BREEDING DRY BEAN FOR RESISTANCE TO BACTERIAL BROWN SPOT DISEASE CONDITIONS IN SOUTH AFRICA

By

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ABSTRACT

Bacterial brown spot (BBS) disease is a major disease in dry beans in South Africa causing yield losses of up to 55%. The overall goal of the research was to improve dry bean production through identifying high yielding and stable cultivars, with resistance to the BBS disease, classifying or detecting mega environments for dry bean production and to conduct pre-breeding trials that will provide information that will contribute to BBS disease breeding in South Africa.

Four hundred and twenty three Andean Diversity Panel (ADP) dry bean genotypes were screened for grain yield and BBS disease resistance in three regions. The plants were inoculated with three isolates of BBS strains or inoculum at 21, 28 and 36 days after planting. Disease severity was rated at 7, 14 and 21 days after the first infection and the relative area under disease progress curve (RAUDPC) was calculated. The analysis of variance revealed significant differences ($P<0.001$) in grain yield and BBS severity for genotype, environment and genotype by environment interaction (GEI). Genotypes were classified as resistant, moderate resistant and susceptible based on BBS severity and RAUPDC. The study identified 21.0% of the genotypes as resistant and 41.6% as moderately resistant to BBS disease. The RAUDPC was significantly ($P<0.001$) negatively associated with grain yield ($r= -0.55$). The small seeded genotypes showed lower RAUDPC than the medium and the large seeded, and genotypes with an indeterminate growth habit showed lower RAUDPC than those with a determinate growth habit. Genotypes ADP-0592, ADP-0790, ADP-0120 and ADP-0008 were selected for both resistance to BBS disease resistance and high seed yield across three environments. The best genotypes had grain yield above 1.45 t ha^{-1} across sites, and above1.85 t ha^{-1} at individual sites, and had grain yield above the grand mean (0.87 t ha^{-1}) and the best performing cultivar (1.13 t ha^{-1}), and mean BBS severity below the grand mean (39.85) and the best performing cultivar (31.67). These genotypes can be useful sources of genetic resistance for future dry bean improvement.

Fourteen dark red kidney (DRK) bean genotypes were evaluated for grain yield, stability and BBS severity across six environments. The additive main effect and multiplicative interaction (AMMI) and genotype plus genotypes by environment interaction were analysed. The analysis of variance showed significant ($P<0.001$) effects for grain yield and BBS severity for genotype, environment and genotype by environment interaction (GEI). The interaction principal components (IPCA1 - 4) for grain yield and IPCA1 for BBS severity were significant ($P<0.001$, $P<0.01$). Genotypes G12 (1.46 t ha^{-1}) was broadly adapted for both high yield and low BBS severity across six environments, while genotypes G08 (1.77), G06 (1.70), G03 (1.62), G02

(1.56), G05 (1.48) and G04 (1.45 t ha^{-1}) had specific adaption for high grain yield and low BBS severity. These genotypes recorded mean yields above the grand mean and the best check both two genotypes with 1.43 t ha^{-1}, and mean BBS severity below grand mean (31.90) and the best check (48.89). The GGE biplot identified three mega-environments for grain yield and BBS severity across six tested environments.

Heritability and gene effects controlling BBS disease resistance were estimated in a cross between a susceptible commercial cultivar RS7 and a resistant genotype A55. The parents (P1 and P2), F1, F2, BCP1 and BCP2 were used in the generation mean analysis. The generations were inoculated with BBS disease and rated for BBS severity using CIAT scale. The analysis of variance for BBS severity showed a significant difference ($P<0.001$) between generations. The data for reaction to BBS severity did not fit a simple additive-dominance model. The digenic interaction model was significant different ($P<0.001$) for mean [m], additive [d], dominance [h], additive x additive [i], and dominance x dominance [l]). The dominance [h] and dominance x dominance[l] gene effects had the inverse signal, showing the existence of duplicate epistasis. The positive signal of dominance x dominance [l] interaction showed unidirectional dominance gene effects. The broad and narrow sense heritability were both moderate. The existence of gene dispersion suggest that the selection for BBS resistance, especially in initial generations, would be complex using conventional breeding methods. The dispersed gene should be brought together and the resistance can be fixed and exploited in progressive or later generation stages for the development of genotypes with high grain yield, stable and BBS disease resistant.

DECLARATION

I, Venancio Alexandre Salegua, declare that

1. The research reported in this study, excluding where otherwise designated, is my innovative research.
2. This study has not been proposed for any degree or examination at any other university.
3. This study does not include other persons' data, pictures, graphs or other information, without exactly recognized as being obtained from other persons.
4. This study does not include other persons' writing, unless exactly recognized as being obtained from other investigators. Where other written sources have been cited, then:

a. Their arguments have been re-written but the general information credited to them has been referenced.

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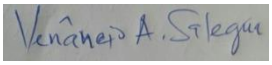
Signature  Date: 6th March 2019

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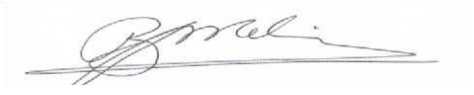
As the candidate's supervisor I agree to the submission of this dissertation

Signature  Date: 6th March 2019

Prof Rob Melis (Supervisor)

Signature  Date: 6th March 2019

Dr Deidré Fourie (Co-Supervisor)

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DEDICATION

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ABBREVIATION

[d]	Additive effects
[h]	Dominance effects
[i]	Additive x additive effects
[j]	Additive x dominance effects
[l]	Dominance x dominance effects
[m]	Mean of the generation
ADP	Andean Diversity Panel
AEC	Axis environment coordinate
AFLP	Amplified Fragment Length Polymorphism
AMMI	Additive Main Effect and Multiplicative Interaction
ANOVA	Analysis of Variance
ARC	Agricultural Research Council
ARC-GCI	Agricultural Research Council-Grain Crops Institute
ARC-GCI-BP	Agricultural Research Council-Grain Crop Institute Breeding Program
ASV	AMMI Stability Value
BBS	Bacterial brown spot
BCP1	Backcross with parent one
BCP2	Backcross with parent two
CIAT	Centre for International Tropical Agriculture
CV	Coefficient of variation
DF	Degrees of freedom
DF	Days to flowering
DM	Days to maturity
DNA	Deoxyribonucleic acid
DRK	Dark red kidney
F1	F1 generation
F2	F2 generation
G	Gram
G	Genotype
GEI	Genotype by environment interaction
GGE	Genotype main effects and genotype by environment interaction
H_b	Broad sense heritability
h_n	Narrow sense heritability
IMCDA	Improved Masters in Cultivar Development for Africa

IPCA	Interaction principal components axis
KASP	Kompetitive allele specific PCR
LSD	Least significance difference
MAS	Marker assisted selection
Masl	Metres above sea level
MS	Mean sum of squares
N	North
P1	Parent one
P2	Parent two
PABRA	Pan-African Bean Research Alliance
PCA	Principal component analysis
PCR	Polymerase chain reaction
Pr	Probability
Pss	Pseudomonas syringae ps syringae
QTL	Quantitative trait loci
R	Correlation coefficient
RAPD	Random amplified polymorphic DNA
RAUDPC	Relative area under disease progress curve
RS7	Red speckled sugar beans
S	South
SD	Standard deviation
SE	Standard error
SNP	Single Nucleotide Polymorphism
SS	Sum of squares
SSA	Sub Saharan Africa
t ha^{-1}	Tons per hectare
Ton	Ton
UKZN	University of KwaZulu-Natal
USA	United State of America
VA	Additive variance
VAD	Interaction between additive and dominance variance
VBCP1	Variance of backcross with parent one
VBCP2	Variance of backcross with parent two
VD	Dominance variance
VE	Environmental variance
VF1	Variance of F1 generation

VF2	Variance of F2 generation
VG	Genetic variance
VP	Phenotypic variance

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Common bean or dry bean (*Phaseolus vulgaris L.*) , (2n = 2x = 22, belonging to subtribe Phaseolinae, tribe Phaseoleae, family Fabaceae) is the third most important grain legume worldwide, surpassed only by soybean and groundnut and the main source of protein and natural fibre (FAO, 2014; Cichy *et al.*, 2015). It is grown on all continents in tropical, semi-tropical and Mediterranean climates, notable between 52 ^{o}N and 32 ^{o}S up to the altitude of 3000 m (Kimani *et al.*, 2005). Among the 70 occurring *Phaseoulus* genus species, only five has been domesticated, namely, dry bean (*P. vulgaris L*.), yardlong bean (*P. dumosus Macfad.),* runner bean (*P. coccineus L*.), tepary bean (*P. acutifolius A Gray*) and lima bean (*P. lunatus L*.) (Singh and Singh, 2015). Brazil is the biggest dry bean producer worldwide, followed by Europe and Africa (Katungi *et al.*, 2009). The average grain yield in South Africa is 1.40 t ha^{-1} (Dlamini *et al.*, 2017). This average grain yield is low when compared with North America (~ 3.00 t ha^{-1}) (Kimani *et al.*, 2005; FAO, 2014). The production in Africa is concentrated in East and Southern Africa and Kenya is Africa's leading producer with 412 382 tons from 910 478 ha (Katungi *et al.*, 2009). In South Africa, the dry bean is a main food crop for small and commercial farmers with the greatest production in the Free State (43%), Mpumalanga (24%), Limpopo (10%) provinces (Muedi, 2015). The mean annual dry bean production in South Africa is about 56 thousand tons from 48 thousand ha (Muedi, 2015). The dry bean annual demand for South Africa is about 100,000 tons and the country needs to import in order to to eliminate the deficit (Cichy *et al.*, 2015). The red specked sugar bean is the main seed type with a 75% market share, while the small white canning bean accounts for 20% of the (Muedi, 2015).

1.2 Importance of dry bean

In Sub-Saharan Africa, dry bean is an important source of protein , containing between 10-25% protein (Muedi, 2015). Due to its high protein content it is often called "*the meat for the poor*" as it can replace meat protein (Kimani *et al.*, 2005). The leaves, immature fresh pods, fresh seeds and dry grains are also eaten (Singh and Miklas, 2015). The dry bean is a major source of income for emerging and commercial farmers in South Africa (Dlamini *et al.*, 2017). Dry bean can increase the soil fertility through fixing atmospheric nitrogen in the soil, and so reduces the amount of inorganic fertilizers to be applied (Farid and Navabi, 2015). Dry bean

contributes to the treatment and prevention of diabetes, low blood, heart, and obesity (Singh and Miklas, 2015).

1.3 Production constraints

The average dry bean yield in South Africa is 1.40 t ha^{-1} (Dlamini *et al.*, 2017). The low yields have been attributed to several biotic and abiotic factors such as unreliable rainfall, high temperatures, poor soil fertility, and pest and diseases (Jung *et al.*, 2003; Navarro *et al.*, 2007; Fao, 2012). Among the diseases, bacterial brown spot (BBS), caused by a bacterium *Pseudomonas syringae* pv.*syringae (Pss)* is a common disease of dry bean in both smallholder and commercial fields (Muedi *et al.*, 2015). The disease infection is favoured by wet environments and the disease attacks both leaves and pods, causing necrotic spots, thereby reducing the photo-synthetically active area and loss in seed quality. Management strategies to control BBS disease have been identified as crop rotation (cultural control method), the use of preventive copper-based bactericides and antibiotics (chemical control), and resistant cultivars. The use of resistant cultivars, however, reduces the need for either rotation and or high priced bactericides, the latter often beyond the reach of smallholder farmers. Therefore, the most reliable, cost-effective and sustainable method to manage the disease is the use of resistant cultivars (Singh and Miklas, 2015). The BBS symptoms emerge as water-soaked spots, which enlarge and dry up, and are surrounded by a narrow yellow or light green zone. Figure 1.1 shows the symptoms of BBS in greenhouse at Potchefstroom.

Figure 1.1 Symptoms of bacterial brown spots (BBS) on dry bean leaves

1.4 Problem and justification

The BBS disease is a major disease in South Africa resulting in yield losses and poor and seed quality. The local commercial cultivars have no resistance to BBS disease. Therefore, there is a need to identify sources of resistance to BBS, study the inheritance of BBS resistance and to develop locally adapted, high yielding and BBS resistant cultivars.

1.5 Research objectives

The general objective of the research aimed to breed dry bean for resistance to bacterial brown spot disease in South Africa, through identifying high yielding and stable cultivars with resistance to the BBS disease. The specific objectives for the research or study are as follows:

- To screen 423 Andean Diversity Panel (ADP) dry bean lines for resistance to BBS disease, under field conditions in South Africa.
- To evaluate the grain yield, stability and BBS disease of fourteen Dark Red Kidney (DRK) dry bean lines across six environments.
- To estimate the heritability and gene effects controlling BBS disease resistance in dry bean.

1.6 Research hypothesis

i. There are BBS disease resistant dry bean lines among 423 Andean genotypes screened under field condition in South Africa

ii. The grain yield performance, stability and BBS disease of the dry bean line are affected by genotype x environment interaction

iii. The gene effects controlling BBS disease resistance and heritability in dry bean can be estimated.

1.7 Dissertation outline

The University of KwaZulu-Natal dissertation has adopted the format of Crop Science Journal. Each chapter is follows the layout of a standard research paper. The system of

referencing is based on “Crop Science Journal”. The framework of the research study is as follows.

Chapter 1: General introduction
Chapter 2: Literature review
Chapter 3: Screening Andean Diversity Panel (ADP) dry bean lines for resistance to bacterial brown spot disease, under field conditions in South Africa.
Chapter 4: Grain yield, stability and bacterial brown spot disease resistance of Dark Red Kidney (DRK) dry bean lines across six environments in South Africa
Chapter 5: Heritability and gene effects controlling the bacterial brown spot disease resistance in a dry bean cross.
Chapter 6: General overview of the study (research results, findings and ways forwards).

1.8 Reference

Buruchara, R., R. Chirwa, L. Sperling, C. Mukankusi, J. C. Rubyogo, R. Mutonhi et al. 2011. Development and delivery of bean varieties in Africa: the Pan-Africa Bean Research Alliance (PABRA) model. African Crop Science Journal 19: 227-245.

Cichy, K. A., T. G. Porch, J. S. Beaver, P. Cregan, D. Fourie, R. P. Glahn et al. 2015. A Phaseolus vulgaris diversity panel for Andean bean improvement. Crop Science 55: 2149-2160.

Dlamini, T., L. Nalley, F. Tsiboe, A. Barkley and A. Shew. 2017. The economic impact of the South African Agricultural Research Council's dry beans breeding program. Journal of Agricultural and Applied Economics 49: 232-250.

FAO, I. 2014. WFP. The State of Food Insecurity in the World 2014 strengthening the enabling environment for food security and nutrition. Rome. Italy.

Fao, W. 2012. IFAD. 2012. The state of food insecurity in the world: Forum for Development Studies 43: 1-63.

Farid, M. and A. Navabi. 2015. N2 fixation ability of different dry bean genotypes. Canadian Journal of Plant Science 95: 1243-1257.

Jung, G., H. Ariyarathne, D. Coyne and J. Nienhuis. 2003. Mapping QTL for bacterial brown spot resistance under natural infection in field and seedling stem inoculation in growth chamber in common bean. Crop Science 43: 350-357.

Katungi, E., A. Farrow, J. Chianu, L. Sperling and S. Beebe. 2009. Common bean in Eastern and Southern Africa: a situation and outlook analysis. International Centre for Tropical Agriculture 61: 12-17

Kimani, P., R. Buruchara, K. Ampofo, M. Pyndji, R. Chirwa and R. Kirkby. 2005. Breeding beans for smallholder farmers in Eastern, Central, and Southern Africa: Constraints, achievements, and potential. PABRA Millennium Workshop: p. 11.

Muedi, H. T., D. Fourie and N. W. McLaren. 2015. Distribution and severity of bacterial brown spot on dry beans in South Africa: an update. South African Journal of Science 111: 1-6.

Muedi, H. T. H. 2015. Reaction of selected common bean germplasm accessions to bacterial brown spot in South Africa. Crop Protection 77: 87-93

Navarro, F., P. Skroch, G. Jung and J. Nienhuis. 2007. Quantitative trait loci associated with bacterial brown spot in loci. Crop Science 47: 1344-1353.

Serfontein, J. 1994. Occurrence of bacterial brown spot of dry beans in the Transvaal province of South Africa. Plant Pathology 43: 597-599.

Singh, B. and B. Singh. 2015. Breeding perspectives of snap bean (*Phaseolus vulgaris L.*). Vegetable Science 42: 1-17.

Singh, S. P. and P. N. Miklas. 2015. Breeding common bean for resistance to common blight: A review. Crop Science 55: 971-984.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The literature review discusses the origin and domestication, taxonomy, botany and genetic diversity of dry bean (*Phaseolus vulgaris .L*). The epidemiology, mechanism of inheritance, sources of resistance and methods for resistance screening for bacterial brown spot (BBS) are also reported. The SNP KASP marker platform for dry bean genetic genotyping and for identification of the genes of resistance to BBS is discussed. Furthermore, the path analysis, correlation analysis, genetic effects (additive, dominant and no-allelic interactions) and different methods used to estimate heritability are reviewed. The effect of GEI for broad and specific adaptation, and the methods for identification of cultivars with high mean performance and relatively stable yields, has been described.

2.2 Origin and domestication of dry bean

Dry bean (*Phaseolus vulgaris .L*) originated from Central and Southern America (Bellucci *et al.*, 2014). The wild dry bean had diverged into the following two main gene pools, the Mesoamerican (Central of America) and the Andean (South America) (Singh and Miklas, 2015). Furthermore, within these gene pools there are six races, namely three Mesoamerican (Mesoamerica, Durango, and Jalisco) and three Andean races (Peru, Nueva Granada, and Chile), which can be distinguished by morphological and biochemical characteristics (Blair *et al.*, 2006). The dry bean has been domesticated over time from a wild relative of dry bean through continuous selection, hybridization, backcrossing, natural or artificial mutation and migration (Bellucci *et al.*, 2014). The features such as growth habit, seed size, seed retention and maturity distinguish the modern cultivated dry bean from their ancestral wild form (Bellucci *et al.*, 2014). The Andean beans are commonly grown in Africa, Europe and North Eastern United States, while Mesoamerican beans are mainly grown in South America (Bellucci *et al.*, 2014). The domestication history of the dry bean is well known and its wild progenitor has been identified (Bellucci *et al.*, 2014). However, the wild relative and cultivated descendants display contrasting differences for many traits and generally give viable and fertile progeny (Broughton *et al.*, 2003). There are several important attributes that are lost in the domestication in dry bean, such as the loss of seed dispersal ability and seed dormancy, which is crucial for adaptation to a cultivated environment (Broughton *et al.*, 2003). The ancestral relatives are conditioned by the presence of fibres in the pods, both in the sutures (‘string’)

and the walls, however, loss of these fibres leads to indehiscence of the pods and lack of seed dispersal at maturity (Broughton *et al.*, 2003; Bellucci *et al.*, 2014). Each domesticated species constitutes a primary gene pool with its wild ancestral forms, and secondary and tertiary gene pools that may exist, depending on the phylogenetic events that lead to the formation of the biological species (Singh and Miklas, 2015). Different species of Phaseolus have been maintained in more than 245 gene banks of various countries (Cichy *et al.*, 2015). CIAT Colombia has the mandate for global germplasm collection and conservation of Phaseolus species and hosts the world's largest and most diverse collections (Singh and Miklas, 2015).

2.3 Taxonomy of dry bean

Dry beans belong to the family Fabaceae (Leguminosae), order Fabales, sub-family Papilionoideae and genus Phaseolus (Singh and Singh, 2015; Rodriguez *et al.*, 2016). The genus Phaseolus comprises of about 70 species including five domesticated species (Table 2.1), namely: dry bean (*P. vulgaris L*.), yearlong bean (*P. dumosus Macfad.),* runner bean (*P. coccineus L*.), tepary bean (*P. acutifolius A Gray*) and lima bean (*P. lunatus L*.) (Bellucci *et al.*, 2014; Singh and Singh, 2015). Amongst the five domesticated species, *Phaseolus vulgaris* is the most important economically and accounts for more than 90% of the cultivated Phaseolus worldwide (Singh and Singh, 2015). Dry bean is a true autogamous diploid species with 22 chromosomes (2n=2x=22) and with a haploid genome (Singh and Singh, 2015). (Singh and Singh, 2015). Dry bean is self-pollinating crop with stigma and anthers in the same flower (Cichy *et al.*, 2015).

Table 2.1 Cultivated species of genus Phaseolus and their preferred agro-ecological conditions

Phaseolus species	Common name	Altitude (m)	Temp (°C)	Rainfall	Cycle (days)
P. vulgaris L	Common bean, dry bean, shell bean, snap bean, French bean	50-3000	14-26	400-1600	70-330
P. polyanthus Gre Enman	Yearlong bean	800-2600	14-24	1000-2600	110-365
P. coccineus L	Runner or scarlet runner bean	1400-2800	13-22	400-2600	90-365
P. acutifolius A. Gray	Tepary bean	50-1900	20-32	200-400	60-110
P. lunatus L	Lima bean	50-2800	16-26	0-2800	90-365

Source: Singh and Singh (2015).

2.4 Morphology of dry bean

Dry beans have a shallow primary root system, which does not go beyond 20 cm (Debouck, 1991). The roots can form nodules, which are distributed on the lateral roots of the upper and middle parts of the root system (Graham and Ranalli, 1997). The nodules are usually 2 to 5 mm in diameter and are colonized by Rhizobium bacteria, which fix atmospheric nitrogen (Graham and Ranalli, 1997). The plant can be either erect, semi-prostrate or prostrate, but tends to grow vertically, either when the bean is growing alone or with support (Graham and Ranalli, 1997). The primary leaves of dry bean are unifoliate and secondary leaves are trifoliate, and these are inserted at the nodes of the stem and branches (Debouck, 1991). Flower initiation is within 28-42 days from the day of planting, even though it may take longer than this in the case of climbing beans grown at higher altitude, which flower after 55 days (Debouck, 1991). The dry bean is a self-pollinated crop, and each flower have ten stamen and a single stigma, and flowers have different colours, such as white, pink and purple (Graham and Ranalli, 1997). Each pod can have 3 to 10 seeds with various shapes and colours, with seed size ranging from 50 mg per seed in wild related species to 200 mg per seed in large seeded cultivars (Graham and Ranalli, 1997).

2.4.1 Dry bean growth habit

Most of dry bean cultivars and landraces grown in the highlands of Mexico, Central America and the Andes have indeterminate growth habits and are photoperiod sensitive (Singh and Singh, 2015). However, photoperiod insensitive genotypes with bush growth habit have evolved during the course of domestication and dissemination that allowed its spread into non-traditional areas (Singh and Singh, 2015). Broadly, dry bean stems can be bush type or pole types, depending on the growth and twining habits (Kwak *et al.*, 2012). Three genes govern the stem growth habit. Long stems is dominant over short stem, an indeterminate growth habit is dominant over the determinate growth habit, and the twining tendency is dominant over the non-twining tendency (Singh and Singh, 2015). In the bush cultivars, the stem growth ceases when an inflorescence emerges and these dry bean plants have few nodes and short internodes, whereas in the indeterminate type, the stem remains vegetative and continues to develop, forming more nodes and internodes even during the reproductive phase (Kwak *et al.*, 2012; Singh and Miklas, 2015). Dry bean genotypes are classified into four growth habits, namely, I, II, III and IV (Singh and Miklas, 2015). The type I has a determinate growth habit whereby the growth of the stem stops once the inflorescence has developed. The plants are usually short with few branches (Kwak *et al.*, 2012). The type II is indeterminate and has an erect growth habit has an erect stem with more nodes and internodes than type I, and continues to grow during flowering. The type III plants have an indeterminate growing habit with branches relatively weak and open, semi-prostrate and a pod load largely concentrated in the basal part of the plant and they posses a weak climbing ability. The type IV beans have an indeterminate growth habit with stem and branches very well and excessively long, possessing strong climbing ability and plants have a climbing growth habit and need to be supported (Singh and Miklas, 2015).

2.5 Genetic diversity of dry bean

Genetic resources of Phaseolus species exist as a complex order of major and minor gene pools, races and intermediate types, with occasional crossing between wild relatives and domesticated species (Singh and Singh, 2015). During the domestication of the two dry bean gene pools, intensive selection and dispersal have resulted in a large genetic diversity of the crop species (Cabral *et al.*, 2011; Singh and Singh, 2015). There is greater genetic diversity in the Mesoamerican gene pool as compared to the Andean gene pool (Cichy *et al.*, 2015). Furthermore, in Africa, approximately half of the beans produced are from the Andean gene pool (Cichy *et al.*, 2015). In East and Southern Africa, Andean beans are preferred and an estimated 73 and 83% of beans are of Andean origin, respectively (Cichy *et al.*, 2015). The

higher diversity found in the Mesoamerican compared with the Andean gene pool has been confirmed in studies using molecular markers (Cabral *et al.*, 2011; Bellucci *et al.*, 2014). Blair *et al.* (2006) used SSR markers to detect genetic diversity within a representative set of 43 dry bean cultivars and wild accession (both gene pools), and it was observed that the microsatellites were useful for distinguishing genotypes from the two gene pools, for distinguishing between the races within each gene pool, and for separating wild accessions from cultivars. SSR molecular markers were also used to evaluate 604 accessions from the CIAT germplasm collection (primary and secondary centres of diversity), and it was shown that dry beans have a very significant population structure (Blair *et al.*, 2009). Random amplified polymorphic DNA marker (RAPD) has been used to analyse a collection of landraces of dry bean and to determine the genetic structure of the Middle American gene pool of cultivated beans, and it was demonstrated that the Mesoamerican germplasm of dry bean has more genetic diversity than the Andean gene pool (Beebe *et al.*, 2000). Mavromatis *et al.* (2010) also used RAPD markers to study genetic diversity of the morphological, agronomical and physicochemical traits along with molecular data analysis, and it was registered that genetic similarity estimated from molecular analysis with RAPDs seemed not to be related with the seed morphological characteristics and agronomic performance. Rosales-Serna *et al.* (2005) used an amplified fragment length polymorphism (AFLP) marker to examine the genetic relationships between Andean races based on the genotyping of 112 cultivars developed in Mexico. They observed that utilization of contrasting parents for specific crosses contributes to a broadening of the genetic bases of dry bean. Furthermore, 4935 SNP markers were used by Cichy *et al.* (2015) to analyse genetic diversity of 396 ADP dry bean lines and the average diversity estimates were determined for germplasm subsets of interest. Genetic diversity studies were also used to identify genetic variation of 347 accessions of dry bean on the basis of 100 seed mass in three categories; large (>40 g per 100 seeds), medium (25-40 g per 100 seeds), and small (<25 g per 100 seeds) (Cichy *et al.*, 2015). The large dry bean seeded accessions belonged to the Andean gene pool, while the medium and the small dry bean seeded accessions belonged to the Mesoamerican gene pool (Cichy *et al.*, 2015).

