Application of molecular markers in breeding rust resistant South African dry bean cultivars

PY Hadzhi

orcid.org 0000-0003-1105-6459

Dissertation submitted in fulfilment of the requirements for the degree *Master of Science in Environmental Sciences* at the North-West University

Supervisor:	Dr CMS Mienie
Co-supervisor:	Dr HTH Muedi
Assistant Supervisor:	Mr LA Madubanya

Graduation May 2019
26051826

Table of contents

DECLARATION

I declare that the dissertation submitted by me for the degree Magister Scientiae in Environmental studies at the North-West University (Potchefstroom Campus), Potchefstroom, North-West, South Africa, is my own independent work and has not previously been submitted by me at another university.

Signed in Potchefstroom, South Africa

Signature:

Date:

ACKNOWLEDGEMENTS

This study would not have been possible without the support and guidance of many individuals who contributed in a substantial manner throughout my Masters programme.

I would like to extend my gratitude to the University of North West, Potchefstroom Campus for allowing me to pursue my Master's degree. Special thanks to the School of Biological Sciences, this study would not have been possible without the funding opportunity that was afforded to me by the Agricultural Research Council under the Professional Development Programme (PDP).

To my supervisors, Dr C.M.S. Mienie and Dr H.T.H Muedi, thank you for believing in me, for the guidance, mentorship and support throughout the duration of my Masters programme.

I am thankful to my dear mom Constance, dad Willie Hadzhi and younger sisters Aluwani, Lufuno and Ronewa for the love, encouragement, prayers and unconditional faith in me in the pursuit of this Master's degree.

ABSTRACT

Common bean (*Phaseolus vulgaris* L.*)* is a widely cultivated legume crop and also an important component in the human diet as it is a great source of protein. It has a wide phenotypic variability especially for traits such as seed types (size and colour) and plant growth habit. Plant breeding has been used to improve traits of importance in common beans and this includes improving disease resistance in market type cultivars. The aim of this study was to phenotypically screen selected varieties for reaction to rust and to validate the use of the sequence characterized amplified regions (SCAR) molecular markers SK14, Ur4-$SA_{1079/800}$, SI19, and SAE19 for rust resistant genes *Ur-3*, *Ur-4*, *Ur-5* and *Ur-11* in the common bean breeding populations of interest. International germplasm varieties Mexico 235, CAL 143, Flor de Mayo, Cornell 49242, G 21212, G 5828, BelDakMi-RMR-22, Mexico 309, Nep-2, Mexico 54 and BAT 332 showed complete resistance to South African rust races in field evaluations at different localities. Popular South African cultivars including red speckled sugar beans and small seeded types were mostly susceptible to rust disease. Cultivars Mkuzi, Kamiesberg, Tygerberg, Teebus-RR1, Teebus-RCR2, PAN 123 and OPS-KW1 were resistant to rust. Large seeded cultivars Sederberg, Werna and RS 7 were moderately resistant. International rust resistant genotypes Nep-2, BelDakMi-RMR-23 and Mexico 309 and the susceptible South African cultivars Bonus and RS6 were used as the parents for in crosses. The crosses were then screened for resistance in a greenhouse against rust races 1, 3, 5 and 11 following standard artificial inoculation procedures. Following hybridization, the parents, F_1 and F_2 plants characteristics were observed of an individual that showed the interaction of the genotype with the environment (phenotyped). The first trifoliate leaves of the $F_{1:2}$ populations were harvested for DNA extraction and molecular analyses of the targeted resistance genes. The SCAR markers were tested for presence in the parents and linkage in the segregating populations. In the RS6 x BelDakMi-RMR-23 population, gene *Ur-11* could be followed with marker SAE19 (64% linkage), but *Ur-3* (SK14) and *Ur-4* ($SA_{1079/800}$) marker selection cannot be used in subsequent generations. Close linkage (91.5%) of the SK14 marker to the *Ur-3* gene was observed in the Bonus x Nep-2 population. Marker SI19 will be applicable for marker selection of the *Ur-5* gene (80% linkage) present in the RS6 x Mexico 309 population. These crosses will now be used to stack the resistance genes in one or more cultivars utilizing the validated markers for selection of putative resistant progeny.

Key words: Common bean, Disease resistance, Rust

LIST OF ABBREVIATIONS

AFLPs	Amplified Fragment Length Polymorphisms
AMMI	Additive Main Effect and Multiplicative Interaction
ANOVA	Analysis of Variance
ARC-GC	Agricultural Research Council-Grain Crop
ASV	Stability Value
CBB	Common bacterial blight
CIAT	International Centre for Tropical Agriculture
CTAB	Cetyl trimethylammonium bromide
CV	Coefficient of Variation
DAFF	Department of Agriculture, Forestry and Fisheries
DF	Degrees of freedom
DNA	Deoxyribonucleic acid
InDel	Insertion-deletion
LSD	Least significant difference
MAS	Marker assisted selection
MS	Mean sum of squares
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
RAPDs	Random Amplified Polymorphic DNA
RFLPs	Restriction Fragment Length Polymorphisms
RR	Rust Resistance
RSS	Red Speckled Sugar
SAS	Statistical Analysis Software
SCAR	Sequence Characterised Amplified Region
SS	Sum of Squares
SNP	Single Nucleotide Polymorphism
SW	Small white
TE	Tris-HCl/EDTA
™	Trade mark

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 - INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the most cultivated legume crops in the world (Petry *et al*., 2015). The crop has significant nutritional, economic and social importance, making it popular through diverse social dynamics (Petry *et al*., 2015). The crop is well documented as a rich source of protein (15.2-36.0%), and fibre. It has been reported to lower the risk of chronic diseases such as, diabetes and cancer (Holden and Haytowitz, 1998). Dry bean is a regular part of South African dishes in both the rural and urban areas, making it an important part of the diet. Dry bean consumed in South Africa is mainly produced locally; however, imports also form a significant part of the market. (DAFF, 2017). Production of dry bean in South Africa has increased from approximately 35 445 ton/ha in 2015/16 to 68 525 ton/ha in 2016/17 (Department of Agriculture, Forestry and Fisheries, 2017). In South Africa, dry bean production is most prevalent in the humid eastern and central parts of the country (DAFF, 2017). Small white (SW) canning beans are planted widely and forms a big part of the canning industry with a growing market as a convenience and health food (DAFF, 2015). Large seeded types in particular red speckled sugar (RSS) beans are most popular and are sold in retail quantities in supermarkets (DAFF, 2015).

1.1 Problem statement

Dry bean production is hampered by numerous factors including diseases such as common bean rust, which is caused by an airborne fungal pathogen called *Uromyces appendiculatus* (Kelly *et al*., 2003). Numerous control methods for rust disease are available; however, they each have important wide-ranging disadvantages including contamination to the environment and high purchase costs. The disease thrives well in many dry bean-producing areas of South Africa where environmental conditions such as mist and cool temperatures prevail (Liebenberg *et al*., 2002). Rust spreads mainly through wind dispersal and contaminations of clothes and working equipment. *U. appendiculatus* overwinters in dry bean debris, leading to new infections the next season if a susceptible host is planted.

The pathogen has numerous existing races with new races developing rapidly. This phenomenon results in short-lasting disease resistance in dry beans, especially resistance governed by single genes and not multiple genes. Through the years, dry bean cultivars lacking resistance to the disease have been cultivated intensively, mainly by subsistence

farmers who generally are reluctant to adopt the newly released dry bean cultivars which offer improved resistance to rust and better yield. This practice usually results in reduced yield potential of cultivars in areas that are prone to the disease.

1.2 Motivation of the study

Quantitative resistant sources and different single genes can control a large range of variable and complex bean pathogens (Schwartz and Pastor-Corrales, 1989; Wortmann *et al*., 1998). There are specific rust resistance genes that have previously been identified as ideal for dry bean producers in South Africa. These genes, *Ur-3*, *Ur-4*, *Ur-5* and *Ur-11* when stacked together in a single dry bean breeding line, will offer the desired broad-spectrum resistance to the rust pathogen in South Africa. The genes are collectively a strategic combination of Andean and Mesoamerican rust resistance sources, which present an opportunity to overcome the phenomenon of pathogen- and host co-evaluation where pathogenic strains or races are understood to overcome bean types with which they share the origin. Stacking of genes based on phenotypic screening only, will however, be impossible to carry out because of the complexity of the pathogenic rust races needed for inoculation. The presence of specific resistance genes is also difficult to assess in greenhouse studies. Molecular markers are undoubtedly a powerful tool in enhancing plant breeding of the modern times. The application of SCAR markers has predominantly been used across the globe in marker-assisted breeding with great success. However, genetic differences between breeding populations cause variability in the applicability of markers for selection of the desired superior plants. Validation of SCAR markers in disease resistance breeding programs is therefore a crucially important step towards making reliable single plant selections.

1.3 Aim of the study

The aim of the study is to develop dry bean lines with stacked resistance to the rust disease and validate molecular markers associated with each of the resistance genes.

1.4 Objectives of the study

The objectives of the study are

- To screen selected common bean germplasm and cultivars for their reaction to rust disease.
- To develop segregating populations from crosses of resistant and susceptible parents.
- To validate SCAR molecular markers associated with targeted rust resistance genes in the breeding populations.

CHAPTER 2 – LITERATURE REVIEW

2.1 Origin and domestication of common bean

The genus *Phaseolus* is classified in the family Fabaceae and contains about 70 species, all native primarily to Mesoamerica. Domesticated species include *Phaseolus vulgaris* (kidney bean, small white beans, common bean, green bean, etc.*), Phaseolus acutifolius* (tepary bean) and *Phaseolus coccineus* (runner bean) which are all agronomically important in the world (Marechal *et al*., 1978).

Common bean (*Phaseolus vulgaris* L.) originated in America. The crop has two wild cultivated gene pools derived from the Andes mountain of South America (Andean genepool) and from the entire Mesoamerican Corridor through Central America (Mesoamerican genepool) (Blair *et al*., 2006; Blair *et al.*, 2009). The diversity in wild accessions of the species is divided into various sub-populations of species from specific geographical regions (Chacon *et al*., 2007; Miklas and Singh, 2007). The number of sub-populations has been discussed since the division of wild *P. vulgaris* is not as simple as the domesticated beans, which are easily separated into Andean and Mesoamerican genepools (Blair *et al*., 2012). The Andean common beans are large seeded while the Mesoamerican is small seeded (Singh, 2001). The morphological and molecular differences among the two groups of wild accessions are not clear among races in the cultivated types and rely on differences such as seed size, growth habit, flower colouration, bracteole size, seed protein (phaseolin), seed colour and molecular marker evaluations (Chacon *et al*., 2007; Gepts and Debouck, 1991: Gepts and Bliss, 1985; Koening and Gepts, 1989; Kwak and Gepts, 2009; Rossi *et al*., 2009).

Andean beans are presently predominantly grown in Africa, Europe and North Eastern United States while Mesoamerican beans are predominately grown in South Western United States which indicates that the distribution of these two gene pools followed two different routes (Gepts and Bliss, 1985). Studies with Andean wild and cultivated common beans showed that the marker system showed no grouping of wild accessions with the Andean genepool (Beebe *et al*., 2001). The population structure of cultivated common bean is documented and shows a comparison of the genetic diversity of the two gene pools based on multiple molecular studies (Blair *et al*., 2006) and also demonstrated that Mesoamerican beans have a wide genetic diversity when compared to Andean beans (Santalla *et al*., 2004). Some studies have further suggested equal diversity or greater diversity in one or

the other gene pool, with relative diversity in each cultivated genepool subjected to different estimates (Blair *et al*., 2009; 2012; Bitocchi and Nanni, 2012; Bitocchi *et al*., 2013; Gioia *et al*., 2013). However, the Andean beans reportedly have more remarkable morphological diversity compared to the Mesoamerican beans (Santalla *et al*., 2004). The other prominent and interesting feature of these two gene pools is that hybridization can result in weak a F_1 plant which is reported to be an attribute of independent evolution (Gepts and Bliss, 1985, Gepts and Debouck, 1991).

2.2 Economic and Nutritional Importance

Common bean is grown and consumed in many parts of the world, mostly in South-, Central- and North America, Africa, India, Asia and also in Europe and Australia (FAO, 2018). It occupies approximately 85% of the area planted to *Phaseolus spp*. in the world (Singh, 2001). Over 30% of dry bean is produced in Latin America and Africa (Hnatowich, 2000). The world leader in production of dry bean is India, followed by Brazil and Myanmar (Choudhary *et al*., 2018). In Eastern and Southern Africa beans are produced by over 20 countries covering over four million hectares, where major producers include Ethiopia and Rwanda, (Asfaw *et al*., 2009; Wortmann *et al*., 1998), with the most important dry bean producer Tanzania, followed by Kenya and Uganda (Awori *et al* ., 2017). Production of bean is important for the food security of Uganda where beans are a major source of protein for most families (Broughton *et al*., 2003). Fifty percent of grain produced is used for direct human consumption (Blair *et al*., 2009). The total area under production worldwide is estimated to be over 18 million ha (Graham and Ranalli, 1997).

Dry bean is presently regarded as one of the most important field crops in South Africa on account of its high protein content (22-24%) and nutritional benefits (Liebenberg *et al*., 2002). Dry beans contributed an estimated amount of R1 039 million to the gross value of field crops for the 2016/17 season, which is 102.1% more than the R514 million of the previous season (DAFF, 2017). The contribution of different types of dry beans to total local production in 2016/17 is estimated to be as follows: light speckled kidney beans 45 597 tons (67%), white pea beans 20 700 tons (30%), large white kidney beans 1 300 tons (2%) and other dry beans 600 tons (1%), mainly cariocas (DAFF, 2017). The estimated commercial crop of 68 525 tons for 2016/17 is 93.3% more than the previous crop of 35 445 tons. The average yield for the 2016/17 crop is approximately 1.52 t/ha - an increase of 47.6% from the 1.03 t/ha of the previous season. An estimate of 126 370 tons of dry beans was expected to be consumed locally during the 2017/18 marketing season (April to

March), which is 34.3% more than the 94 078 tons in 2016/17. Consumption projected per capita was 2.06 kg, which is 29.6% more than the 1.59 kg in 2016/17 (DAFF, 2017). Commercially available dry bean cultivars have a growth period that ranges from 103 to 121 days after planting, which could fluctuate by at least ten days, depending on the weather conditions (Fourie *et al*., 2011).

Dry bean production provides farming households with both income and food security, combating nutritional deficiencies. Due to its high protein content, common bean has been described as “the meat for the poor” as it is the best substitute for expensive animal protein (Kimani *et al*., 2001). Although much of the staple diets of Sub-Saharan Africa are largely cereal based, providing the much needed energy, common bean on the other hand is rich in protein, complex carbohydrates, folic acid, dietary fibre, zinc and iron (Beebe *et al*., 2000). It is low in fat and sodium, contains potassium, vitamin A, ascorbic acid, has flavones and niacin - the latest being an ingredient for the prevention and treatment of diseases such as low blood sugar and obesity (Holden and Haytowitz, 1998; Huang *et al*., 1991; Vorster and Venter, 1994), and minerals such as phosphorus (Broughton *et al*., 2003).

Benefits of consuming common beans include decreased absorption of dietary cholesterol due to plant sterols, increased fibre intake to control blood glucose concentrations and decreased body weight which lowers Type 2 diabetes through reduction in glycaemic load of a meal and providing slowly digestible carbohydrates (Hutchins *et al*, . 2012). Regular consumption of beans typically reduces colon cancer by 50-75%, prevents malnutrition helps to improve the immune system and increases the CD4 count in HIV positive persons (Mensack *et al*., 2010). It is also a relatively inexpensive food source (Pachico, 1993).

Leguminous food crops are harvested fresh to eat raw or cooked, as dried seeds for long term storage and cooking (Beebe *et al*., 2014). Dry bean is considered the most important leguminous crop in terms of its nutritional value. The leaves, green pods, fresh seeds and dry grains of this crop are consumed (Beebe *et al*., 2014). Common bean production also improves soil fertility by fixing nitrogen in the soil, an intrinsic feature of legume crops (Rondon *et al*., 2007) and is thus a preferred choice for crop rotation.

