicated that a negative maternal gene effect is an indication that the stronger parent is a poor maternal parent. However, additive x additive maternal epistatic effects were predominant over additive maternal effects and positive. This indicates that Teebus-RCR 2 is a good maternal parent. The presence of fixable maternal effects in both crosses is encouraging in that it can be fixed and exploited in breeding for resistance.

Narrow sense heritability is the most important due to its implication in breeding programmes (Acquaah, 2009). The magnitude of heritability of a trait determines the selection method. Low heritable traits do not permit early generation selection. Selection for high heritable traits is effective in F2. Unlike high heritable traits, low heritable traits are influenced by the environment. Besides determining the method of selection to be deployed, the success of selection also depends on the magnitude of heritability. Selection for traits with high values of narrow sense heritability is likely to be successful and easy. Selecting low heritable trait is rigorous, difficult and with low chances of success (Sleper and Poehlman, 2006; Ajay et al., 2012). Heritability values are also used in determining the response to selection in plant breeding programmes (Nyquist and Baker, 1991; Sleper and Poehlman, 2006; Said, 2014). When dealing with low to moderate heritable traits, breeders rely on transgressive segregants to register progress (Sleper and Poehlman, 2006). Using values of genetic means generated from generation mean analysis to estimate heritability values has been discouraged (Hallauer and Miranda, 1981). Variance components have been recommended to be used in estimating heritability of traits in crop improvement programme. Kearsey and Pooni (1998) indicated that variance components reveal the true genetic variation, while means reveal the breeding potential of source material.

Narrow sense heritability for resistance to CBB in both crosses was moderate. These results agree with those reported previously by other investigators on inheritance of CBB resistance (e Silva et al., 1989; Arnaud-Santana et al., 1994; Tryphone et al., 2012). This is an indication that resistance to CBB is controlled by multiple genes (Tryphone et al., 2012). The implication of these results in a breeding programme is that selection could be delayed to advanced stages where plants have reached some homozygosity. High resistance could be fixed through selection of transgressive segregants that are more resistant than the more resistant parent.

The presence of markers for a particular trait in breeding populations, eases the process of selection if utilised in the breeding programme (O'Boyle et al., 2007), particularly in traits with low to moderate heritability like CBB. Indirect selection using markers is reported to be of value when the marker positively correlates with phenotypic data (Tryphone et al., 2012). In this study the SU91 and BC420 SCAR markers were both present in Teebus-RCR 2. These results are

consistent with previous results (Fourie and Herselman, 2002; Vandemark et al., 2008). Common bacterial blight resistance in Teebus-RCR 2 was derived from XAN159, which has both markers (Vandemark et al., 2008). The presence of these two QTL markers linked to CBB resistance in Teebus-RCR 2 is evidence that resistance was successfully transferred into Teebus, a commercial parent cultivar for Teebus-RCR 2 (Vandemark et al., 2008). The study also established that both markers were absent in RS 7. BC420 is reported to be linked to the *V* gene which affect the seed colour of red speckled beans (Mutlu et al., 2005). Its absence in RS 7 confirms these previous results, since RS 7 maintained the red speckled colour. The suggested reason for the absence of SU91 in RS 7 is that the original source of resistance in the cultivar might not have SU91. RS 7, despite the absence of the two markers, exhibited good resistance in the greenhouse study. This suggest that resistance might be conditioned by other QTL linked markers or minor genes not linked to these major known QTL linked markers (Viteri et al., 2014). Since RS 7 is a good source of resistance (greenhouse data) there is a need of conducting further investigations to establish the QTL markers in this genotype so that MAS can also be utilised.

The presence of markers in segregating generations derived from a cross between Teebus-RCR 2 and Teebus-RR 1 indicates that resistance was successfully transferred into these progenies. There was a direct relationship between numbers of plants with markers and the mean phenotypic reaction to the disease. Generations with low disease mean score had higher number of plants with markers. This confirms the relationship between the markers and CBB resistance (Mutlu et al., 2005). The significant relationship between the expected and observed CBB reaction indicates that resistance to CBB in Teebus-RCR 2 is governed by several genes. For marker assisted selection in CBB resistance breeding to be effective, high levels of positive correlation between markers and CBB resistance is needed (Yu et al., 2004). The indirect selection through using markers allows for early generation selection and improved response to selection (Xu, 2010). The insignificant correlation between the expected and observed plants with the marker BC420 indicates that it is not an effective marker in CBB resistance breeding. This support the need to test the marker before using it in the breeding programme (Park et al., 1999; Fourie, 2002a).