2.6 Dry bean production and consumption

Dry bean is an important source of protein, vitamins, minerals, fibre and calories in tropical and subtropical countries (Latin America, East and Southern Africa) (Singh and Miklas, 2015). This crop provides a cheap source of protein and higher prices compared to cereals and is a cash crop for many farmers (FAO, 2014). The dry bean 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. It is cultivated in

monoculture, intercropping and rotations (Broughton *et al.*, 2003). The production is largely concentrated in Latin America, Eastern and Southern Africa (Broughton *et al.*, 2003). The total area under production worldwide is about 28 million ha, producing about 20 million ton with average yield among 0.49 to 0.73 t ha^{-1} (Muedi, 2015). Brazil is the world's largest dry bean producer and Kenya is leading the production in Africa (412 381 tons from 910 478 ha) (Muedi, 2015). The average dry bean yields in Africa are below half a ton per hectare compared to those obtained in South Africa with 1.40 t ha^{-1} mean yield and ranges between 0.9 to 2.90 t ha^{-1} for small scale farmers (Dlamini *et al.*, 2017). The red speckled sugar beans are produced by emerging and commercial farmers in Mpumalanga, Free State, KwaZulu-Natal, Limpopo and Gauteng provinces (Muedi, 2015). The average production between 2011 to 2014 was about 50 000 ton and the consumption was 100 000 ton, which implies that South Africa is a net dry bean importer (Muedi, 2015). Dry bean is considered a 'poor man's meat' and plays a particularly important role in the diet of the poor (Singh and Miklas, 2015). In Eastern and Southern Africa, the dry bean consumption is higher than in Latin America reaching up to 66 kg per person in some rural areas of Kenya, whereas, in Rwanda and Burundi, the average of consumption exceeds 40 kg per person per year (Singh and Singh, 2015).

2.6.1 Dry bean yield constraints

The high yields losses in dry bean have been attributed to several biotic (diseases and pests) and abiotic factors (drought, heat and low soil fertility) (FAO, 2014). The main production constraints are diseases (fungal and bacterial) and pests (Broughton *et al.*, 2003). (Bellucci *et al.*, 2014). Important diseases include angular leaf spot (ALS), anthracnose, ashy stem blight, bean golden yellow mosaic virus (BGYMV), bacterial brown spot (BBS), common bacterial blight (CBB), fusarium root rot (FRR), halo blight (HB), root rots, rust, web blight and white mold (Miklas *et al.*, 2006; Singh and Schwartz, 2010). Pests of economic importance include bean pod weevil, bruchids, thrips, stem maggot and aphid (Miklas *et al.*, 2006). The agronomic constraints are late planting, poor weed management, continuous cropping and use of unimproved seed (Broughton *et al.*, 2003). (Muedi *et al.*, 2015). The climatic factors such as rainfall amount and distribution, temperature, and incident radiation have significant influence on dry bean yields (Cichy *et al.*, 2015).

2.6.2 Agronomic and cultural practices

Each dry bean row was 5 m long and is planted with a 76 cm and 7.5 cm of inter-row and intra-row, respectively (Bellucci *et al.*, 2014). The cultural practices varied somewhat across

production locations, and each bean trial is produced under normal farming practices for a given region (Dlamini *et al.*, 2017). The nitrogen, phosphorus, and potassium fertilizers are applied to the fields at an average with rate of 42.30 kg ha^{-1}, 22.30 kg ha^{-1} and 18.40 kg ha^{-1}, respectively (Dlamini *et al.*, 2017). The experiment were weeded and harvested mechanically or manually (Muedi, 2015)..

2.7 Bacterial brown spot disease

Bacterial brown spot (BBS) disease, caused by *Pseudomonas syringae pv syringae* (*Pss*) is an important disease of dry bean (*Phaseolus vulgaris* L.) worldwide (Harveson *et al.*, 2015). *Pseudomonas syringae pv syringae* is a common epiphytic bacterium that colonizes many species (Hirano and Upper, 2002). This disease is of economic importance due to its epidemiology and yield losses (Bastas and Sahin, 2017). The disease has been widely reported in the USA, Brazil and Canada. Presently, BBS is prevalent in regions such as Algeria, Asia, Australia, Egypt, Europe, Ethiopia, Kenya, Lesotho, Malawi, Mauritius, Morocco, New Zealand, Tanzania, Tunisia, Uganda and Zimbabwe and South Africa (Singh and Schwartz, 2010). Bacterial brown spot has been reported to cause yield and economic losses of more than 20 % in dry bean production worldwide (Bastas and Sahin, 2017).

2.7.1 Epidemiology of bacterial brown spot

Humid conditions and temperatures between 28 to 32°C favour bacterial brown spot disease epidemics. Seed transmission is a significant primary source of inoculum to start an epidemic (Navarro *et al.*, 2007; Harveson *et al.*, 2015). Sources of inoculum include infected seed, crop debris, infected soils and infected weed hosts (Muedi *et al.*, 2015). The wind and rain are the most important modes of dissemination of *Pss* (Kimani *et al.*, 2005). The BBS pathogen can enter bean plants through openings such as stomata in leaves, leaf margins, and wounds of plants that are created by wind-blown soil particles, leaf insects or humans (Muedi *et al.*, 2015). The pathogen is readily transmitted mechanically, especially when the plants are wet (Navarro *et al.*, 2007). Symptoms caused by BBS emerge as water-soaked spots, which enlarge and dry up, and are surrounded by a narrow yellow or light green zone (Koutsika-Sotiriou and Traka-Mavrona, 2008). When the lesion matures, it typically develops brown spots and dead tissue in the centre may fall out, producing a shot-hole appearance (Bastas and Sahin, 2017). On infected pods water-soaked spots can also develop as round and initially water-soaked spots, later becoming darker green, depressed, brown and necrotic (Muedi *et al.*, 2015). Infected seeds initially have water-soaked spots which later become brown and corrugated (Koutsika-Sotiriou and Traka-Mavrona, 2008). Stem lesions emerge when the

disease becomes systemic (Muedi *et al.*, 2015). Methods for controlling BBS include pathogen-free seed (produced in arid environments and tested free of *Pss*), crop rotation (a minimum rotation of two years), deep ploughing, removal of crop debris, the use of disease resistant cultivars and control of weed hosts, especially hairy vetch (Duncan *et al.*, 2014; Muedi *et al.*, 2015; Bastas and Sahin, 2017). Chemical seed treatments and foliar sprays have provided erratic control of the disease (Bastas and Sahin, 2017). Therefore, the use of resistance cultivars and disease free certified seed are important interventions in reducing the spread of disease (Navarro *et al.*, 2007).

2.7.2 Genetic variability of the bacterial brown spot pathogen

Little research on the genetic variability of the BBS pathogen has been conducted in different dry bean growing regions for the development of BBS resistance (Navarro *et al.*, 2007). Fifteen isolates of the BBS pathogen have so far been used, and the pathogen is capable of causing disease on more than 200 different plants species (Scortichini *et al.*, 2003). Different techniques have been used to study diversity of the *Pseudomonas syringae pv. syringae (Pss)* strains (Young, 2010), including biochemical, physiological, and pathogenicity methods. Recently, several studies on the 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) (Scortichini *et al.*, 2003). The rep- PCR technique is useful in studying genetic diversity of bacterial pathogens due to its ability in fingerprinting gram-negative bacteria (Young, 2010).

2.7.3 The influence of the host on pathogen

There are two main outcomes expected when a pathogen infects a host plant; the host plant can be susceptible or resistant (Urrea and Harveson, 2014). A susceptible host plant is unable to recognize the pathogen or to offer a desired protection mechanism that could restrict the development and spread of the pathogen, whereas a resistant host plant has the ability to hinder the growth, development and spread of the pathogen (Parlevliet, 2002; Urrea and Harveson, 2014). There are two types of mechanisms to restrict disease development in a plant, namely physical barriers and chemical barriers (Agrios, 2005b). The host plant with physical barriers does not allow the pathogen to penetrate the plant through the thick cuticle layer, the size and location of stomata and through others organelles (Vanderplank, 2012; Urrea and Harveson, 2014). A host plant with chemical barriers releases chemical compounds that can inhibit pathogen development, such as phenols and tannins, which offer

a good chemical protection mechanism in plants (Agrios, 2005a). There are two types of host plant resistances, namely non-host resistance and host resistance (Parlevliet, 2002). The non-host resistance is a form of resistance where plants are not considered host of the pathogen (Agrios, 2005a; Urrea and Harveson, 2014). The host plant resistance has been called true resistance, which is genetically controlled through incompatibility between host plant and the pathogen (Agrios, 2005a). True resistance can be horizontal resistance or vertical resistance (Parlevliet, 2002). Horizontal resistance is the form of resistance that is non-race specific, quantitative and controlled by many genes, and is also called polygenic resistance (Jung *et al.*, 2003; Vanderplank, 2012). Resistance to BBS is an example of a polygenic resistance since it is controlled by more than one gene (Jung *et al.*, 2003). In horizontal resistance, a single gene cannot play a role in resistance alone, but in combination with other genes (Vanderplank, 2012). Horizontal resistance does not protect plants from being infected, but slows the development of the disease and slows the spread of the disease in the field (Vanderplank, 2012). Horizontal resistance is generally more durable and difficult to overcome (Agrios, 2005b; Vanderplank, 2012). The vertical resistance or monogenic resistance is a race-specific form of resistance usually controlled very few genes, between one to three genes (Vanderplank, 2012). In monogenic resistance, the host plant can be resistant to some races of the pathogen and susceptible to other races of the same pathogen (Parlevliet, 2002; Urrea and Harveson, 2014). The vertical resistance is characterized by incompatibility between the host plant and a pathogen race (Vanderplank, 2012). When the host plant has been attacked, it responds with a hypersensitive reaction (Parlevliet, 2002). This reaction results in a rapid localized death of host plant cells and tissue as response to infection (Parlevliet, 2002). The vertical resistance is easy to overcome due to mutations of the pathogen or the arrival of a new strain from elsewhere (Agrios, 2005b; Vanderplank, 2012).

2.7.4 Sources of resistance to bacterial brown spot

The availability of good sources of resistance is the essential requirement for a successful resistance breeding programme (Singh and Schwartz, 2010). The sources of resistance can include genotypes from primary, secondary and tertiary gene pools (Singh and Miklas, 2015). The primary gene pool often possesses a low level of resistance, while genotypes from the secondary and tertiary gene pools possess an intermediate or high level of resistance, respectively. Several BBS resistant dry and green bean breeding lines have been developed more than 30 years ago, however, there are no recent reports of active public breeding efforts for resistance to BBS in dry bean (Singh and Schwartz, 2010). The green bean ‘Hystyle’ was

the first cultivar with useful field resistance; hence, resistance from Hystyle was transferred to cultivars such as 'Hercules' and 'Titan' (Singh and Schwartz, 2010). Resistance from small-seeded dry beans such as A 55 and Puebla 152, and green beans such as Hystyle, and *P. coccineus* are important sources of resistance that can be used to develop BBS resistant locally adapted dry bean cultivars (Navarro *et al.*, 2007).

2.7.5 Mode of inheritance of BBS resistance

Several genetic studies have established that the inheritance to BBS resistance is quantitatively inherited and the mode of gene action is mainly through additive, often dominant and epistasis effect (Miklas *et al.*, 2006). Singh and Schwartz (2010) reported that multiple recessive genes control resistance to BBS in dry bean and the heritability estimate for field reaction was lower than that for the greenhouse evaluation, using the stem inoculation method. The most relevant inheritance study of BBS resistance was done in a field experiment using the *Pseudomonas siringae pv siringae (Pss)* seedling stem inoculation method in a Belneb RR-1 × A55 RIL segregating dry bean population (Navarro *et al.*, 2007). The authors identified genomic regions located in several linkage groups associated with BBS resistance.

2.7.6 Marker assisted selection

Genotypic screening using marker-assisted breeding (MAS) has advantages over classical breeding (Cichy *et al.*, 2015). MAS has the ability to screen for resistance at the seedling stage, identify resistance genes even when there are disease escapes. It has a high efficiency in screening for environment dependent traits with a few plants, and several generation per year can be tested (He *et al.*, 2014). MAS also saves time, resources, space and money, and makes the breeding effort more efficient, effective, reliable and cost-effective compared to the more conventional plant breeding (He *et al.*, 2014). Selection can also be done in the absence of a reliable inoculation and scoring methods for a disease (Jung *et al.*, 2003). Jung et al. (2003) studied quantitative trait loci (QTLs) related to BBS resistance through stem inoculation of a recombinant inbred line population derived from the cross between susceptible line (Belneb RR-1) and resistant line (A 55) and found the QTL RAPD marker O10.650 significantly associated with variation in *Pss* severity. Confirming the efficacy of markers prior to their use in a breeding programme is of importance (Fourie, 2002).

2.7.7 Screening for resistance to bacterial spot in the field

In order to achieve an optimum inoculation a highly concentrated inoculum of between 10 million to 100 million cells per millimetre from an aggressive isolate is required. Isolates from infected leaves can be cultured on a yeast-extra-dextrose-calcium-carbonate nutrient agar at 27^{o}C for two to three days (Muedi, 2015). The BBS field screening consists of inoculation with isolates of Pss (1x10^{8} cfu/ml) using a mistblower at 21, 28 and 36 days after planting, whereby the plants are rated seven days after the first inoculation and repeated weekly for three consecutive weeks. A one (immune) to nine (susceptible) CIAT scale is generally used (Muedi, 2015). The field screening with or without inoculation is required to identify resistance reactions that may have been missed in greenhouse studies (Singh and Schwartz, 2010).

2.8 Path and correlation analysis

Path analysis measures the direct and indirect effects of one variable upon another and permits the separation of the correlation coefficient into components of direct and indirect effect (Ramteke *et al.*, 2010). The correlation analysis provides information about the degree of relationship between important plant traits and is a good index to predict the yield response in relation to the change of a particular character (Ramteke *et al.*, 2010). To determine the inter-relationships among grain yield components, a better understanding of both the direct and indirect effects of the specific components needs to be attained (Chaudhary and Joshi, 2005). The correlation, although very useful in quantifying the size and direction of trait associations, can be misleading if the high correlation between two traits is a consequence of the indirect effect of other traits (Ramteke *et al.*, 2010). Each correlation coefficient between a predictor variable and the response variable can be partitioned into direct and indirect effects, which involves the product of a correlation coefficient between two predictor variables with the appropriate path coefficient in the path diagram (Dawo *et al.*, 2007). Path analysis and correlations have been estimated for different yield characters in dry bean which revealed what selection method was effective for a population with broad genetic variability and those with a high narrow sense heritability (Dawo *et al.*, 2007).

2.9 Generation mean analysis

The reliable choice of the mating design and good parent selections are important factors in a successful breeding (Dabholkar, 1999). Mating designs are used in generating genetic information on the mode of gene action. These included general combining ability (additive on

top of good level of dominance and epistasis effects) and specific combining ability (dominance on top of good level of additive and epistasis effects), associated with the trait and determines the genetic gain in breeding. The bi-parental mating design, which simply involves mating of two parents selected from large population, is the simplest design (Akhshi *et al.*, 2014). The generation mean analysis (GMA) is used to estimate the type of gene action associated with the inheritance of the trait by establishing the relationship between generations (Hayman and Mather, 1955). The GMA is used to explain the additive and dominance model and digenic or non-allelic interaction (epistasis) (Kearsey and Pooni, 1998). The GMA is a useful technique in plant breeding for estimating gene interaction effects such as mean [m], additive [d] and dominance [h] and the digenic or non-allelic interactions additive x additive [i], additive x dominance [j] and dominance x dominance [l], responsible for inheritance of quantitative traits (Dabholkar, 1999). If the variation within families of linear regression ANOVA is significant, hence there are parameters apart from mean and additive effects, then these parameters have to be included in the analysis through multiple linear regression (Kearsey and Pooni, 1998). However, the ratio of 20:50:30 are recommended for non-segregating (P1, P2 and F1), F2 and BCF1s, respectively (Mather and Jinks, 2013). Thus, the means are adjusted in the regression analysis by weight according the sample size (Kearsey and Pooni, 1998). The scaling test has been developed that establishes generation relationships between means and variances (Akhshi *et al.*, 2014). The scale was limited to six generations only and used for the six basic generation (Kearsey and Pooni, 1998). Though the scaling test is limited to six generations, it accounts for additive, dominance and epistasis gene actions (Hayman, 1958). The joint scaling test was developed to address the weakness and is not limited to a specified number of generations (Kearsey and Pooni, 1998). The digenic non-allelic interaction (epistasis) broadly classified in complementary (the same sign of [h] and [l]) and duplicate (the opposite sign of [h] and [l]), while, the positive [d] indicates gene association and negative [d] reveals gene dispersion (Hayman and Mather, 1955).

2.9.1 Variance components in the generation mean analysis and heritability

The breeder is interested in the amount of heritable variation, and the magnitude and importance of variation (Kearsey and Pooni, 1998). The environment variation is the main source of variation for non-segregating generations (P1, P2 and F1). The variation in the segregating generation (BCP1, BCP2 and F2) is affected not only by environment factors, but also by the genetic effects (additive, dominance), maternal effects and gene interaction (epistasis or non-allelic interaction) (Jatothu *et al.*, 2013). This variation originates from segregation, random assortment and recombination of alleles (Hayman and Mather, 1955).

The heritability can be estimated through the broad and narrow sense heritability. The broad sense heritability is the proportion of phenotypic variation due to genetic factors, while the narrow sense measures the proportion of the variation which is due to the additive effects of genes (Akhshi *et al.*, 2014). Heritability estimates provide an indication of the expected response to selection in a segregating population (Ramteke *et al.*, 2010). The narrow sense heritability is estimated through the ANOVA, regression of covariance between offspring family mean and the mean of their parents (Akhshi *et al.*, 2014). The covariance is used to estimate all the three components of variance (additive variance, dominance variance and environmental variance) (Akhshi *et al.*, 2014). The relationship between offspring family mean and their mid-parental value obtained from the regression involving a full-sibs family gives a slope that is equivalent to narrow sense heritability (Akhshi *et al.*, 2014). The heritability less than 30% is considered low, 30- 60% is moderate and more than 60% is considered high (Robinson *et al.*, 1949).

2.10 Stability of genotype performance

The changes in the relative performance of genotypes across different environments are referred to as genotype by environment interaction (GEI) and are due to changes in the genotype, the environment or both (Mortazavian *et al.*, 2014). There are crossover GEI (genotype rank change) and non-crossover GEI (genotype non rank change)) (Agyeman *et al.*, 2015). The GEI is useful to identify the stability of performance and the crossover GEI suggests that the target environments may be divided into different mega-environments (Maqbool *et al.*, 2015). The methods for evaluating stability have been proposed, reflecting different aspects of GEI are univariate and multivariate methods (Oladosu *et al.*, 2017).

2.11 Genetic stability estimates

The stability parameters are useful in characterizing genotypes by showing their relative performance in various environments (Mortazavian *et al.*, 2014). Univariate models for stability evaluation are; cultivar superiority, static stability, mean ranks, Wricke's ecovalence, difference of pairs ranks and variances of ranks. These are considered stable when the stability coefficient is not significantly different from zero (Oladosu *et al.*, 2017). The others stability methods are regression slope, deviation from the regression, Shukla's stability variance, and Kang's stability statistic (Chipeta *et al.*, 2017); The genotype is considered stable if the regression coefficient (slope) is approximating unity (Yan *et al.*, 2007). Genotypes

with a slope greater than unity, have higher sensitivity to environmental change and have specific adaptation (Yan *et al.*, 2007). However, there is no consensus among breeders as to which methodology is the best for stability analysis (Abuali *et al.*, 2014).

2.12 Multivariate measure of stability

The multivariate approaches are genotype main effect plus the genotype by environment interaction biplot (GGE biplot) and additive main effects and multiplicative interaction (AMMI) ANOVA or biplot (Gauch and Zobel, 1996). The GGE biplot and AMMI are multivariate approaches used to analyse the GEI and these techniques are powerful tools for extracting patterns of interactions (Gauch and Zobel, 1996).

2.12.1 Additive main effect and multiplicative interaction analysis

The AMMI analysis is a very powerful multivariate technique for quantifying GEI and combines the analysis of variance of the genotype and environment main effects with the interaction of principal component analysis (IPCA) of the GEI (Abuali *et al.*, 2014; Oladosu *et al.*, 2017). The AMMI analysis has been shown to be effective because it captures a large portion of the GEI sum of squares, clearly separating main and interaction effects (Abuali *et al.*, 2014). The larger IPCA scores, regardless of signage, indicates specific adaptation, while the lower IPCA scores, regardless of signage, indicates broad adaptation (Dia *et al.*, 2016). The different signage of IPCA scores indicates crossover GEI , and a similar positive and negative signal of two IPCAs indicate positive and negative interactions, respectively (Mortazavian *et al.*, 2014). However, stability is meaningful only when associated with high trait means, whereby an ideal genotype has both high trait mean and relatively stable performance (Abuali *et al.*, 2014). The interaction principal components analysis (IPCAs) and AMMI stability value (ASV) are used for stability evaluation in the AMMI ANOVA (Purchase *et al.*, 2000). The failure of the AMMI model to measure stability, which is essential in quantifying and ranking yield stability of genotypes across environment, resulted in the development of ASV (Purchase *et al.*, 2000). The lower ASV indicates that the genotype has a wide adaptation and a high ASV indicates specific adaptation (Dia *et al.*, 2016). The AMMI biplot analysis is used to show the performance of genotypes across environment for broad and specific adaptation (Oladosu *et al.*, 2017). All genotypes close to the centre of the AMMI biplot have revealed general adaptation across the testing environments and genotypes far apart have specific adaptation over the environment (Abuali *et al.*, 2014).

2.12.1 Genotype main effect plus genotype by environment interaction biplot

This model is used when the environments are the main source of variation in relation the genotypes and the GEI (Oladosu *et al.*, 2017). This technique allows the detection of GEI in terms of the crossover effect resulting from great changes in the ranking of the genotypes across the environments (Yan *et al.*, 2007). The first interaction principal component analysis (IPCA1) represents responses of the genotypes that are proportional to the environments, which are associated with the GEI without change of the range (Dia *et al.*, 2016). The second interaction of principal component analysis (IPCA2) provides information about cultivation locations that are not proportional to the environments, indicating that those are responsible for the GEI crossover interaction (Yan *et al.*, 2007). There is non-crossover when there is no change in the rank of performance in all environments, while the crossover shows a shift in yield ranking of genotypes across the environments (Abuali *et al.*, 2014). The GGE biplot has specifically been used for mega-environment to show the which-won-where pattern based on genotype mean performance and stability across a range of environments (Oladosu *et al.*, 2017). The ideal genotype has a high mean performance and stability and is located almost on the average environment at first coordinate abscissa and has a near-zero projection on to the AEC ordinate and the closest of the direction of array (Maqbool *et al.*, 2015).

2.13 Reference

Abuali, A. I., A. A. Abdelmula, M. M. Khalafalla, N. B. Hamza, A. H. Abdalla and A. E. Idris. 2014. Assessment of yield stability and adaptability of parental inbred lines and f1-hybrids of grain maize (*Zea mays L.*) Using AMMI Analysis. British Biotechnology Journal 4. 339-349.

Agrios, G. 2005a. Plant pathology. 5th ed. Elsevier Academic Press. New York, United States of America.

Agrios, G. N. 2005b. Plant Pathology. 5th ed. Department of Plant Pathology. University of Florida. United States of America.

Agyeman, A., E. Parkes and B. Peprah. 2015. AMMI and GGE biplot analyses of root yield performance of cassava genotypes in forest and coastal ecologies. International Journal of Agricultural Policy and Research 3: 122-132.

Akhshi, N., K. Cheghamirza, H. Ahmadi and F. Firouzabadi. 2014. Generation mean analysis to estimate genetic parameters for morphological traits in common bean (*Phaseolus vulgaris L.*). Journal of Biodiversity and Environmental Sciences 3: 254-257.

Bastas, K. K. and F. Sahin. 2017. Evaluation of seedborne bacterial pathogens on common bean cultivars grown in central Anatolia region, Turkey. European Journal of Plant Pathology 147: 239-253.

Beebe, S., P. W. Skroch, J. Tohme, M. Duque, F. Pedraza and J. Nienhuis. 2000. Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. Crop Science 40: 264-273.

Bellucci, E., E. Bitocchi, D. Rau, M. Rodriguez, E. Biagetti, A. Giardini et al. 2014. Genomics of origin, domestication and evolution of *Phaseolus vulgaris*. Genomics of plant genetic resources. Springer 1. 483-507.

Blair, M. W., L. M. Díaz, H. F. Buendía and M. C. Duque. 2009. Genetic diversity, seed size associations and population structure of a core collection of common beans (*Phaseolus vulgaris L.*). Theoretical and Applied Genetics 119: 955-972.

Blair, M. W., M. Giraldo, H. Buendia, E. Tovar, M. Duque and S. E. Beebe. 2006. Microsatellite marker diversity in common bean (*Phaseolus vulgaris L.*). Theoretical and Applied Genetics 113: 100-109.

Broughton, W. J., G. Hernandez, M. Blair, S. Beebe, P. Gepts and J. Vanderleyden. 2003. Beans (*Phaseolus spp.*)–model food legumes. Plant and Soil 252: 55-128.

Cabral, P., T. Soares, A. Lima, F. De Miranda, F. Souza and L. Gonçalves. 2011. Genetic diversity in local and commercial dry bean (*Phaseolus vulgaris*) accessions based on microsatellite markers. Genetics and Molecular Research 10: 140-149.

Chaudhary, R. R. and B. K. Joshi. 2005. Correlation and path coefficient analyses in sugarcane. Nepal Agriculture Research Journal 6: 28-34.

Chipeta, M. M., R. Melis, P. Shanahan, J. Sibiya and I. R. Benesi. 2017. Genotype X environment interaction and stability analysis of cassava genotypes at different harvest times. JAPS, Journal of Animal and Plant Sciences 27: 901-919.

Cichy, K. A., T. G. Porch, J. S. Beaver, P. Cregan, D. Fourie, R. P. Glahn et al. 2015. A *Phaseolus vulgaris* diversity panel for Andean bean improvement. Crop Science 55: 2149-2160.

Dabholkar, A. 1999. Elements of bio metrical genetics. 1 th ed. Concept publishing company. New Delhi. India.

Dawo, M. I., J. M. Wilkinson, F. E. Sanders and D. J. Pilbeam. 2007. The yield and quality of fresh and ensiled plant material from intercropped maize (*Zea mays*) and beans (*Phaseolus vulgaris*). Journal of the Science of Food and Agriculture 87: 1391-1399.