2.3 Climatic Requirements

Dry bean is an annual crop which thrives well in warm climatic conditions. It grows optimally at temperatures between 18 to 24°C. The maximum temperature during flowering should

not exceed 30°C for small seeded dry bean and 26°C for large white kidney bean (Liebenberg *et al*., 2002). High temperatures during the flowering stage lead to abscission of flowers and a low pod set, resulting in yield losses. Day temperatures below 20°C cause a delay in plant maturity and empty matured pods develop. Cultivated under rain fed conditions, the crop requires a minimum of 400 to 500 mm of rain during the growing season, but an annual total of 600 to 650 mm is considered optimal (Liebenberg *et al*., 2002). Cultivation is performed in areas with altitudes more than 1000 m above sea level, concentrated in cooler highlands and warmer mid-elevation areas, however the cropping area is being extended to lower elevations due to population pressure (Katungi *et al*., 2009).

In South Africa, dry bean production is most prevalent in the humid eastern and central parts of the country. Large seeded bean types, mostly red speckled sugar (RSS) beans, are the most popular and most consumed locally (DAFF, 2017). Dry beans in South Africa are produced in the following areas: Mpumalanga/Gauteng (Middelburg, Nigel, Delmas and Ermelo), Free State (Bethlehem, Fouriesburg, Harrismith and Kroonstad), North-West (Lichtenburg, Koster, and Brits), Limpopo (Thabazimbi, Koedoeskop), Kwazulu-Natal (Kokstad, Vryheid, Bergville, and Winterton) and Northern Cape (Kimberley, Douglas). Limpopo, North-West, Free State, Kwazulu-Natal and Northern Cape are commercial production areas while the Lowveld in the Mpumalanga province and the Western Cape are seed production areas (DAFF, 2017).

2.4 Production challenges of common bean

Factors that contribute to production challenges in the world are classified into two broad categories, namely biotic and abiotic. Abiotic challenges that affect production of dry bean include low fertile soils, drought and water stress which have the capacity to reduce yield and may cause complete crop failure (Kelly and Miklas, 1999; Thung and Rao, 1999). High temperatures have an effect on the total bean yield and production (Kelly and Miklas, 1999; Liebenberg *et al*., 2002; Singh, 1999; Singh and Munoz, 1999). Other abiotic factors such as frosts, low temperatures and hail (Brick and Grafton, 1999) as well as air pollution and soil disorder like soil erosion, soil crusting and compaction have a negative effect on common bean yield (Thung and Rao, 1999). In some cases, abiotic factors can trigger biotic stress (Thung and Rao, 1999). The most predominant climatic related factor is inadequate rainfall, which result in moisture deficit where under severe conditions the problem causes complete crop loss (Wortmann, 1998). Soil production limiting factors includes essential

nutrient deficiency such as low nitrogen, phosphorus and potassium, poor exchangeable bases and aluminium, zinc and manganese toxicity (Kimani *et al.,* 2001).

Diseases and insect pests are major biotic factors that limit common bean production. A lot of disease-causing pathogens attack common bean and cause considerable economic loss (Vieira *et al.,* 2010). Diseases are reported to have more severe impact in the tropics than in cool temperate climates. Rust is among the most economically important and destructive diseases of dry bean (Liebenberg and Pretorius, 2010; Miklas *et al*., 2006; Steadman *et al*., 2002). The farming system of the tropics where two to three planting cycles are achieved in a year, results in a continuity of inoculum which attributes to severity in disease under field conditions. Insect pests are of greater economic importance in Africa and Latin America than in USA and Europe (Graham and Ranalli, 1997).

Within the species P*. vulgaris* may vary in a number of phenotypic features. *P. vulgaris* seed can display different sizes, types and colours, with the most well-known being small white (SW) and red speckled sugar (RSS) beans (Liebenberg *et al*., 2002). Differences occur in adaptability, growth habit, disease resistance and other characteristics (Liebenberg *et al*., 2002). The RSS bean cultivars are widely grown nationwide and are popular within the small scale farmer community (Liebenberg *et al*., 2002). Teebus is a well-adapted and predominant small white cultivar used for canning and considered to have superior canning quality (Fourie, 2015), however, it is susceptible to rust (Fourie, 2015). Dry bean production is hampered by numerous factors including diseases such as common bean rust (Kelly *et al*., 2003), amongst many other fungal, bacterial and viral diseases.

Apart from abiotic and biotic factors agronomic factors such as late planting, poor weed management, mono-cropping and use of unimproved seed have been reported to limit common bean production (Kimani *et al*., 2001).

2.5 Common bean diseases

Diseases are regarded as one of the most important production constraints that lead to decreased common bean production world-wide. Different types of bacterial, fungal and viral pathogens affect crops during different development growth stages of the plant and cause yield loss (Schwartz and Pastor-Corrales, 1989; Schwartz *et al*., 2005). The major bacterial diseases are common bacterial blight, halo blight and bacterial brown spot.

The common bean diseases include angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), bacterial common bean diseases includes bacterial brown spot (*Pseudomonas syringae pv. syringae*), root rots (*Aphanomyces*, *Fusarium, Pythium, Rhizoctonia*, *Thielaviopsis* spp.) and rust (*U. appendiculatus*) (Beebe and Pastor-Corrales, 1991; Miklas *et al*., 2006, Singh and Schwartz, 2010). Insect pests that are of economic importance in common bean production include, bean pod weevil (Subfamily Brichinae), bruchids (Coleoptera: *Bruchidae*) and aphids (*Aphis fabae* and *A. craccivora*) (Graham and Ranalli, 1997; Miklas *et al*., 2006; Wortmann, 1998).

2.5.1 Rust

The disease develops and thrives in cool, humid tropical and sub-tropical areas as well as humid temperate areas (De Souza *et al*., 2008). Temperatures favouring germination of spores differ; however optimum infection takes place at 17ºC (Harter *et al.,* 1935) or between 18-21ºC (Shands and Schein, 1962) with a decrease in the number of pustules per leaf at incubation temperature of between 21-25ºC (Mendes and Filho, 2008). The development of rust disease is influenced by temperature, where changes of 5-6ºC for 4-8 hrs can significantly influence the epidemiology of the disease, interspersed with dryer periods. Night temperature of 20-26ºC for instance, inhibits pustule development (Schein, 1961).

U. appendiculatus, an obligate parasite, is autoecious and macrocyclic, completing its entire life cycle on the common bean (Harter and Zaumeyer 1941). Initially rust symptoms germinate on the surface of the leaf and germ tube as small yellow or white, slightly raised spots on the upper or lower surface of the leaf and grows parallel to the epidermis until it reaches a stoma. Once the stoma, appressorium forms where the physical topography of the host appears to play a key role (Allen *et al*., 1991). Disease symptoms can be seen on both sides of the leaves, also on stems and pods (McMillan and Schwartz, 1994; McMillan *et al*., 2003). The lesions later enlarge and turn brown as the fungus sporulates to form uredinia or pustules that are usually surrounded by a chlorotic halo (Liebenberg, 2003) and a ring of secondary pustules which may develop on susceptible genotypes (Liebenberg and Pretorius, 2010). Aust *et al*. (1984) indicated that bean pustules have the potential to release more than 20 000 urediniospores per pustule per day. These spores usually intensify in quantity during the reproduction stage of the crop and therefore play an important role in the spread of the disease, especially when windy conditions alternate with wet weather (Stavely, 1991; Stavely and Pastor-Corrales, 1989). To survive the winter

period, urediniospores become hardened and turn black, and are in this form called teliospores (Aust *et al*., 1984).

2.5.2 Distribution and social importance of rust

Rust pathogen spores are distributed all over the world and can effectively cause major production problems in the humid tropical, subtropical areas and periodic severe epidemics in humid temperate regions (Pastor-Corrales, 2004). Rust is an air borne fungal disease which easily distributes over large areas. Its economic impact is high when the plants are infected during pre-flowering and flowering development stages (Pastor-Corrales, 2004). The yield losses that are being measured worldwide in greenhouse and field conditions can vary from 18 to 100% reduction of grain yield in dry beans while reduction in pod quality has also been reported (De Jesus *et al*., 2001). Field experiments and surveys have ranked bean rust as a major disease in some of the African countries (Lindgren *et al*., 1995; Monda *et al*., 2003; Wahome *et al*., 2011). In South Africa, rust incidence is common in areas with favourable conditions, mainly the eastern part of the country and losses of common bean due bean rust (Liebenberg, 2003).

2.5.3 Disease control Measures of common bean rust

The control of common bean rust is based on three main strategies of integrated disease management: fungicides applications, host resistances and other kinds of cultural or breeding practice methods. The use of resistant cultivars is a major component of bean rust integrated management (Liebenberg *et al.,* 2002). Disease control management practices for rust include crop rotation, soil incorporation of infected bean debris which may bear viable urediniospores and teliospores overwintering from rust spores, planting during the recommended dates, growing cultivars which are resistant, and timely spraying of fungicides (De Souza *et al*., 2008; Mmbaga *et al*., 1996). Beans are normally rotated with maize and sunflower in South Africa in order to be able to add stability and a bit of security to the soil. Planting dates may be adjusted in some of the production areas to decrease the incidence of rust by minimizing the exposure to moderate to cool temperatures and long dew periods during the critical pre-flowering to flowering stage of plant development (Stavely and Pastor-Corrales, 1989).

As a biological control measure, the bacteria *Bacillus subtilis* can be sprayed on the field of dry bean at a sequence of at least three times a week where 75% reduction in rust severity was recorded (Baker *et al*., 1983) and is currently the most promising biological control method for sustainable agriculture (Wang *et al.,* 2018). Allen (1982) and Grabski and Mendgen (1986) reported that the fungus, *Verticillium lecanii* (Zimm.) *viegas* penetrates, invades, and kills urediniospores and teliospores of *U*. *appendiculatus*, and colonizes pustules.

Fungicides such as chlorothalonil and some dithiocarbamates are effective in controlling rust, especially when sprayed at the appropriate time (Lindgren *et al*., 1995; Schwartz *et al*., 1994) when the symptoms are first seen and low severity, however fungicides have no limited curative activity (McGrath, 2014). Bean rust has been controlled by dusting plants every 7-10 days with sulphur at a rate of 25-30 kg/ha after pustule first appears. A seven to fourteen-day spray schedule can be recommended for other preventive chemicals such as chlorothalonil, maneb, mancozeb, bitertanol, triadimefon, propriconazole, triphenylphosphite, and oxycarboxin (Stavely and Pastor-Corrales, 1989). Punch C (trademark of Du Pont) is currently the preferred fungicide used to control rust at the Agricultural Research Council-Grain Crops (ARC-GC) of South Africa in the research trials (Liebenberg *et al*., 2002). Fungicides are costly in the subsistence production systems of Africa and Latin America, where most of the world's common bean production occurs (Broughton *et al*., 2003; Wortmann *et al*., 1998). Due to the relatively high cost of the fungicide treatments, there is a decrease in profits and with variable effectiveness of cultural practices, more attention has been given to host resistance (Jochua *et al*., 2008).

Breeding for resistance is regarded as the most cost effective and beneficial control measure for many diseases including rust (Coyne and Schuster, 1975). This approach is also harmless to the environment and it is an economically cost effective strategy when compared to chemical control (De Souza *et al*., 2005a, 2007a, 2007b, 2013). Resistance to rust has been reported in many international common bean varieties that are part of the germplasm collection of the ARC-GC (Liebenberg, 2003; Liebenberg *et al*., 2005).

2.6 Dry bean genetics

Common bean is a diploid plant with n=11 chromosomes estimated to have a genomic size of 514 Mb (Blair *et al*., 2018). Blair *et al*. (2018) used an array of 812 single nucleotide polymorphisms (SNP) and 265 previously mapped markers, including dominant type

markers AFLP and RAPD as well as co-dominant type markers including RFLP's from the Bng and D series and SSRs from BM and BMd series resulting in a map of 1 000 markers in a well-known Recombinant Inbred Line (RIL) reference mapping population (BAT93 x Jalo EEP558) (Blair *et al*., 2018). The SNP map was corrected by adding legacy markers as chromosomal anchor so as to identify each chromosome (Blair *et al*., 2018). Variability in genetic and physical distance ratio ranged from 1.24 cM/Mb for Pv09 to 2.87 cM/Mb for Pv02, which showed that for each linkage group the genetic map size was generally well correlated with the physical length of the chromosome (Blair *et al*., 2018). The map covered all eleven linkage groups (LG). The genetic distance of the final map was 1097.5 cM with an average length of the linkage groups 99.8 cM and an average distance between markers of 1.35 cM. Linkage disequilibrium levels within the Mesoamerican gene pool where found to be stronger while the Andean genepool was said to decay more rapidly (Blair *et al*., 2018). The recombination rate across the genome was 2.13 cM/Mb, but recombination was found to be highly repressed around centromeres (Blair *et al*., 2018).

2.6.1 Genetics of rust resistance in common bean

Common bean germplasm can be classified into two gene pools or centres of origin, Andean and Mesoamerican based on the level of divergence at the molecular level (Gepts, 1993) and the existence of reproductive isolation (Coyne, 1965; Koinange and Gepts, 1992; Sprecher and Khairallah, 1989). Each of these two gene pools had been subdivided into races primarily based on comprehensive analysis of germplasm within the Andean and the Mesoamerican centres (De Souza *et al*., 2002; Liebenberg and Pretorius, 2004a, 2004b). Several genes which are derived from Mesoamerican rust resistance (RR) sources (*Ur-3, Ur-5, Ur-7 a*nd *Ur-11*), and the Andean (*Ur-4, Ur-6, Ur-9,* and *Ur-12*) gene pool have been shown to confer resistance to different races of dry bean rust (Kelly *et al.,* 2003; Miklas *et al*., 2002; Miklas *et al*., 2006; Stavely, 2000; Pastor-Corrales, 2001; Wright *et al.,* 2008). The genes of interest for the current study *Ur-3*, *Ur-5* and *Ur-11* rust resistance genes are from the small and medium seeded beans of the Mesoamerican gene pool, while *Ur-4* and *Ur-6* are from the large seeded beans of the Andean gene pool (Pastor-Corrales *et al*., 2007). The *Ur-3* gene was found to be effective in South Africa, where rust is the most devastating disease on dry beans (Liebenberg, 2003).

Rust resistance in common bean is determined by specific host pathogen interactions which are mostly controlled by a gene for gene model (Finke *et al*., 1986; Stavely, 1984; Stavely and Pastor-Corrales, 1989). Plant Introduction (PI) 181996 is a Mesoamerican bean variety

that contains rust resistance gene *Ur-11,* which confers resistance to almost all known rust races (Stavely, 1990). However, this vigorous climbing bean is poorly adapted in South Africa and has an unmarketable small black seed. The *Ur-11* gene has been introduced successfully into the well-adapted, rust susceptible South African cultivars, for instance the SW canning bean Teebus and several RSS beans (Liebenberg *et al.*, 2002). Limited gene pyramiding with other RR genes has also been undertaken (Liebenberg *et al*., 2006) to ensure more stable resistance.

Resistance to bean rust is mainly controlled by major single dominant genes (Alzate-Marin *et al*., 2004; Correa *et al*., 2000; De Souza *et al*., 2007a, 2007b, 2007c; Faleiro *et al*., 2000). Resistance can also be controlled by single recessive genes (Luann-Finke,*et al*., 1986; Zaiter *et al*., 1989). De Souza *et al*. (2013) reported that at least 14 major dominant RR genes have been identified (*Ur-1* to *Ur-14*). In addition to these 14 named genes, other important unnamed RR genes have also been identified in varieties such as Dorado (Miklas *et al*., 2000, 2002) CNC (Rasmussen *et al*., 2002) and PI 260418 (Pastor-Corrales, 2005; Pastor-Corrales *et al*., 2008).