4.5. Conclusion

The study has established that common bacterial blight is governed by additive, dominance and epistatic gene effects. It has also been found that the disease is a moderately heritable trait and governed by many genes. Maternal effects play a significant role in common bacterial blight resistance breeding. Furthermore, resistance in Teebus-RCR 2 is linked to two markers (SU91 and BC420), however only SU91 could be used for indirect selection. The overall implications of

these finding in CBB resistance breeding programme is that it will affect the selection strategy to be deployed and also the choice of a female parent in resistance breeding programme. Backcross breeding, recombinant breeding, delayed selection, choosing a resistant parent as a female parent and using MAS, especially in crosses involving Teebus-RCR 2, would register positive result in CBB resistance breeding programme.

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CHAPTER 5
OVERVIEW OF THE STUDY

Dry bean is an important legume crop worldwide. In South Africa dry bean is a major source of plant protein and also an important source of income among growers. The crop is mainly, produced in the Free State, Mpumalanga, Gauteng, KwaZulu-Natal, North West, Limpopo and Eastern Cape provinces, all exhibiting different agro-ecological conditions such as temperature, humidity, soil fertility and rainfall. Crop productivity is low and national demand outstrip production. The main contributing factors to low production includes both abiotic and biotic stresses. Yield instability due to environmental changes and common bacterial blight disease, caused by *Xanthomonas axonopodis* pv *phaseoli* (Xap) (Smith) Vauterin, Hoste, Kosters & Swings and its fuscans variant, *X*. *fuscans* sbsp. *fuscans* (Xff) are considered to be major contributing factors to low productivity. Breeding for stable high yielding and common bacterial blight resistant cultivars is of importance in South Africa. The study aimed at investigating yield stability, adaptation and breeding for common bacterial blight resistance in dry bean. This chapter highlights the study objectives with summary of major findings and their implications in South Africa dry bean breeding.

Yield stability and adaptation analysis of South African dry bean cultivars

Data from the National Dry Bean Cultivar Trials (NCT) conducted during the 2014/2015 growing season in 21 locations in three countries, namely South Africa, Swaziland and Lesotho, was used to investigate grain yield stability and adaptability of thirty South African dry bean cultivars. Data was subjected to additive main effect and multiplicative interaction model (AMMI). The findings were as follows:

- The presence of crossover genotype by environment interaction effects.
- Cultivars G6 and G14 were identified by AMMI as high yielding and stable with mean yield 1.46 t ha^{1} and 1.45 t ha^{-1} respectively. These genotypes were selected for general adaptation.
- Cultivars G26 and G29 were identified as high yielding in selected environments. These genotypes were selected for specific adaptation to high potential environments.
- Environments with similar response were identified by AMMI bi-plot. For example KN and NP had similar response.

Evaluation of newly introduced dry bean germplasm for resistance to common bacterial blight and for yield

The reaction to CBB and yield was evaluated in the field using 60 dry bean genotypes of diverse genetic background, of which 39 were from the Andean Diversity Panel (ADP) and 13 from the Phaseolus Improvement Cooperative (PIC). Five were from the Agricultural Research Council-Grain Crop Improvement (ARC-GCI) dry bean breeding programme and three were from Pro-Seed, a seed company based in KwaZulu-Natal South Africa. Lines from the ARC-GCI and Pro-Seed were used as checks in the study. The findings were as follows;

- The reaction of dry bean genotypes to CBB varied across the two environments and between genotypes. At Cedara the reaction among genotypes was 30% resistant, 42% moderate and 28% susceptible, while at Potchefstroom the figures were 27, 15 and 58% respectively. Seven ADP lines showed resistant reaction across the two locations (0531, 0041, 0211, 0790, 0427, 0036 and 0544). Only three PIC lines, M-139, M-145 and M-197 showed resistant reaction to CBB across the two locations.
- Only five of the eight local cultivars which were included as checks (Werna, RS 7, Teebus-RCR 2, Caledon and Ukulinga) were resistant.
- Yield ranged from 2.91 t ha^{-1} to 0.08 t ha^{-1} with ADP-0041 and ADP-0611 recording the highest and lowest yield respectively. Five of the eight local dry bean genotypes were among the top ten high yielding genotypes and these were Caledon (2.36 t ha^{-1}), Teebus-RCR 2 (2.30 t ha^{-1}), Teebus-RR 1 (2.29 t ha^{-1}), Tygerberg (2.17 t ha^{-1}) and Werna (1.79 t ha^{-1}). ADP-0790 and Teebus RRI were the only two genotypes among the top ten high yielding that were susceptible to CBB.
- Genotypes ADP-0041, ADP-0790, M-125, ADP-0096, ADP-0544 and M-191 were selected as both high yielding and resistant. These genotypes had RAUDPC values of less than 2000 and yielded above 1.7 t ha^{-1}. Genotypes ADP- 0055, ADP-0099 and ADP-0103 were selected on the basis of yield. These genotypes showed susceptible reaction to CBB, but yielded above 1.7 t ha^{-1}.