Debouck, D. G. 1991. Systematics and morphology. Common beans: Research for Crop Improvement 1: 55-118.

Dia, M., T. C. Wehner, R. Hassell, D. S. Price, G. E. Boyhan, S. Olson et al. 2016. Genotype× environment interaction and stability analysis for watermelon fruit yield in the United States. Crop Science 56: 1645-1661.

Dlamini, T., L. Nalley, F. Tsiboe, A. Barkley and A. Shew. 2017. The economic impact of the South African Agricultural Research Council's dry beans breeding program. Journal of Agricultural and Applied Economics 49: 232-250.

Duncan, R. W., R. L. Gilbertson, M. Lema and S. P. Singh. 2014. Inheritance of resistance to the widely distributed race 6 of *Pseudomonas syringae pv. phaseolicola* in common bean pinto US14HBR6. Canadian Journal of Plant Science 94: 923-928.

FAO, I. 2014. WFP. The state of food insecurity in the World 2014 strengthening the enabling environment for food security and nutrition. Rome. Italy.

Fourie, D. 2002. Distribution and severity of bacterial diseases on dry beans (*Phaseolus vulgaris L.*) in South Africa. Journal of Phytopathology 150: 220-226.

Gauch, H. and R. Zobel. 1996. AMMI analysis of yield trials (genotype-by-environment interaction). Jornal Press 4. 85-122.

Graham, P. and P. Ranalli. 1997. Common bean (*Phaseolus vulgaris L.*). Field Crops Research 53: 131-146.

Harveson, R. M., H. F. Schwartz, C. A. Urrea and C. D. Yonts. 2015. Bacterial wilt of dry-edible beans in the central high plains of the United State of America: past, present, and future. Plant Disease 99: 1665-1677.

Hayman, B. 1958. The separation of epistatic from additive and dominance variation in generation means. Heredity 12: 371-372.

Hayman, B. and K. Mather. 1955. The description of genic interactions in continuous variation. Biometrics 11: 69-82.

He, J., X. Zhao, A. Laroche, Z.-X. Lu, H. Liu and Z. Li. 2014. Genotyping-by-sequencing, an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Frontiers in Plant Science 5: 484-485.

Hirano, S. and C. Upper. 2002. Effect of pathogenicity-associated genes on field fitness of *Pseudomonas syringae pv. syringae*. Phyllosphere microbiology. Am. Phytopath. Soc. Press 1: 81-99.

Jatothu, J. L., K. S. Dangi, S. K. Sagi, H. Bhandari, M. Tripathi and B. Chaudhary. 2013. Estimation of gene effects based on joint scaling test and sequential model fit scheme for quantitative traits in sesame (*Sesamum indicum L.*). Journal of Agricultural Science 5: 224-227.

Jung, G., H. Ariyarathne, D. Coyne and J. Nienhuis. 2003. Mapping QTL for bacterial brown spot resistance under natural infection in field and seedling stem inoculation in growth chamber in common bean. Crop Science 43: 350-357.

Kearsey, M. J. and H. S. Pooni. 1998. The genetical analysis of quantitative traits. Stanley Thornes (Publishers) Limited. New York. USA

Kimani, P., R. Buruchara, K. Ampofo, M. Pyndji, R. Chirwa and R. Kirkby. 2005. Breeding beans for smallholder farmers in Eastern, Central, and Southern Africa: Constraints, achievements, and potential. PABRA Millennium Workshop: p. 11.

Koutsika-Sotiriou, M. and E. Traka-Mavrona. 2008. Snap bean and vegetables II. Springer 2: 27-83.

Kwak, M., O. Toro, D. G. Debouck and P. Gepts. 2012. Multiple origins of the determinate growth habit in domesticated common bean (*Phaseolus vulgaris. L*). Annals of Botany 110: 1573-1580.

Maqbool, M. A., M. Aslam, H. Ali, T. M. Shah and B. M. Atta. 2015. GGE biplot analysis based selection of superior chickpea (*Cicer arietinum L.*) inbred lines under variable water environments. Pakistan. Jornal. Botanic 47: 1901-1908.

Mather, K. and J. L. Jinks. 2013. Biometrical genetics: The study of continuous variation. Chapman and Hall, London. United Kingdom.

Mavromatis, A., I. Arvanitoyannis, A. Korkovelos, A. Giakountis, V. Chatzitheodorou and C. Goulas. 2010. Genetic diversity among common bean (*Phaseolus vulgaris L.*) Greek landraces and commercial cultivars: nutritional components, RAPD and morphological markers. Spanish Journal of Agricultural Research 8: 986-994.

Miklas, P. N., J. D. Kelly, S. E. Beebe and M. W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147: 105-131.

Mortazavian, S., H. Nikkhah, F. Hassani, M. Sharif-al-Hosseini, M. Taheri and M. Mahlooji. 2014. GGE biplot and AMMI analysis of yield performance of barley genotypes across different environments in Iran. Journal of Agricultural Science and Technology 16: 609-622.

Muedi, H. T., D. Fourie and N. W. McLaren. 2015. Distribution and severity of bacterial brown spot on dry beans in South Africa: an update. South African Journal of Science 111: 1-6.

Muedi, H. T. H. 2015. Reaction of selected common bean germplasm accessions to bacterial brown spot in South Africa. Crop Protection 77: 87-93.

Navarro, F., P. Skroch, G. Jung and J. Nienhuis. 2007. Quantitative trait loci associated with bacterial brown spot in loci. Crop Science 47: 1344-1353.

Oladosu, Y., M. Y. Rafii, N. Abdullah, U. Magaji, G. Miah, G. Hussin et al. 2017. Genotype× Environment interaction and stability analyses of yield and yield components of established and mutant rice genotypes tested in multiple locations in Malaysia. Acta Agriculturae Scandinavica, Section B. Soil and Plant Science 7: 1-17.

Parlevliet, J. E. 2002. Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica 124: 147-156.

Purchase, J., H. Hatting and C. Van Deventer. 2000. Genotype× environment interaction of winter wheat (*Triticum aestivum L.*) in South Africa: II. Stability analysis of yield performance. South African Journal of Plant and Soil 17: 101-107.

Ramteke, R., V. Kumar, P. Murlidharan and D. K. Agarwal. 2010. Research note study on genetic variability and traits interrelationship among released soybean varieties of India [*Glycine max (L.) Merrill*]. Electronic Journal of Plant Breeding 1: 1483-1487.

Robinson, H., R. E. Comstock and P. Harvey. 1949. Estimates of heritability and the degree of dominance in corn. Agronomy Journal 41: 353-359

Rodriguez, M., D. Rau, E. Bitocchi, E. Bellucci, E. Biagetti, A. Carboni et al. 2016. Landscape genetics, adaptive diversity and population structure in *Phaseolus vulgaris*. New Phytologist 209: 1781-1794.

Rosales-Serna, R., S. Hernandez-Delgado, M. Gonzalez-Paz, J. Acosta-Gallegos and N. Mayek-Perez. 2005. Genetic relationships and diversity revealed by AFLP markers in Mexican common bean bred cultivars. Crop Science 45: 1951-1957.

Scortichini, M., U. Marchesi, M. Dettori and M. Rossi. 2003. Genetic diversity, presence of the syrB gene, host preference and virulence of *Pseudomonas syringae pv. syringae* strains from woody and herbaceous host plants. Plant Pathology 52: 277-286.

Singh, B. and B. Singh. 2015. Breeding perspectives of snap bean (*Phaseolus vulgaris L.*). Vegetable Science 42: 1-17.

Singh, S. P. and P. N. Miklas. 2015. Breeding common bean for resistance to common blight: A review. Crop Science 55: 971-984.

Singh, S. P. and H. F. Schwartz. 2010. Breeding common bean for resistance to diseases: a review. Crop Science 50: 2199-2223.

Urrea, C. A. and R. M. Harveson. 2014. Identification of sources of bacterial wilt resistance in common bean (*Phaseolus vulgaris L.*). Plant Disease 98: 973-976.

Vanderplank, J. E. 2012. Disease resistance in plants. 1 th ed. Elsevier Academic. Florida. United State of America.

Yan, W., M. S. Kang, B. Ma, S. Woods and P. L. Cornelius. 2007. GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop Science 47: 643-653.

Young, J. M. 2010. Taxonomy of *Pseudomonas syringae*. Journal of Plant Pathology 92: 5-14.

CHAPTER 3
SCREENING ANDEAN DIVERSITY PANEL DRY BEAN LINES FOR RESISTANCE TO BACTERIAL BROWN SPOT DISEASE UNDER FIELD CONDITIONS IN SOUTH AFRICA

Abstract

Bacterial brown spot (BBS) disease caused by *Pseudomonas syringae* pv. *syringae* (*Pss*) is an important disease of dry bean (*Phaseolus vulgaris* L.) with grain yield losses more than 55%. This study aimed to identify BBS disease resistant genotypes from 423 Andean Diversity Panel (ADP) dry bean lines under field conditions across three sites viz. Warden and Middelburg under natural infestation, and Potchefstroom under artificial inoculation. Plants were inoculated with BBS disease using three isolates at 21, 28 and 36 days after planting and disease scoring was done at 7, 14 and 21 days after inoculation following a modified 1-9 CIAT scale. The BBS severity percentage and relative area under disease progress curve (RAUDPC) were applied to quantify the reaction of bean genotypes to BBS disease. The study identified 21.03% of evaluated germplasm as resistant and 41.63% as moderately resistant to BBS disease. Genotypes ADP-0592, ADP-0790, ADP-0120 and ADP-0008 were selected for both resistance to BBS disease and higher seed yield across three environments. Genotypes ADP-0546, ADP-0630, ADP-0183 and ADP-0279 were selected for both yield and BBS resistance at Warden, whereas ADP-0038, ADP-0721, ADP-0790 were selected for both traits at Middelburg and lastly ADP-0120 and ADP-0079 were selected for both traits at Potchefstroom. The best genotypes selected for both yielding and BBS resistance had grain yield above 1.45 t ha^{-1} across sites, and above1.85 t ha^{-1} at individual sites, and out-yielded the best performing cultivar (1.13 t ha^{-1}) and the grand mean (0.87 t ha^{-1}). The RAUDPC was highly significantly ($P<0.001$) negatively strong correlated with grain yield ($r= -0.55$) at Potchefstroom. Medium seeded genotypes showed low RAUDPC than the large seeded, and indeterminate growing habit genotypes showed low RAUDPC than determinate growth habit. These genotypes can be useful sources of genetic resistance for future dry bean improvement for South African bean market.

Keys words: Screening Andean Diversity Panel (ADP) Dry bean lines, reaction to BBS disease and correlation among related traits.

3.1 Introduction

Sub-Saharan Africa (SSA) is a nutritionally unstable region with reported malnutrition-related challenges (Dlamini *et al.*, 2017), where millions of people in the region depend on cereal based foods that are deficient in proteins, vitamins and several other micronutrients. Most people in the region face household food shortages leading to hunger and starvation (Singh and Miklas, 2015). The adoption of legumes with increased levels of proteins can boost nutritional security in the region (Singh and Miklas, 2015). Dry bean (*Phaseolus vulgaris* L.) provides between 10 and 25% of protein and 71% of starch (Broughton et al., 2003) to the daily diet (Broughton *et al.*, 2003). The average mean yield in South Africa is about 1.40 t ha^{-1} (Dlamini *et al.*, 2017), which is low when compared with North America (~3.00 t ha^{-1}) (Kimani *et al.*, 2005; FAO, 2014).

Dry bean cultivation is affected by several biotic constraints including bacterial brown spot (BBS), a bacterial disease, that occurs worldwide and is particularly serious in South Africa, being present in all the dry bean growing regions (Singh and Miklas, 2015). The BBS disease has been extensively described in the USA, Brazil and Canada (Harveson and Schwartz, 2007; Singh and Schwartz, 2010). The disease, caused by *Pseudomonas syringae* pv.*syringae* (*Pss*), is seed-borne and largely affects the foliage and to a smaller extend the pods (Singh and Miklas, 2015), and is especially severe when beans are grown in a mono-cropping systems (Muedi *et al.*, 2015). It has been reported that BBS disease can cause up to 55% of yield losses where conditions are conducive to the disease (Serfontein, 1994; Muedi *et al.*, 2015). Symptoms may initially appear as small water saturated wounds on leaves and pods and subsequently develop into elliptical, necrotic brown wounds encircled by a thin yellow-green part (Kimani *et al.*, 2005; Muedi *et al.*, 2015). Sources of infection include infected seed, wind, contaminated farm implements and soil (Harveson *et al.*, 2015). Infected seed is an important way of dissemination of *Pss* (Kimani *et al.*, 2005), however, wind, rain and overhead irrigation have similarly been recognized as effective dispersion methods of *Pss* (Navarro *et al.*, 2007). Humidity over 95% and temperatures between 28 and 32°C are favourable conditions for BBS disease, and these conditions are common in the central and eastern regions of South Africa, where dry beans are widely gown on a commercial scale (Harveson and Schwartz, 2007).

The BBS disease control includes planting of certified disease free seed, crop rotation, resistant cultivars and control of host plants (Navarro *et al.*, 2007). Resistant cultivars have been recognized as the best way to control the disease in a sustainable way (Harveson *et al.*, 2015). An important study of the inheritance of BBS resistance was undertaken by Navarro

et al. (2007). This research used the *Pss* seedling stem inoculation in dry bean segregating population of the cross Belneb RR-1 × A55 RIL and found a number of genomic regions situated in several linkage groups that were related to BBS resistance (Navarro *et al.*, 2007). The large-seeded dry and green bean cultivars originating from Andean gene pool are highly susceptible to BBS disease, while, the small and medium seeded cultivars of Mesoamerican gene pool origin are more tolerant to BBS disease (Singh and Schwartz, 2010). The identification of genetic resources resistant to BSS disease will assist in the development of cultivars with improved resistance to BBS to the benefit of South African farmers. This study aimed to screen 423 Andean Diversity Panel (ADP) dry bean lines for resistance to bacterial brown spot (BBS) disease, under field conditions in South Africa.

3.2 Methodology

3.2.1 Genetic materials and experimental sites

Four hundred and twenty three Andean Diversity Panel (ADP) dry bean lines maintained by the Agricultural Research Council Grain Crops Institute Program (ARC-GCIP) were included in the study. The ADP consisted of genotypes from both the Andean gene pool, local commercial cultivars, breeding lines, and landraces from Africa, the Caribbean and America (Cichy *et al.*, 2015). These materials were screened for resistance to BBS disease under artificial infection at Potchefstroom and under natural infection at Warden and Middelburg during the 2017/2018 growing season. Potchefstroom is located in the North West province of South Africa at an altitude about 1349 m, latitude 26.74^{o} S and longitude 27.08^{o} E. Warden is situated in the Free State province and is at an altitude of about 1720 m, latitude 28. 31^{o} S and longitude 29.12^{o} E and Middelburg is located in Mpumalanga province at an altitude about 1277 m, latitude 31.47^{o} S and longitude 25.03^{o} E (Muedi *et al.*, 2015). The genetic materials are presented in Appendix 1.1.

3.2.2 Weather data

The weather data across three experimental sites is indicated in Table 3.1. The mean temperatures ranged from 25.0^{o}C (Warden) to 28.2^{o}C (Middelburg), whereas the Middelburg had the lowest rainfall (346.34 mm) and Warden the highest (687.7 mm).

Table 3.1 The weather data of three experimental sites

Month	Potchefstroom		Warden		Middelburg	
	Temp (ºC)	Rainfall (mm)	Temp (ºC)	Rainfall (mm)	Temp (ºC)	Rainfall (mm)
October-2017	26.39	56.13	24.89	49.53	24.90	28.45
November-2017	29.12	69.34	27.05	82.04	29.30	19.56
December-2017	29.29	62.48	26.06	208.79	32.01	10.03
January-2018	31.04	47.24	28.39	110.49	32.74	111.51
February-2018	27.68	68.33	26.75	66.80	30.68	77.47
March-2018	27.54	58.93	23.63	131.52	29.21	22.10
April-2018	25.33	35.56	22.90	12.95	24.75	62.74
May-2018	22.78	11.28	20.32	25.65	21.61	14.48
Average	27.40	-	25.00	-	28.15	-
Total	-	409.29	-	687.77		346.34

Source: Agricultural Research Council (2018).

3.2.3 Experimental design

The experiment was established in an alpha lattice design with three replicates with 35 incomplete blocks (33 with ADP lines and 2 with border rows) and each with 46 plots (42 with ADP and 4 with border rows) at all the three locations. Each replicate contained 11 incompletes blocks (IBLK). The last IBLK contained one plot with 3 rows of ADP lines, 10 rows of A 55 (resistant to BBS) as control and 33 rows of RR-1 as BBS spreader. Each plot had one row with 5 m length and 2 m of pathway. Each row had 75 plants with a 76 cm and 7.5 cm of inter-row and intra-row, respectively (Bellucci *et al.*, 2014). Two borders rows (RR-1 as spreader or source of inoculum) were planted around the four sides of the experiment, and the trial was weeded mechanically. Irrigation was applied when required. The nitrogen, phosphorus, and potassium fertilizers were applied to the fields at an average with rate of 42.30 kg ha^{-1}, 22.30 kg ha^{-1} and 18.40 kg ha^{-1}, respectively.

3.2.4 Bacterial brown spot disease inoculation

Middleburg and Warden are hotspots of the BBS disease and the experiments relied on natural infestation, while in Potchefstroom artificial inoculation of BBS disease was applied. The inoculum was made from 48 to 72 hours old cultures grown on King's B medium (Muedi, 2015). Three isolates (BV 6.3, BV 3.3.2 and BV 27.1) with the highest level of aggression based on bean pod assays were mixed and suspended in water for inoculation. The suspension was

adjusted to 1 x 10^8 CFU/ml and was used immediately for the field inoculation (Muedi, 2015). To increase the development of BBS the experiment was irrigated before the inoculum application on the leaves, and plants were sprayed at 21, 28 and 36 days after planting through a mechanical inoculum sprayer (Stihl mistblower SR 430) during the morning hours.

3.2.5 Data collection

The BBS severity was evaluated seven days after the initial infestation and the rating was done weekly for three consecutive weeks (Muedi *et al.*, 2015). The BBS severity was evaluated based on the percentage diseased leaf area for the total plot using a standardised CIAT scale of 1 (resistant or immune) to 9 (susceptible or disease) (Petersen *et al.*, 2015). The BBS severity were converted in percentages 1 = 5%, 2 =15%, 3 = 25%, 4 = 35%, 5 = 45%, 6 = 55%, 7 = 65%, 8 = 75% and 9 = 85%. The BBS severity percentages were used to calculate the relative area under disease progress curve (RAUDPC) (Muedi *et al.*, 2015). Several other traits were measured, namely, days to flowering, days to maturity, growth habit, seed size and grain yield. The days to flowering, days to maturity and grain yield data were collected from the experiments across three evaluated sites, while the seed size and the growth habit data were obtained through the secondary data. The relationship between secondary data and RAUDPC were visually presented.

3.2.6 Data analysis

The data were analysed using unbalanced analysis of variance in in Genstat 18th edition (Gilmour *et al.*, 2015). Means were separated by the least significant difference (LSD) at P = 0.05. The relationship between BBS reaction, growth habit and seed size was performed using the phenotypic secondary and the primary data of the relative area under disease progress curve (RAUDPC). The model for the combined ANOVA of multi-environment trials included additive terms for main effects of genotype and environment, as well as the genotype by environment interaction term (Equation 3.1).

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \in_{ij} \qquad \text{Equation 3.1}$$

Where Yij is the yield of the genotype i in environment j and *k*th replication;, μ is overall yield mean, αi and βj are genotypic and environmental effect, $(\alpha\beta)ij$ is the effect of interaction between the ith genotype and jth environment, $\in ij$ is the mean random error of the ith genotype and ej environment.

3.2.7 Disease reaction and relative area under the disease progress curve

The relative area under the disease progress curve (RAUDPC) for each genotype was calculated using the percentage severity scores as a dependant variable (Campbell and Madden, 1990) and was calculated as follows.

$$\text{RAUDPC}=\sum_{i=1}^{n}[(X_{i+1}+X_i)/2]\ x\ [t_{i+1}-t_i] \qquad \text{Equation 3.2}$$

Where: X_i is the percentage of disease severity rating at data i; X_{i+1} is the percentage of disease severity rating at data i+1; t_i is the time in days of each rating after the inoculation day i; t_{i+1} is the time in days of each rating after the inoculation day i+1; and n is total number of observations.

The RAUDPC data were subjected to analysis of variance and means were separated using Fischer's LSD (P≤ 0.05). Furthermore, the correlation between reaction to BBS, DF, DM, RAUDPC and grain yield was performed for the Potchefstroom evaluation trial.

3.3 Results

3.3.1 Combined analysis of variance across three sites

The combined analysis of variance for BBS severity and grain yield (t ha^{-1}) is exposed in Table 3.2. The mean squares for genotype, environment, genotype and environment interaction (GEI) showed significant differences (P < 0.001) for both BBS severity and grain yield (t ha^{-1}). The source of variation for BBS severity were partitioned for environment, genotype and GEI were 4.32%, 28.08% and 49.29%, of the total sum of squares, respectively, whereas for grain yield (t ha^{-1}) as 20.92%, 24.93% and 44.95%, respectively. The mean BBS severity and yield were 38.85 and 0.87 t ha^{-1}, respectively. There were significant GEI effects for BBS disease (P < 0.001) and grain yield (P < 0.001). Significant differences observed indicated the rank changes per environment and thus further analysis was done to establish which genotypes performed better or worse in particular environments.

Table 3.2 Combined analysis of variance showing mean squares for BBS severity and grain yield across three experimental sites

Source	DF	BBS severity	Yield (t ha^{-1})
		MS	MS
Environment	2	18624.09***	89.52***
Rep	2	571.62***	0.11**
Block	10	3722.37***	1.93***
Rep. Block	20	71.36	0.22***
Genotypes	412	587.34***	0.52***
Genotype*Environment (GEI)	844	503.31***	0.46***
Residual	2516	46.93	0.02
Total	3806	226.45	0.23
LSD		10.97	0.24
CV		17.19	17.61
Mean		39.85	0.87

* P < 0.05, ** P < 0.01, *** P < 0.001; LSD= Least significant difference, Mean=The mean, CV=Percentage coefficient of variation, MS=Mean of square, BBS=Bacterial brown spot, Yield=Grain yield (t ha^{-1}).

3.3.2 Analysis of variance for individual sites

The analysis of variance for BBS severity and grain yield (t ha^{-1}) for the individual sites is presented in Table 3.3. There were significant differences (P<0.05) among genotypes for BBS severity, RAUDPC and grain yield (t ha^{-1}) at three sites (Table 3.3). The source of variation for BBS severity, RAUDPC were partitioned for genotype, block, replication and interaction between replication and block. Genotypes was the main source of variation with 73.23, 69.86 and 72.80% of BBS severity and 86.68, 84.66 and 84.47 of grain yield of the total sum square in Warden, Middelburg and Potchefstroom, respectively. The genotype variation for RAUDPC was 72.80% at Potchefstroom (Table 3.3). The variation for the blocks were 4.39, 20.61, 10.54% for BBS severity and 1.94, 10.71 and 7.12% for grain yield of total sum square in Warden, Middelburg and Potchefstroom, respectively. The variation for the replication were 0.63, 0.06, 0.18 for BBS severity and 0.09, 0.02 and 0.04% for grain yield in Warden, Middelburg and Potchefstroom, respectively. The RAUDPC variation were 10.54 for block and 0.18 for replication of total sum square.

Table 3.3 Analysis of variance showing mean for bacterial brown spot disease severity, RAUDPC and grain yield for individual sites

Source	DF	Warden		Middelburg		Potchefstroom		
		BBS severity	Grain yield	BBS severity	Grain yield	BBS severity	RAUDPC	Grain yield
		MS	MS	MS	MS	MS	MS	MS
Rep	2	650.59***	0.09*	123.01	0.02	198.96**	87741.0 **	0.06
Block	10	908.30***	0.38**	8260.34***	2.32***	2288.68***	1009309.0***	1.89***
Rep. Block	20	54.15	0.02	136.59***	0.02	52.4	23107.0	0.03
Gen	412	367.43***	0.42***	679.57***	0.45***	383.65***	169192.0***	0.52***
Residual	824	53.26	0.02	42.75	0.01	42.14	18584.0	0.04
Total	1268	163.04	0.15	316.08	0.17	171.23	75514.0	0.21
LSD		11.7	0.22	10.48	0.18	10.40	218.5	0.32
CV		16.54	17.32	17.8	17.00	16.78	16.78	17.03
Mean		44.13	0.80	36.73	0.64	38.68	812.34	1.16

* P<0.05, ** P<0.01, *** P<0.001; LSD=Least significant difference, Mean=The mean, SE=Standard error between predicted means, CV=Percentage coefficient of variation, MS = Mean of squares, BBS= Bacterial brown spot, RAUDPC = Relative area under disease progress curve and Grain yield=Grain yield in t ha^{-1}.

3.3.3 Mean of BBS severity and grain yield across three sites

The mean BBS severity and the grain yield of the best 15 performing genotypes and five the worst performing genotypes across three environments are indicated in Table 3.4. The mean BBS severity ranged from 15 (ADP-0592) to 63.15 (ADP-0310). While, the grain yield of genotypes ranged from 1.66 (ADP-00097) to 0.32 t h^{-1} (ADP-0577). Four genotypes of the best 15 performing, namely ADP-0592, ADP-0790, ADP-0120 and ADP-0008 had both a low BBS severity and a high grain yield across three environments (Table 3.4). Genotypes selected across three sites had the mean grain yield above the grand mean (0.87 t ha^{-1}), and the best performing cultivar (1.13 t ha^{-1}) and mean BBS severity below the grand mean (39.85) and the best performing cultivar (31.67) (Table 3.4).

Table 3.4 The mean of BBS severity and grain yield of the best 10 performing genotypes and the worst five performing genotypes across three sites

Item	ADP	BBS severity	ADP	Yield (t ha^{-1})
The best 10 performing genotypes	ADP-0592	15.00	ADP-0097	1.66
	ADP-0790	16.11	ADP-0522	1.62
	ADP-0432	19.44	ADP-0079	1.59
	ADP-0454	21.30	ADP-0122	1.58
	ADP-0796	21.30	ADP-0592	1.56
	ADP-0126	22.78	ADP-0545	1.55
	ADP-0008	23.15	ADP-0790	1.55
	ADP-0125	23.15	ADP-0120	1.53
	ADP-0079	23.52	ADP-0630	1.51
	ADP-0120	24.63	ADP-0008	1.46
The worst 5 performing genotypes	ADP-0391	57.22	ADP-0587	0.39
	ADP-0609	57.22	ADP-0639	0.38
	ADP-0203	58.33	ADP-0310	0.38
	ADP-0652	61.30	ADP-0417	0.34
	ADP-0310	63.15	ADP-0577	0.32
	LSD	10.97	LSD	0.25
	Mean	39.85	Mean	0.87
	SE	5.59	SE	0.15
	CV	17.19	CV	17.61

LSD= Least significant difference, Mean=The mean, SE=Standard error between predicted means, CV=Percentage of coefficient of variation, ADP=Andean Diversity Panel lines, BBS= Bacterial brown spot and Grain yield=Grain yield in t ha^{-1}.