Twelve rust differential lines are used for characterising the RR genes which are effective against different rust races in greenhouse studies. These lines contain the most important rust resistance genes *(Ur-3, Ur-5, Ur- 11* and *Ur-13)* of Mesoamerican origin are available at Agricultural Research council – Grain Crop (ARC-GC) (Liebenberg, 2003). Previous reports indicate that the RR genes (*Ur-4, Ur-6, Ur-7* and *Ur-8)* of Andean origin were generally susceptible and thus ineffective in Africa, reflecting the historical preference for large seeded beans in Africa and co-evolution of host and pathogen (Liebenberg and Pretorius, 2010). In Africa, presumably due to the predominance of large-seeded beans, the most effective sources of disease resistance in particular, rust resistance are of Mesoamerican origin (Jochua *et al*., 2004; Liebenberg, 2003).

2.6.2 Breeding Dry Bean for Rust Resistance

Introducing suitable, multiple resistance genes into locally adapted, high yielding and stable cultivars, remain the most cost effective control measure and are widely utilized in the control of rust (Coyne and Schuster, 1975; Stavely and Kelly, 1996). However, in areas where successful breeding has not yet been undertaken, the disease remains a problem in the favourable climate, or where races virulent on previously resistant materials have appeared (Stavely and Kelly, 1996).

Mmbaga *et al*. (1996) and Stavely (1984) indicated that bean cultivars that have a single rust resistance gene have been developed, with less durability due to the likely appearance of races of the pathogen with new or different virulence soon after their release. *U. appendiculatus* is among the most pathogenically variable plant pathogens with the most diversity in virulence and its race components in a population can vary within a matter of time, space, and more than one race can simultaneously occur on the same leaf (Jochua *et al*., 2008; Groth *et al*., 1995).

The range variability and complexity among bean pathogens can be controlled by single genes or by quantitative trait loci (QTLs). Combining these resistance sources into commercial cultivars is a major challenge for bean breeders (Adam-Blondon *et al*., 1994). The pyramiding of different rust resistance genes in the same genetic background could be the best approach in order to obtain bean cultivars with durable and wide spectrum resistance (Alzate-Marin *et al*., 2005; Coyne and Schuster, 1975; Miklas *et al*., 1993) and remains the most cost effective control measure for the rust pathogen (Liebenberg *et al.,* 2005). Combining multiple disease resistance genes has been the most widespread application for pyramiding (i.e. combining multiple qualitative resistance genes together into a single genotype) (Collard and Mackill, 2008). In terms of hybridization between bean lines from the two origins, it has been discovered that weak F_1 plants are formed, a phenomenon that is attributed to independent evolution (Gepts and Bliss, 1985, Gepts and Debouck, 1991).

2.7 Molecular marker technology

Molecular-genetic maps and Quantitative Trait Loci (QTL) mapping are tools used for the localization of genomic regions that control single and complex inheritance, making the genetic architecture of the traits of interest such as resistance to disease possible (Lynch and Walsh, 1998). It is interesting to have maps fully saturated with markers that indicate genes and QTLs' locations from the breeding perspective (Hanai *et al*., 2007). This information could be used in helping breeders understand the effects and mode of action of loci that control the traits of interest in the breeding programs when producing new cultivars through marker assisted selection (MAS) breeding (Bassi *et al*., 2017).

Molecular markers exploit the discovery that Mendelian genetic factors which lie close together on a chromosome are usually co-transmitted from one parent to progeny (Tanksley et al., 1989; Collard and Mackill, 2008). If the desired genes are tightly linked to a DNA marker, the segregating population of plants can be screened in the seed or seedling stage for the presence of the genes of interest before the traits are expressed (Tanksley *et al*., 1989).

The detection of genes and QTLs controlling traits are possible due to analysis of genetic linkage, based on the principle of genetic recombination when meiosis takes place (Tanksley, 1993). The construction of linkage maps is composed of genetic markers for specific populations (Collard and Mackill, 2008). The F_2, F_3 and backcross segregating populations are frequently used for the purpose of marker development, however populations such as recombinant inbred (RI) lines, and double haploids (DH) have an advantage in that they are homozygous and can therefore be maintained over a long period of time for continuous use and produced permanently (Collard and Mackill, 2008). The latter two populations are generally preferred because they can be replicated and repeated on experiments (Collard and Mackill, 2008). Collard *et al*. (2005) reported that major advantages of using RI and DH populations are that they produce homozygous or true breeding lines that can be multiplied and produced without genetic change occurring.

Genetic markers represent genetic differences between individuals or species. Generally, they are used for labelling and tracking the genetic variations in the DNA samples. They act as a sign or flags and they are used as chromosome land marks to facilitate the introgression of chromosome regions with genes associated with economically important traits (Kordrostami and Rahimi, 2015). The phenotype of the traits of interest is not affected by the genetic markers because they are located near or linked to genes controlling the target traits (Tryphone *et al*., 2013). To evaluate DNA polymorphism, various types of molecular markers are utilized and are classified as either hybridization based or polymerase chain reaction (PCR) based markers. DNA markers are useful particularly when they reveal the difference between individuals of the same species or different species (Choudhary *et al*., 2018; Collard *et al.,* 2005), are practically unlimited in numbers and are also not affected by the environmental factors and development stages of the plant (Winter and Kahl, 1995).

The presence of disease resistance genes may not always be easy to assess conclusively from the host plant reaction and the presence of genes may be masked by epistasis of

resistance genes (Young and Kelly, 1996). Single gene resistance can be overcome by newly emerging pathogen types, which is a common occurrence in resistance breeding and the pyramiding of several complementary genes is widely seen as a way to obtain more durable host plant resistance (Sanglard *et al*., 2009). To achieve this pyramiding of resistance genes, without potentially loosing sources of resistance already present but defeated by local races, or hypostatic to newly introduced resistance genes, a MAS approach is proposed (Young and Kelly, 1996). Genetic markers originate from DNA sequence polymorphisms and can be used to distinguish the parental origin of alleles (Andersen and Lübberstedt, 2003). Marker-assisted selection offers a better option to overcome problems of masking hypostatic genes and inadequate inoculation techniques, resulting in disease escape in conventional screening (Stavely, 2000). It has also been possible to identify linkage between markers and quantitative trait loci controlling complex traits such as stress tolerance (Schneider *et al*., 1997).

The discovery of restriction fragment length polymorphisms (RFLP) by Botstein *et al*., (1980), created hope in plant molecular breeding, however the technique was time and cost ineffective. RFLPs flag polymorphisms in restriction enzyme recognition sites because they result in differential lengths of DNA product, which are then separated by gel electrophoresis (Botstein *et al*., 1980). They are particularly useful in that they are co-dominant, meaning they can distinguish heterozygotes from homozygotes (Botstein *et al.,* 1980). With the advent of the polymerase chain reaction (PCR), public and commercial research institutes switched to PCR based molecular markers (Earthington *et al.,* 2007). These include random amplified polymorphic DNA (RAPD) developed by Williams *et al*. (1990), amplified fragment length polymorphism (AFLP) developed by Vos *et al*. (1995) and microsatellites or short sequence repeats (SSR) as utilised in maize by Taramino and Tingey (1996). AFLPs and RAPDs were highly favoured due to their ease of application in the laboratory, but unfortunately their reproducibility between different laboratories are low (Taramino and Tingey, 1996).

Simple sequence repeat markers (SSR) are hyper variable, numerous and fairly evenly distributed co-dominant markers consisting of differing numbers of short (usually less than 100 bases) repeated sequences and can be easily assayed using PCR (Sunnucks, 2000). SSR markers along with the advancement in automation of molecular genotyping dramatically reduced the cost per data point (defined as the genotype of one genetic sample revealed by one marker) (Earthington *et al*., 2007). Genome-wide scans consisting of 300-400 SSRs at an average spacing of around 10 cM was a norm (Earthington *et al*., 2007; Sunnucks, 2000).

Allele specific associated primers and SCAR markers offer an additional refinement over RAPD markers since they allow a number of marker analyses and eliminate problems of reproducibility associated with RAPDs (Kelly and Miklas, 1998). These markers produce a single polymorphic band that is more reproducible across different laboratories, are easier to score, and applicable to use with low-quality DNA obtained through speedy DNA extraction procedures (Kelly and Miklas, 1998). Their development however is costly and labour intensive. Several SCAR markers have been developed for *Ur-* genes for rust resistance (Melmaiee *et al*., 2013; Mienie *et al*., 2005; Miklas *et al*., 2002; Park *et al.,* 2004; Queiroz *et al*., 2004). A number of problems have been encountered in the development of SCAR markers. For instance, SCAR markers crops did not show the same polymorphism as they did when using RAPD markers (Kelly and Miklas, 1998). The SCAR markers are being extensively employed by scientists at CIAT to ensure that most of the genes are present in all new bean germplasm targeted for production (Kelly *et al*., 2003). Although the markers have many negative issues, once validated in a specific breeding population, these markers are very reliable and easy to apply.

2.7.1 Application of molecular markers in dry bean breeding

Development of molecular markers in common bean began in the 1990s, where most of the molecular markers were derived from RFLPs (Adam-Blondon *et al*., 1994; Vallejos *et al*., 1992) and RAPDs (Freyre *et al*., 1998). Amplified Fragment Length Polymorphisms (AFLPs) have also been used to develop a Sequence Characterized Amplified Region (SCAR) for a rust resistance gene, *Ur-13* (Mienie *et al*., 2005) and genes associated with resistance to other bean pathogens such as bacterial blight (Naidoo *et al.,* 2003). However, single nucleotide polymorphism (SNP), insertion-deletion (InDel) detection and genotyping have become more feasible on a whole genome scale and widely applied to diversity and plant association studies (Thudi *et al*., 2012; Varshney *et al*., 2014). Molecular markers which are available for dry bean include 23 RAPD and 5 SCAR markers that are linked to 15 different rust resistance genes and QTL. Other markers are available for genes conditioning resistance to major pathogens of common bean including bean common mosaic necrosis virus (Teran *et al*., 2013.), common bacterial blight (*Xanthomonas axonopodis pv*. *phaseoli*) (Fourie, *et al* 2002), halo bacterial blight (*Pseudomonas syringae pv phaseolicola*) (Liebenberg *et al*., 2005), angular leaf spot (*Phaeoisariopsis griseola*) (Kelly, 1995) and anthracnose (*Colletotricum lindemuthianum*) (Goncalves-Vidigal *et al*., 2011; Tryphone *et al*., 2013) Bacteria brown spot (Fourie 2002).

Several authors reviewed the potential of using MAS in breeding common bean (Kelly and Miklas, 1999). Using specific races of the bean rust pathogen in multiple individual race inoculations provides a reliable means of detecting the rust resistance genes of selected *U. appendiculatus* that produce proven reactions in the presence of the rust resistance genes of choice (Groth and Roelfs, 1982). However, when breeding for disease resistance, the use of MAS helps prevent the introduction of exotic pathotypes of a pathogen into the environment and thus does not require secure quarantined facilities, which would otherwise be needed to screen for the presence of hypostatic resistant genes (Kelly and Miklas, 1999).

Molecular markers have been used extensively in assistance in the different filial generations of common bean breeding programs that were aimed to develop rust resistant cultivars (McClean *et al*., 1995; Faleiro *et al*., 1999). It is particularly important when developing bean breeding lines with multiple resistance (Bernardo, 2008).

Several molecular markers were developed into SCAR markers (Melmaiee, *at el*., 2013; Mienie *et al*., 2005; Miklas *et al*., 2002; Park *et al.,* 2004; Queiroz *et al*., 2004) and have previously been used to identify rust resistance genes in common bean. Two SCAR markers have been published for the *Ur-11* gene which are SAE19 (Johnson *et al*., 1995; Miklas *et al*., 2002; Queiroz *et al*., 2004) and UR11-GT2 (Boone *et al*., 1999; Miklas *et al*., 2002). Molecular markers have been developed for *Ur-3* (De Souza *et al*., 2003; Hurtado-Gonzales *et al*., 2017; Queiroz *et al.,* 2004), *Ur-4* (Mienie *et al*., 2004; Miklas *et al*., 2002; Miklas *et al*., 1993) and the SI19 marker for *Ur-5* (Haley *et al.,* 1993; Melotto *et al*., 1998; Miklas *et al*., 2002). However, there is a limitation to some of the molecular markers with low utility across different genetic backgrounds or gene pools of the common bean (Steadman *et al*., 2002). Close linkage of *Ur-11* and *Ur-3* genes possibly explains the lack of reproducibility of its published markers (Miklas *et al*., 2002; Steadman *et al*., 2002).

The *Ur-5* gene has been detected effectively using a co-dominant SCAR marker reproducible across snap bean cultivars (Mesoamerican gene pool) which was also effective against the East African rust races on the Andean gene pool (Wasonga, 2010). The SCAR marker $SA14_{1079/800}$ was developed from the RAPD marker $OA14_{1100}$ (Mienie *et al*., 2004), resulting in a co-dominant marker amplifying two different sized bands in resistant and susceptible plants. This marker can only be used in Mesoamerican genotypes as it is positive in all South African cultivars from Andean origin, except for genotype KW 780 (Liebenberg *et al*., 2004a). Broad utility of the *Ur-11* markers is essential to facilitate transfer of the gene to bean genotypes of Andean and Mesoamerican gene pools

(Wasonga, 2010). However, the published SCAR markers for the *Ur-11* gene lack reproducibility across different common bean genetic backgrounds (Wasonga, 2010). Lack of reproducibility of the markers lead to the necessity of validating the markers in different breeding populations.

Although *Ur-11* is genetically dominant over many other less effective resistance genes, it cannot be detected in the presence of a combination of the other RR genes such as *Ur-3* and *Ur-5* genes. However, the genes in Teebus are unknown and originated from the Mesoamerican gene pool (Liebenberg *et al*., 2002; Liebenberg and Liebenberg, 1998, 2000a, 2000b; Liebenberg *et al*., 2005). The best option to detect individual resistance genes where more than one gene is present, is by using molecular markers. The *Ur-3* gene has been mapped on Chromosome Pv11 of common bean (Stavely, 1998; Miklas *et al*., 2002). Therefore, inheritance of resistance and phenotypic data has revealed that these two genes (*Ur-3* and *Ur-11*) have a strong close link (Kelly, 1995), although reports later demonstrated the independence of the two genes and revealed that these two genes were linked in repulsion phase and are different from one another (Stavely, 1998). Rust resistance genes named *Ur-6* and *Ur-7* are also on Pv11 as well as two other unnamed genes (*Ur-Dorado53* and *Ur-BAC 6*). However, they are not tightly linked to *Ur-3* and *Ur-11* as compared to the link between the two genes (*Ur-3* and *Ur-11*) (Miklas, 2002; Kelly *et al*., 2003). The *Ur-3* gene is epistatic to the *Ur-11* gene and this makes it difficult to distinguish between the two genes when inoculating with races of the rust pathogen (Stavely, 1998). The *Ur-5* gene originated from the Mesoamerican gene pool and has a broad resistance against many races of the bean rust pathogen although mostly to rust races that are known to originate from the Andean genepool (Pastor-Corrales, 2006). The *Ur-3, Ur-5* and *Ur-11* genes (Mesoamerican) already have closely linked DNA markers identified (Hurtado-Gonzales *et al*., 2017), however a marker for *Ur-11*, originating specifically from BelDakMi-RMR-23 has been developed (Pastor-Corrales *et al*., 2007).

The pyramiding of different resistance genes will prolong the life of a bean cultivar by creating a more durable resistance complex against the highly variable rust pathogen (Jung *et al*., 1998; Mmbaga *et al*., 1996). Markers have been useful in the maintenance of hypostatic rust resistance genes in the presence of epistatic resistance genes (Stavely *et al*., 1994). However, molecular markers may not be reproducible across different breeding populations and need to be validated for use in a specific cross (Collard and Mackill, 2008).