Heritability and efficacy of marker assisted selection in breeding for common bacterial blight resistance in South Africa dry bean germplasm

The study to investigate mode of gene action, heritability and efficacy of marker assisted selection in common bacterial blight resistance was conducted using two susceptible cultivars Teebus-RR 1 and Tygerberg and two resistant cultivars Teebus-RCR 2 and RS 7. All parental genotypes

were selected from the local germplasm. The four parents were crossed using a simple biparental mating design. Teebus-RCR 2 was crossed to Teebus-RR 1 and RS 7 to Tygerberg with reciprocals. In total eight generations were generated. The generations were as follows: P1, P2, F1, RF1, F2, RF2, BCP1 and BCP2. Generations for each cross were evaluated separately in the greenhouse under artificial inoculation. Leaf samples were collected for molecular analysis. Two SCAR QTL linked markers BC420 and SU91 were used to test the efficacy of marker assisted selection. The findings were as follows;

- Dominant gene action predominated additive gene action indicating the presence of gene dispersion. This implies that selection could not be effective in early generations and also the need to bring the dispersed genes together for easy development of transgressive segregants.
- The presence of duplicate epistasis is an indication that variability will be very low in segregating populations resulting in selection difficulties.
- Additive by additive gene effects were significant in both crosses which implies that transgressive segregants of higher resistance than parents can be achieved in advanced generations and also resistance can be exploited and fixed.
- Narrow sense heritability was moderate (36 and 59%) in both crosses and this implies that resistance to CBB is conditioned by several genes.
- Maternal effects were significant and governed by fixable gene effects. This implies that choice of female parent is important and also that resistance governed by maternal effects can be fixed and exploited.
- The two QTL linked SCAR markers BC420 and SU91 are present in Teebus-RCR 2 only.
- Only SU91 QTL linked SCAR was found to be effective in MAS breeding for CBB resistance involving Teebus-RCR 2.

Breeding implications and recommendations

Yield stability and adaptation study: The dry bean breeding programmes in South Africa usually release cultivars first before testing in multi-location trials. Several released cultivars are withdrawn from the list every year following their poor performance in national cultivar trials. Among the contributing factors of poor performance are, inappropriate testing sites prior to release and yield instability. In this study, through subjecting the data to AMMI analysis, high yielding cultivars that showed general adaptation and specific adaptation were identified. Sites with similar response were also identified. The identification of these sites implies that in future it

could be possible for the national cultivars trials to drop certain sites in order to reduce the cost associated with multi-location trials. On the other hand, the identification of representative (informative) environment implies that breeding programmes should utilise these locations in preliminary variety selection trials.

Disease and yield evaluation study: The fundamental basis of a successful breeding programme lies in the genetic resources of available germplasm (Beebe and Corrales, 1991). The identified superior genotypes from the introduced germplasm could be ideal in improving dry bean production in the country in a number of ways; firstly the genotypes could be used in developing pre-breeding populations for yield and CBB resistance breeding. Secondly genotypes could be utilised directly as parental genotypes in both yield and CBB resistance breeding and lastly these superior introduced genotypes could be released as commercial cultivars. The study established that introduced germplasm has a potential to improve dry bean production. Therefore it is recommended that the germplasm should be evaluated for other important traits over multiple seasons.

Heritability and efficacy of marker assisted selection: A clear understanding of mode of gene action conditioning expression of traits of interest is fundamental in breeding programmes. It helps in developing breeding strategies for crop improvement (Zalapa et al., 2006). The overall implications of these findings in CBB resistance breeding is that it will affect the selection strategy to be deployed and also the choice of a female parent in resistance breeding programme. Backcross breeding, recombinant breeding, delayed selection, choosing a resistant parent as a female parent and using MAS, especially in crosses involving Teebus-RCR 2, could be options which could register positive results in a CBB resistance breeding programme. In this study, the selected progenies have been advanced to F3 generation. Pedigree selection will be employed up to F7 so that market class cultivars that are high yielding and resistant to CBB might be identified.

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