3.3.4 Mean of BBS disease severity and grain yield for each sites

The 10 best performing genotypes for mean BBS severity, RAUDPC and grain yield and 10 the worst performing genotypes at the three sites are indicated in Table 3.5. Genotypes with BBS severity less than 35 in three sites were regarded as resistant to BBS disease, between 35.0 and 48.3 as moderate, and greater than 48.3 as susceptible to the BBS disease. As a result, 17.5%, 50.6% and 49.4% genotypes were selected as resistant, moderate and susceptible reaction to BBS disease, respectively, in Warden. In Middelburg 24.6% of genotypes showed a resistant reaction, while 49.2 and 26.2% showed a moderate and susceptible reaction to BBS disease, respectively. At Potchefstroom, 21.0% were resistant to BBS disease, and 25.1 and 53.9% as moderate and susceptible, respectively. The RAUDPC ranged from 1948.30 for ADP-0733 to 105.00 for ADP- 0798. The mean BBS severity were 44.1, 36.7% and 38.68, while the mean grain yield were 0.8, 0.64 and 1.16 t ha-1 for Warden, Middelburg and Potchefstroom, respectively.

3.3.5 Relation between BBS disease reaction and grain yield

The grain yield ranged from 2.47 (ADP-0546) to 0.18 t h^{-1} (ADP-0746) in Warden, from 2.13 (ADP-0239) to 0.17 t ha^{-1} (ADP-0644) in Middelburg and from 2.31 (ADP-0517) to 0.16 t ha^{-1} (ADP-0242) in Potchefstroom (Table 3.5). The genotypes ADP-0546, ADP-0630, ADP-0183 and ADP-0279 had both low mean BBS severity and high grain yield of the best 10 performing genotypes in Warden. The genotypes ADP-0038, ADP-0721, ADP-0790 had both low mean BBS severity and high grain yield of the best 10 performing genotypes in Middelburg. Genotypes ADP-0120 and ADP-0079 had low mean BBS severity, low RAUDPC and high grain of the best 10 performing genotypes in Potchefstroom (Table 3.5). Genotypes with RAUDPC smaller than 665.0 were regarded resistant to BBS, genotypes with RAUDPC between 668.0 and 875.00 were regarded as moderate resistance, and genotypes with RAUDPC higher than 898.3 were regarded susceptible to BBS disease. Therefore, 16.50% of the genotypes exhibited resistance to BBS disease, while 41.60% and 41.80% of the genotypes showed a moderate and susceptible reaction, respectively. The best local commercial cultivar had a mean BBS severity of 45.0, 45.0 and 5.0, with grain yield of 1.28, 0.55 and 1.56 t ha^{-1} in Warden, Middelburg and Potchefstroom, respectively. The genotypes selected for both traits had mean BBS severity below and a grain yield above the best local commercial cultivar, with the exception of Potchefstroom where the genotype selected for both (ADP-0120 and ADP-0079) had approximately the same mean BBS severity and RAUDPC as the best local commercial cultivar (ADP-0798).

Table 3.5 Mean of BBS severity, RAUDPC and grain yield of the best 10 performing genotypes and the worst 10 performing genotypes across individual sites

Warden				Middelburg				Potchefstroom					
ADP	BBS Severity	ADP	Grain yield	ADP	BBS Severity	ADP	Grain yield	ADP	BBS severity	ADP	RAUDPC	ADP	Grain yield
546	5.00	546	2.47	038	8.33	239	2.13	120	5.00	798	105.00	517	2.31
478	15.00	063	2.31	551	8.33	057	2.07	126	5.00	797	105.00	554	2.23
376	18.33	183	2.01	125	8.33	621	2.05	432	5.00	716	105.00	522	2.19
279	18.33	473	2.00	721	8.33	038	2.03	716	5.00	120	105.00	060	2.17
611	18.33	658	1.99	592	8.33	522	2.03	790	5.00	790	105.00	008	2.17
355	18.33	101	1.97	790	8.33	192	2.03	797	5.00	126	105.00	120	2.16
585	18.33	673	1.93	510	8.33	790	2.03	798	5.00	432	105.00	097	2.15
767	18.33	279	1.91	093	11.67	737	1.98	125	6.11	125	128.30	532	2.13
183	18.33	337	1.90	740	11.67	721	1.98	455	6.11	455	128.30	527	2.13
630	18.33	630	1.89	035	11.67	684	1.94	079	7.22	079	151.70	079	2.12
074	65.00	730	0.27	207	68.33	581	0.19	587	58.33	587	1225.00	672	0.29
203	65.00	640	0.26	759	71.67	481	0.18	609	58.33	609	1225.00	479	0.29
095	68.33	531	0.24	770	71.67	640	0.18	623	58.33	623	1225.00	598	0.29
049	68.33	078	0.24	644	71.67	207	0.18	598	59.44	598	1248.30	459	0.28
640	68.33	639	0.24	788	71.67	595	0.18	610	59.44	610	1248.30	376	0.28
549	71.67	654	0.24	659	71.67	379	0.18	777	59.44	777	1248.30	280	0.26
105	71.67	681	0.21	379	71.67	604	0.18	220	61.67	220	1295.00	587	0.25
797	71.67	721	0.20	581	71.97	765	0.18	672	61.67	672	1295.00	190	0.23
078	71.67	549	0.20	652	75.00	279	0.17	310	66.11	310	1388.30	279	0.17
721	75.00	746	0.18	595	75.00	644	0.17	733	92.78	733	1948.30	242	0.16
LSD	11.70		0.22	LSD	10.48		0.18	LSD		10.40	219.00		0.32
Mean	44.13		0.80	Mean	36.73		0.64	Mean		38.68	812.00		1.16
SE	5.96		0.11	SE	5.34		0.09	SE		5.30	111.00		0.16
CV	16.50		17.30	CV	17.80		17.0	CV		16.80	16.80		17.00

LSD= Least significant difference, Mean=The mean, SE=Standard error between predicted means, CV=Percentage of coefficient of variation, ADP=Andean Diversity Panel lines, BBS = Bacterial brown spot, RAUDPC = Relative area under disease progress curve and Grain yield=Grain yield in t ha^{-1}.

3.3.6 Correlation among traits

The correlations among traits at Potchefstroom, namely the relative area under disease progress curve (RAUDPC), grain yield (t ha^{-1}), DF and DM of the 423 ADP dry bean genotypes is presented in Table 3.6. The RAUDPC was highly significant and negatively correlated with grain yield (r= -0.55***), DF (r=-0.27***) and DM (r=-0.47***). Grain yield was highly significant and positively correlated with DF (r=0.09***) and DM (r=0.33***). Days to flowering-DF was highly significant and positively correlated with DM (r=0.31***) and grain yield (r=0.09***).

Table 3.6 Correlation between days to flowering, days to maturity, RAUDPC and grain yield at Potchefstroom

Traits	**DF**	**DM**	**RAUDPC**	**Grain yield (t ha^{-1})**
DF	1			
DM	0.31***	1		
RAUDPC	-0.27***	-0.47***	1	
Grain yield (t ha^{-1})	0.09***	0.33***	-0.55***	1

* P<0.05; ** P<0.01; *** P<0.001. DF = Days to flowering, DM = Days to maturity, RAUDPC = Relative area under disease progress curve, Grain yield = Grain yield in t ha^{-1}.

3.3.7 Relationship between morphological traits and reaction to BBS disease

The relationship between seed size, growing habit and reaction to bacterial brown spot in Potchefstroom is displayed in Figure 3.1. The medium seeded genotypes showed the lowest RAUDPC mean compared with the large seeded. In addition, the genotypes with an indeterminate growth habit showed a lower RAUDPC mean than those with a determinate growth habit.

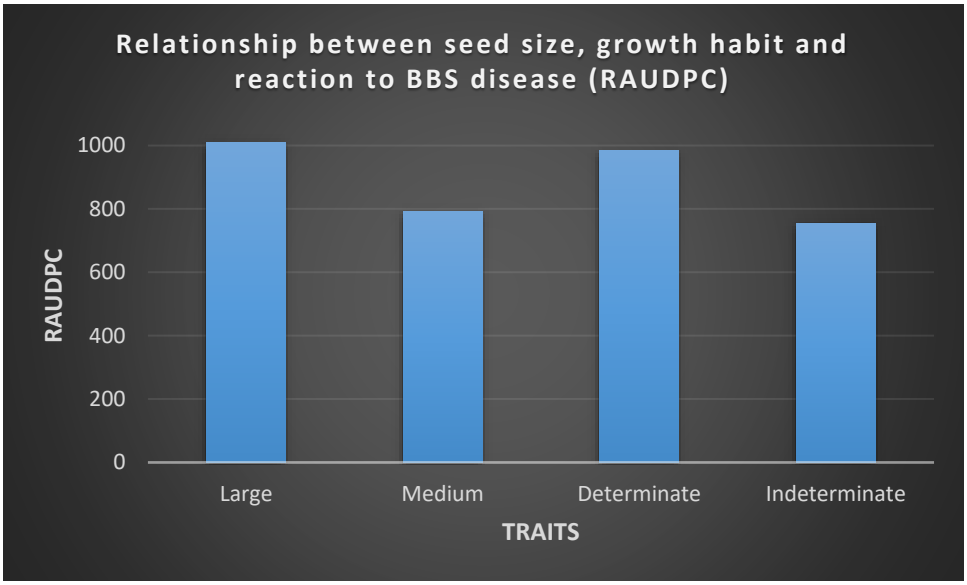


Figure 3.1 Relationship between seed size, growth habit and reaction to BBS disease (RAUDPC) at Potchefstroom

3.4 Discussion and conclusion

Bacterial brown spot is an economically important disease to dry bean production globally. This study aimed to identify BBS disease resistance and high grain yield performance amongst 423 Andean Diversity Panel (ADP) dry bean lines. The analysis of variance indicated high variability among selected genotypes for BBS disease resistance, and grain yield performance both across and at individual environments.

The study revealed that 21.0% of genotypes were resistant, while 41.6% were moderately resistant to BBS disease. These genotypes can be useful sources of genetic resistance for future dry bean improvement for the South African bean market. The high variability among genotypes implies better selection criteria based on resistance and yield performance. The GEI was also significant which implies rank changes on genotypic performances across different sites. Significant GEI can imply that selection of genotypes is environment specific, hence, genotypes can be selected for a particular environment.

Genotypes ADP-0592, ADP-0790, ADP-0120 and ADP-0008 were selected for both high disease resistance and high grain yield across three environments. Genotypes ADP-0546, ADP-0630, ADP-0183 and ADP-0279 were selected for both high BBS disease resistance as well as high yield in Warden. Genotypes ADP-0038, ADP-0721, ADP-0790 were best

performing at Middelburg while, genotypes ADP-0120 and ADP-0079 were better performing at Potchefstroom. Genotypes selected had the mean grain yield above the grand mean (0.87 t ha^{-1}) and the best performing cultivar (1.13 t ha^{-1}), and mean BBS severity below the grand mean (39.85) and the best performing cultivar (31.67). These genotypes had either broad or specific adaption with lower mean BBS severity and higher grain yield, than the best local commercial cultivar (ADP-0798).

The results indicated that RAUDPC was significantly and negatively correlated with grain yield (r= -0.55***), days to flowering (r = -0.27*** and days to maturity (r = -0.47***). This implies that disease occurrence and severity is affected by maturity period and that grain yield is negatively correlate with BBS severity of the ADP genotypes. The results from the study corroborates with findings by Muedi *et al.* (2015) who indicated that the higher RAUDPC the lower the grain yield will be. The small seeded genotypes had the lower RAUDPC mean than the medium and the large seeded dry bean genotypes. Research by Navarro *et al.* (2007) also reported that the seed size had an effect on resistance to the BBS disease The genotypes with an indeterminate growth habit had lower RAUDPC means than those with a determinate growth habit. Singh *et al.* (1991) also suggested that genotypes with indeterminate genotypes had higher disease resistance than determinate genotypes because longer vegetative growth of indeterminate genotypes increases plant resistance.

The study identified 21.03% and 41.63% genotypes with resistance to moderate resistance to BBS disease, respectively. Several genotypes were adapted to specific environments. Genotypes ADP-0592, ADP-0790, ADP-0120 and ADP-0008 were selected across the three environments. Genotypes ADP-0546, ADP-0630, ADP-0183 and ADP-0279 were selected for both high BBS disease resistance and high yield at Warden. Genotypes ADP-0038, ADP-0721, ADP-0790 were the best performing at Middelburg, while genotypes ADP-0120 and ADP-0079 were the best performers at Potchefstroom. Genotypes selected had mean grain yield above the grand and the best performing cultivar, and mean BBS severity below the grand mean and the best performing cultivar. These materials can be used as sources of resistance to BBS future breeding programmes.

3.5 References

Agrios, G. N. 2005. Plant Pathology. 5th ed. Department of Plant Pathology. University of Florida. United States of America.

Bellucci, E., E. Bitocchi, D. Rau, M. Rodriguez, E. Biagetti, A. Giardini et al. 2014. Genomics of origin, domestication and evolution of *Phaseolus vulgaris*. Genomics of plant genetic resources. Springer 1. 483-507.

Broughton, W. J., G. Hernandez, M. Blair, S. Beebe, P. Gepts and J. Vanderleyden. 2003. Beans (*Phaseolus spp.*)–model food legumes. Plant and Soil 252: 55-128.

Campbell, C. L. and L. V. Madden. 1990. Introduction to plant disease epidemiology. John Wiley and Sons 38.187-188.

Cichy, K. A., T. G. Porch, J. S. Beaver, P. Cregan, D. Fourie, R. P. Glahn et al. 2015. A *Phaseolus vulgaris* diversity panel for Andean bean improvement. Crop Science 55: 2149-2160.

Dlamini, T., L. Nalley, F. Tsiboe, A. Barkley and A. Shew. 2017. The economic impact of the South African Agricultural Research Council's dry beans breeding program. Journal of Agricultural and Applied Economics 49: 232-250.

FAO, I. 2014. WFP. The State of Food Insecurity in the World 2014 strengthening the enabling environment for food security and nutrition. Rome. Italy.

Fourie, D. 2002. Distribution and severity of bacterial diseases on dry beans (*Phaseolus vulgaris L*.) in South Africa. Journal of Phytopathology 150: 220-226.

Gilmour, A., B. Gogel, B. Cullis, S. Welham and R. Thompson. 2015. Asreml user guide release and structural specification. VSN International Limited 4: 7-9.

Harveson, R. M. and H. F. Schwartz. 2007. Bacterial diseases of dry edible beans in the Central High Plains. Plant Health Prog 10: 1094.

Harveson, R. M., H. F. Schwartz, C. A. Urrea and C. D. Yonts. 2015. Bacterial wilt of dry-edible beans in the central high plains of the US: past, present, and future. Plant Disease 99: 1665-1677.

Katoh, K., K. Misawa, K. i. Kuma and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059-3066.

Kimani, P., R. Buruchara, K. Ampofo, M. Pyndji, R. Chirwa and R. Kirkby. 2005. Breeding beans for smallholder farmers in Eastern, Central, and Southern Africa: Constraints, achievements, and potential. PABRA Millennium Workshop: p. 11.

Muedi, H. T., D. Fourie and N. W. McLaren. 2015. Distribution and severity of bacterial brown spot on dry beans in South Africa: an update. South African Journal of Science 111: 1-6.

Muedi, H. T. H. 2015. Reaction of selected common bean germplasm accessions to bacterial brown spot in South Africa. Crop Protection 77: 87-93.

Navarro, F., P. Skroch, G. Jung and J. Nienhuis. 2007. Quantitative trait loci associated with bacterial brown spot in loci. Crop Science 47: 1344-1353.

Payne, S. L. B. 2014. The Art of Asking Questions: Studies in Public Opinion, 3th ed. Princeton University Press. Pakistan.

Petersen, S., J. H. Lyerly, M. L. Worthington, W. R. Parks, C. Cowger, D. S. Marshall et al. 2015. Mapping of powdery mildew resistance gene Pm53 introgressed from Aegilops speltoides into soft red winter wheat. Theoretical and applied genetics 128: 303-312.

Serfontein, J. 1994. Occurrence of bacterial brown spot of dry beans in the Transvaal province of South Africa. Plant pathology 43: 597-599.

Singh, S. P., J. Gutierrez, A. Molina, C. Urrea and P. Gepts. 1991. Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. Crop Science 31: 23-29.

Singh, S. P. and P. N. Miklas. 2015. Breeding common bean for resistance to common blight: A review. Crop Science 55: 971-984.

Singh, S. P. and H. F. Schwartz. 2010. Breeding common bean for resistance to diseases (review). Crop Science 50: 2199-2223.

3.6 Appendices

Appendix 3.1: The genetic material screened for resistance to bacterial brown spot (BBS) across three sites in South Africa

Entry	ADP	Seed size	Growth Habit	Entry	ADP	Seed size	Growth habit	Entry	ADP	Seed size	Growth habit
1	3	medium	Vine	142	279	Large	Bush	283	609	large	Bush
2	4	Large	Bush	143	280	Large	Bush	284	610	large	Bush
3	5	medium	Vine	144	288	medium	Vine	285	611	large	Bush
4	6	Large	Bush	145	303	Large	Bush	286	612	medium	Vine
5	7	medium	Vine	146	310	medium	Vine	287	613	medium	Bush
6	8	medium	Vine	147	324	medium	Vine	288	615	medium	Vine
7	9	medium	Vine	148	337	medium	Vine	289	616	medium	Vine
8	11	medium	Vine	149	345	medium	Vine	290	617	medium	Vine
9	12	medium	Vine	150	354	medium	Vine	291	618	medium	Vine
10	13	Large	Bush	151	355	Large	Bush	292	619	medium	Vine
11	14	medium	Vine	152	367	medium	Vine	293	620	medium	Vine
12	15	medium	Bush	153	368	medium	Vine	294	621	medium	Vine
13	16	medium	Vine	154	376	medium	Vine	295	622	large	Bush
14	17	medium	Vine	155	379	medium	Vine	296	623	large	Bush
15	18	medium	Vine	156	383	medium	Vine	297	624	medium	Vine
16	19	Large	Vine	157	391	medium	Vine	298	625	large	Bush
17	21	medium	Vine	158	392	medium	Vine	299	626	medium	Vine
18	22	medium	Vine	159	413	medium	Vine	300	629	medium	Vine
19	23	medium	Bush	160	417	medium	Vine	301	630	medium	Bush
20	24	medium	Vine	161	428	medium	Vine	302	631	medium	Vine
21	25	medium	Vine	162	429	medium	Vine	303	633	medium	Bush
22	26	medium	Vine	163	430	medium	Bush	304	634	large	Bush
23	27	medium	Bush	164	431	medium	Vine	305	635	medium	Vine
24	28	medium	Vine	165	432	medium	Vine	306	636	medium	Vine
25	29	medium	Bush	166	435	medium	Vine	307	637	medium	Vine
26	30	medium	Bush	167	438	medium	Vine	308	638	large	Bush
27	31	Large	Bush	168	439	Large	Bush	309	639	medium	Bush
28	32	medium	Vine	169	440	medium	Vine	310	640	medium	Vine
29	33	medium	Vine	170	441	medium	Vine	311	641	medium	Vine
30	34	medium	Vine	171	442	medium	Vine	312	642	large	Bush
31	35	medium	Vine	172	443	medium	Vine	313	644	medium	Vine
32	36	medium	Vine	173	445	medium	Vine	314	645	medium	Bush
33	37	medium	Vine	174	446	medium	Vine	315	646	medium	Vine
34	38	medium	Vine	175	447	medium	Vine	316	647	medium	Vine
35	40	Large	Vine	176	449	medium	Vine	317	648	large	Bush
36	41	Large	Vine	177	450	medium	Vine	318	649	medium	Vine
37	42	medium	Vine	178	452	Large	Bush	319	650	medium	Vine
38	43	medium	Vine	179	453	medium	Vine	320	652	large	Vine
39	44	medium	Vine	180	454	medium	Vine	321	653	medium	Vine

Entry	ADP	Seed size	Growth Habit	Entry	ADP	Seed size	Growth habit	Entry	ADP	Seed size	Growth habit
40	45	medium	Bush	181	455	medium	Vine	322	654	medium	Vine
41	46	medium	Vine	182	456	medium	Vine	323	655	medium	Bush
42	48	medium	Vine	183	457	medium	Vine	324	656	medium	Bush
43	49	Large	Bush	184	458	medium	Vine	325	657	large	Vine
44	50	medium	Vine	185	459	Large	Bush	326	658	medium	Vine
45	51	medium	Vine	186	460	Large	Bush	327	659	large	Vine
46	52	medium	Vine	187	461	medium	Vine	328	660	medium	Vine
47	53	Large	Bush	188	462	medium	Vine	329	661	medium	Vine
48	54	medium	Bush	189	464	medium	Vine	330	662	large	Vine
49	55	medium	Vine	190	465	medium	Vine	331	663	medium	Vine
50	56	medium	Vine	191	466	medium	Vine	332	664	large	Vine
51	57	medium	Vine	192	467	medium	Vine	333	665	medium	Vine
52	85	medium	Vine	193	468	medium	Vine	334	666	medium	Vine
53	59	medium	Bush	194	469	Large	Bush	335	668	medium	Vine
54	60	medium	Bush	195	470	medium	Vine	336	670	medium	Vine
55	61	medium	Bush	196	471	medium	Vine	337	672	large	Bush
56	62	medium	Bush	197	472	medium	Vine	338	673	medium	Bush
57	63	medium	Bush	198	473	medium	Vine	339	674	medium	Vine
58	64	medium	Bush	199	474	medium	Vine	340	675	medium	Vine
59	65	Large	Bush	200	475	Large	Bush	341	677	medium	Vine
60	66	medium	Bush	201	476	medium	Vine	342	678	medium	Vine
61	67	Large	Bush	202	478	medium	Vine	343	679	large	Vine
62	69	medium	Bush	203	479	medium	Vine	344	680	medium	Vine
63	70	medium	Bush	204	481	medium	Vine	345	681	medium	Vine
64	71	medium	Bush	205	482	medium	Vine	346	682	medium	Vine
65	72	Large	Bush	206	483	medium	Vine	347	683	medium	Vine
66	74	Large	Bush	207	508	medium	Vine	348	684	medium	Vine
67	75	medium	Bush	208	510	medium	Vine	349	685	medium	Vine
68	76	medium	Bush	209	512	medium	Vine	350	686	medium	Vine
69	77	medium	Bush	210	513	medium	Vine	351	716	large	Vine
70	78	medium	Bush	211	514	Large	Bush	352	717	large	Vine
71	79	medium	Vine	212	515	medium	Vine	353	718	large	Vine
72	81	medium	Vine	213	516	medium	Vine	354	719	medium	Vine
73	82	Large	Bush	214	517	medium	Vine	355	720	medium	Vine
74	83	medium	Vine	215	518	medium	Vine	356	721	medium	Vine
75	84	Large	Bush	216	520	medium	Vine	357	722	medium	Vine
76	85	medium	Bush	217	521	medium	Vine	358	723	medium	Vine
77	86	medium	Bush	218	522	medium	Vine	359	724	medium	Vine
78	87	medium	Bush	219	523	medium	Vine	360	725	medium	Vine
79	88	medium	Vine	220	524	Large	Bush	361	726	medium	Vine
80	89	medium	Vine	221	525	Large	Bush	362	727	medium	Vine
81	90	medium	Bush	222	527	medium	Vine	363	728	medium	Vine
82	92	medium	Bush	223	528	medium	Vine	364	729	medium	Vine
83	93	Large	Bush	224	529	medium	Vine	365	730	medium	Vine

Entry	ADP	Seed size	Growth Habit	Entry	ADP	Seed size	Growth habit	Entry	ADP	Seed size	Growth habit
84	94	Large	Bush	225	530	medium	Bush	366	731	medium	Vine
85	95	Large	Bush	226	531	medium	Vine	367	732	medium	Vine
86	96	medium	Vine	227	532	medium	Vine	368	733	large	Bush
87	97	medium	Vine	228	534	Large	Bush	369	734	medium	Vine
88	98	medium	Vine	229	535	medium	Vine	370	735	medium	Vine
89	99	medium	Bush	230	536	medium	Vine	371	736	medium	Vine
90	100	medium	Vine	231	537	medium	Vine	372	737	medium	Vine
91	101	medium	Bush	232	538	medium	Vine	373	739	large	Vine
92	102	medium	Vine	233	540	medium	Vine	374	740	medium	Vine
93	103	medium	Vine	234	543	medium	Vine	375	741	medium	Vine
94	105	medium	Vine	235	544	medium	Vine	376	742	medium	Vine
95	10.6	medium	Bush	236	545	medium	Vine	377	743	medium	Vine
96	107	medium	Bush	237	546	Large	Vine	378	744	medium	Vine
97	108	medium	Bush	238	549	medium	Vine	379	745	medium	Vine
98	109	medium	Bush	239	550	Large	Bush	380	746	large	Vine
99	110	medium	Vine	240	551	medium	Vine	381	747	medium	Vine
100	111	Large	Bush	241	554	medium	Vine	382	748	medium	Vine
101	113	medium	Vine	242	555	medium	Vine	383	750	medium	Bush
102	117	medium	Vine	243	556	medium	Vine	384	751	medium	Vine
103	118	medium	Vine	244	557	medium	Vine	385	752	medium	Vine
104	119	medium	Vine	245	559	medium	Vine	386	753	medium	Vine
105	120	medium	Vine	246	560	medium	Vine	387	757	medium	Vine
106	121	medium	Vine	247	561	medium	Vine	388	758	large	Vine
107	122	medium	Vine	248	562	medium	Vine	389	759	medium	Vine
108	123	medium	Vine	249	564	medium	Vine	390	760	large	Vine
109	125	medium	Vine	250	566	medium	Vine	391	761	medium	Vine
110	126	medium	Vine	251	567	medium	Vine	392	762	medium	Vine
111	127	medium	Vine	252	570	medium	Vine	393	765	medium	Vine
112	166	medium	Vine	253	571	medium	Vine	394	767	medium	Vine
113	180	Large	Bush	254	572	medium	Vine	395	768	large	Vine
114	183	medium	Vine	255	574	medium	Vine	396	769	medium	Vine
115	186	medium	Vine	256	575	medium	Bush	397	770	large	Vine
116	188	Large	Bush	257	576	medium	Vine	398	771	medium	Vine
117	190	medium	Vine	258	577	medium	Vine	399	772	medium	Vine
118	192	Large	Bush	259	578	medium	Vine	400	773	medium	Vine
119	199	medium	Vine	260	579	Large	Bush	401	774	medium	Vine
120	203	Large	Vine	261	580	Large	Bush	402	775	medium	Vine
121	205	medium	Vine	262	581	medium	Vine	403	776	medium	Vine
122	206	medium	Vine	263	583	medium	Vine	404	777	large	Bush
123	207	Large	Vine	264	585	Large	Bush	405	778	large	Vine
124	208	medium	Vine	265	586	Large	Vine	406	779	medium	Vine
125	211	medium	Vine	266	587	Large	Bush	407	780	medium	Vine
126	212	medium	Vine	267	589	medium	Vine	408	781	medium	Vine
127	213	medium	Vine	268	590	Large	Bush	409	783	medium	Vine