2.7.2 Population Development

To develop new cultivars with resistance to rust certain breeding methods have been selected and widely used (Coyne and Schuster, 1975). The breeding lines could be crossed to germplasm that have plant resistance (Jung *et al*., 1998), which impart race non-specific resistance to complement and develop a more durable and higher level of rust resistance (Mmbaga and Steadman, 1992). A backcross breeding method has been used widely on the RR of some cultivars in order to obtain improved desirable cultivars, however, attempts to develop cultivars have been complicated by the great pathogenic variation of the fungus (Alvarez-Ayala and Schwartz, 1979; Correa-Victoria, 1987; Nietsche *et al*., 1997; Sartorato *et al.,* 1991; Sartorato and Rava, 1984; Villegas, 1959). A backcross-breeding program is used to transfer favourable traits from a donor plant into an adapted variety (Ragimekula *et al*., 2012). The backcrossing method was first described in 1922 and between the 1930's and 1960's it was then widely used (Stoskopf *et al*., 1993). Backcrossing consists of three levels of selections in which markers may be applied. Markers can be used in the context of Marker assisted backcrossing (MABC), to either control the target gene (foreground selection) or to accelerate reconstruction of the recurrent parent genotype (background selection) and to select backcross progeny having the target gene with tightly linked flanking markers in order to minimize linkage drag (recombination selection) (Ragimekula *et al*., 2012). MABC is efficient if a single allele is transferred into a different genetic background, for example, in order to improve an existing variety for a specific trait (Ragimekula *et al*., 2012).

2.7.3 Validation of markers

In order for MAS to be successful the markers must meet some requirements: 1) The marker must be tightly linked to the gene of interest; 2) must be stable and reliable in the selected breeding population and 3) the assay must be easy and cost effective to use (Tryphone *et al*., 2013). The utility of markers linked to resistance genes must be demonstrated outside the original mapping population (Miklas, 2002). Reasons why previously developed markers may not be useful or have restricted utility include: 1) linkage intensity may vary across different genetic backgrounds, 2) the gene is not expressed in certain genetic backgrounds, 3) the marker may be difficult to assay due to differences in PCR equipment and protocols, 4) markers can occur in a gene-pool specific pattern (Miklas *et al*., 2002). Thus the efficiency of the marker to predict the phenotype of progeny in a new breeding population must be validated.

Marker validation involves testing the reliability of the markers to predict phenotype (Ogbannaya *et al*., 2000). This will show if the marker can be used in routine screening for MAS (Ogbannaya *et al*., 2000; Sharp *et al*., 2001). Markers are validated by testing for the presence of the markers on a range of cultivars and other important genotypes (Sharp *et al*., 2001; Spielmeyer *et al.,* 2003) and also testing their effectiveness in determining the target phenotypes in independent populations and different genetic backgrounds, even when a single gene controls a particular trait (Yu *et al.,* 2000). In breeding programs, markers that are useful should be able to reveal polymorphism in different populations derived from a range of different parental genotypes (Langridge *et al*., 2001).

2.7.4 Pyramiding of resistance genes

The process of simultaneously combining genes from more than two parents into a single genotype is known as pyramiding. This process is possible through conventional breeding but it is usually not easy to identify the plants containing more than one gene at an early generation (Collard and Mackill, 2008). In order to pyramid disease resistance genes that have similar phenotypic effects, and for which the matching races are often available, MAS might even be the only practical method, especially where one gene masks the presence of the other genes (Sanchez *et al*., 2000; Walker *et al*., 2002). Molecular markers are also independent of environmental conditions which can have a large impact on phenotyping plants during a breeding program.

CHAPTER 3 – REACTION OF SELECTED COMMON BEAN GERMPLASM AND SOUTH AFRICAN DRY BEAN CULTIVARS TO RUST (*UROMYCES APPENDICULATUS*) IN THE FIELD

3.1 Introduction

Rust, caused by an airborne fungus *Uromyces appendiculatus*, is one of the most widespread and destructive fungal diseases affecting common bean (*Phaseolus vulgaris* L.) all over the world where the crop is grown (Allen, 1995; Stavely and Pastor-Corrales, 1989; Steadman, 1995). The disease is known to develop in humid tropical and subtropical areas as well as humid temperate regions (De Souza *et al*., 2008), causing enormous amounts of damage and yield loss (Liebenberg and Pretorius, 2010; Lindgren *et al*., 1995; De Souza *et al*., 2008). In South Africa, rust of dry bean occurs in every dry bean growing season, accompanied by high levels of incidence and severity in areas where favourable weather conditions persist such as the eastern half of the country (Liebenberg, 2003). Rust of dry bean thrives well in extended wet and cool weather conditions whereas the significance of the disease severity can be escalated by use of susceptible cultivars (De Souza *et al*., 2008).

A number of control methods for the rust disease have been considered to manage the disease, although with limited success and at times raising concerns to the environment. The methods include the application of fungicides, practicing crop rotation with non-host plants in at least a two-year cycle, promoting sanitation in and around the fields, and the use of genetic resistance in market type dry beans (Schwartz *et al*., 2011). Concerns surrounding the use of fungicides are of serious nature, including cost ineffectiveness, contamination to the ecosystem and its biodiversity, and exacerbating the effects of climate change through mechanized application procedures. Rust spores can travel considerably long distances from infected neighbouring fields to uninfected fields through wind, thereby compromising the effectiveness of crop rotation approach (Schwartz *et al*., 2011). The overwintering form of rust spores, teliospores, remain dormant for extended periods and only become active when a susceptible host plant is available and the weather becomes ideal, which poses an important challenge to sanitation efforts. A sanitation approach may

further be complicated by the fact that *U. appendiculatus* has a wide range of host plants including weeds. (Schwartz *et al*., 2011). It has been observed and reported that single gene resistance to plant diseases in general, rust in particular, may not last for a long period of time since new pathogenic strains develop from time to time through natural mutation in the field. In this way, single gene rust resistance is easily broken, causing a once resistant cultivar to become susceptible (Schwartz *et al*., 2011).

Disease resistance is however considered the most reliable, environmentally safe and long-lasting disease control method (Mmbaga *et al*., 1996), especially where important rust resistance genes are identified and pyramided in the same dry bean cultivar. Pyramiding multiple rust resistance genes in the same cultivar creates a broad spectrum disease resistance base which minimizes the chances of genetic resistance being overcome by the ever changing pathogenic strains and races (Collard and Mackill, 2008). Resistance to rust has been reported in each of the twelve international rust differential cultivars and other common bean varieties that are part of the international germplasm collection of the ARC-GC (Liebenberg, 2003). Collectively, these materials contain a wide range of characterized and not-yet-characterized rust resistance genes that are important for use even in the major common bean producing countries in the world.

In South Africa, the *Ur-3, Ur-4, Ur-5* and *Ur-11* rust resistance genes have been carefully identified as important rust resistance sources for the local dry bean production industry (Liebenberg *et al*., 2005). Important factors such as the major rust races occurring in the field, the genes that offer resistance to such races, and the dominant market dry bean types that may have evolved with such races were taken into consideration (Liebenberg *et al*., 2006). Different bean types of different gene pools may possess variable disease resistance genes. This phenomenon is a significant aspect to assist in the development of a breeding strategy, especially when resistance genes are introduced into bean types of a different gene pool (the Andean (large seeded) and Mesoamerican (small seeded) (Liebenberg *et al*., 2006)). This strategy helps overcome disease pathotypes/strains that have evolved with a specific gene pool over a long period of time. As the pathogen populations are greatly diverse and characterized by many races (Ochoa *et al*., 2007), resistance conferred by multiple genes is therefore very important. The South African commercial dry bean cultivars are also part of the germplasm of the ARC-GC and some were mainly bred for resistance to rust, however pyramided rust resistance need to be a continuous exercise to ensure effective genetic resistance to rust through all the dry bean growing seasons.

For effective and comprehensive analysis of multi-locality and multi-season data, the additive main effect and multiplicative interaction (AMMI) model has been used before (Adjebeng-Danquah *et al.*, 2017). The AMMI model helps to fit the sum of several multiplicative terms and not only one multiplicative term in assessing the performance of genotypes in different environments (Adjebeng-Danquah *et al.*, 2017). It is a hybrid analysis that incorporates both the additive and multiplicative components of the two-way data structure (Mukherjee *et al*., 2013). The model has mainly been used for yield analysis, however it has also been considered in analysing disease reaction data (Mukherjee *et al*., 2013). The principal component axis (PCA) scores of the AMMI analysis are used to determine stability of the genotypes across different locations (Adjebeng-Danquah *et al.*, 2017). AMMI biplot analysis is considered to be an effective tool to diagnose the genotype-by-environment interaction (GEI) patterns graphically (Mukherjee *et al*., 2013). The aim of the study was therefore to screen selected international common bean germplasm as well as the South African commercial dry bean cultivars for their reaction to rust in the field in order to identify rust resistance sources containing genes of interest.

3.2 Materials and Methods

3.2.1 Trial establishment

Thirty-one selected international germplasm varieties (Table 1) from the collection at the ARC-GC, Potchefstroom, South Africa and twenty-five South African commercial dry bean cultivars (Table 3) (including one rust spreader) were evaluated for reaction to rust under field conditions at three localities. Trials were conducted at Cedara (2015/16 and 2016/17 seasons), Potchefstroom (2015/16 season) and Makhathini (2016/17 season). Trials at Cedara were solely rain-fed and evaluated under natural infection while artificial inoculations were carried out and irrigation (when necessary) was done for Potchefstroom and Makhathini trials. Potchefstroom (26°43′00″S27°06′00″E, 1335 m altitude) is located in the North West province where summer temperatures ranged from 23ºC to 31ºC average of 27ºC in 2015/2016. Cedara (29.54’S; 30.26’E, 1068 m altitude) and Makhathini (27°22′41″S31°37′08″E, 73 m altitude) research stations are located in the KwaZulu-Natal province of South Africa where temperatures ranged from 21ºC to 29ºC in 2015/2016, with average temperatures of 25ºC and from 21ºCto 28ºC, (average of 25ºC) in 2016/2017 while temperatures ranging from 15ºC to 32ºC, (average of 24ºC) were recorded for Makhathini in 2016/2017 season. The average temperatures changes as a result of the combination of

high day temperatures (32, 2ºC) and low night temperatures (15.6ºC), which greatly retarded development of the common bean rust.

Trials were either manually or mechanically planted in 5 m long 4-row plots, with 75 cm inter-row and 7.5 cm intra-row spacing, giving a total plot area of 3.75 m^2. Trials were arranged in a randomized complete block design (RCBD) with three replications. Thirty-five seeds were planted per row. Following seedbed preparations, approximately 250-300 kg/ha 3:2:1 (25) N:P:K fertilizer was applied. Where necessary, a pre-emergence herbicide, Bateleur Gold ((Syngenta Group Company), active ingredients: Flumetsulam (20 g/L) and Smetplachlor (640 g/L)) at 1.7 L/ha, was applied directly after planting to control weeds while insect pests were controlled using Endosulfan (active ingredient: Endosulfan (34%)) at 250 ml/100 L H_2O/ha, when required.

3.2.2 Inoculum preparation

A mixture of four rust races (1, 3, 5 and 11) where used and four rust isolates, that were purified and increased from single-pustule isolates (R12/01-J, R12/01-M, R13/02-B and R13/02-H), was used to prepare the inoculum for field trial inoculations. Rust races 1, 3, 5 and 11 were increased on a susceptible variety Pinto 114 in order to obtain enough spaces for inoculation of the differential cultivars. All four isolates were collected from Cedara during the 2013/2014 dry bean growing season and carefully maintained at -80°C at ARC-GC. Fourteen days prior to the preparations of suspensions, isolates were first increased on the highly susceptible variety Pinto 114 in the greenhouse. Fresh spores were subsequently harvested and used immediately. Where long distance transportation was required, the harvested spores were kept in well closed cryotubes containing silica gel to prevent hydration of urediniospores. The cryotubes were placed in a cooler bag containing ice to prevent premature germination of urediniospores.

3.2.3 Inoculations

The inoculum suspension contained approximately 5mg of spores per 20 L of tap water was measured by a 10000mL (10L) Laboratory plastic beaker. First inoculations were done during a third trifoliate leaf stage (V3) and repeated twice every other week using a STIHL SR 430 backpack mist blower at minimal pressure (90 kpa). Inoculations were carried out in the early hours of the morning when the weather was cool in order to stimulate or enhance spore germination.

3.2.4 Evaluation of rust resistance

3.2.4.1 Inoculation technique

Artificial rust inoculations in the field are done using freshly produced urediniospores. In order to enhance the success rate of infections on the common bean plants, trials are irrigated so that there is adequate moisture on the plants for spore attachment on the plant and subsequent germination. Also, inoculations are done in the morning hours when the temperatures are cooler, which is an important requirement for maximal spore germination rate. Urediniospores are mixed in tap water and transferred to plants using a hand-pumped backpack mist blower at minimal pressure to avoid damage to the plants. Inoculations are done 14 and 21 days after planting.

Inoculations of rust is often done when the primary leaves of the bean plant reach approximately 2/3 of their full development stage at 10 days after sowing under greenhouse conditions (20±5°C). The standard concentration of inoculum is 2.0×10^4 urediniospores /mL of distilled water containing 0.05% Tween-20. The inoculum mixture can be applied on both leaf surfaces using a manual atomizer spray (e.g., atomizer De Vilbiss atomizer no 15º) connected to an electric compressor. After inoculation the plants are transferred to a mist chamber at a temperature of 20±1°C and relative humidity of >95% where they are kept for approximately 48hr under a fluorescent light regime. Plants are inoculated with different isolates or races and kept in a mist chamber in order to enhance infection. After this period the plants are transferred to a greenhouse (20±5°C), where they are kept until symptom evaluation, about 14 days after inoculation (Carrijo *et al*., 1980; De Souza *et al*., 2005a, 2005b, 2007a, 2007b).

Inoculation can also be conducted using bean plant excised leaves, as reported by De Souza *et al*. (2009). In this alternative method, after inoculation, each leaf must be placed in a Petri dish on a sterile filter paper moistened with distilled water. The dishes are incubated in the incubator at 20°C, under a 12-hour light regime and moist conditions until disease symptom evaluation at about 10 days after inoculation.

Both inoculation methods are efficient for evaluating the reaction of the common bean to *U. appendiculatus*. The only difference observed on the results by the two methods is that the number of pustules are higher in the conventional method and disease symptoms appear

earlier in the alternative method (De Souza *et al*., 2009). The conventional inoculation method is more appropriate for spore multiplication and the excised leaf method can be used in the cases where the same plant need to be assayed several times. Another advantage of the alternative procedure refers to costs and safety; the whole method can be conducted in the laboratory without the need for exposing other plants to the pathogen, while the tested plants are still able to grow and produce seeds (De Souza *et al*., 2009).

3.2.4.2 Disease symptom evaluation

Disease symptoms can be evaluated by one of two available rating scales, depending on the purpose of the study. Van Schoonhoven and Pastor-Corrales (1987) recommended a rating scale of 1 to 9 when the symptoms are visible on the plant leaves, where 1 represents immunity to the disease and 9 represents highly susceptible or a dead plant as a result of the disease. The disease rating score described above is used in both the field and greenhouse studies and can further be simplified in the following manner: reaction rating score of 1 to 3 are basically considered resistant, 4 to 6 intermediate resistant and 7 to 9 susceptible. In the greenhouse, the reaction of the rust disease can also be determined on the basis of a rust grading scale of 1-6 adopted for the new inoculation procedure (Steadman *et al*., 2002). The scale considers six infection degrees 1-no pustules (immunity); 2-presence of necrotic spots without sporulation; 3-pustules undergoing sporulation with a diameter of < 300 µm (cultivar considered resistant to the rust pathogen); 4-pustule undergoing sporulation with a diameter ranging from 300 µm to 499 µm (cultivar considered susceptible to the rust pathogen); 5-pustules undergoing sporulation with a diameter ranging from 500 µm to 800 µm; and 6-pustules undergoing sporulation with a diameter of>800 µm (cultivar considered susceptible to the rust pathogen). This rust scoring approach allows for measurement of pustules and necrotic areas surrounding the pustules in a controlled environment, which would not be feasible under field conditions due to many other possible causes of injuries to the plant besides rust. The degree of infection is determined approximately 10-15 days after the inoculation when the pustules are sporulating up to 50%. The lesions in both surfaces of the primary leaves should be determined visually by at least two evaluators.

3.2.5 Disease ratings and harvesting

Disease ratings on the foliage were first done approximately 18 days after first inoculation and also repeated twice every other week. A disease rating scale of 1-9 (Van Schoonhoven

and Pastor-Corrales, 1987) where 1 to 3 is tolerant, 4 to 6 is intermediate and 7 to 9 highly susceptible was used. The disease ratings synchronised with the flowering and pod development stages on each plant individually, where rust was more severe on susceptible varieties. At maturity, trials were harvested manually and yield data recorded per rep.

3.2.6 Statistical Analysis

Data on cultivars and germplasm were respectively pooled and analysed using GenStat for windows 19th Edition (Yan and Kang, 2003) Statistical Analysis Software. Analysis of Variance (ANOVA) was conducted on disease ratings and yield data (Nau, 2005). The 25 cultivars and 3 locality-seasons, as well as the 31 germplasm varieties and 3 locality-seasons were used as treatments. Means were separated using Fischer's LSD ($P \leq 0.05$) (SAS Institute, Inc. (1999), SAS/STAT, User Guide version 14.1). Bartlett's Test was used to determine homogeneity of the variance in rust ratings and yield. Correlation coefficients were calculated using Microsoft Excel 2016 in order to determine a relationship between mean yields and mean disease rating for all three localities. The AMMI determined using VSN international 2017 GenStat for Windows 19th Edition (Yan and Kang, 2003) following the equation used by SHOLIHIN (2011). The PA biplots were constructed using Microsoft excel program (SHOLIHIN, 2011) of 2016.

3.3 Results

Rust rating scores and the mean separations of the international germplasm varieties are presented in Table 1. Germplasm varieties Mexico 235, CAL 143, Flor de Mayo, Cornell 49242, G 21212, G 5828, BelDakMi-RMR-22, Mexico 309, Nep-2, Mexico 54 and BAT 332 showed good rust resistance with ratings between 1 and 3 across different seasons and localities. Varieties Pinto114 and GN 1140 were most susceptible to rust with highest severity ratings of 8 and 7, respectively in Cedara and Makhathini during the 2015/2016 season. Comparison of means amongst germplasm varieties showed highly significant differences ($P \leq 0.01$) as indicated with ANOVA analysis (Table 2).

Table 1: Reaction of selected common bean germplasm varieties to rust in the field

Variety	Potch 15/16		Cedara 15/16		Cedara 16/17		Makhathini 16/17		Pooled	
	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS
Mexico 235	1.0	a	1.0	a	1.0	a	1.0	a	1	a
CAL 143	1.0	a	1.0	a	1.0	a	1.0	a	1	a
Flor de Mayo	1.0	a	1.0	a	1.0	a	1.0	a	1	a
Cornell 49242	1.0	a	1.0	a	1.0	a	1.0	a	1	a
G 21212	1.0	a	1.0	a	1.0	a	1.0	a	1	a
G 5828									1	a
BelDakMi-RMR-22	1.0	a	1.0	a	1.0	a	1.0	a	1	a
Mexico 309	1.0	a	1.0	a	1.0	a	1.0	a	1	a
Nep-2	1.0	a	1.0	a	1.0	a	1.0	a	1	a
Mexico 54	1.0	a	1.0	a	1.0	a	1.0	a	1	a
BAT 332	1.0	a	1.0	a	1.0	a	1.0	a	1	a
PAN 72	1.0	a	1.0	a	1.0	a	1.3	a	1.1	ab
BelMiNeb-RMR-7	1.3	b	1.0	a	1.0	a	1.0	a	1.1	ab
BelMiNeb-RMR-1	2.0	c	1.0	a	1.0	a	1.0	a	1.2	ab
BelMiDak-RMR-10	2.0	c	1.0	a	1.0	a	1.0	a	1.2	ab
A 240	2.0	c	2.3	b	1.0	a	1.0	a	1.5	abc
BelDakMi-RMR-19	1.0	a	1.0	a	1.0	a	1.0	a	1.5	bcd
CNC	1.0	a	1.0	a	1.0	a	2.3	b	1.7	cde
Ecuador 299	1.0	a	1.0	a	1.0	a	5.0	ef	1.8	cde
KW 814	1.0	a	1.0	a	2.7	cd	5.0	ef	1.9	cdef
Aurora	1.0	a	1.0	a	3.0	a	3.0	cd	2.0	def
Don Timoteo	3.0	d	3.7	d	1.7	abc	1.0	a	2.1	ef
A 55	1.0	a	1.0	a	1.3	ab	4.0	cd	2.1	ef
Golden Gate Wax	1.0	a	1.0	a	3.3	de	4.7	df	2.3	fg
Montcalm	2.0	c	3.0	c	2.3	bcd	3.7	c	2.7	gh

Variety	Potch 15/16		Cedara 15/16		Cedara 16/17		Makhathini 16/17		Pooled	
	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS
G 5686	1.0	a	1.0	a	4.0	e	5.7	f	3.0	h
VAX 4	2.0	c	3.3	cd	1.7	abc	5.0	ef	3.1	h
BBSR 28	3.0	d	3.3	cd	3.3	de	5.0	ef	3.7	i
GN 1140	4.7	e	3.7	d	8.7	f	6.7	g	4.9	j
Pinto 114	5.3	f	6.3	e	8.0	f	8.0	h	6.6	k

LSD P<0.05; MR=Mean Rating; MS=Mean Separation

Table 2: Analysis of variance of rust ratings on the selected international common bean germplasm varieties

Source	SS	DF	MS	F-Ratio	P-Value
Between groups	147.877	29	5.09922	50.10	0.000
Within groups	6.10667	60	0.101778		
Total (corr.)	153.984	89			

The disease rating and the mean separation of the South African cultivars are presented in Table 3. Cultivars Mkuzi, Kamiesberg, Teebus-RCR2, OPS-KW1, Tygerberg, PAN 9292, CAR 2008, and Teebus-RR1 showed strong rust resistance with rust rating ranging between 1 and 3. Cultivars DBS 360, OPS-RS4, PAN9249, Bonus, Sederberg, Kranskop HR-1, DBS 310, DBS 830 and PAN 116 showed intermediate reaction ratings of 4 to 6 while the control varieties Pinto114 and Teebus were the most susceptible with ratings of 7 to 9. Comparison of means amongst the South African dry bean cultivars showed highly significant differences as indicated in the ANOVA analysis (Table 4).

A legend for abbreviations used in the graphs (Figures 1-4) is presented in Table 5. A biplot for rust ratings on South African dry bean commercial cultivars is presented in Figure 1 as principal component 1 (PC1) and PC2. For all the graphs presented in these results, the top right area of the graph is the first quadrant (+), the bottom right area is the second quadrant (+), the bottom left area is the third quadrant (-), and the top left area is the fourth quadrant (-). The convex hull in the legend for figure1 means the enclosure of all the common bean materials evaluated for rust reaction, sectors of convex hull refers to the quadrant where the evaluated materials are distributed while mega environments refers to the environments associated with higher rust severity. The AMMI model for rust ratings indicates that PC1 and PC2 explained 68.57% and 17.43%, respectively, of the total

genotype x environment (GxE) interaction. Bartlett's Test for homogeneity revealed significant variance among cultivars in rust ratings (P=0.0012) and yield (P=0.0114) at P≤0.05 level of significance. The four environments distributed in two of four quadrants, with the first sector containing C14 (Cedara, 2015/2016 season) and P14 (Potchefstroom, 2015/2016 season) while the second quadrant contains C15 (Cedara, 2016/2017 season) and M15 (Makhathini, 2016/2017 season). The effects of the interaction are significant at P<0.05 (Table 4). Although variety Pinto 114 appears to be adapted specifically to P14 locality, the variety appears to be highly susceptible across environments in general. Cultivars PAN 123, PAN 116, PAN 9249, Kamiesberg, Sederberg, Kranskop-HR1 appear to be well-adapted across the four different environments. Cultivars Kamiesberg, PAN 123, CAR 2008, PAN 9249, Teebus-RR1, Teebus-RCR2 and Tygerberg are some of the cultivars that appear to have shown resistance to the rust disease since they are in the negative quadrants of the biplot graph.

Table 3: Reaction of the South African dry bean cultivars to rust in the field

Cultivar	Potch 15/16		Cedara 15/16		Cedara 16/17		Mak 16/17		Pooled	
	MR	MS	MR	MS	MR	MS	MR	MS	MR	MS
Mkuzi	1.0	a	1.0	a	1.3	a	1.0	a	1.0	a
Kamiesberg	1.7	ab	1.0	a	1.0	a	1.0	a	1.2	ab
RS7	2.7	cde	4.3	efg	4.7	cd	1.0	a	4.2	ad
Teebus-RCR2	1.0	a	1.0	a	1.0	a	2.0	bc	1.3	ab
DBS 360	4.0	gh	3.7	ef	5.0	cd	5.3	de	4.5	def
OPS-RS4	3.0	def	3.0	de	4.3	bc	4.7	cd	3.8	c
PAN 9249	3.7	fgh	4.7	fgh	5.7	d	5.3	a	4.8	fgh
OPS-KW1	2.3	bcd	2.0	bc	1.0	a	1.0	a	1.6	b
Tygerberg	2.0	bc	2.0	bc	1.0	a	1.3	ab	1.6	b
PAN 9292	2.0	bc	1.0	ab	1.3	a	1.0	a	1.4	ab
CAR 2008	1.0	a	2.7	cd	1.3	a	1.0	a	1.5	b
Werna	2.7	cde	4.7	fgh	5.0	cd	4.3	c	4.2	cd
Teebus	5.0	i	5.3	hi	5.3	cd	7.3	fg	5.8	i
Bonus	3.7	fgh	5.0	ghi	5.7	d	6.7	f	5.3	h
RS5	3.3	efg	4.7	fgh	3.3	b	5.3	de	4.7	cd
Teebus-RR1	1.0	a	1.0	a	1.0	a	1.7	ab	1.2	ab
Sederberg	3.0	def	4.3	fgh	5.0	cd	4.7	cd	4.3	de
Kranskop-HR1	3.3	efg	4.0	fg	4.7	cd	5.0	cde	4.3	de
DBS 310	3.7	fgh	4.7	fgh	5.3	cd	5.0	cde	4.7	efg
DBS 830	2.3	bcd	4.3	fgh	4.7	cd	5.3	de	4.2	cd
PAN 116	6.7	j	3.0	de	5.7	a	5.7	e	5.3	h
RS6	4.3	hi	4.3	efg	5.0	cd	5.0	cde	4.7	efg
PAN 123	1.0	a	1.0	a	1.0	a	1.0	a	1.0	a
Kranskop	3.7	fgh	5.7	i	5.3	cd	5.3	de	5.0	gh
Pinto 114	5.0	i	8.0	j	8.0	e	8.0	g	7.3	j

LSD P<0.05; MR=Mean Rating; MS=Mean Separation

Table 4: Analysis of variance of rust ratings on the South African dry bean cultivars

Source	SS	DF	MS	F-Ratio	P-Value
Between groups	78.5368	24	3.27236	195.91	0.000
Within groups	0.835153	50	0.0167031		
Total (corr.)	79.3719	74			

Table 5: Legend for locality, seasons, cultivars and germplasm in AMMI biplot graphs (Figure 1-4)

Locality and season		Cultivars		Germplasm	
C14	Cedara 2015/2016 season	Bonus	Bo	A 240 AR	AR
P14	Potchefstroom 2015/2016 season	CAR 2008	CA	A 55	A5
C15	Cedara 2016/2017 season	DBS 310	DB1	Aurora	Au
M15	Makhathini 2016/2017 season	DBS 360	DB6	BAT 332	BA
		DBS 830	DB8	BBSR 28	BB
		Kamiesberg	Ka	BelDakMi-RMR19	Be9
		Kranskop	Kr	BelDakMi-RMR-22	Be2
		Kranskop-HR1	Kr1	BelMiDak-RMR-10	Be0
		Mkuzi	Mk	BelMiNeb-RMR-1	Be1
		OPS-KW1	OP	BelMiNeb-RMR-7	Be7
		PAN 116	PA6	CAL 143	CA
		PAN 123	PA3	CNC	CN
		PAN 9249	PA9	Cornell 49242	Co
		PAN 9292	PA2	Don Timoteo	Do
		Pinto 114	Pi4	Ecuador 299	Ec
		RS4	RS4	Flor de Mayo	Fl
		RS5	RS5	G 21212	G2

Locality and season	Cultivars		Germplasm	
	RS6	RS6	G 2858	G8
	RS7	RS7	G 5686	G5
	Sederberg	Se	GN 1140	GN
	Teebus	Te	Golden Gate Wax	GW
	Teebus-RCR2	Te2	KW 814	KW
	Teebus-RR1	Te1	Mexico 235	M2
	Tygerberg	Ty	Mexico 309	M3
	Werna	We	Mexico 54	M5
			Montcalm	Mo
			Nep-2	NE
			PAN 72	PA
			Pinto 114	Pi
			VAX 4	VA

The yield performance of the dry bean cultivars for the four localities evaluated is shown in a biplot graph (Figure 2). The PC1 and PC2 obtained from all four localities accounted for 47.37% and 25.82%, respectively, for yield of the commercial cultivars in two seasons. The four environments distributed in two sectors, where the first sector contains C14 (Cedara 2015/2016), C15 (Cedara 2016/2017) and P14 (Potchefstroom 2015/2016) while the second sector contains M15 (Makhathini 2015/2016) environments. The largest environmental vector was observed for Makhathini 2015/2016, followed by Potchefstroom 2015/2016 and Cedara 2015/2016, implying that Makhathini accounted for a greater variation in yield amongst the cultivars. Cultivars RS5, Teebus-RR1, Teebus-RCR2, Werna, Kamiesberg and CAR 2008 appear to be specifically adapted to all the four environments since the yield is closer to zero. Cultivars Teebus-RCR2, RS5, RS7, Kamiesberg and CAR 2008 are adapted across all the environments and both the seasons regarding yield. Variety Pinto 114 lies in the negative quadrants, implying that the variety had low yield. Pooled data of both seasons and all environments amongst the commercial dry bean cultivars produced a correlation coefficient value of -0.443 between the rust disease ratings and yield.