Entry	ADP	Seed size	Growth Habit	Entry	ADP	Seed size	Growth habit	Entry	ADP	Seed size	Growth habit
128	214	medium	Vine	269	591	medium	Vine	410	784	medium	Vine
129	220	Large	Bush	270	592	Large	Bush	411	785	medium	Vine
130	224	Large	Vine	271	595	medium	Bush	412	786	medium	Vine
131	225	medium	Vine	272	596	medium	Vine	413	788	medium	Vine
132	232	medium	Vine	273	597	medium	Vine	414	789	medium	Vine
133	239	medium	Vine	274	598	Large	Bush	415	790	medium	Vine
134	242	Large	Bush	275	599	Large	Bush	416	791	medium	Vine
135	247	medium	Vine	276	601	medium	Vine	417	792	medium	Vine
136	225	medium	Vine	277	602	Large	Bush	418	793	medium	Vine
137	267	medium	Vine	278	603	medium	Vine	419	794	medium	Vine
138	269	medium	Vine	279	604	Large	Bush	420	795	medium	Vine
139	271	medium	Vine	280	605	Large	Bush	421	796	medium	Vine
140	272	medium	Vine	281	606	Large	Bush	422	797	medium	Vine
141	277	Large	Vine	282	608	medium	Vine	423	798	medium	Vine

The source of origin of Andean Diversity Panel lines (ADP lines): 3-185=Africa, 186-368=CIAT core, 369-433=US core, 434-442=Carribean, 443-481=East Africa, 482-591=CIAT Africa, 592-724=North America, 725-751=East Africa, 752-798=Southern Africa

CHAPTER 4

GRAIN YIELD, STABILITY AND BACTERIAL BROWN SPOT DISEASE OF DARK RED KIDNEY DRY BEAN GENOTYPES ACROSS SIX ENVIRONMENTS IN SOUTH AFRICA

Abstract

Dry bean (*Phaseolus vulgaris L*.) is grown under an extensive range of agro-climatic conditions and is an important source of protein and income globally. This study aimed to identify and evaluate yield performance, stability and bacterial brown spot (BBS) disease resistance of fourteen Dark Red Kidney genotypes across environments Carolina, Clarens, Cedara, Middelburg, Potchefstroom and Warden in South Africa. The univariate and multivariate models, additive main effect multiplicative interaction (AMMI) and genotype plus genotypes by environment interaction-GGE biplot analysis were used to evaluate the grain yield performance, stability and BBS disease resistance. The AMMI analysis of variance revealed that mean squares for grain yield and BBS severity for environment, genotype and genotype by environment interaction were highly significant ($P<0.001$). The interaction principal components (IPCA1 - 4) for grain yield and IPCA1 for BBS severity were highly significant ($P<0.001$, $P<0.01$). Genotype G12 (1.46 t ha^{-1}) showed a broad adaptation for both high grain yield, low BBS severity and was stable across six environments, while genotypes G08 (1.77), G06 (1.70), G03 (1.62), G02 (1.56), G05 (1.48) and G04 (1.45 t ha^{-1}) had specific adaption for high grain yield, low BBS severity and were unstable. These genotypes recorded mean grain yield above the grand mean and the best performing cultivar both with (1.43 t ha^{-1}), and BBS severity below the grand mean (31.90) and the best performing cultivar (48.89). The GGE biplot identified three mega-environments for grain yield and BBS severity across the six environments. The AMMI analysis and GGE biplot found similar mean performance and stability of the genotypes over six sites. These genotypes can be released for broad and specific adaptation across tested environments and similar environments or can be used as parents in a breeding programme to improve the grain yield and BBS disease resistance of dry bean for farmers in South Africa.

Keys: AMMI stability value (ASV), Grain yield and BBS severity and broad and specific adapted

4.1 Introduction

Dry bean (*Phaseolus vulgaris L*.) (2n=2x=22), is the third important source of protein and income crops worldwide and is produced under an extensive series of agro-climatic conditions, surpassed only by soybean *(Glycine max (L.) Merr*.) and peanut (*Arachis hypogea L*.) (González *et al.*, 2006; Dia *et al.*, 2016). The crop is grown between 52 ^{o} N and 32 ^{o} S up to an altitude of 3000 m (Kimani *et al.*, 2005). Southern and Eastern Africa are main production regions with approximately 3.7 million ha of arable land per year under dry beans (Kimani *et al.*, 2005). In South Africa this crop is largely grown in the Free State (43%), Mpumalanga (23%), Limpopo (10%) provinces with the remaining produced in the KwaZulu-Natal, Gauteng, North West and Eastern Cape provinces (Muedi *et al.*, 2015). These agro-ecological regions are different in terms of temperature, rainfall and soil fertility (Muedi *et al.*, 2015). The mean grain yield in South Africa is 1.40 t ha^{-1} (Dlamini *et al.*, 2017). This mean grain yield is low compared with North America (~ 3.00 t ha^{-1}) (Kimani *et al.*, 2005; FAO, 2014). The grain yield losses caused by *Pseudomonas syringae* pv.*syringae* (*Pss*) can be up to 55% (Serfontein, 1994; Muedi *et al.*, 2015). The most popular grain types grown in South Africa are red speckled sugar (75% of the local market share) and small white canning beans (20% of the local market share), with the large white kidney beans, alubia, painted lady and cariocas making up the niche markets (Muedi, 2015). The genotype by environment interaction (GEI) reveals the changes of comparative performance of genotypes over sites because of genotype, the environment, or both (Mortazavian *et al.*, 2014). The GEI complicates the identification and selection of suitable genotypes for specific environment or across environments. The GEI analysis is used to identify lines that perform consistently well over a range of sites for broad and specific adaption (Dia *et al.*, 2016). The statistical methods for stability evaluation include univariate and multivariate analysis (Chipeta *et al.*, 2017). The AMMI analysis is used for quantifying GEI which has the capacity to extract genotype and environment effect, and uses the interaction of principal component analysis (Abuali *et al.*, 2014; Oladosu *et al.*, 2017). The failure of the AMMI analysis to generate expectation for stability measure (ranking genotypes) guided or directed to the development of the AMMI stability value (ASV) (Purchase *et al.*, 2000). A lower ASV reveals that a genotype has a wide adaptation and higher ASV reveals that a genotype has specific adaptation (Agyeman *et al.*, 2015). The genotype main effect plus genotype by environment interaction biplot is multivariate analysis tool, which is based on the genotype mean performance and stability over a range of sites (Dia *et al.*, 2016; Oladosu *et al.*, 2017). Broad and specific adapted dry bean cultivars with bacterial brown spot (BBS) disease resistance would offer South African farmers a sustainable way of improving yields. This study aimed to evaluate the grain yield performance, stability parameters and resistance

to bacterial brown spot (BBS) disease of Dark Red Kidney (DRK) dry bean lines across six environments in South Africa.

4.2 Methodology

4.2.1 Genetic material

Twelve Dark Red Kidney (DRK) dry bean lines, coded G01-G12 from the Agricultural Research Council-Grain Crops Institute Breeding Program (ARC-GCI-BP) were included (Table 4.1). All genetic materials had red seed and a Type II growth habit as described in the field book evaluation (Agricultural Research Council-GCIP, 2018). The type II is indeterminate and has an erect growth habit with an erect stem with more nodes and internodes than type I, and continues to grow during flowering (Kwak *et al.*, 2012). The main selection criteria were yield, resistance to shattering, lodging, disease resistance and seed quality. Genotypes G13 and G14, well known dark red kidney beans in the USA known as Montcalm and AC Calmont, respectively, were included as checks (Table 4.1).

Table 4.1 Genetic material used in this study

No	Line ID	Seed colour	100 seed weight (g)	Growth habit	Origin
1	G01	Red	56.40	Type II	ARC-GCI-BP
2	G02	Red	61.13	Type II	ARC-GCI-BP
3	G03	Red	55.83	Type II	ARC-GCI-BP
4	G04	Red	63.37	Type II	ARC-GCI-BP
5	G05	Red	50.83	Type II	ARC-GCI-BP
6	G06	Red	62.70	Type II	ARC-GCI-BP
7	G07	Red	53.63	Type II	ARC-GCI-BP
8	G08	Red	52.67	Type II	ARC-GCI-BP
9	G09	Red	51.57	Type II	ARC-GCI-BP
10	G10	Red	55.27	Type II	ARC-GCI-BP
11	G11	Red	68.57	Type II	ARC-GCI-BP
12	G12	Red	72.30	Type II	ARC-GCIP-BP
13	G13 (Montcalm)	Red	69.60	Type II	ARC-GCIP
14	G14 (AC Calmont)	Red	55.40	Type II	ARC-GCIP

Source: Agricultural Research Council-GCIP (2018), ARC-GCI-BP= Agricultural Research Council-Grain Crops Institute Breeding Program and ARC-GCIP= Agricultural Research Council-Grain Crops Institute Program.

4.2.2 Experimental sites and the weather data

The experimental sites, weather data, altitude, latitude and longitude are indicated in Table 4.2. The localities were Warden and Clarens (Eastern Free State), Middelburg and Carolina (Mpumalanga), Cedara (KwaZulu-Natal) and Potchefstroom (North West). The mean temperature was lowest at Carolina (24.4^{o}) and highest at Middelburg (28.2^{o}C), whereas Middelburg had the lowest rainfall (349 mm) and Cedara had the highest rainfall (931 mm).

Table 4.2 Experimentals sites and the weather data across six environments

Month	North West				Free-State		KwaZulu-Natal		Mpumalanga			
	Potchefstroom		Warden		Clarens		Cedara		Middelburg		Carolina	
	Temp	Rain	Temp	Rain	Temp	Rain	Temp	Rain	Temp	Rain	Temp	Rain
Oct-17	26.39	56.13	24.89	49.53	23.40	59.00	22.70	145.00	24.90	28.45	24.10	4.00
Nov-17	29.12	69.34	27.05	82.04	25.30	46.00	24.10	136.00	29.30	19.56	25.50	54.00
Dec-17	29.29	62.48	26.06	208.79	25.70	117.00	23.50	98.00	32.01	10.03	25.10	155.00
Jan-18	31.04	47.24	28.39	111.49	27.40	114.00	27.10	65.00	32.74	111.51	26.50	89.00
Feb-18	27.68	68.33	26.75	69.80	25.40	53.00	26.50	228.00	30.68	77.47	25.80	79.00
Mar-18	27.54	58.93	23.63	131.52	23.30	146.00	25.70	156.00	29.21	22.10	24.80	119.00
Apr-18	25.33	35.56	22.90	12.95	22.10	18.00	25.30	65.00	24.75	62.74	23.60	9.00
May-18	22.78	11.18	20.32	25.65	29.10	26.00	21.50	38.00	21.61	14.48	20.10	23.00
Average	27.40	-	25.00	-	25.20	-	24.60	-	28.15	-	24.40	-
Total	-	409.29	-	687.77	-	579.00	-	931.00	-	346.34	-	532.00
Latitude		26.74		28.31		28.5		29.54		31.47		27.95
Longitude		27.08		29.11		28.58		30.26		25.03		29.43
Altitude		1349		1720		1849		1068		1277		1782

Source: Agricultural Research Council (2018). Temp =Temperature (ºC); Rain = The rainfall (mm); Latitude=Latitude (º); Longitude =Longitude (º) and Altitude= meters above the sea level(m).

4.2.3 Experimental design

The experimental design was an alpha-lattice design with three replicates. Each incomplete block (IBLK) had seven plots of DRK dry bean lines. Each row had 75 plants with a 76 cm and 7.5 cm of inter-row and intra-row spacing, respectively. Two border rows were planted around the four sides of the experiment and the weeds were controlled manually. Irrigation was applied whenever required. The fertilizer was applied at a rate of 42.3 kg ha^{-1} N, 22.3 kg ha^{-1} P, and 18.4 kg ha^{-1} K.

4.2.4 Data collection and analysis

The grain yield per plot was weighed and converted to tons per hectare (t ha^{-1}), while the BBS severity was rated and converted to the percentage of leaf area diseased for the total plot using a standardised CIAT scale of 1 (resistant or immune) to 9 (susceptible or disease) (Petersen *et al.*, 2015). The scores transformed into percentages 1 = 5%, 2 =15%, 3 = 25%, 4 = 35%, 5 = 45%, 6 = 55%, 7 = 65%, 8 = 75% and 9 = 85%. Data were analysed using the unbalanced analysis of variance in Genstat 18th edition (Payne et al., 2014), whereas grain yield performance and stability were analysed using univariate and multivariate stability parameters. The means were separated by the least significant difference (LSD) at P = 0.05. The genotypes, environments, replications and blocks were analysed as random effects and the mean performance as fixed effect. The univariate stability parameters such as the cultivar superiority measure, and the Wricke's ecovalence and multivariate parameters such as AMMI analysis and GGE biplot were used for stability analysis.

4.2.5 Analysis of variance

Data analysis were performed using analysis of variance (ANOVA) across locations and at each location (Payne, 2014). The model contains additive terms for main effects of genotype and environment, as well as the genotype by environment interaction term (Equation 4.1).

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \in_{ij} \qquad \text{Equation 4.1}$$

Where Yij is the yield of the genotype i in environment j, μ is overall yield mean, αi is the genotype effects, βj is the environmental effects, $(\alpha\beta)\, ij$ is the effect of interaction between i and j, $\in ij$ is the mean random error of i and j.

4.2.6 Additive main effect and multiplicative interactions

The additive main effect and interaction (AMMI) analysis partitioned the covariance components into additive (ANOVA) and multiplicative (biplot) effects (Gauch Jr, 1988). The biplot allows the visualized relationship between IPCA and the means of genotypes and environment (Gauch and Zobel, 1996). The large IPCA scores, regardless of the signal, revealed the specific adapted genotype and the low IPCA scores (positive/negative) revealed a broad adapted genotype (Gauch, 2006). The opposite signage of IPCA scores shows crossover GEI (Gauch and Zobel, 1996). The model bellow contains additive terms for main effects of genotype and environment together, as well as extra additive terms that accounts for interaction (Payne et al., 2014).

$$Y_{ij} = \mu + g_i + e_j + \sum_{n=1}^{N} \alpha_n \gamma_{in} \delta_{jn} + \in_{ijk} \quad \text{Equation 4.2}$$

Where Y_{ij} is the yield of the genotype (i=1,..,I) in the j environment (j=1,..,J); μ is the grand mean; g_i and e_j are the genotype and environment deviations from the grand mean, respectively; $\boldsymbol{\alpha}_n$ is the eigenvalue of the IPCA analysis axis n; $\boldsymbol{\gamma}_{in}$ and δ_{jn} are the genotype and environment principal components scores for axis n; N is the number of principal components retained in the model and $\in_{ijk}$ is the error term.

4.2.7 AMMI stability value

AMMI stability values (ASV) (Equation 4.3) were used to identify cultivars that showed specific or general adaptation across environments (Purchase, 1997).

$$ASV = \sqrt{\left[\left(\frac{SSIPCA1}{SSIPCA2}\right)(IPCA1\ score)\right]^2 + (IPCA2\ score)^2} \quad \text{Equation 4.3}$$

Where: ASV = AMMI's stability value, SS = sum of squares, IPCA1 = interaction of principal component analysis one and IPCA2 = interaction of principal component analysis two.

4.2.8 Genotype main effect plus genotype by environment interaction

The GGE biplot identifies mega environment (which-won-where), yield performance, stability and BBS disease severity percentage (Yan *et al.*, 2000). The GGE biplot was calculated using the equation 4.4 (Yan *et al.*, 2000).

$$Y_{ij} = \mu + e_j + \sum_{n=1}^{N} \alpha_n \gamma_{in} \delta_{jn} + \in_{ijk} \quad \text{Equation 4.4}$$

Where Y_{ij} is the yield of the i genotype (i=1,..,I) in the j environment (j=1,..,J); μ is then grand mean; e*j* are the environment deviations from the grand mean; $\boldsymbol{\alpha}_n$ is the eigenvalue of the IPCA analysis axis n; $\boldsymbol{\gamma}_{in}$ and δ_{jn} are the genotype and environment principal components scores for axis n, respectively; N is the number of principal components retained in the model and $\in_{ijk}$ is the error term.

4.3 Results

4.3.1 Analysis of variance

The analysis of variance across sites indicated that the mean squares for genotypes, environments and genotype by environment interaction (GEI) were significant (P<0.001) for grain yield and BBS severity (Table 4.3). The environmental mean squares had the highest total sum of squares (68.29) followed by GEI (15.81) and lastly genotype (5.62%), while the BBS severity was partitioned as 4.26%, 65.84 and 9.13 for environment, genotype and GEI, respectively (Table 4.3).

Table 4.3 Analysis of variance for grain yield and BBS severity of dark red kidney dry bean across six environments

Source		Grain yield (t ha^{-1})		BBS severity	
	DF	SS	MS	SS	MS
Environments	5	102.38	20.48***	2082.94	416.59***
Env. Rep	12	2.20	0.18**	691.67	57.64
Env. Rep. Block	18	2.90	0.16**	3660.71	203.37**
Genotype	13	8.43	0.65***	32164.91	2474.22***
GEI	65	23.70	0.37***	4462.06	68.65**
Residual	138	10.32	0.08	5794.46	41.99
Total	251	149.93	0.6	48856.75	194.65
LSD 5%			0.44		10.45
CV			19.11		19.32
Mean			1.43		33.53
SE			0.22		5.29

* P < 0.05, ** P < 0.01, *** P < 0.001, DF = Degrees of freedom, CV = Coefficient of variation, SS = Sum of squares, MS = Mean of squares, BBS=Bacterial brown spot, GEI=Genotype by environment interaction, LSD=Least significance difference, Grain yield=Grain yield (t ha^{-1}) and SE=Standard error of difference between predicted means, Env=Environment, Rep=Replication.

4.3.2 Mean grain yield and BBS severity across six environments

The grain mean and BBS severity across six environments are exposed in Table 4.4. The mean grain yield of genotypes and BBS severity were 1.43 t ha^{-1} and 33.53, respectively. The least significance difference (LSD) for grain yield and BBS severity were 0.44 and 10.45, respectively. The dry bean genotypes performed differently across the locations. Potchefstroom had the highest mean yield and the lowest BBS severity and Middelburg had the lowest mean yield and the highest BBS severity. Genotypes G08 (1.77), G06 (1.70), G03 (1.62), G02 (1.56), G05 (1.48), G12 (1.46), and G04 (1.45 t ha^{-1}) had a grain above the grand mean and the best performing cultivar (both 1.43 t ha^{-1}) and a BBS severity less the grand mean (31.90) and the best performing cultivar (48.89) across six environments.

Table 4.4 Mean grain yield and BBS severity across six environments

Genotypes	North West		Free-State				KwaZulu-Natal		Mpumalanga				Mean grain yield	Mean BBS severity
	Potchefstroom		Warden		Clarens		Cedara		Middelburg		Carolina			
	Yield	BBS Severity	Yield	BBS Severity	Yield	BBS severity	Yield	BBS severity	Yield	BBS severity	Yield	BBS severity		
G01	2.79	48.33	1.50	60.00	1.31	48.33	1.13	45.00	0.87	60.00	0.64	40.00	1.37	46.39
G02	2.83	21.67	1.05	20.00	1.12	21.67	1.28	28.33	1.79	18.33	1.27	23.33	1.56	22.22
G03	3.02	18.33	1.25	33.33	1.24	25.00	1.00	23.33	2.02	25.00	1.21	23.33	1.62	24.72
G04	2.67	21.67	1.26	28.33	1.69	25.00	1.15	21.67	1.05	25.00	0.88	26.67	1.45	24.72
G05	2.81	28.33	1.20	20.00	1.09	28.33	1.16	20.00	1.53	21.67	1.11	25.00	1.48	23.89
G06	2.74	21.67	1.57	25.00	1.23	18.33	1.30	23.33	2.01	21.67	1.34	30.00	1.70	23.33
G07	3.41	25.00	1.44	28.33	1.06	31.67	0.10	33.33	0.68	35.00	0.53	26.67	1.35	30.00
G08	3.02	21.67	1.34	20.00	1.42	18.33	1.20	25.00	1.20	25.00	2.42	20.00	1.77	21.67
G09	2.40	25.00	1.02	28.33	0.84	26.67	0.94	28.33	0.51	31.67	1.10	25.00	1.13	27.50
G10	3.24	35.00	1.42	51.67	0.55	33.33	0.38	41.67	0.47	53.33	0.43	26.67	1.08	35.56
G11	2.91	41.67	1.05	60.00	1.18	40.00	1.23	43.33	0.53	56.67	0.76	38.33	1.28	39.44
G12	2.48	25.00	1.30	36.67	1.26	20.00	1.25	25.00	1.30	31.67	1.15	35.00	1.46	28.89
G13	2.96	51.67	1.07	53.33	0.94	51.67	1.41	48.33	0.70	58.33	1.04	50.00	1.35	48.89
G14	2.53	45.00	1.68	60.00	2.09	46.67	0.94	55.00	0.67	61.67	0.67	53.33	1.43	49.44
Mean	2.84	30.72	1.30	37.50	1.22	31.07	1.03	32.98	1.10	37.50	1.04	31.67	1.43	31.90

Yield=Grain yield (t ha^{-1}), BBS=Bacterial brown spot, G01-G14=Genotypes

4.3.3 AMMI analysis for grain yield and bacterial brown spot severity

The AMMI analysis of variance with four principal components analysis (IPCAs) for grain yield and one for BBS severity are shown in Table 4.5. The IPCA 1-4 axes were significant (P<0.001, P<0.01) and explained 43.56%, 23.47%, 21.72% and 8.19% for grain yield of the total GEI sum of squares, respectively. The four IPCAs accounted for 96.94% of GEI and the residual 3.06% the GEI sum of squares was not significant. The mean squares for IPCA 1 and IPCA 2 cumulatively contributed 67.03% of the total GEI. The IPCA1 explained 61.13% for BBS severity of the total GEI sum of squares and the residual 38.38% was not significant.

Table 4.5 AMMI analysis of variance for grain yield and bacterial brown spot severity of DRK dry bean lines across six environments

Source	Grain yield				BBS severity			
	DF	MS	Treat exp (%)	GEI explained (%)	DF	MS	Treat exp (%)	GEI exp (%)
Total	251	0.60	-	-	251	194.60	-	
Treatment	83	1.64***	-	-	83	501.10***	-	
Genotype	13	0.68***	6.46	-	13	2658.90***	83.11	
Environment	5	20.48***	75.10	-	5	416.60***	5.01	
Replication	12	0.18**	1.61	-	12	57.60	0.17	
GEI	65	0.39***	18.44	-	65	76.00**	11.88	
IPCA 1	17	0.64***	-	43.56	17	177.70***	-	61.13
IPCA 2	15	0.39***	-	23.47	-	-	-	
IPCA 3	13	0.42***	-	21.72	-	-	-	
IPCA 4	11	0.19**	-	8.19	-	-	-	
Residual	9	0.09	-	3.06	48	40.00	-	38.88
Error	156	0.07	7.61		156	42.10	15.90	

* P < 0.05, ** P < 0.01, *** P < 0.001, DF = Degrees of freedom, CV = Coefficient of variation, SS = Sum of squares, MS = Mean of squares, Threat exp (%) =Treatment explained in %, GEI exp=GEI explained in %, IPCA= Interaction principal component axis scores and BBS=Bacterial brown spot.

4.3.4 Mean yield, BBS severity and AMMI stability value

The mean, IPCAs scores and AMMI stability value (ASV) of grain yield and BBS severity are presented in Table 4.6. The ASV for grain yield ranged between 0.14 (G09) to 1.19

(G14). The grain yield ranged from 1.08 t ha^{-1} (G10) to 1.77 t ha^{-1} (G08), while the BBS severity ranged from G08 (21.67 to G14 (53.06). Genotypes G09 (0.14), G13 (0.23), G04 (0.44), G12 0.46), G05 (0.54), G11 (0.55) and G01 (0.57) revealed broad adaptation, while genotypes G10 (1.09), G07 (1.05), G02 (0.91), G06 (0.88), G03 (0.86) and G08 (0.74) revealed specific adaptation. Furthermore, the genotypes G12 (1.46 t ha^{-1}) had both low ASV for grain yield and BBS severity, while genotype G08 (1.77), G06 (1.70), G03 (1.62), G02 (1.56), G05 (1.48) and G04 (1.45 t ha^{-1}) had high ASV and high grain yield, and revealed specific adaptation.

Table 4.6 The mean, IPCA scores and ASV of grain yield and BBS severity of each DRK dry bean lines evaluated across six environments

Genotype	Grain yield						BBS severity	
	Mean	IPCAg[1]	IPCAg[2]	IPCAg[3]	IPCAg[4]	ASV	Mean	IPCAg[1]
G01	1.37	0.30	-0.09	0.18	0.07	0.57	50.28	-1.92
G02	1.56	-0.49	0.11	0.16	0.18	0.91	22.22	1.96
G03	1.62	-0.45	0.22	0.37	-0.18	0.86	24.72	0.10
G04	1.45	0.12	-0.37	0.16	0.06	0.44	24.72	0.81
G05	1.48	-0.29	0.11	0.15	0.07	0.55	23.89	2.18
G06	1.70	-0.47	0.03	0.33	-0.07	0.88	23.33	1.50
G07	1.35	0.52	0.42	0.05	0.05	1.05	30.00	0.51
G08	1.77	-0.39	-0.13	-0.80	-0.39	0.74	21.67	0.80
G09	1.13	-0.03	-0.13	-0.39	0.03	0.14	27.50	0.35
G10	1.08	0.47	0.64	-0.01	-0.42	1.09	40.28	-2.94
G11	1.28	0.30	-0.02	-0.19	0.36	0.55	46.67	-2.43
G12	1.46	-0.22	-0.23	0.07	0.09	0.46	28.89	0.07
G13	1.35	0.10	0.14	-0.32	0.45	0.23	52.22	0.16
G14	1.43	0.52	-0.7	0.24	-0.29	1.19	53.06	-0.94

IPCA= Interaction principal component axis scores, ASV = AMMI stability value, Mean =Mean grain yield (t ha^{-1}) and BBS=Bacterial brown spot.