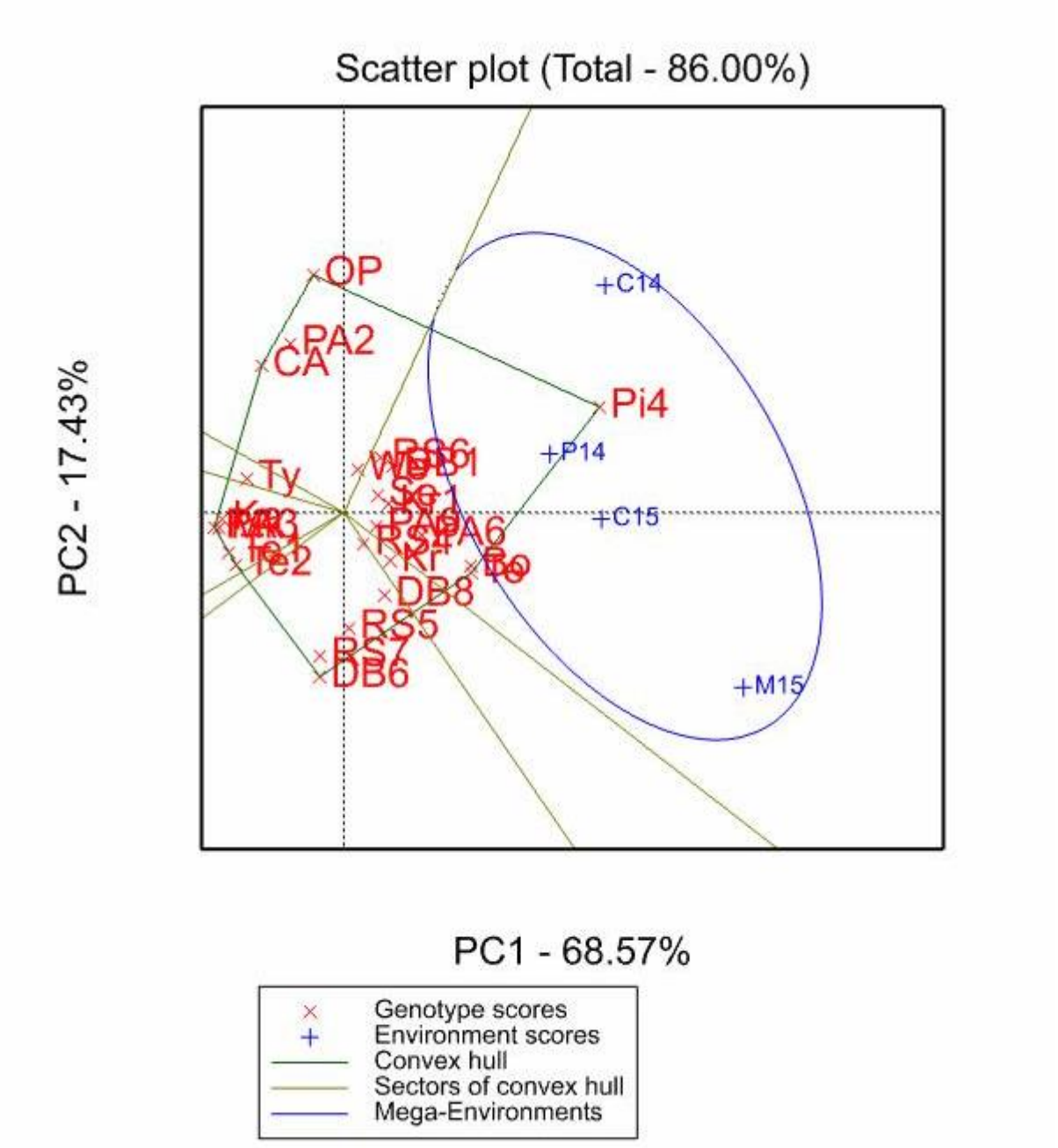

Figure 1: AMMI model biplot of disease ratings of 24 South African commercial dry bean cultivars and a spreader in 4 environments and 2 seasons

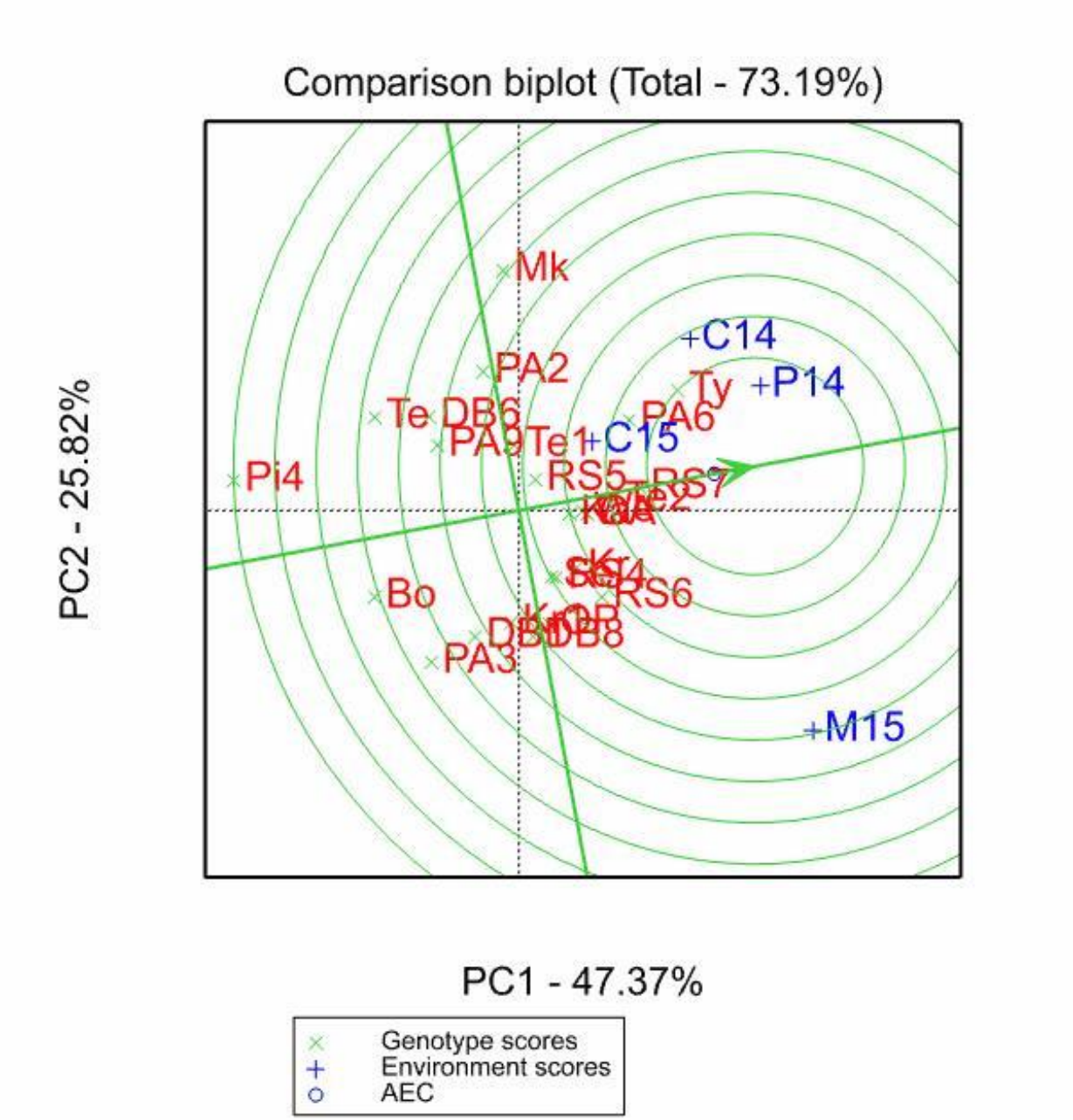

Figure 2: AMMI model Biplot of yield of 24 South African commercial dry bean cultivars and a spreader in 4 environments and 2 seasons

Bartlett's Test for homogeneity revealed significant variance (P=0.0001) for both rust ratings and yield among germplasm materials at P≤0.05 level of significance. PC1 and PC2 obtained from all four localities for rust ratings of germplasm are presented in Figure 3. Observed characters accounted for 78.02% and 14.70%, respectively, of the variation of the total disease ratings among germplasm evaluated in two seasons (Figure 3). Figure 3

shows that the four environments are distributed in two, positive quadrants, where the first quadrant contains Cedara (2015/2016 season), Cedara (2016/2017 season) and Potchefstroom (2015/2016 season) while the second quadrant contains Makhathini (2016/2017 season). Germplasm GN 1140 and Pinto 114 appear to be environment specific towards Potchefstroom (2015/2016) and Cedara (2015/2016) regarding resistance to rust while Montcalm, BBSR 28 and PAN 72 are some of the germplasm that do not appear to be environment specific.

The yield performance of the common bean germplasm is shown in a biplot graph (Figure 4). The PC1 and PC2 obtained from all four localities accounted for 41.68% and 34.55%, respectively, for yield of the germplasm in two seasons. The four environments distributed in two sectors, where the first sector contains Potchefstroom (2015/2016) while the second sector contains Cedara (2015/2016), Cedara (2016/2017) and Makhathini (2016/2017) environments. The largest environmental vector was observed for Potchefstroom (2015/2016), followed by Cedara (2015/2016), and Cedara (2016/2017). Genotypes G 21212 and PAN 72 are among the best performing in all localities and also better adapted across environments. Pooled data of both seasons and all environments amongst the germplasm produced a correlation coefficient value of -0.434 between the rust disease ratings and yield.

Makhathini (2016/2017) provided more information regarding the cultivar differences on yield variation as indicated in Figure 2. Cultivars Tygerberg, PAN 116, Pinto 114, Teebus-RCR2, RS5, Kamiesberg and RS7 are among the best performing in all localities and also better adapted across environments. This implies that the cultivars are basically the most favoured by climatic conditions in all the localities tested. The three environments Cedara (2015/2016), Cedara (2016/2017 season) and Potchefstroom (2015/2016) appear close to one another, indicating that they are positively correlated and that the cultivar performances in these environments are closely related, with the performance of cultivars being comparable in these environments.

Potchefstroom (2015/2016) provided more information regarding the germplasm differences on yield variation due to its longest vector (Figure 4). Germplasm G 2858, Mexico 309, BelDakMi-RMR-19, BAT 332 and PAN 72 are among the best performing in all localities and also better adapted across environments. The two environments, Makhathini (2016/2017 season) and Cedara (2016/2017 season) appear close to each other, indicating that they are positively correlated and that the germplasm performance in these environments are closely related.

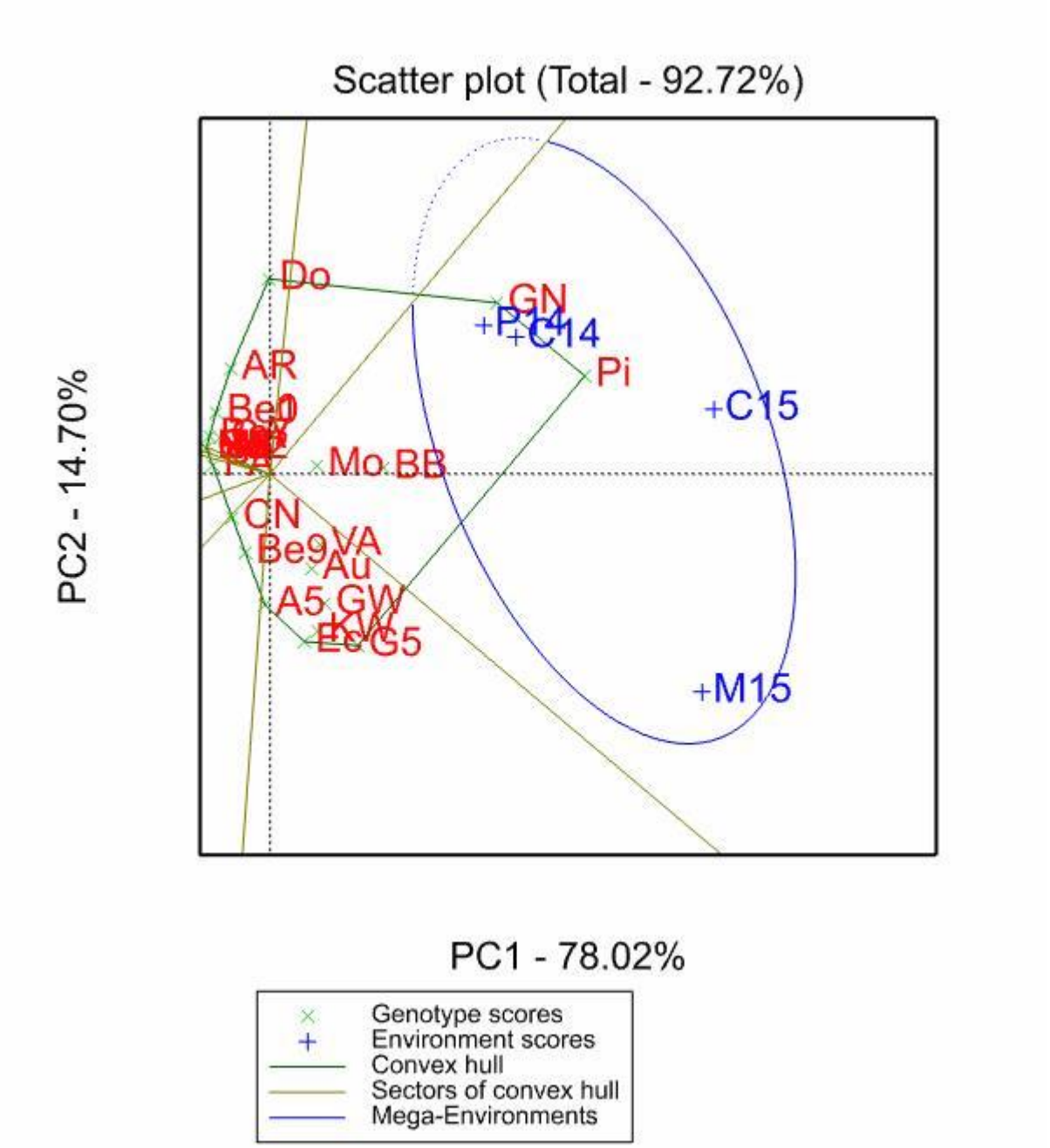

Figure 3: AMMI model Biplot of disease ratings of 30 selected international common bean germplasm in 4 environments and 2 seasons

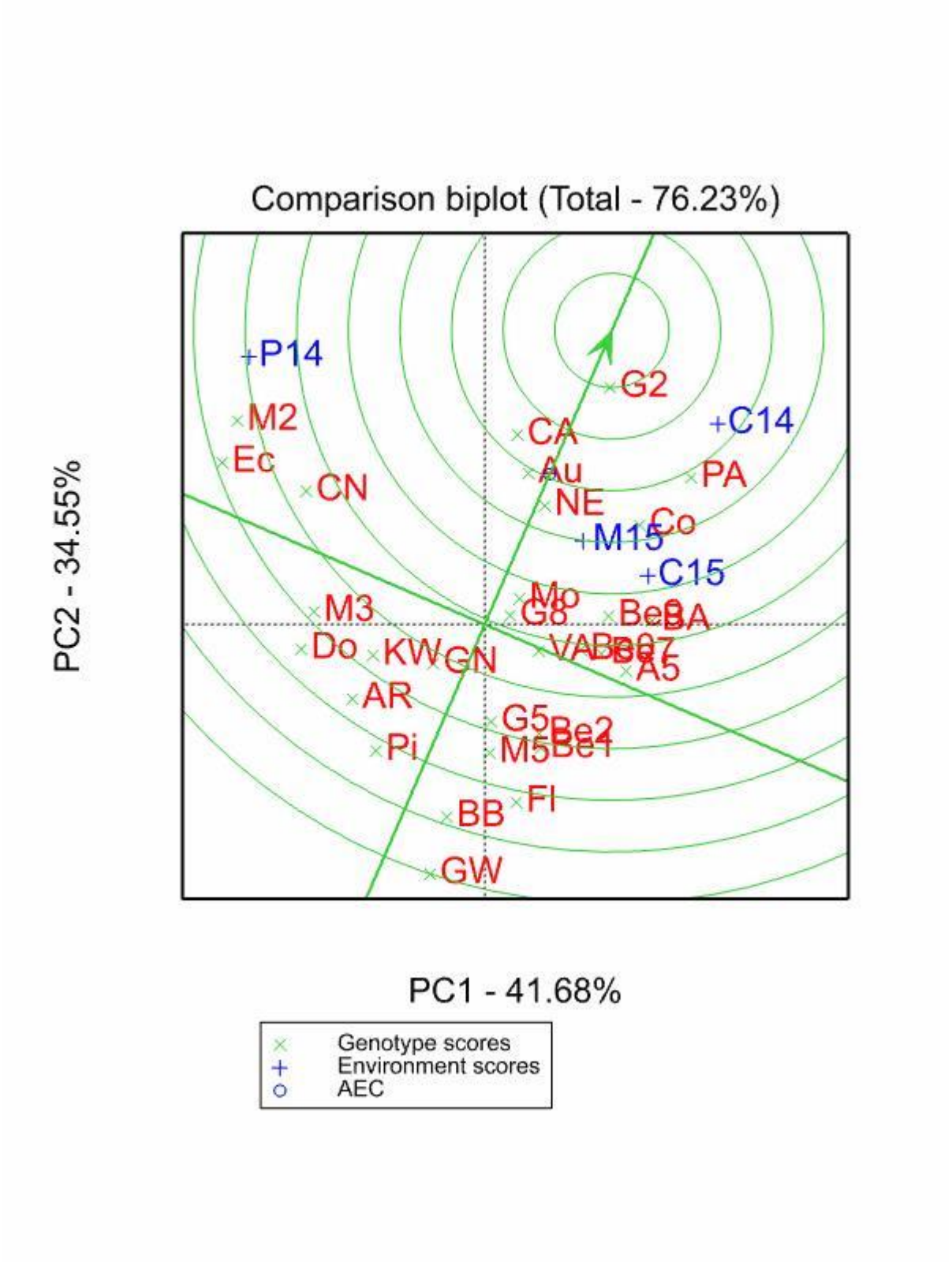

Figure 4: AMMI model Biplot of yield of 30 selected international common bean germplasm in 4 environments and 2 seasons

3.4 Discussion

The reaction of the South African dry bean cultivars and germplasm to rust under field conditions was observed for two different seasons and three localities in this study. The majority (90%) of the selected international germplasm tested showed complete resistance (immunity) to the rust disease under field conditions in South Africa (Table 1). The varieties included the twelve international rust differential cultivars which were all resistant except GN 1140. Most of these germplasm varieties contain rust resistance genes that have been characterized before (De Souza *et al*., 2011). De Souza *et al*. (2011) reported that variety CNC was resistant to rust in Brazil, however the resistance gene has not been named yet. Pastor-Corrales (2004) reported rust resistance in BelMiNeb-RMR-1 (*Ur-5, Ur-6*, & *Ur-7*), BelMiNeb-RMR-7 (*Ur-3, Ur-4* & *Ur-11*), BelMiDak-RMR-10 (*Ur-4* & *Ur-11*), BelDakMi-RMR-19 (*Ur-3, Ur-4, Ur-6* & *Ur-11*) and BelDakMi-RMR-22 (*Ur-3, Ur-4, Ur-6* & *Ur-11*). It is most likely the stacking of the multiple rust resistant genes in these germplasm varieties that render the resistance to field rust even under South African climatic conditions. Rust resistance in small seeded Mexico 309 (*Ur-5*), PI 51051 and CNC has previously been reported in South Africa in the field (Liebenberg *et al*., 2005).