4.3.5 Mean yield and AMMI stability value score for environment

The mean yield of genotypes over environments ranged from 1.04 t ha^{-1} at Carolina to 2.84 t ha^{-1} at Potchefstroom (Table 4.7). The grain yield ranking over environments indicated that Potchefstroom had the highest yield (2.84), followed by Warden and Clarens, while Carolina(1.04 t ha^{-1}) had the lowest grain yield. Potchefstroom had the lowest BBS severity (30.71) and Middelburg and Warden had the highest BBS severity (37.50). The AMMI stability value (ASV) for grain yield ranged between 0.15 (Cedara) to 1.66 (Middelburg),

while for BBS severity ranged between 0.64 (Cedara) to -3.17 (Warden) (Table 4.7). Cedara had the lowest ASV for grain yield and BBS severity and was the most stable environment, while Warden, Middelburg and Carolina had the highest ASV and were the least stable environments.

Table 4.7 The mean, IPCAs scores and ASV for grain yield and BBS severity of DRK dry bean lines evaluated in each environment

Environment	Grain yield						BBS severity	
	Mean	IPCAe[1]	IPCAe[2]	IPCAe[3]	IPCAe[4]	ASV	Mean	IPCAe[1]
Carolina	1.04	-0.63	-0.12	-0.85	-0.27	1.17	31.67	2.91
Cedara	1.10	0.04	-0.13	-0.13	0.79	0.15	32.74	0.64
Clarens	1.22	0.41	-0.81	0.20	-0.11	1.11	31.07	1.41
Middelburg	1.10	-0.89	0.21	0.72	-0.05	1.66	37.50	-3.04
Potchefstroom	2.84	0.53	0.82	-0.14	-0.01	1.28	30.71	1.24
Warden	1.30	0.53	0.03	0.19	-0.36	0.99	37.50	-3.17

IPCA= Interaction principal component axis scores, ASV = AMMI stability value, Mean=Mean grain yield (t ha^{-1}) and BBS=Bacterial brown spot.

4.3.6 Rank of genotypes per environment

The best four performing genotypes for grain yield and BBS severity for each environment are exhibited in Table 4.8. The rank of genotypes performance changed across the environments and indicated the crossover GEI for grain yield and BBS severity.

Table 4.8 Ranking of the best four dark red kidney (DRK) dry bean lines for grain yield and BBS disease severity over six environments

Env	Ranking based on grain yield						Ranking based on BBS severity					
	Mean grain yield	IPCA score	1st	2nd	3rd	4th	Mean BBS severity	IPCA score	1st	2nd	3rd	4th
Carolina	1.04	-0.63	G08	G06	G02	G03	31.67	2.91	G13	G14	G01	G11
Cedara	1.10	0.04	G13	G02	G11	G06	32.74	0.64	G14	G13	G01	G11
Clarens	1.22	0.41	G04	G04	G01	G08	31.07	1.41	G13	G14	G01	G11
Middel	1.10	-0.89	G06	G03	G02	G05	37.50	-3.04	G01	G14	G11	G13
Potch	2.84	0.53	G07	G10	G13	G08	30.71	1.24	G13	G14	G01	G11
Warden	1.30	0.53	G14	G07	G08	G01	37.50	-3.17	G01	G14	G11	G13

Env=Environment, Potch=Potchefstroom, Middel=Middelburg, Mean =Mean grain yield (t ha^{-1}) and BBS=Bacterial brown spot.

4.3.7 Mean grain yield vs IPCA1

AMMI 1 with IPCA 1 and grain yield over six environments are presented in Figure 4.1. The genotypes with no or little interactions have low IPCA 1 scores, while larger IPCA scores indicates that they were highly interactive. Environments close to zero have less discriminating ability. High potential environments and high-yielding genotypes are observed in quadrants 2 and 3. Genotypes with IPCA 1 scores close to zero had low interaction over sites, whereas the genotypes with great IPCA 1 scores, either positive or negative signal were greatly interactive. The lower potential environments and the lower yielding genotypes were observed to the left of the vertical line, and in contrast, high yielding genotypes are to the right of the vertical lines. Genotypes G04 and G12 were both high yielding and stable, while genotypes G08, G6, G02, G03 and G05 were high yielding and unstable. Potchefstroom, Middleburg and Clarens were further away from the origin and were therefore unstable environments, though most discriminating.

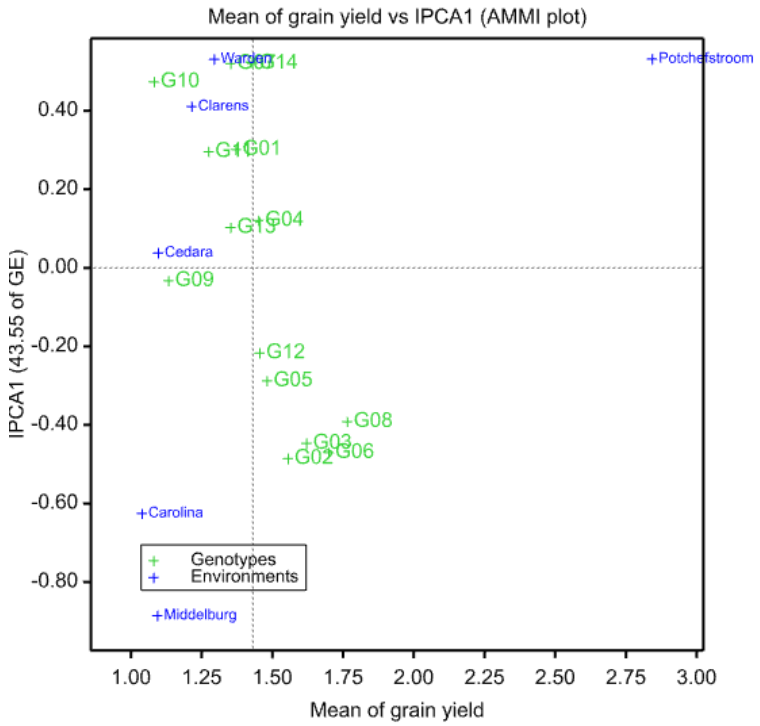


Figure 4.1 AMMI biplot showing the genotype adaptation based on grain yield and IPCAs scores across six environments

4.3.8 Mean bacterial brown spot severity vs IPCA1

AMMI 1 with IPCA 1 and BBS severity across six environments are exposed in Figure 4.2 . The genotypes with no or little interactions have low IPCA 1 scores, while larger IPCA scores indicates that they were highly interactive. Environments close to zero have less discriminating ability. High potential environments and BBS resistant genotypes are observed in quadrants 1 and 4. Genotypes with IPCA 1 scores nearby zero had low interaction over locations, whereas the genotypes with large IPCA 1 scores, either positive or negative signal were highly interactive. The lower potential environments and the genotypes with low BBS severity were observed to the left of the vertical line, and in contrast, genotypes with high BBS severity are to the right of the vertical lines. Several genotypes scattered on the left of the quadrant and were BBS disease resistant. Genotypes G13, G014, G11 and G10 had high BBS severity and were unstable, while G08, G06, G02, G5, G07 and G12 had low BBS severity and were stable. Warden, Middleburg and Carolina were further away from the origin and were therefore very unstable, though most discriminating.

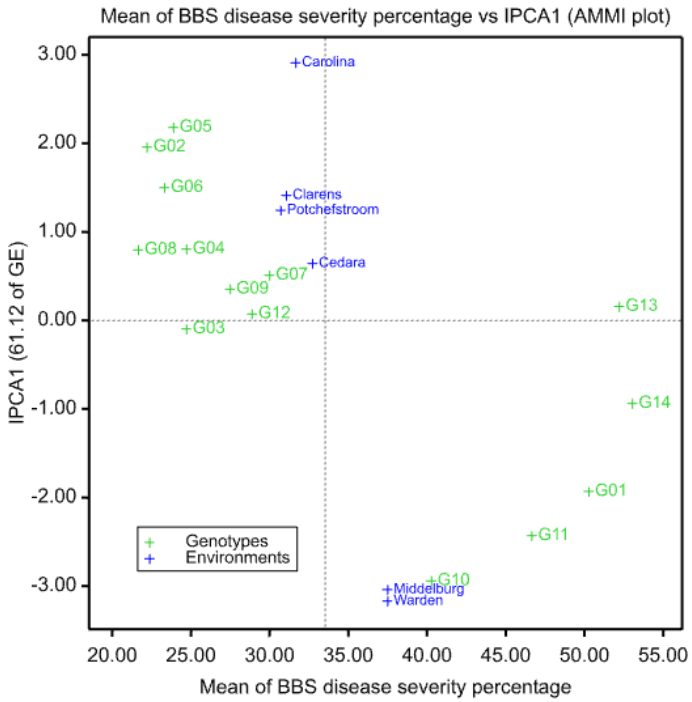


Figure 4.2 AMMI biplot showing the genotype adaptation based on BBS severity across six environments

4.3.9 Mean grain yield of IPCA1 vs IPCA2

The AMMI biplot analysis indicates that the initial two IPCAs scores components explained 67.01% of the total variation (Figure 4.3). Potchefstroom and Clarens had long vectors in the biplot. Cedara had a short vector and was therefore the most stable environment. Genotype G4 had specific adaptation for Clarens. Genotypes G10 and G07 had specific adaptation for Potchefstroom. Genotypes G02, G03, G05 and G06 performed better at Middelburg. Genotypes G11 and G01 performed well at Warden. Genotypes G09, G12, G04 and G05 were close to centre of the biplot and showed broad adaptation (Figure 4.3).

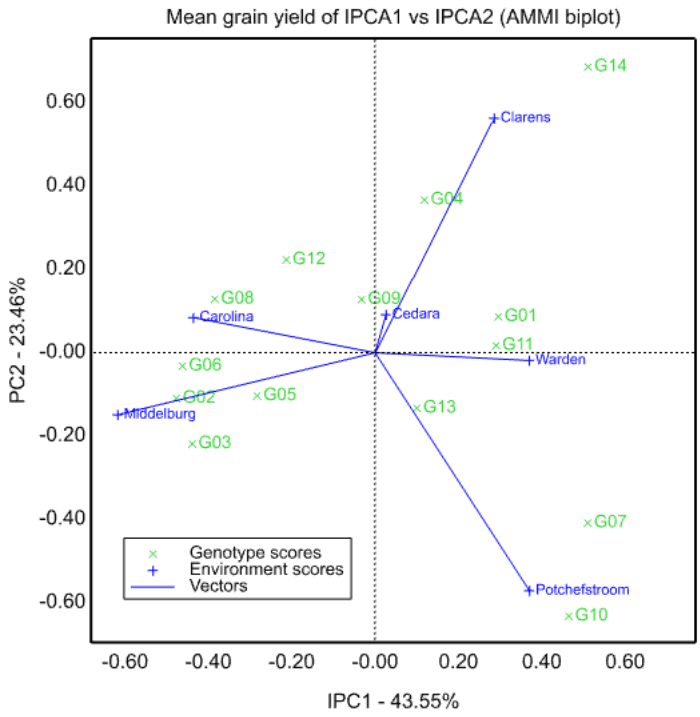


Figure 4.3 Mean IPCA1 and IPCA2 for yield across six environments

4.4 GGE biplot for grain yield and BBS severity

4.4.1 Mega-environments for grain yield

The winning genotypes and mega-environments for grain yield are shown in Figure 4.4. The polygon view was constructed by genotypes G10, G14, G6 and G3, which were furthest from the centre. The IPCA 1 and 2 explained 67.13% of the total variation. The GGE biplot indicated the presence of three mega-environments. Potchefstroom formed its own mega-environment, while Middleburg, Carolina and Cedara formed another one. The last mega-environment comprised Warden and Clarens. The first mega-environment combined environment Potchefstroom with genotypes G07, G10, G13, G11 and G09. The second mega-environment handled environments Cedara, Carolina and Middelburg with genotypes such as G06, G08, G12, G02, G03 and G05 performing well, while G14, G04 and G01 were adapted to the third mega-environment. Genotypes G10 and G14 performed poorly across all environments.

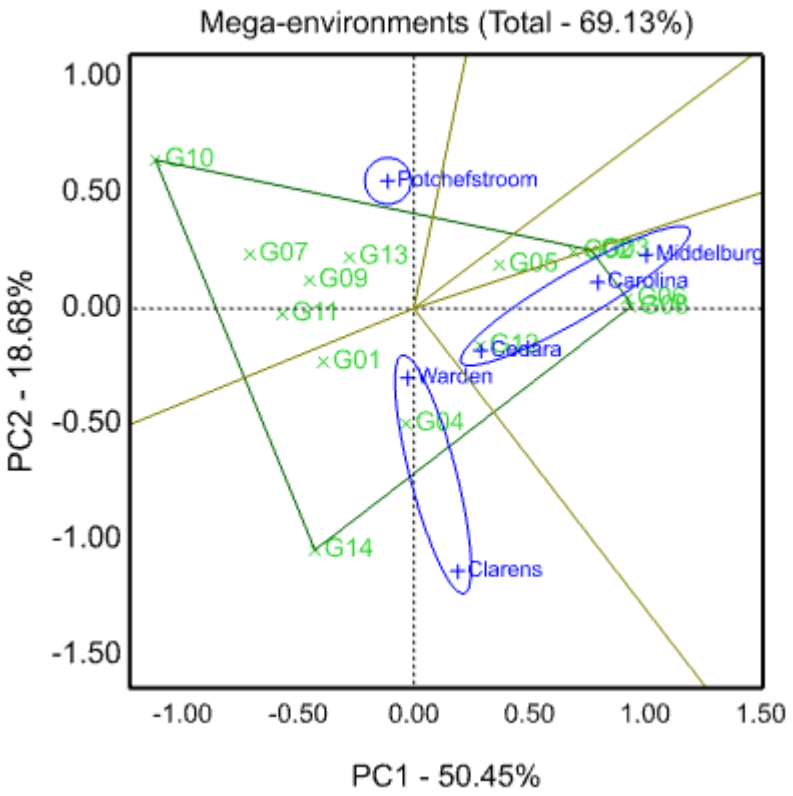


Figure 4.4 Mega-environment for grain yield

4.4.2 Mega-environments for BBS disease severity

The winning genotypes and mega-environments for BBS severity are presented in Figure 4.5. The polygon view was constructed by genotypes G10, G11, G01, G14, G13, G05, G02, G06 and G03, which were furthest from the centre. The IPCA 1 and 2 explained 67.13% of the total variation. The GGE biplot indicated the presence of three mega-environments for BBS severity. Warden formed its own mega-environment, while Carolina, Clarens and Potchefstroom formed another one. The last mega-environment comprised Cedara and Middelburg. The first mega-environment combined environment Warden with genotype G10.

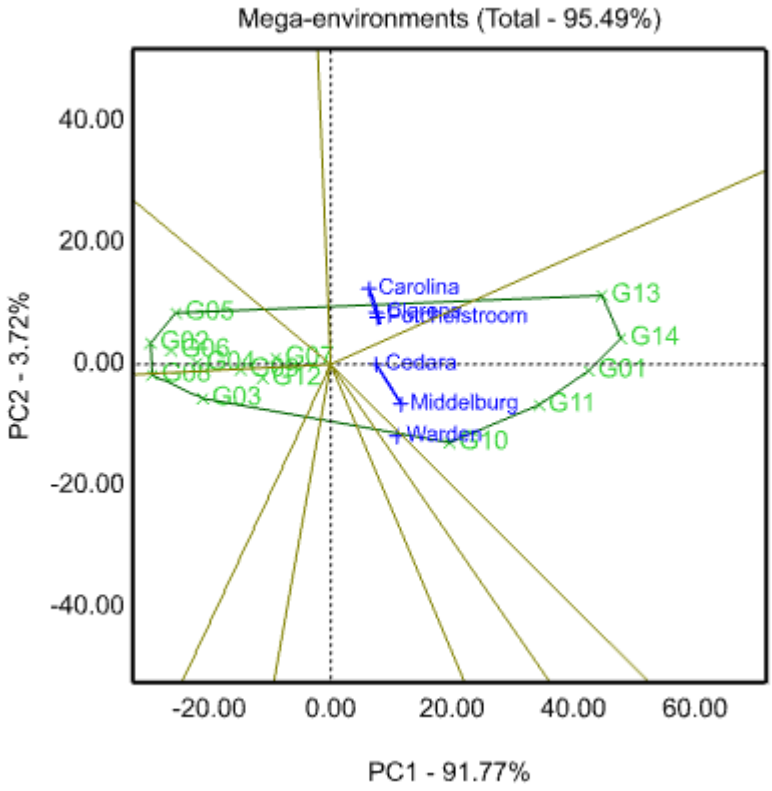


Figure 4.5 Mega-environments for bacterial brown spot severity

4.4.3 Mean grain yield performance vs stability

The GGE biplot shows the average environment coordinate (AEC) abscissa for the genotypes and interaction with the six environments for grain yield (Figure 4.6). The further away the genotype is from the AEC ordinate, the more unstable and vice versa. The ideal genotype had higher mean yield performance and higher stability over environments. Genotypes were observed above and below the AEC ordinate line. Genotypes G04 to G08 were above AEC and had high mean performance, whereas G14 to G10 were below AEC had low mean performance (Figure 4.6). Genotypes such as G09, G12, G07, G13 and G11 had short vectors running from the AEC indicating that they were relatively stable while genotypes G14, G06, G08, G03 and G02 had the longest vectors and highly interactive with the environment.

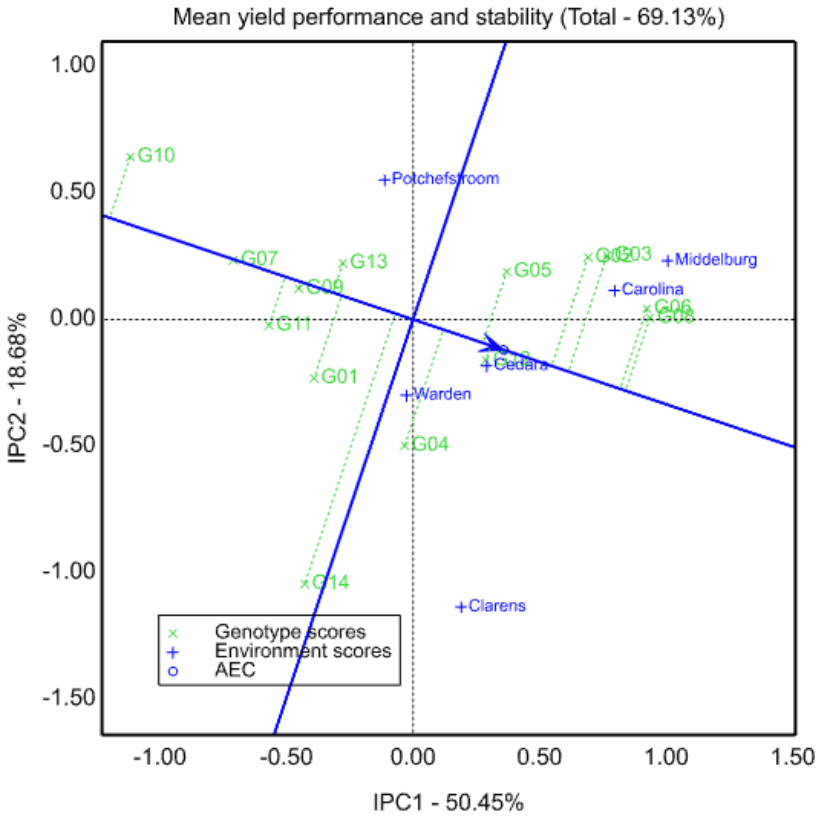


Figure 4.6 Mean grain yield performance and stability across six tested environments

4.4.4 Mean bacterial brown spot severity vs stability

The GGE biplot shows the average environment coordinate (AEC) abscissa for the genotypes and interaction with the six environments for BBS severity (Figure 4.7). The further away the genotype is from the AEC ordinate, the more unstable and vice versa. The ideal genotype had low mean BBS severity and high stability over environments. Genotypes were observed above and below the AEC ordinate line. Genotypes G07 to G08 were below AEC and had low mean BBS severity, while G10 to G14 were above AEC as had high mean BBS severity (Figure 4.7). Genotypes such as G08, G03, G04, G09, G12, G07 and G13 had short vectors running from the AEC indicating that they were relatively stable while genotypes G02, G05, G06, G10, G11, G01 and G14 had the longest vectors and highly interactive with the environment.

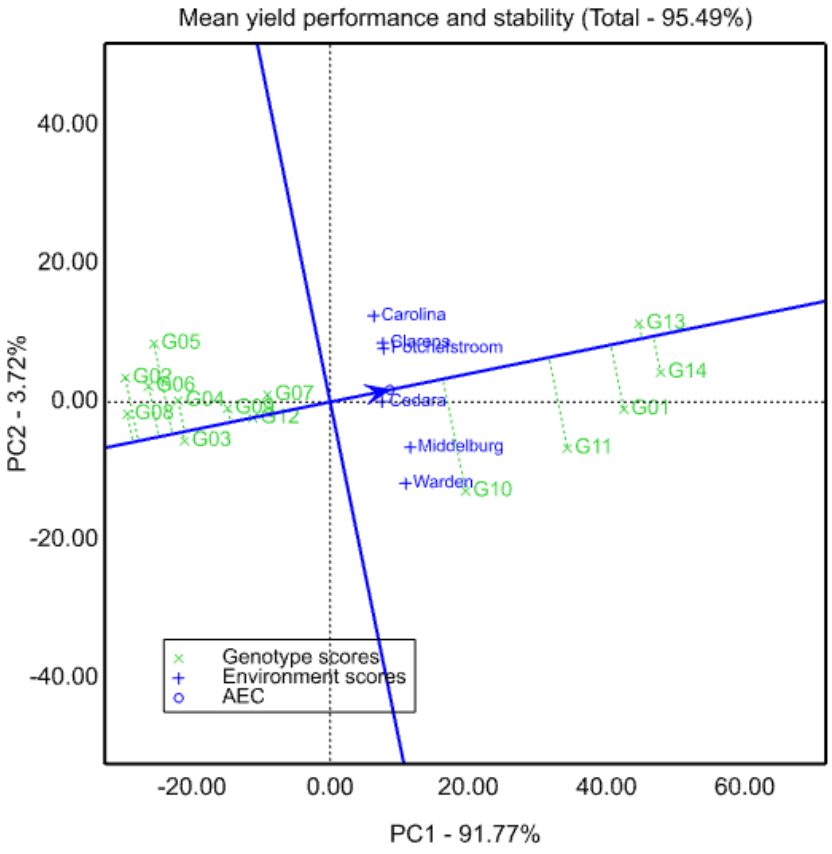


Figure 4.7 Mean bacterial brown spot disease severity and stability over six environments

4.5 Stability coefficients

The yield stability coefficients and ranking of genotypes over six locations are given in Table 4.9 . A genotype was considered stable when the univariate stability coefficient of grain yield did not differ significantly from zero. Large stability coefficients indicated genotypes with specific adaptation to high yielding environments, while low stability coefficient indicated genotypes with broad adaptation over environments. Genotype, G12, had the lowest static stability (0.26) and Wricke's ecovalence stability coefficient (0.20) of grain yield (t ha^{-1}), while genotype G08 had the lowest cultivar superiority, difference of pair of ranks and variances of ranks stability coefficient of grain yield of 0.12, 2.43 and 4.38, respectively (Table 4.9). Genotype G06 had the lowest mean ranks stability coefficient of grain yield of 4.17 (Table 4.9).

Table 4.9 Yield stability coefficients and genotypes ranking over six environments

Gen	EC1	R1	EC2	R2	EC3	R3	EC4	R4	EC5	R5	EC6	R6
G01	0.47	9	0.58	7	0.25	3	7.50	7	4.07	8	11.50	7
G10	0.82	14	12.67	14	11.56	12	3.00	13	5.80	13	30.30	14
G11	0.54	11	0.71	12	0.34	6	9.00	12	4.00	7	10.40	5
G12	0.32	6	0.26	1	0.20	1	6.50	5	3.40	4.5	11.10	6
G13	0.46	8	0.67	11	0.36	7	7.67	9	4.93	11	16.67	11
G14	0.49	10	0.62	10	13.53	14	8.00	10	5.87	14	29.60	13
G02	0.26	4	0.46	6	0.54	8	6.17	4	4.47	10	14.57	10
G03	0.22	3	0.59	9	0.71	10	5.58	3	4.23	9	11.84	8
G04	0.35	7	0.43	4	0.29	5	7.50	7	3.40	4.5	9.10	4
G05	0.30	5	0.45	5	0.21	2	7.50	7	2.87	3	5.50	2
G06	0.20	2	0.34	2	0.63	9	4.17	1	3.67	6	12.17	9
G07	0.55	12	11.18	13	0.77	11	8.33	11	5.47	12	22.27	12
G08	0.12	1	0.59	8	13.22	13	4.25	2	2.43	1	4.38	1
G09	0.61	13	0.43	3	0.26	4	12.33	14	2.53	2	7.07	3

Gen=Genotypes, R1-R6=Ranking one to six, EC1=Cultivar superiority, EC2=Static stability, EC3=Wricke's Ecovalence, EC4=Mean Ranks, EC5=Differences of pairs ranks and EC6=Variances of ranks.

4.6 Discussion and conclusion

This study evaluated fourteen Dark Red Kidney dry bean genotypes for grain yield and BBS disease resistance across six environments. The significance of main effects of genotypes and environments indicated broad adaptation of some genotypes across tested sites, while GEI significance indicated that some genotypes were specifically adapted to certain environments.

The two DRK cultivars used as checks viz. G13 and G14, both had low grain yield of 1.35 and 1.43 t ha^{-1}, respectively, and high BBS severity of 48.89 and 49.44, respectively. Genotypes G12 (1.46 t ha^{-1}) had a high yield, low BBS severity and was stable, and revealed broad adaptation across six environments, while, G08 (1.77), G06 (1.70), G03 (1.63), G02 (1.56), G05 (1.48) and C04 (1.45 t ha^{-1}) had a high grain yield, low BBS severity and was unstable, and therefore revealed specific adaptation. These genotypes had grain yields above the grand mean and the best check both with 1.43 t ha^{-1} and with BBS severity below the grand mean (31.90) and the best performing cultivar (48.89). These genotypes selected for broad and specific adaptation had both high grain yield and low BBS severity than the grand mean and the best local check. Mortazavian *et al.* (2014) indicated that the genotypes with a high mean

performance and low ASV revealed specific adaptation and those that had both high mean performance and high stability, revealed broad adaptation.