Many SA cultivars of the most popular large seeded dry bean type of Andean origin (Singh *et al*., 1991a, b), RSS beans Sederberg, Kranskop, PAN 9292, Werna, Kamiesberg, DBS 830 and Kranskop HR1, were susceptible to rust in this study (Table 3). The Mesoamerican small seeded type (Singh *et al*., 1992) beans PAN 116, Teebus, Teebus RCR2, Teebus RR1, OPS-KW1, and PAN 123 and the carioca types Mkuzi and CAR 2008, also showed susceptibility to rust in this study. Cultivars Mkuzi, Kamiesberg, Tygerberg, Teebus-RR1 (possibly containing rust resistance gene *Ur-3*), Teebus-RCR2, PAN 123, OPS-KW1 (*Ur-3*) were resistant to rust in this study. Cultivar Sederberg, possibly containing (*Ur-11*), Werna and RS7 were moderately resistant while Kranskop (*Ur-13*) was moderately susceptible. These findings correlate with that of the dry bean National Cultivar Trials conducted annually in the field in South Africa (Fourie, 2015).

The two major gene pools existed in the Andean and the Mesoamerican within *P-vulgaris* has led to co-evolution of several pathogens and the host (Pastor-Corrales, 1996). Large seeded Andean bean types in Africa as a whole are generally preferred, more particularly in South Africa and have undoubtedly contributed to the predominance of Andean types of rust races in Africa (Liebenberg, 2003). In the past years, increasing levels of rust have been observed on these cultivars (Kranskop, Sederberg, Kamiesberg, Jenny, Werna,

Teebus, and Pinto 114) and a number of isolates have been collected to which they are susceptible (Liebenberg,2003), and it further indicates that this resistance may not be able to continue to provide sufficient protection.

High disease intensity was observed in the two seasons at Cedara (naturally infected trials), allowing for a clear distinction between the susceptible and resistant reactions in all the materials evaluated. Resistant cultivars often react to haustoria formation with a hypersensitive reaction (Mendgen, 1978). The negative correlation values between the rust ratings and yield indicate that the yield of the South African dry bean cultivars (-0,443) and the selected germplasm (-0,434) decrease as rust severity increases. Yield losses of common bean rust ranging between 18-100% as a result of rust infections has previously been reported (De Jesus *et al*., 2001; Lindgren *et al*., 1995). Other previous studies of the impact of rust on yield have confirmed bean yield losses of up to 100% (Lindgren *et al*., 1995; De Souza *et al.*, 2008).

The most favourable locations for rust development amongst the cultivars were Cedara (2015/2016 season) and Makhathini (2016/2017 season) as indicated by the largest environmental vectors compared to the other localities (Figure 1). The least favourable environment for rust development was Potchefstroom (2015/2016 season) as indicated by the smallest environmental vector. Locations Cedara (2015/2016) and Makhathini (2016/2017 season), as indicated in Figure 1, provided more information about differences amongst cultivars. Figure 1 further shows that all the localities appear in the two positive quadrants (2 & 3), indicating that the localities had high rust density. Rust spores were observed on the leaves of the susceptible cultivars Teebus, Pinto 114, Kranskop, Jenny, Werna, Kamiesberg and RS6 in Cedara (2015/2016) and Makhathini (2016/2017). The fact that the four environments are all in the positive quadrants of the graph imply that they are positively correlated and performance of genotypes comparable in these environments. Cultivars (RS5, RS7, and DBS 360) appear further away from zero, showing high interactions and that they are more adapted to a specific locality (Makhathini) rather than across all four environments. Cultivars RS6, DBS 310, Werna and Sederberg appear more close to zero, indicating that they have small interactions and are therefore better adapted to the four environments (Tarakanovas and Ruzgas, 2006). Resistance to rust in cultivar Kranskop (*Ur-13*) was previously reported (Liebenberg and Pretorius, 2004) and in this study Kranskop appears to be broadly adapted across the four environments with a moderately resistant reaction.

The frequent development of new rust races in the field presents a great risk for resistance conferred by a single gene to be overcome by the disease. McMillan *et al*. (2003) indicated that during the sexual stage of the life cycle of *U. appendiculatus*, the fungus may mutate to form new rust races that did not exist before. The reaction between the standard differential cultivars showed a clear and wide resistance spectrum towards *U. appendiculatus,* with differences in reaction between the Andean cultivars and the Mesoamerican cultivars. High rust incidence and severity were observed amongst different types of cultivars that were planted in this study. This may quickly result in the loss of resistance among cultivars if the new races are more virulent. It is therefore of utmost importance to continuously search for new sources of resistance under the field conditions where new rust races may emerge.

Araya *et al*. (2004), using phenotypic (virulence diversity) and genotypic (RAPD) analyses of 90 rust isolates demonstrated that rust strains may co-evolve with the bean type of the same origin or gene pool. This parallel evolution of the bean rust pathosystem was also reported by Sandlin *et al*. (1999). In order to overcome a situation where rust resistance genes may not be effective due to co-evolution with the rust pathogen, it is therefore important to introduce genes from a different centre of domestication or gene pool (Borelli *et al*., 2018). Germplasm varieties of Mesoamerican origin have generally demonstrated more emphatic resistance to rust throughout the study. It is therefore presumed that rust resistant genes of Mesoamerican origin would perform better if introduced in beans of Andean origin. This approach could greatly improve the aspect of rust resistance among South African dry bean cultivars, especially when employed with a gene-stacking technique using the identified germplasm as sources of resistance.

3.5 Conclusions and recommendations

Field resistance to rust largely exists amongst the selected international germplasm varieties and to a lesser extent amongst the South African commercial dry bean cultivars. The germplasm varieties, containing known rust resistance genes of interest (*Ur-3, -5* and *-11*) for the South African dry bean industry were resistant across different seasons and localities. The findings of this study further demonstrate great prospects to adequately improve rust resistance and the general genetic base of both the large seeded and the small seeded dry bean cultivars in the breeding program of the ARC-GC. It is therefore recommended that more diverse rust resistance genes should be introduced, using a genetic pyramiding approach, into the South African commercial dry bean cultivars of the

dominant market classes. This will directly benefit farmers and all other stakeholders involved in the production and consumption chain. However, growing resistant cultivars should remain a sustainable strategy for managing diseases in the South Africa.

CHAPTER 4 - VALIDATION OF RUST RESISTANCE SCAR MARKERS IN DRY BEAN BREEDING LINES

4.1 Introduction

Common bean (*Phaseolus vulgaris* L.*)* is a widely cultivated legume crop, which is an important component of the human diet on account of its high protein content (Machado *et al*., 2008). It has a wide phenotypic variability especially for traits such as seed types (size and colour) and plant growth habit. Morphological and biochemical traits are important in classifying common bean lines into two geographically distinct gene pools, namely the Andean (large-seeded) and the Mesoamerican (small-seeded) gene pools (Garzon and Blair, 2014; Jochua *et al*., 2008). Multiple lines of evidence show that wild common bean were organized in these two geographically isolated and genetically differentiated wild gene pools that diverged from a common ancestral wild population (Singh and Schwartz, 2010). A high level of resistance to rust has been identified in the Andean and Mesoamerican varieties (Liebenberg, *at el.,* 2006).

Genetic resistance is a widely used strategy to control rust disease of common bean, hence host plant resistance is known to be the most effective bean rust management strategy among several others (Mmbaga *et al*., 1996; Liebenberg and Pastor-Corrales, 2010). In order to track disease resistance traits, it is essential to develop a breeding population from parental materials that have known and contrasting phenotypic reactions to a disease. Different breeding populations such as mapping populations, double haploids as well as segregating populations have been extensively used before in the development and validation of plant molecular markers (Collard *et al*., 2005). Marker validation in a marker assisted selection (MAS) breeding process is important, especially when markers may not reproduce consistent outcomes across different breeding populations (Collard and Mackill, 2008).

Molecular markers have been used for tracking disease resistance genes in agricultural crops to aid the gene pyramiding process (Ragimekula *et al*., 2012). These markers have become important tools for genetic analysis and crop improvement in the dry bean industry (Ragimekula *et al*., 2012). Different types of molecular markers in common bean have been developed and used, including but not limited to restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length

polymorphism (AFLP), inter simple sequence repeat (ISSR), microsatellites or simple sequence repeat (SSR), expressed sequence tag (EST), cleaved amplified polymorphic sequence (CAPS), diversity arrays technology (DArT), single nucleotide polymorphism (SNP), and sequence characterized amplified region (SCAR) (Doveri *et al*., 2008; Garzon and Blair, 2014; Rodriguez *et al*., 2011). Each marker system has its own advantages and disadvantages and there are various factors to be considered when selecting one or more of these markers (Panigrahi, 2011; Semagn *et al*., 2006).

A number of major disease resistance genes are evaluated by the linkage analysis and many of these genes have been molecularly tagged in common bean, but mostly with other types of markers such as SCAR markers (Garzon and Blair, 2014). Numerous SCAR markers have been successfully used to amplify regions of interest in molecular plant breeding programs, including SCAR markers SK14 and SAE19, which successfully amplified 600 bp and 800 bp sequences derived from common bean variety Sierra (Melmaiee *et al*., 2013).

Resistance to bean rust can be controlled by a series of several single dominant genes (Kelly *et al*., 1996), however these genes have since been pyramided into single lines that contain two to four genes (Liebenberg and Pastor-Corrales, 2010). A few of the commercial dry bean cultivars from the Agricultural Research Council – Grain Crops contain single dominant rust resistance genes including *Ur-3, Ur-11*, and *Ur-13*.

A combination of the *Ur-3* and *Ur-11* rust resistance genes proved difficult to be overcome by *U. appendiculatus* races in America (Liebenberg and Pastor-Corrales, 2010). Liebenberg and Pastor-Corrales, (2010) further reported that the *Ur-13* rust resistant gene present in most of the Andean origin South African dry bean cultivars has proved to be useful, however, the gene needs protection by other Mesoamerican genes. Previous studies have indicated the specific and important rust resistant genes that are important for Africa and Southern Africa, including South Africa (Liebenberg and Pastor-Corrales, 2010). Rust resistant genes *Ur-3, Ur-5* and *Ur-11*, of the Mesoamerican origin, have previously been identified as the most useful in the African continent (Liebenberg and Pastor-Corrales, 2010). The *Ur-4* rust resistant gene of the Andean origin is reportedly resistant to a considerably high number of rust races maintained in Beltsville in America (Liebenberg and Pastor-Corrales, 2010). The objective of this study was therefore to validate the use of the molecular SCAR markers linked to the *Ur-3*, *Ur-4*, *Ur-5* and *Ur-11* rust resistant genes using the F_2 dry bean breeding populations for selection purposes. After this initial validation, the markers can be used for selection of the resistant genes in subsequent generations through

Marker assisted selection (MAS) technique. MAS for disease resistance in common bean provides opportunities to breeders that were not feasible with the traditional breeding methods (Adam-Blondon *et al*., 1994). Several authors have reviewed the potential of using MAS in breeding common bean (Kelly and Miklas, 1999). The MAS technique allows for efficient and more accurate selection of the best performing individual plants in a segregating population.

4.2 Materials and methods

4.2.1 Greenhouse screening and crosses

The rust resistant genotypes Nep-2, BelDakMi-RMR-23, Mexico 309 and the susceptible South African cultivars, Bonus and RS6, were used for screenings to specific rust races and also for crosses as parental materials in this study. The three aforementioned genotypes belong to the Mesoamerican gene pool and the two South African cultivars belong to the Andean gene pool and therefore exhibit contrasting phenotypic reactions to rust with respect to the *Ur-3*, *Ur-4*, *Ur-5* and *Ur-11* genes, respectively. Plants were grown in the greenhouse in 8 cm diameter pots containing sterilized potting soil which contained equal amounts of artificial mixture of a pure material, e.g. pine bark and vermiculite with two seeds planted per cultivar in a pot and a total of four pots per cultivar. Temperature in the greenhouse ranged between 24-26^{o}C during the day and between 18-22^{o}C during the night with approximately 12 hr day time period. Irrigation was done manually at least twice a week using water can.

When the primary leaves reached two-thirds expansion, plants were inoculated with different rust races (1, 3, 5 and 11), using only one race at a time in order to accurately observe the reaction of the plants to individual races. Plants were inoculated on both sides of the leaves using a manual atomizer spray (De Vilbiss n$^{\circ}$15) at minimal pressure. Freshly harvested urediniospores of the above-mentioned rust races were suspended in tap water-Tween-20 (40 µL/L) solution that was adjusted to 2x10^{4} spores per ml. Inoculated plants were left untouched for approximately 1 hr before being moved to a dew chamber operating at >95% relative humidity with ±20^{o}C ambient temperature. Plants were transferred back to the same greenhouse that was used for planting 48 hrs after inoculation. In cases where two different races were used within the space of two weeks, plants were separated according to rust race and placed in different, properly demarcated greenhouse

compartments in order to prevent contamination. Results of the rust reaction of the inoculated materials were collected 14 days after inoculation using the 1 to 9 CIAT disease rating scale where 1 is resistant and 9 susceptible (Van Schoonhoven and Pastor-Corrales, 1987). The inoculated primary leaves were subsequently severed and discarded. Plants were transferred to 10L pots containing sterilized sandy-loam soil in order to prevent soil-borne diseases and allowed to grow further.

Using the same plants that were previously inoculated and rated, crosses were made in the greenhouse between the resistant genotypes and the susceptible South African cultivars through the emasculation and pollination method. At maturity, seed of the parents and that of the F_1 were harvested.

Seed of all five parental materials as well as all the F_1 individuals of the three populations were planted in the greenhouse and subjected to similar maintenance measures as previously indicated. The four rust races (1, 3, 5 and 11) were suspended in a mixture due to the fact that each F_1 seed is a unique genotype and therefore cannot be planted more than once. Rust races were mixed primarily to confirm the phenotypic status of the F_1 populations compared to their resistant and susceptible parents for *Ur-3*, *Ur-4*, *Ur-5* and *Ur-11*. The mixed rust spore suspension was also adjusted to a $2x10^4$ spores per ml concentration in the same solution as indicated previously. Plants were inoculated at the same growth stage and subjected to handling procedures and greenhouse conditions as previously described. Disease ratings were also recorded 14 days after inoculation and plants were transferred to 10L pots containing similar dry bean growth medium as described before. Plants were allowed to grow to maturity and seed harvested.