The AMMI analysis of variance revealed that the IPCA 1- 4 axes were greatly significant ($P<0.001$, $P<0.01$). Four IPCAs explained 43.56%, 23.47%, 21.72% and 8.19% of the total sum square GEI of DRK dry bean genotypes, respectively. Four IPCAs accounted for 96.94% of GEI and 3.06% of the remained pooled GEI. Mortazavian *et al.* (2014) found that IPCA1 (28.62%), IPCA2 (24.79%), IPCA3 (13.85%) and IPCA4 (10.17%) accounted 77.43 % of the variation of GEI in their analysis of grain and stability in barley tested within sites in Iran. Furthermore, Mohammadi *et al.* (2015) in study of yield stability of durum wheat genotypes, found that IPCA1 (33.74%), IPCA2 (19.00%), IPCA3 (15.48%) and IPCA4 (10.10%) account for 78.32% of the variation of GEI for grain yield in wheat.

The genotype G07 had a positive signal IPCA1 score of 0.52, and had specific adaptation to Potchefstroom with a positive signal IPCA1 score of 0.53 and Warden with a positive signal IPCA1 score of 0.53. Similarly, G02, with a negative signal IPCA1 score of -0.49, had adaptation to Carolina with a negative signal IPCA1 score of -0.63. However, many genotypes showed this relationship between the signal of IPCA1 of genotype and IPCA1 of environment. Karimizadeh *et al.* (2016) reported that genotype with high IPCA1 scores were adapted to specific site with IPCA1 scores of the similar or identical sign.

This study grouped the six environments in three mega-environments. The first mega-environment for grain yield was Potchefstroom with genotypes G07, G10, G13, G11 and G09. The second included Cedara, Carolina and Middelburg with genotypes such as G06, G08, G12, G02, G03 and G05, while genotypes G14, G04 and G01 were adapted to the third mega-environment, which included Clarens and Warden. However, for BBS severity Warden formed its own mega-environment, while Carolina, Clarens and Potchefstroom formed another one, and the last mega-environment included Cedara and Middelburg. Kendal (2016) indicated that sites with identical reactions to genotype performance were assembled together (mega-environments) and substituted the representative environment of the region, in which the genotype was grown.

Genotype G12 (1.46 t ha^{-1}) had both high yield, low BBS severity and stable, and revealed broad adaptation across six environments, while G08 (1.77), G06 (1.70), G03 (1.63), G02 (1.56), G05 (1.48) and C04 (1.45 t ha^{-1}) had high grain yield, low BBS severity and unstable,

and revealed specific adaptation. These genotypes had grain yield above the grand mean and the best performing cultivar both with 1.43 t ha^{-1} and with BBS severity below the grand mean (31.90) and the best performing cultivar (48.89). These genotypes can be recommended as cultivars for release or used as parents in a breeding programme to improve the grain yield and BBS resistance of the dark red kidney beans.

4.7 Reference

Abuali, A. I., A. A. Abdelmula, M. M. Khalafalla, N. B. Hamza, A. H. Abdalla and A. E. Idris. 2014. Assessment of yield stability and adaptability of parental inbred lines and f1-hybrids of grain maize (*Zea mays L.*) Using AMMI Analysis. British Biotechnology Journal 4. 339-349.

Agyeman, A., E. Parkes and B. Peprah. 2015. AMMI and GGE biplot analyses of root yield performance of cassava genotypes in forest and coastal ecologies. International Journal of Agricultural Policy and Research 3: 122-132.

Chipeta, M. M., R. Melis, P. Shanahan, J. Sibiya and I. R. Benesi. 2017. Genotype X environment interaction and stability analysis of cassava genotypes at different harvest times. Journal of Animal and Plant Sciences 27: 901-919.

Dia, M., T. C. Wehner, R. Hassell, D. S. Price, G. E. Boyhan, S. Olson et al. 2016. Genotype× environment interaction and stability analysis for watermelon fruit yield in the United States. Crop Science 56: 1645-1661.

Dlamini, T., L. Nalley, F. Tsiboe, A. Barkley and A. Shew. 2017. The economic impact of the South African Agricultural Research Council's dry beans breeding program. Journal of Agricultural and Applied Economics 49: 232-250.

FAO, I. 2014. WFP. The State of Food Insecurity in the World 2014 strengthening the enabling environment for food security and nutrition. Rome. Italy.

Gauch, H. and R. Zobel. 1996. AMMI analysis of yield trials (genotype-by-environment interaction). Jornal Press 4. 85-122.

Gauch, H. G. 2006. Statistical analysis of yield trials by AMMI and GGE. Crop Science 46: 1488-1500.

Gauch Jr, H. G. 1988. Model selection and validation for yield trials with interaction. Biometrics 44: 705-715.

González, A., A. Monteagudo, P. Casquero, A. De Ron and M. Santalla. 2006. Genetic variation and environmental effects on agronomical and commercial quality traits in the main European market classes of dry bean. Field Crops Research 95: 336-347.

Karimizadeh, R., A. Asghari, R. Chinipardaz, O. Sofalian and A. Ghaffari. 2016. Determining yield stability and model selection by AMMI method in rain-fed durum wheat genotypes. Turkish Jornal Field Crops 21: 174-183.

Kendal, E. 2016. GGE biplot analysis of multienvironment yield trials in barley (*Hordeum vulgare L.*) cultivars. Ekin Journal of Crop Breeding and Genetics 2: 90-99.

Kimani, P., R. Buruchara, K. Ampofo, M. Pyndji, R. Chirwa and R. Kirkby. 2005. Breeding beans for smallholder farmers in Eastern, Central, and Southern Africa: Constraints, achievements, and potential. PABRA Millennium Workshop: p. 11.

Mohammadi, M., P. Sharifi, R. Karimizadeh, J. ALT JAFARBY, H. Khanzadeh, T. Hosseinpour et al. 2015. Stability of grain yield of durum wheat genotypes by AMMI model. Agriculture and Forestry Sumarstvo 61: 181-182.

Mortazavian, S., H. Nikkhah, F. Hassani, M. Sharif-al-Hosseini, M. Taheri and M. Mahlooji. 2014. GGE biplot and AMMI analysis of yield performance of barley genotypes across different environments in Iran. Journal of Agricultural Science and Technology 16: 609-622.

Muedi, H. T., D. Fourie and N. W. McLaren. 2015. Distribution and severity of bacterial brown spot on dry beans in South Africa: an update. South African Journal of Science 111: 1-6.

Muedi, H. T. H. 2015. Reaction of selected common bean germplasm accessions to bacterial brown spot in South Africa. Crop Protection 77: 87-93.

Oladosu, Y., M. Y. Rafii, N. Abdullah, U. Magaji, G. Miah, G. Hussin et al. 2017. Genotype× Environment interaction and stability analyses of yield and yield components of established and mutant rice genotypes tested in multiple locations in Malaysia. Acta Agriculturae Scandinavica, Section B. Soil and Plant Science 7: 1-17.

Payne, S. L. B. 2014. The Art of Asking Questions: Studies in Public Opinion. 3th ed. Princeton University Press. Pakistan.

Petersen, S., J. H. Lyerly, M. L. Worthington, W. R. Parks, C. Cowger, D. S. Marshall et al. 2015. Mapping of powdery mildew resistance gene Pm53 introgressed from *Aegilops speltoides* into soft red winter wheat. Theoretical and Applied Genetics 128: 303-312.

Purchase, J., H. Hatting and C. Van Deventer. 2000. Genotype× environment interaction of winter wheat (*Triticum aestivum L.*) in South Africa: II. Stability analysis of yield performance. South African Journal of Plant and Soil 17: 101-107.

Purchase, J. L. 1997. Parametric analysis to describe genotype x environment interaction and yield stability in winter wheat. University of the Free State. South Africa.

Serfontein, J. 1994. Occurrence of bacterial brown spot of dry beans in the Transvaal province of South Africa. Plant Pathology 43: 597-599.

Singh, S. P. and H. F. Schwartz. 2010. Breeding common bean for resistance to diseases (review). Crop Science 50: 2199-2223.

Yan, W., L. Hunt, Q. Sheng and Z. Szlavnics. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Science 40: 597-605.

Yan, W., M. S. Kang, B. Ma, S. Woods and P. L. Cornelius. 2007. GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop Science 47: 643-653.

CHAPTER 5
HERITABILITY AND GENE EFFECTS CONTROLLING BACTERIAL BROWN SPOT DISEASE RESISTANCE IN DRY BEAN

Abstract

Dry bean (*Phaseolus vulgaris L*.) is grown over a wide range of agro-climatic conditions and is an important source of protein and income worldwide. This study aimed to evaluate the heritability and the gene effects influencing BBS disease resistance in a dry bean cross between a resistance donor parent A55 (P1) and a susceptible commercial cultivar RS7 (P2). A generation mean analysis experiment involving the two parents, their F1, F2, and two backcross generations was conducted at Potchefstroom, South Africa. The six generations were grown in a randomized complete block design with two replications, and inoculated with BBS isolates at two weeks after planting. BBS disease scoring was done two weeks after inoculation using a 1-9 CIAT scale, with 1 representing highly resistant and 9 representing highly susceptible. Data were recorded for BBS severity on individual plants, namely 20 plants for each of non-segregating generations, 50 plants for each backcross generation and 100 plants for the F2 generation. The analysis of variance showed significant differences among generations ($P<0.001$). The joint scaling test parameters A and C were significant ($P<0.001$), revealing that data did not fit a simple additive-dominance model, and indicating that the digenic interactions model was involved. The digenic interaction model showed five highly significant ($P<0.001$) parameters *viz*. mean [m], additive [d], dominance [h], additive x additive [i], and dominance x dominance [l]). The dominance [h] and dominance x dominance [l] gene effects had inverse signal, showing the existence of duplicate epistasis. The negative and significant additive x additive [i] showed alleles dispersion in parents. The positive signal of dominance x dominance [l] interaction showed unidirectional leading (dominance). The existence of significant non-additive gene effects, joined with moderate broad and narrow-sense heritability, suggest that the selection for BBS resistance, especially in initial generations, would be complex using conventional breeding methods.

Keys words: Generation mean, BBS severity and epistasis or digenic interaction model

5.1 Introduction

Dry bean (*Phaseolus vulgaris L*.) is an important source of protein, natural fibre and calories in tropical and subtropical countries (FAO, 2014). This crop provides a cheap source of vegetal protein and fetches higher prices compared to cereals, resulting in increased incomes for farmers (Wortmann, 1998; FAO, 2014). Red speckled sugar beans (RSS) is the most widely produced grain type in South Africa with 75% of the local market share (Muedi, 2015). The average grain yield in South Africa is about 1.40 t ha^{-1} (Dlamini *et al.*, 2017). The mean grain yield is low compared with North America (~ 3.00 t ha^{-1}) (Kimani *et al.*, 2005; FAO, 2014). The low grain yield is attributed to several biotic and abiotic factors (Navarro *et al.*, 2007). The bacterial brown spot (BBS), caused by *Pseodomonas syringae pv. syringae* is an important disease of dry bean, and can cause grain yield losses up to 55% in South Africa field farmers (Serfontein, 1994; Muedi *et al.*, 2015). Resistant cultivars offer the best way to reduce grain yield losses by the BBS disease (Singh and Miklas, 2015).

The generation mean analysis (GMA) is a simple procedure for evaluating the main gene effects such as mean [m], additive [d] and dominance [h] and the digenic or non-allelic interactions such as additive x additive [i], additive x dominance [j] and dominance x dominance [l], key factors of genetic inheritance for quantitative traits (Dabholkar, 1999; Mather and Jinks, 2013). The digenic non-allelic interaction (epistasis) is broadly classified as complementary (the same sign of [h] and [l]) and duplicate (the inverse sign of [h] and [l]), while the positive [d] indicates gene association and negative [d] reveals gene dispersion (Hayman and Mather, 1955). A key inheritance study of BBS resistance was done in a field experiment using the dry bean plant stem inoculation method at seedling stage in a Belneb RR-1 × A55 RIL segregating dry bean population (Navarro *et al.*, 2007). The authors identified genomic regions situated in various linkage clusters involved with BBS resistance. However, stem inoculation may not be the best inoculation method, and leaf inoculation, which was done in this experiment, may be more appropriate since the disease mostly enters the leaves.

The selection accuracy is mainly influenced by the additive genetic or heritable variance, effect of the environment and the genotype by environment interaction (Akhshi *et al.*, 2014). The selection based on phenotype is effective with large genetic variability and higher narrow sense heritability (Malik *et al.*, 2007). Heritability provision gives an idea of the expected response to selection in a segregating generations (Ramteke *et al.*, 2010). The narrow sense heritability is the most important because it provides the breeding value (additive gene effects) (Ramteke *et al.*, 2010). The heritability is estimated through variance components and the regression of the offspring mean on parental mean values (Jatothu *et al.*, 2013). The main

sources of resistance to BBS are from the primary gene pool, including known donors such as Puebla 152, Hystyle, A 55, BBSR 17 and BBSR 28 (Muedi *et al.*, 2015). This study aimed to estimate heritability and gene effects controlling bacterial brown spot (BBS) disease resistance in dry bean.

5.2 Methodology

5.2.1 Genetic materials

The experiment was conducted as a preliminary genetic study in the greenhouse with controlled conditions (27^{o}C /19^{o}C day/night cycle of 12 hour each). Crosses were performed between A55 (P1) and RS7 (P2) in a greenhouse at the Agricultural Research Council - Grain Crops Institute (ARC-GCI) at Potchefstroom, South Africa (S 26. 74^{o} and E 27.08^{o} at an altitude of 1349 m) during the 2017/18 growing season. The donor parent (A55) is a small seeded line from the CIAT core collection and RS7 is a medium seeded commercial cultivar. Both parents have an indeterminate growing habit. The donor, A55 is black seeded with pink flowers, while RS7 is red speckled seeded with white flowers. The A55 had higher plant height than the RS7 plants. The RS7 flowered later than A55, hence both parents were planted three times (four days of difference) in order to synchronise days to flowering. The F1 plants had pink flowers (similar with A55) and red speckled seed (similar with RS7) and the F1 plants were taller than both parents.

The development of planting materials for evaluation was done in the period spanning from February to August 2018. The crosses were performed between RS7, a commercial cultivar susceptible to BBS, and A55, a donor parent resistant to BBS using the emasculation and pollination method to obtain F1 generation. The F1 was backcrossed to both parents (P1 and P2) in order to obtain BCP1 and BCP2 generations, respectively. The F1 was selfed to obtain F2 seed (Figure 5.1).

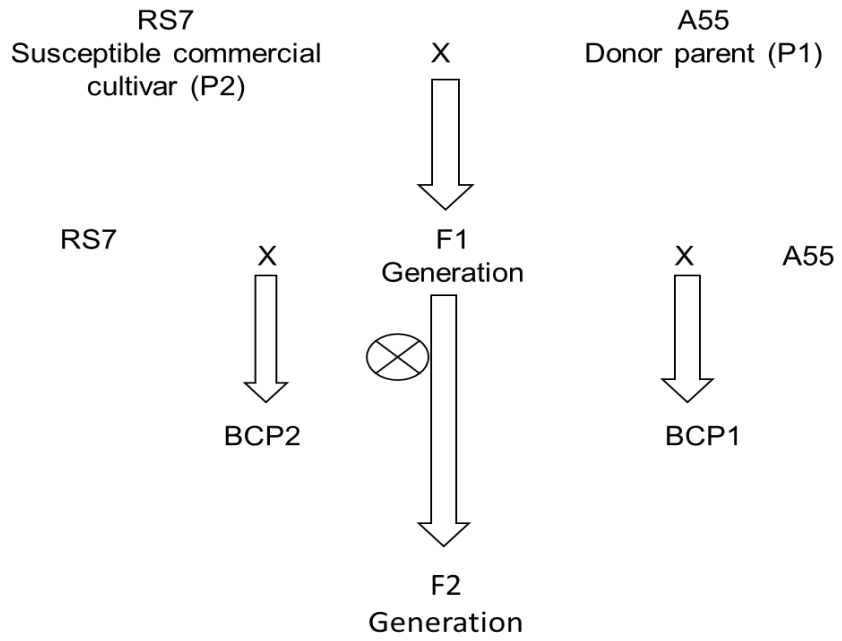


Figure 5.1 Development of planting material for generation mean analysis (GMA)

5.2.2 Bacterial brown spot disease inoculation

Three aggressive *Pss* isolates (BV 6.3, BV 3.3.2 and BV 27.1) from the ARC-GC collection were used in the study. Inoculum was prepared by suspending a 24- to 48-hr-old isolates in sterile distilled water and adjusting it with a spectrophotometer to contain approximately 10^8 CFU/ml.

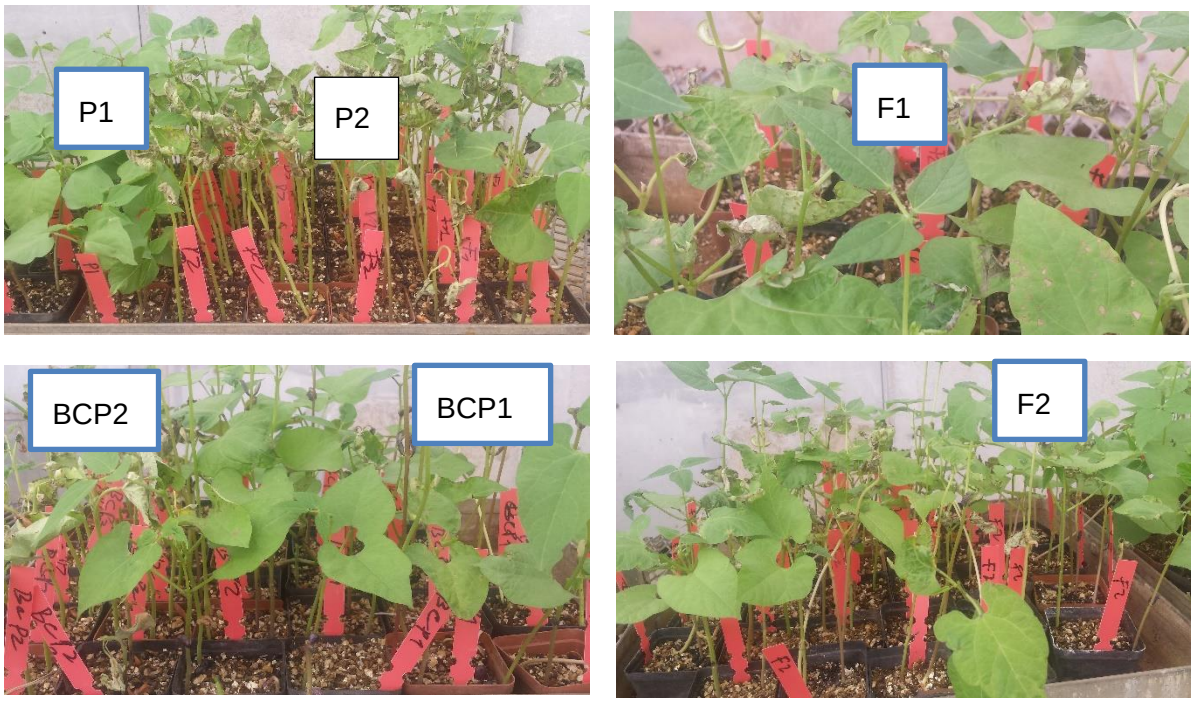
5.2.3 Evaluation of plant materials

The evaluation for the GMA was conducted in a randomised complete block design (RCBD) with two replications, in a greenhouse during September 2018. Seeds of the respective progenies were planted in 8 cm ofdiameter plastic pots in sterile commercial potting soil and maintained in a greenhouse at a 27^{o}C /19^{o}C day/night cycle of 12 hr each. Vermiculite was added on top of the soil medium to reduce evaporation. The experiment consisted of six generations namely P1, P2, F1, F2, BCP1 and BCP2. The number of plants in segregating generations (F2, BCP1 and BCP2) were higher than the non-segregating generations (P1, P2 and F1). The data were recorded from 20 plants for each parent (P1, P2) and F1, 50 plants for each backcross (BCP1 and BCP2) and 100 plants of F2 in each replicate. The

generations (P1, P2, F1, BCP1, BCP2, and F2) were planted at the same time in the greenhouse using planting trays. Seedlings were inoculated with *Pss* using an airbrush by spraying the bacterial suspension over the entire leaf area until completely wet. Inoculated plants were kept in a humidity chamber (19^{o}C±1^{o}C, RH=100%) for 48 hr before being transferred to a greenhouse (18^{o}C night/25^{o}C day, RH=70%). Irrigation was applied when required and no fertilisers were applied.

5.2.4 Data collection and analysis

Figure 5.2 shows the six basic generation means evaluated for BBS disease reaction at Potchefstroom. Data were collected for bacterial brown spot (BBS) severity 14 days after inoculation using a standardized CIAT disease severity scale of 1 (resistant or immune) to 9 (highly susceptible) (Petersen *et al.*, 2015). The BBS severity were transformed in percentages as follows 1 = 5%, 2 =15%, 3 = 25%, 4 = 35%, 5 = 45%, 6 = 55%, 7 = 65%, 8 = 75% and 9 = 85% (Petersen *et al.*, 2015). The data were analysed using unbalanced analysis of variance in Genstat 18th edition (Payne, 2014). The variance components were analysed using SAS version 9.4 (Hayman and Mather, 1955). The means were compared using the least significant difference (LSD) test with a level of significance of 5%.



P1=Parent one, P2=Parent two, F1=F1 generation, F2= F2 generation, BCP1=backcross with parent one and BCP2=backcross with parent two

Figure 5.2 The bacterial brown spot severity scoring of the generations

5.2.5 Analysis of genetic effects

The six parameters model proposed by Hayman (1958) was used for evaluation of genetic parameters from the means of generations (Equation 5.1). The type of digenic interaction (epistasis) was calculated when the leading or governing gene effect [d] was significant ($P<0.05$). When the gene effects had the equal sign, it was regarded complementary epistasis, while an opposite sign indicated a duplicate epistasis (Dvojković *et al.*, 2010). The means of several generations were not equal because the difference of family sizes were large and the means were corrected through weighting such as defined by Kearsey and Pooni (1998).

$$\mu_1 = m + [d]x_{i1} + [h]x_{i2} + [i]x_{i1}^2 + [j]x_{i2}^2 + [l]x_{i1}x_{i2} \quad \text{Equation 5.1}$$

Where: μ = mean of the generation; m = phenotypic mean of parents; [d] = additive or stabilizer gene effect; [h] = dominance or leading gene effect; [i] = additive x additive gene effects; [j] = additive x dominance gene effects; [l] = dominance x dominance gene effects; xi1 and xi2 = coefficients assigned for each generation.

The unbalanced analysis of variance was performed to calculate the mean of the generation, genetic variances, standards errors and to test homogeneity of genetic effects parameters ([m], [d], [h], [i], [j] and [l]) (Piepho and Möhring, 2010). The lack of fit test was performed to observe the adequacy of the generation mean model for assessing genetic effects. The joint scaling test suggested by Mather and Jinks (1982) was performed to ensure the fitted generation mean model. Table 5.1 shows the coefficients that estimate the degree of relationship of several generations used to determine gene effects for the generation means.

Table 5.1 Generalized expectations of the six generation mean

		Genetics effects coefficients				
Generation	**[m]**	**[d]**	**[h]**	**[i]**	**[j]**	**[l]**
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). [m]=Generation mean; [d] =Additive effect; [h] =Dominance effect; [i] =Pooled additive x additive effects; [j] = Interaction effect of [d] and [h]; and [l] = Pooled dominance x dominance effects.

5.2.7 Genetic variance components

The analysis of variance of an unbalanced model was used to calculate the mean of the generations and genetic variances (Mather and Jinks, 2013). The variance components were analysed using formulas described by Kearsey and Pooni (1998). The phenotypic variance was regarded equal to the variance of the F2 generation. The variance components were determined according to the following five formulas:

i) VE= $[\mathrm{VP1} + \mathrm{VP2} + 2\mathrm{VF1}]/4$, where VE=environment variance, VP1=variance of parent one, VP2=variance of parent two and VF1=variance of F1 generation.

ii) ii)VG= $\mathrm{VP} - \mathrm{VE}$, where VG=genetic variance, VP=phenotypic variance and VE=environmental variance.

iii) iii) VA=$2\mathrm{VF2} - (\mathrm{VBCP1} + \mathrm{VBCP2})$, where VF2=variance of F2 generation, VBCP1=variance of backcross with parent one, VBCP2=variance of backcross with parent two.

iv) iv) VD=$\mathrm{VBCP1} + \mathrm{VBCP2} - \mathrm{VF2} - \mathrm{VE}$. and

v) v) VAD=$1/2(\mathrm{VBCP2} - \mathrm{VBCP1})$, where VAD=additive and dominance variance.

5.2.8 Broad and narrow sense heritability

The broad-sense heritability *(H_b^2)* was estimated as the ratio of genotypic variance (VG) to phenotypic variance (VP) in the F2 population, while the narrow-sense heritability *(h_n^2)* was estimated as the ratio of additive variance (VA) to phenotypic variance (VP) (Akhshi *et al.*, 2014). The heritability estimates less than 30% considered low, 31- 60% considered moderate and more than 60% considered high (Robinson *et al.*, 1949).

Broad-sense heritability $H_b^2 = \frac{(VA+VD)}{(VA+VD+VE)}$ Equation 5.2

The narrow sense heritability (h_n^2) was estimated using the formula below.

Narrow-sense heritability $h_n^2 = \frac{(VA)}{(VA+VD+VE)}$ Equation 5.3

The dominance (governing) ratio used to evaluate the importance of dominance and additive gene effects as used for inbred line selection was calculate according Kearsey and Pooni (1998).

Dominance ratio DR= $\sqrt{\frac{4VD}{2VA}}$ Equation 5.4

Where: DR=dominance ratio, VD=dominance variance and VA=additive variance.

5.3 Results

5.3.1 Analysis of variances for reaction to bacterial brown spot

The analysis of variance for the bacterial brown spot (BBS) severity among generations from the cross between RS7 and A55 is exposed in Table 5.2. The source of variation for BBS severity were partitioned for the generations and plants in 71.30% and 11.96% of total sum of squares, respectively. The analysis indicated highly significant differences for generations ($P<0.001$). The significant variability observed for generations indicate that some performed better than others and early generation selection can be useful on identifying potential families to advance further in breeding.

Table 5.2 Analysis of variance of BBS severity for the cross between RS7 and A55

Source	DF	SS	MS	Vr	F pr
Replication	1	8.68	8.68	0.12	0.725
Generations	5	38280.91	7656.18	109.96	<.001
Plants	124	6422.19	51.79	0.74	0.951
Residual	129	8981.85	69.63		
Total	259	53693.73	207.31		
Mean		37.51	CV		22.25
LSD		16.51	SE		8.34

DF=degree of freedom, SS=sum square, MS= Generation mean of squares, Vr=variance components, Fpr=Probability, CV = Coefficient of variation, LSD = Least significant difference and SE = Standard error.

5.3.2 Generation mean analysis

The means and variances of the generation means of the cross between RS7 and A55 is presented in Table 5.3. Parent P1 had the lowest mean BBS severity followed by BCP1 with mean BBS severity of 12.62±1.29 and 20.95±1.03, respectively. Parent P2 and BCP2 had the highest mean BBS severity of 54.87± 1.30 and 45.05 ±1.13, respectively. Generations F1 and F2 had a medium mean BBS severity of 39.00±1.31 and 43.23±0.89, respectively, and both F1 and F2 generations had BBS average greater than the mid parent BBS mean. Generation F2 had a higher genetic variance than BCP1 and BCP2. The segregating generation F2, BCP1 and BCP2 had a high genetic variance of 78.57, 52.78 and 63.65, respectively, while the non-segregating generations P1, P2 and F1 had a low genetic variance of 33.21, 33.87 and 34.47, respectively.

Table 5.3 The mean BBS severity and variance of the generation from the cross between RS7 and A55

Generation	N	Mean	Variance	SD	SE
P1	20	12.62	33.21	5.76	1.29
P2	20	54.87	33.87	5.82	1.30
F1	20	39.00	34.47	5.87	1.31
F2	100	43.23	78.57	8.86	0.89
BCP1	50	20.95	52.78	7.27	1.03
BCP2	50	45.05	63.65	7.98	1.13

N=sample size of each of six generation mean, SD=Standard deviation and SE = Standard error.

5.3.3 Distribution of BBS severity in the generation

The bacterial brown spot (BBS) severity over the generations is shown in Figure 5.3. Parent P1 had a low BBS severity mean, while P2 had a high mean BBS severity. Generation F1 had a lower mean BBS severity than F2. Finally, BCP1 had a lower mean BBS severity than BCP2 (Figure 5.3).

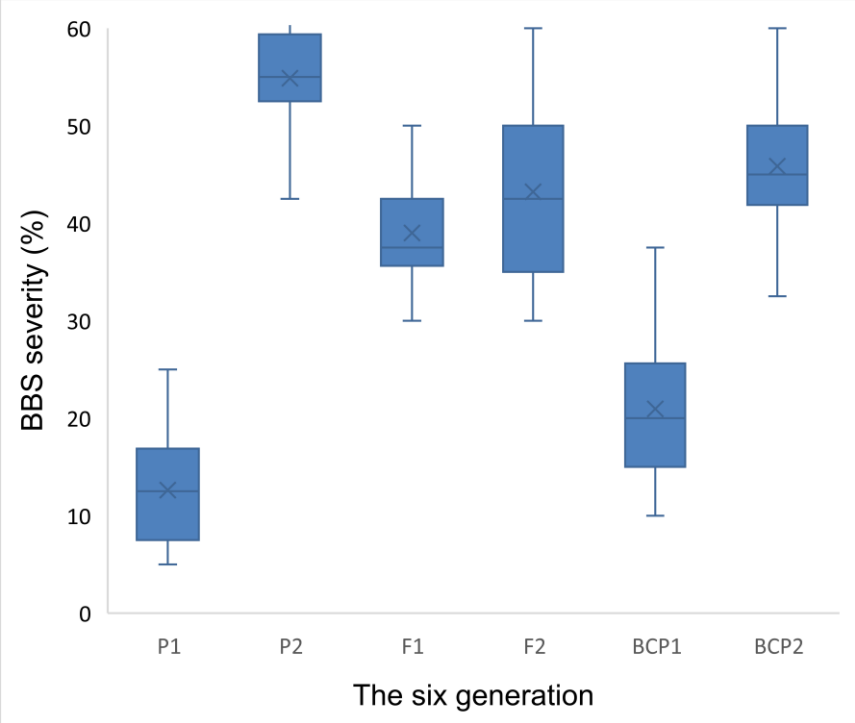


Figure 5.3 The bacterial brown spot (BBS) severity across the six generation mean

5.3.4 The joint scaling test

The joint scaling test for the six generations is displayed in Table 5.4. The scaling test parameter A and C were highly significant (P<0.001). The scaling test revealed the complicating effects such as maternal effects and gene interaction that were involved in the genetic control the BBS severity in this cross, and that the simple additive-dominance model did not adequately explain the genetic control for the cross between RS7 and A55.

Table 5.4 The joint scaling test with three parameters comparing chi-square and t test

Parameters	Scaling test	Chi-square	t calculated	DF	t critic
A	-9.72	7.61***	-3.52	87	2.62
B	-3.77	8.51	-1.29	87	2.62
C	27.43	22.82***	5.74	156	2.58

* P < 0.05, ** P < 0.01, *** P < 0.001; and DF=Degree of freedom

5.3.5 Generation variance components

The variance components of non-segregating and segregating generations is indicated in Table 5.5. The environmental variance was not significant (mean of non-segregation generation), while the variances for the segregating generation (F2, BCP1 and BCP2) were highly significant (P<0.001). The VE (environmental average of P1, P2 and F1), F2, BCP1 and BCP2 were 33.84, 78.57, 52.78 and 63.95, respectively. The F2 generation genetic variance component was higher than BCP1 and BCP2 genetic variance, while the BCP2 generation genetic variance component was higher than the BCP1 genetic variance. The significant variance components of the segregating generation (F2, BCP1 and BCP2) revealed the hereditary variation that exists in the generations derived from the cross between RS7 and A55 controlling BBS disease resistance.

Table 5.5 Generation variance components of non-segregating (P1, P2 and F1) and segregating (F2, BCP1 and BCP2) generations

Generation variance Components	Variance	F calculate	DF	t critic
VE	33.85	1.04	(06, 57)	3.87
F2	78.57***	2.32	(99, 297)	1.38
BCP1	52.78*	1.56	(49, 147)	1.44
BCP2	63.95***	1.88	(49, 147)	1.68

* P < 0.05, ** P < 0.01, *** P < 0.001, VE=Environmental variance [VE= (P1+P2+F1)/3], F2=F2 Generation, BCP1=Backcross with parent one, BCP2=Backcross with parent two and DF=Degree of freedom.

5.3.6 Analysis of variance of the genetic effects

The analysis of variance of the genetics effects of the generation mean is indicated inTable 5.6. The digenic interaction model and genetics parameters were highly significant (P<0.001). The genetics parameters [d], [h], [i], [l] and generation mean model were 27.04, 5.99, 9.74,

5.01 and 71.31% of total sum square, respectively. The significant genetic parameters indicated the existence of gene interaction in the cross between RS7 and A55 in controlling BBS disease. The generation mean had 37.51% of the bacterial brown spot (BBS) severity and 71.31% of the coefficient of determination (R^2) (Table 5.6). The higher the R^2, the greater the precision and accuracy the disease severity estimates.

Table 5.6 Analysis of variance of the genetic effect of the generation mean

Source	DF	SS	MS	F value	Pr>F
Replication	1	8.68	8.68	0.14	0.706
[d]	1	14520.25	14520.25	238.48	<0.001
[h]	1	3217.52	3217.52	52.85	<0.001
[i]	1	5227.53	5227.53	85.86	<0.001
[j]	1	136.16	136.16	2.24	0.136
[l]	1	2690.33	2690.33	44.19	<0.001
Model	6	38289.59	6381.60	104.81	<0.001
Error	25	15404.13	60.89		
Total	259	53693.73			
R^2		71.31		CV	20.80
Mean		37.51		R-MSE	7.80

[d] = Additive gene action, [h] = Dominance gene action, [i] =Additive x additive gene action, [j] = Additive x dominant gene action, [l] = Dominant x dominant gene action, R^2=R-square, Mean=Generation mean, CV=Coefficient of variation, R-MSE=Root mean square standard error, SS=Sum square and MS=Mean square

5.3.7 Gene effects

The estimates of gene effects of the reaction to bacterial brown spot (BBS) disease for the cross between RS7 and A55 is exposed in Table 5.7. The data for reaction to BBS disease did not indicate a simple additive and dominance model, however it was fitted for the non-allelic or digenic interaction model viz additive [d], dominance [h], additive x additive [i], and dominance x dominance [l]) gene effects. The highly significant differences (P<0.001) for the variables of the six generation mean parameter model, revealed that data were adequate and suited the non-allelic or epistasis model well. The [d] and [l] gene effects had opposite signs (both were significant) showing the existence of duplicate epistasis. The negative and significant values of additive x additive [i] gene effects in the cross between RS7 and A55 revealed alleles dispersion in parents for BBS disease. The negative sign of dominance [h] gene effect revealed that reductive alleles including dominant alleles involve governing the

phenotype. The positive sign of dominance x dominance [l] gene effects revealed unidirectional dominance.

Table 5.7 Estimations of gene effects of the reaction to bacterial brown spot severity for the cross between RS7 and A55

Gene action	RS7 x A 55	
	Estimate	**SE**
[m]	43.41***	0.92
[d]	-24.10***	1.56
[h]	-35.65***	4.90
[i]	-40.90***	4.41
[j]	-2.96	1.99
[l]	54.40***	8.18
Epistasis type	Duplicate	-

* P<0.05; ** P<0.01; *** P<0.001. SE = Standard error, [m] = Mean parent, [d] = Additive gene effects, [h] = Dominance gene effects, [i] = Additive x additive gene effects, [j] = Additive x dominant gene effects, [l] = Dominant x dominant gene effects and SE=Standard error.

5.3.8 Variance components and heritability

The broad and narrow sense heritability were moderate with 56.72% and 51.81%, respectively (Table 5.8). The real number of genes governing the BBS disease resistance was not estimated due to significant epistasis interaction. The BBS resistance for this cross revealed a measurable hereditary trait. The genes controlling the BBS disease (dominance ratio below unity) showed on average partial dominance. The dominance ratio determined the importance of dominance effects in relation to the additive effects deviation of genes.

Table 5.8 Variance components, heritability (wide and narrow sense) and dominance ratio for reaction to bacterial brown spot (BBS) disease

Item	Genetic parameters	Parents RS7 x A 55
Variance components	VE	34.00
	VA	40.71
	VD	3.86
	VAD	58.22
Heritability	H^2b (%)	56.72
	h^2n(%)	51.81
Dominance ratio	DR	0.44

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

5.4 Discussion and conclusion

This study estimated the heritability and gene effects controlling the BBS disease resistance in a cross between RS7 and A55 using the generation mean analysis. The genetic effects revealed the existence of the duplicate epistasis and gene dispersion with broad and moderate narrow sense heritability. The selection can be postponed and the gene could be fixed and exploited in later generation.

The large difference in BBS severity between the parents P1 and P2 indicates that the P1 and P2 used inthe cross were divergent for the studied character, which is a requirement for the success a generation mean analysis (Jinks and Mather, 1982). The average BBS severity reaction was lower in the F1 than the F2 generation. The backcross generations had a disease severity mean close to their respective recurrent parent.

The joint scaling test parameters A, B and C showed that the simple additive-dominance model did not adequately explain the genetic effect of the resistance to BBS disease. The digenic interaction model was highly significant (P<0.001). The additive [d], dominance [h], additive x additive [i] and dominance x dominance [l] gene effects had highly significant (P<0.001) influences in controlling the BBS disease.

The positive or negative additive x additive gene effects [i] indicate association and dispersion of alleles in parents (Kearsey and Pooni, 1998). The negative and significant additive x

additive gene effects [i] in this cross revealed alleles dispersion in parents for BBS disease resistance. The significance of additive x additive [i] gene effects indicated the potential that the resistance can be fixed and exploited in later generations.

The interaction is regarded to be complementary when the [h] and [l] have the equal signal and to be duplicating when the signal is opposite (Mather and Jinks, 1982). The dominance [h] and dominance x dominance [l] gene effects had inverse signal, showing the existence of duplicate epistasis. The existence of duplicate epistasis suggested that variability in segregating generations would be reduced until the homozygous generation (Kumar and Patra, 2010).

The signal of dominance gene effects [h] and dominance x dominance gene effects [l] revealed a dominance direct and unidirectional dominance gene effects, respectively (Kearsey and Pooni, 1998). The negative signal of dominance [h] gene effect shows that the reductive alleles are including dominant alleles, while the positive sign of dominance x dominance gene effects [l] shows unidirectional dominance.

The broad and narrow sense heritability were both moderate for BBS disease resistance. The selection for a trait with a high narrow sense heritability is easy, while the selection of a moderate heritable character is more difficult and with a low probability of successes, as breeders would rely on transgressive segregates to register the progress (Ajay *et al.*, 2012).

The existence of gene dispersion combined with moderate narrow-sense heritability suggest that the selection for BBS disease resistance, mainly in early generation, would be complex or difficult using conventional breeding methods. The significance of additive x additive gene effects revealed the opportunity that the resistance can be fixed and exploited in later generations for the advance of genotypes with high grain yield and BBS disease resistance.

5.5 Reference

Ajay, B., B. Gnanesh, K. Ganapathy, M. B. Gowda, P. Prasad, G. Veerakumar et al. 2012. Genetic analysis of yield and quantitative traits in pigeonpea (*Cajanus cajan L. Millsp.*). Euphytica 186: 705-714.

Akhshi, N., K. Cheghamirza, H. Ahmadi and F. Firouzabadi. 2014. Generation mean analysis to estimate genetic parameters for morphological traits in common bean (*Phaseolus vulgaris L.*). Journal of Biodiversity and Environmental Sciences 3: 254-257.

Broughton, W. J., G. Hernandez, M. Blair, S. Beebe, P. Gepts and J. Vanderleyden. 2003. Beans (*Phaseolus spp.*)–model food legumes. Plant and Soil 252: 55-128.

Dabholkar, A. 1999. Elements of bio metrical genetics. 1 th ed. Concept publishing company. New Delhi. India.

Dlamini, T., L. Nalley, F. Tsiboe, A. Barkley and A. Shew. 2017. The economic impact of the South African Agricultural Research Council's dry beans breeding program. Journal of Agricultural and Applied Economics 49: 232-250.

Dvojković, K., G. Drezner and D. Novoselović. 2010. Estimation of some genetic parameters through generation mean analysis in two winter wheat crosses. Periodicum Biologorum 112: 247-251.

FAO, I. 2014. WFP. The State of Food Insecurity in the World 2014 strengthening the enabling environment for food security and nutrition. Rome. Italy.

Hayman, B. 1958. The separation of epistatic from additive and dominance variation in generation means. Heredity 12: 371.

Hayman, B. and K. Mather. 1955. The description of genic interactions in continuous variation. Biometrics 11: 69-82.

Jatothu, J. L., K. S. Dangi, S. K. Sagi, H. Bhandari, M. Tripathi and B. Chaudhary. 2013. Estimation of gene effects based on joint scaling test and sequential model fit scheme for quantitative traits in sesame (*Sesamum indicum L.*). Journal of Agricultural Science 5: 224-227.

Jinks, J. L. and K. Mather. 1982. Biometrical genetics: The study of continous variation. Chapman and Hall, London. United Kingdom.

Kearsey, M. J. and H. S. Pooni. 1998. The genetical analysis of quantitative traits. Stanley Thornes (Publishers) Limited. New York. USA.

Kimani, P., R. Buruchara, K. Ampofo, M. Pyndji, R. Chirwa and R. Kirkby. 2005. Breeding beans for smallholder farmers in Eastern, Central, and Southern Africa: Constraints, achievements, and potential. PABRA Millennium Workshop: p. 11.

Kumar, B. and N. K. Patra. 2010. Genetic analysis of capsule and its associated economic traits in opium poppy (*Papaver somniferum L.*). Journal of heredity 101: 657-660.

Malik, M. F. A., M. Ashraf, A. S. Qureshi and A. Ghafoor. 2007. Assessment of genetic variability, correlation and path analyses for yield and its components in soybean. Pakistan Journal of Botany 39: 405.

Mather, K. and J. L. Jinks. 1982. Diallels. Biometrical Genetics. Springer 1: 255-291.

Mather, K. and J. L. Jinks. 2013. Biometrical genetics: The study of continous variation. Chapman and Hall, London. United Kingdom.

Muedi, H. T., D. Fourie and N. W. McLaren. 2015. Distribution and severity of bacterial brown spot on dry beans in South Africa: an update. South African Journal of Science 111: 1-6.

Muedi, H. T. H. 2015. Reaction of selected common bean germplasm accessions to bacterial brown spot in South Africa. Crop Protection 77: 87-93.

Navarro, F., P. Skroch, G. Jung and J. Nienhuis. 2007. Quantitative trait loci associated with bacterial brown spot in loci. Crop Science 47: 1344-1353.

Payne, S. L. B. 2014. The Art of Asking Questions: Studies in Public Opinion. 3th ed. Princeton University Press. Pakistan.

Petersen, S., J. H. Lyerly, M. L. Worthington, W. R. Parks, C. Cowger, D. S. Marshall et al. 2015. Mapping of powdery mildew resistance gene Pm53 introgressed from Aegilops speltoides into soft red winter wheat. Theoretical and Applied Genetics 128: 303-312.

Piepho, H. and J. Möhring. 2010. Generation means analysis using mixed models. Crop Science 50: 1674-1680.

Ramteke, R., V. Kumar, P. Murlidharan and D. K. Agarwal. 2010. Research Note Study on genetic variability and traits interrelationship among released soybean varieties of India [*Glycine max (L.) Merrill*]. Electronic Journal of Plant Breeding 1: 1483-1487.

Robinson, H., R. E. Comstock and P. Harvey. 1949. Estimates of heritability and the degree of dominance in corn. Agronomy Journal 41: 353-359

Serfontein, J. 1994. Occurrence of bacterial brown spot of dry beans in the Transvaal province of South Africa. Plant Pathology 43: 597-599.

Singh, S. P. and P. N. Miklas. 2015. Breeding common bean for resistance to common blight: A review. Crop Science 55: 971-984.

Wortmann, C. S. 1998. Atlas of common bean (*Phaseolus vulgaris L.*) production in Africa. CIAT. Colombia.

CHAPTER 6
OVERVIEW OF THE STUDY

6.1 Introduction

Dry bean (*Phaseolus vulgaris L*.) is grown under a wide range of agro-climatic conditions and is an essential source of protein and income worldwide. In South Africa the crop is mostly, grown at altitude between 1000 to 1800 m. The mean grain yield is around 1.40 t ha^{-1} (Dlamini *et al.*, 2017). The average grain yield is low when compared with North America (~3.00 t ha^{-1}) (Kimani *et al.*, 2005; FAO, 2014). The demand is higher than the local production (Dlamini *et al.*, 2017). Red speckled sugar beans have 75% of local market share, followed by small white canning (20%). The low grain yield potential of the local varieties, their instability and susceptibility to diseases such as bacterial brown spot (BBS) are factors that contribute to the low productivity (Muedi *et al.*, 2015). The grain yield losses caused by *Pseudomonas syringae pv. Syringae* (BBS disease) can be up to 55% (Serfontein, 1994; Muedi *et al.*, 2015). Therefore, the development of high and stable yielding varieties with resistance to BBS is important. The study aimed to improve dry bean production in South Africa through finding high yielding and stable cultivars with resistance to BBS disease. This chapter summarizes the results presents suggestions for dry bean breeding in South Africa.

6.2 Summary of results

6.2.1 Screening of Andean Diversity Panel dry bean lines for grain yield and bacterial brown spot (BBS) disease, under field conditions.

The reaction to BBS and grain yield were evaluated under field conditions using 423 Andean Diversity Panel (ADP) dry bean lines over three sites (Middelburg, Potchefstroom and Warden). These genetic materials were sourced from the Agricultural Research Council- Grain Crop Improvement, Breeding Program (ARC-GCI-BP). The keys finding were as follows:

- The study identified genotypes with resistance (21.0%) and moderate resistance (41.6%) to the BBS disease.
- Genotypes ADP-0592, ADP-0790, ADP-0120 and ADP-0008 were selected as both high yielding and BBS disease resistant across three environments. These genotypes had grain yield above 1.45 t ha^{-1} and a BBS severity below 25.5.

- Genotypes ADP-0546, ADP-0630, ADP-0183 and ADP-0279 were selected for both high BBS disease resistance as well as high grain yield at Warden. Genotypes ADP-0038, ADP-0721, ADP-0790 were the best performing genotypes at Middelburg, while genotypes ADP-0120 and ADP-0079 were the best performing genotypes at Potchefstroom. These genotypes had grain yield above 1.85 t ha^{-1} and BBS severity below 18.50.

- The above genotypes had higher grain yield and low BBS severity across three tested sites than the grand mean (0.87 t ha^{-1}) and the best performing cultivar (1.13 t ha^{-1}), and mean BBS severity below the grand mean (39.85) and the best performing cultivar (31.67).

- The mediuml seeded dry bean genotypes had lower relative area under disease progress curve (RAUDPC) than the medium and the large seeded, and the indeterminate genotypes had lower RAUDPC mean than determinate genotypes.

6.2.2 Grain yield performance, stability and bacterial brown spot (BBS) disease resistance of fourteen Dark Red Kidney (DRK) dry bean lines across six environments

Fourteen Dark Red Kidney (DRK) dry bean lines were evaluated for grain yield, stability and BBS disease resistance across six environments (Carolina, Clarens, Cedara, Middelburg, Potchefstroom and Warden). These DRK dry bean lines were sourced from the Agricultural Research Council-Grain Crops Institute Breeding Program (ARC-GCI-BP). The AMMI analysis and GGE biplot were performed using Genstat 18th. The main findings were as follows:

- The crossover genotype by environment interaction (GEI) was present among fourteen dark red kidney dry bean over six environments.

- Genotypes G12 (1.46 t ha^{-1}) had both high yield, low BBS severity and was stable, revealing broad adaptation across six environments, while, genotypes G08 (1.77), G06 (1.70), G03 (1.63), G02 (1.56), G05 (1.48) and C04 (1.45 t ha^{-1}) had high grain yield, low BBS severity and unstable, revealing specific adaptation.

- The above genotypes had grain yields above the grand mean and the best performing cultivar both with 1.43 t ha^{-1} and with BBS severity grand mean (31.90) and the best performing cultivar (48.89).

- This study clustered six environments in three mega-environments for grain yield and three for BBS severity. The first mega-environment for grain yield was Potchefstroom with genotypes G07, G10, G13, G11 and G09. The second handled Cedara, Carolina and Middelburg with genotypes such as G06, G08, G12, G02, G03 and G05, while genotypes G14, G04 and G01 were adapted to the third mega-environment that included Clarens and Middelburg environments.

6.2.3 Heritability and gene effects controlling the bacterial brown spot (BBS) disease resistance in a dry bean cross.

This study aimed to estimate heritability and mode of gene effects controlling the bacterial brown spot (BBS) disease resistance in a dry bean cross between a susceptible commercial cultivar RS7 and resistant donor parent A55 (Navarro *et al.*, 2007). These materials were sourced from the Agricultural Research Council-Grain Crops Institute Breeding Program (ARC-GCI-BP). These parents were crossed, backcrossed and selfed and six generations (P1. P2, F1, F2, BCP1 and BCP2) were generated. The generations were inoculated with BBS and rated using the CIAT scale 1-9 (Petersen *et al.*, 2015). The main results were as follows:

- The dominance [h] and dominance x dominance [l] gene effects had opposite signal, showing the existence of duplicate epistasis. The duplicate epistasis indicates that variability will be highly in segregating generations.

- The positive signal of dominance x dominance [l] interaction showed unidirectional dominance.

- The negative significant additive x additive [i] gene effects showed alleles dispersion in parents. The gene dispersion showed that selection could not be effective in initial generation stages and the dispersed genes should be brought together and the resistance can be fixed and exploited in the later generation stages.

- The broad and narrow sense heritability were both moderate with 56.72 and 51.82%, respectively, which indicated several genes conditioned the BBS disease resistance.

6.3 Research findings and ways forward

- **Screening Andean Diversity Panel (ADP) dry bean lines for grain yield and BBS disease**

Several ADP dry bean lines with broad adaptation had both high grain yield and low BBS severity were identified, and these genotypes can be considered as potential sources for both traits. These genotypes could be used as donor parents in a breeding programme for high yield and resistance to BBS. Four, three and two genotypes were selected for specific adaptation for Warden, Middelburg and Potchefstroom, respectively. These genotypes can be recommended for each specific environment. The above genotypes should be evaluated for two season and across sites, before possible release commercial farmers.

- **Grain yield performance, stability and bacterial brown spot (BBS) disease of DRK lines**

The presence of crossover GEI suggested that breeding for broad and specific adaptation is crucial. Several genotypes were high yielding with good resistance to BBS and can be considered for potential release of can be used as parents in a breeding programme. The six environments were clustered into three-mega environment, which can be considered in future trial programmes

- **Heritability and gene effects controlling bacterial brown spot (BBS) disease**

The existence of significant gene dispersion combined with narrow sense heritability suggest that the selection for BBS resistance, especially in initial generations, would be complex using conventional breeding methods. The dispersed gene should be brought together and the resistance can be fixed and utilized or exploited at progressive or later generations for the development of genotypes with high grain yield, stable and BBS disease resistant..

6.4 References

Dlamini, T., L. Nalley, F. Tsiboe, A. Barkley and A. Shew. 2017. The economic impact of the South African Agricultural Research Council's dry beans breeding program. Journal of Agricultural and Applied Economics 49: 232-250.

FAO, I. 2014. WFP. The State of Food Insecurity in the World 2014 strengthening the enabling environment for food security and nutrition. Rome. Italy.

Kimani, P., R. Buruchara, K. Ampofo, M. Pyndji, R. Chirwa and R. Kirkby. 2005. Breeding beans for smallholder farmers in Eastern, Central, and Southern Africa: Constraints, achievements, and potential. PABRA Millennium Workshop: p. 11.

Muedi, H. T., D. Fourie and N. W. McLaren. 2015. Distribution and severity of bacterial brown spot on dry beans in South Africa: an update. South African Journal of Science 111: 1-6.

Navarro, F., P. Skroch, G. Jung and J. Nienhuis. 2007. Quantitative trait loci associated with bacterial brown spot in loci. Crop Science 47: 1344-1353.

Petersen, S., J. H. Lyerly, M. L. Worthington, W. R. Parks, C. Cowger, D. S. Marshall et al. 2015. Mapping of powdery mildew resistance gene Pm53 introgressed from Aegilops speltoides into soft red winter wheat. Theoretical and Applied Genetics 128: 303-312.

Serfontein, J. 1994. Occurrence of bacterial brown spot of dry beans in the Transvaal province of South Africa. Plant Pathology 43: 597-599.