The F_2 seed as well as seed of the parental materials were planted in the greenhouse under similar conditions as described above. At two-thirds first trifoliate leaf expansion, plants were inoculated with fresh urediniospores in a mixed suspension and similar strength as in the case of the F_1 generation. This was also done to verify phenotypic reaction of the F_2 populations, which would allow for appropriate determination or comparison with molecular analyses of the targeted genes. Results of the rust reaction were collected 14 days after inoculation. Subsequently, the primary leaves that were inoculated were severed and discarded. Plants were transferred to 10L pots with similar growth medium as previously described. Phenotypic data on rust ratings for the three F_2 populations were used to test the goodness-of-fit to expected ratios using the chi-squared (χ^2) test of the 2016 Microsoft Excel version. The second trifoliate leaves of the five parental materials and of a total of

230 individual F_2 plants, from all three populations *colle*ctively, were harvested, freeze-dried and ground for DNA extraction.

4.2.2 Molecular analysis

4.2.2.1 DNA Extraction

Genomic DNA of the five parents and all the F_2 individuals was extracted using the CTAB method as described by Kalavachara *et al*. (2000) and Todd (2008), 250mg plant materials were used for the extraction of DNA. The plant materials were ground by the leaf tissuelyser manufactured by Retsch® for 35 seconds at a frequency of 30 1/s. DNA was further purified with1 µl RNase A (10 mg/mL) at 37ºC for 30 min followed by phenol: chloroform:IAA (25:24:1 v/v) extraction (Sambrook *et al*., 1989). After isopropanol precipitation, the DNA pellets were washed twice with 70% ethanol, dried on the laminar flow hood and re-suspended in 200 µl TE (10 mM Tris.HCl, pH 8.0; 1 mM EDTA) buffer. DNA integrity was checked on a 1% agarose gel (m/v) and the concentration and quality were measured with the NanoDrop 2000 (Thermo Scientific, Wilmington, DE) and diluted to a standard concentration of 50 ng/µL.

4.2.2.2 Validation of the SCAR markers

SCAR markers SK14, SI19, Ur4-$SA_{1079/800}$ and SAE19 obtained from IDT (Whitehead Scientific) were utilized to amplify genomic DNA of the five parents (Nep-2, Mexico 309, BelDakMi-RMR-23, Bonus and RS6) and the 220 segregating F_2 population individuals.

The PCR reaction mixture contained 1 µl template DNA, 1 µl of each primer (10 µM), 0.05 mM dNTPs, and 1 x PCR buffer (10 mM TrisHCl, pH 8.4, 50 mM KCI, 1.8 mM $MgCl_2$) and 1 unit of Taq polymerase (Promega Corporation, Madison, WI, USA) in a final reaction volume of 10 µl. The PCR amplification was carried out on a PCRmax Alpha Cycler thermal Cycler (Bibby Scientific). The primer sequences optimized PCR protocols for the four SCAR markers and their associated RR genes are indicated in Table 6.

The amplification products were analysed by adding loading buffer containing 10x GelRed (Biotium) to 10 µl sample and loading onto 1% agarose gel immersed in TAE buffer (40 mM Tris; 20 mM acetic acid; 1 mM EDTA). The gels were run for approximately 1 hour at 90 Volt in order to obtain sufficient separation of bands. Bands were visualised under UV light

and digital images were recorded in the ChemiDoc MP imaging system (Bio-Rad). The presence and absence of different-sized fragments were determined visually and recorded.

4.2.3 Statistical analyses

Chi-square (goodness of fit) analysis was done on phenotypic data using Microsoft Excel 2016 version calculator and reporting the two-tailed P values (http://www.biostathandbook.com/ chigof.html) is used to estimate the confidence interval for a normally distributed population's standard. The linkage of the markers to the genes in these specific populations were analysed using a general linear model of Statistica 64 version 13 (Dell Inc., 2016) at P≤0.05 level of significance. The marker data were used as dependent variable and the rust scores as a continuous variable.

4.3 Results

4.3.1 Greenhouse screenings and crosses

Reaction of the parental materials to different rust races is shown in Table 7. The resistance donor parents (Nep-2, Mexico 309 and BelDakMi-RMR-23) had rating scores of between 1 and 2, indicating resistance to the four races (1, 3, 5 and 11) evaluated. Evaluations were based on the CIAT 1-9 scale with 1 being resistant and 9 susceptible (Van Schoonhoven and Pastor-Corrales, 1987). Rust resistance from Mexico 309 and BelDakMi-RMR-23 appear to be immune while Nep-2 resistance was characterized by a hypersensitive reaction. The South African commercial cultivars showed rating scores of between 5 and 7, indicating susceptibility to all four races evaluated.

Table 6: Target rust resistance genes and their associated SCAR markers with PCR details

Gene	SCAR Marker	PCR program	Primer sequence (5' to 3')	Amplicon length	Reference
Ur-3	SK14	35 cycles of 2min at 94º C,30 sec at 94º C, 1min at 60º C, and 2 min at 72º C; followed by one cycle of 5 min at 72ºC	**Forward**- CCC GCT ACA CAC CAA TAC CTG **Reverse-** CCC GCT ACA CTT GAT AAA ATG TTA G	620 bp	Haley *et al*., 1994 Nemchinova& Stavely, 1998 Miklas *et al*., 2002
Ur-4	Ur-4 $SA_{1079/800}$	35 cycles of 2min at 94º C,5 min at 94º C, 60 sec at 55º C and 90sec at 72º C ; followed by one cycle of 7 minutes at 72º C	**Forward-** CTA TCT GCC ATT ATC AAC TCA AAC **Reverse-** GTG CTG GGA AAC ATT ACC TAT T	1079 and 800 bp	Miklas *et al*. 1993 Mienie *et al*., 2004 Miklas *et al.,* 2002
Ur-5	SI19	34 cycles of 10s at 94º C at 67º C and 120s at 72ºC ;followed by one cycle of 5 minutes at 72º C	**Forwar**d-AAT GCG GGA GAT ATT AAA AGG AAA G R**everse**-AAT GCG GGA GTT CAA TAG AAA AAC C	460 bp	Miklas *et al*., 2000 Melotto *et al*., 1998 Miklas *et al*., 2002
Ur-11	SAE19	94º C 5 min; 35 cycles of 15s at 94º C, 1min at 58º C and 1 min 30s at 72ºC; followed by one cycle of 7 minutes at 72º C	**Forward**- CAG TCC CTG ACA ACA TAA CAC C **Reverse**- CAG TCC CTA AAG TAG TT GTC CCT A	890 bp	Johnson *et al*., 1995 Queiroz *et al*., 2004 Miklas *et al*., 2002

Table 7: Reaction of parental materials to specific rust races[1]

Parents	Gene Pool	Resistance Gene	Race 1	Race 3	Race 5	Race 11
Nep-2	Mesoamerican	*Ur-3*	2	1	1	1
Bonus	Andean	Unknown	6	5	6	7
Mexico 309	Mesoamerican	*Ur-5*	1	1	1	1
RS6	Andean	Possibility of *Ur-13*	7	6	6	5
BelDakMi-RMR-23	Mesoamerican/ Andean	*Ur-11 , Ur-4*	1	1	1	1

1 Van Schoonhoven and Pastor- Corrales, *Rust in Standard system for the evaluation of Bean Germplasm* (CIAT, Cali Colombia), 24-25.

The cross between RS6 x BelDakMi-RMR-23 yielded 4 F_1 seed, while RS6 x Mexico 309, and Bonus x Nep-2 crosses both yielded 3 F_1 seed. The advanced F_1 seed yielded 77 F_2 individuals for the Bonus x Nep-2 cross, 89 F_2 individuals for the RS6 x BelDakMi-RMR-23 cross, 54 F_2 individuals for the RS6 x Mexico 309 cross (Table 8).

Table 8: Genetic segregation pattern of the F_2 populations derived from the cross of susceptible and the resistant parents.

Population F_2	Resistant observed Rust score 1-4	Susceptible observed Rust score 5-9	Expected Ratio	Observed ratio	χ^2	P-value
Bonus x Nep-2	44	33	3:1	1.3:1	12.160	0.000
RS6 x BelDakMi-RMR-23	71	18	3:1	3.9:1	0.843	0.359
RS6 x Mexico 309	25	29	3:1	1:1.16	22.22	0.000

All the F_1 plants of the three populations showed phenotypic reaction consistent with dominant gene resistance to all the rust races evaluated where ratings of between 1 and 3 were observed (data not shown). When the segregating F_2 plants of the three populations were

phenotypically assessed, reactions to the rust races ranged from 1 to 7 (Table 9, 10, 11), indicating segregation of the trait and expression of the resistance gene(s) involved. Phenotypic ratios were determined with rust scores 1-4 classified as highly resistant, resistant and intermediate and 5-9 as susceptible and highly susceptible (Van Schoonhoven and Pastor-Corrales, 1994). Population Bonus x Nep-2 rated 44:33 plants (1.3:1), (χ^2= 12160, P=0.000). The F_2 plants from the RS 6 x Mexico 309 cross containing the *Ur-5* gene segregated in a 1:1.16 (25:29) ratio (χ^2= 22.220, P=0.000). These two populations did not segregate according to the expected ratio of 3:1 (P<0.05) for a single dominant gene. The RS6 x BelDakMi-RMR-23 plants carrying the *Ur-11*, *Ur-3* and *Ur-4* genes segregated in a 3:1 (72:18)(3.9:1) ratio (χ^2 =0.843, P=0.359).

4.3.2 Molecular marker analysis

Four tested markers, namely SAE19 for *Ur-11* (Figure 5), SK14 for *Ur-3* (Figure 6), SI19 for *Ur-5* (Figure 7) and Ur4-$SA_{1079/800}$ for *Ur-4* (Figure 8) amplified the genes of interest in the following F_2 populations: RS6 x BelDakMi-RMR-23 (*Ur-11*) (*Ur-4*), Bonus x Nep-2 (*Ur-3*) and RS6 x Mexico 309 (*Ur-5)*, respectively. The SK14 marker, for the *Ur-3* gene, produced an amplification product of ~ 620 bp on Nep-2 (positive control) and no product was observed from the susceptible cultivar Bonus (negative control) (Figure 6). The SAE19 marker (Figure 5), for the *Ur-11* gene, produced an amplification product of ~ 890 bp on BelDakMi-RMR-23 (positive control) with no band observed in the susceptible parent RS6. The SI19 marker also produced an amplification product of ~460 bp on Mexico 309 (positive control) with no band observed on the susceptible parent (RS6) (Figure 7). The Ur4-$SA_{1079/800}$ marker produced amplification products of both ~ 1079 bp and 800 bp (Figure 8) on BelDakMi-RMR-23, a positive control for the *Ur-4* gene, with no band observed in the susceptible parent RS6. The marker was thus not reacting as a co-dominant marker in this population. However, the $SA_{1079/800}$ marker could be successfully used to amplify the 1079 bp fragment of *Ur-4*, which co-occurs with the *Ur-11* in the BelDakMi-RMR-23 genotype. The SK14 marker did not show any amplification from the resistant parent BelDakMi-RMR-23 and was therefore not useful in detecting the *Ur-3* gene in the RS6 x BelDakMi-RMR-23 cross (results not shown). Segregation patterns were observed for all the SCAR markers in the respective F_2 populations (Table 9, 10, 11).

Linkage of the markers to the resistance genes in these populations were determined using a logistic regression model to validate the use of the markers in the next generations of the crosses. Marker SAE19 was linked to the segregation pattern of the *Ur-11* gene in the RS6 x

BelDakMi-RMR-23 cross with an R^2 value of 0.639 (64%) (p=0.000). However, the $SA_{1079/800}$ marker was not linked significantly (R^2=0.004 (0.4%), p=0.540) to the *Ur-4* gene in this population. The rust scores in the Bonus x Nep 2 population were linked to the SK14 marker for *Ur-3* significantly at a level of R^2=0.915 (91.5%) (p=0.000). Marker SI19 was linked significantly to the *Ur-5* gene in the RS6 x Mexico 309 population with R^2=0.802 (80%) (p=0.000).

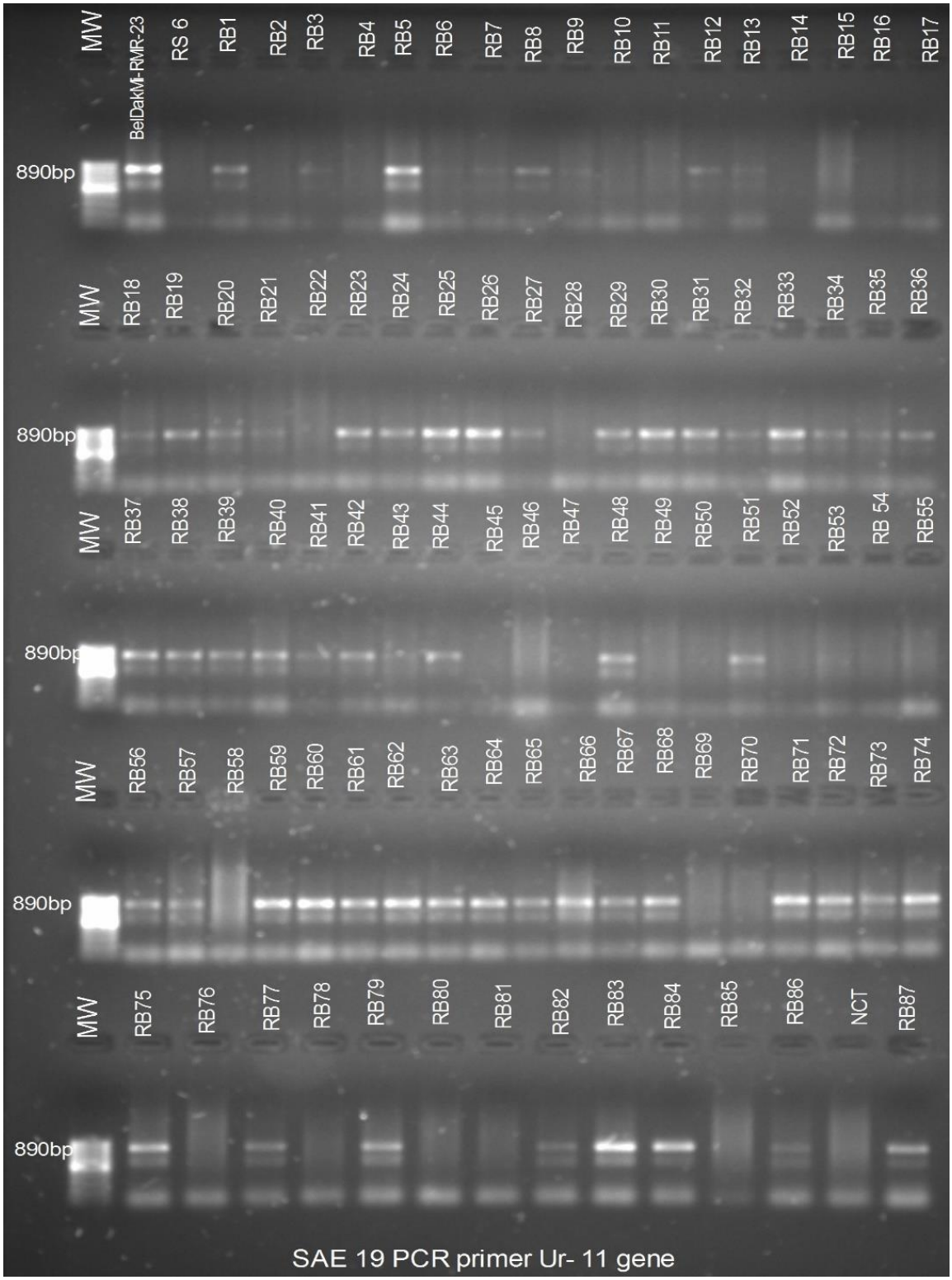

Figure 5: Agarose gel of the parents and F_2 population from the RS6 x BelDakMi-RMR-23 cross using marker SAE19 amplifying an 890 bp fragment linked to *Ur-11* rust resistance gene. MW: 100 bp ladder. NCT: No template control.

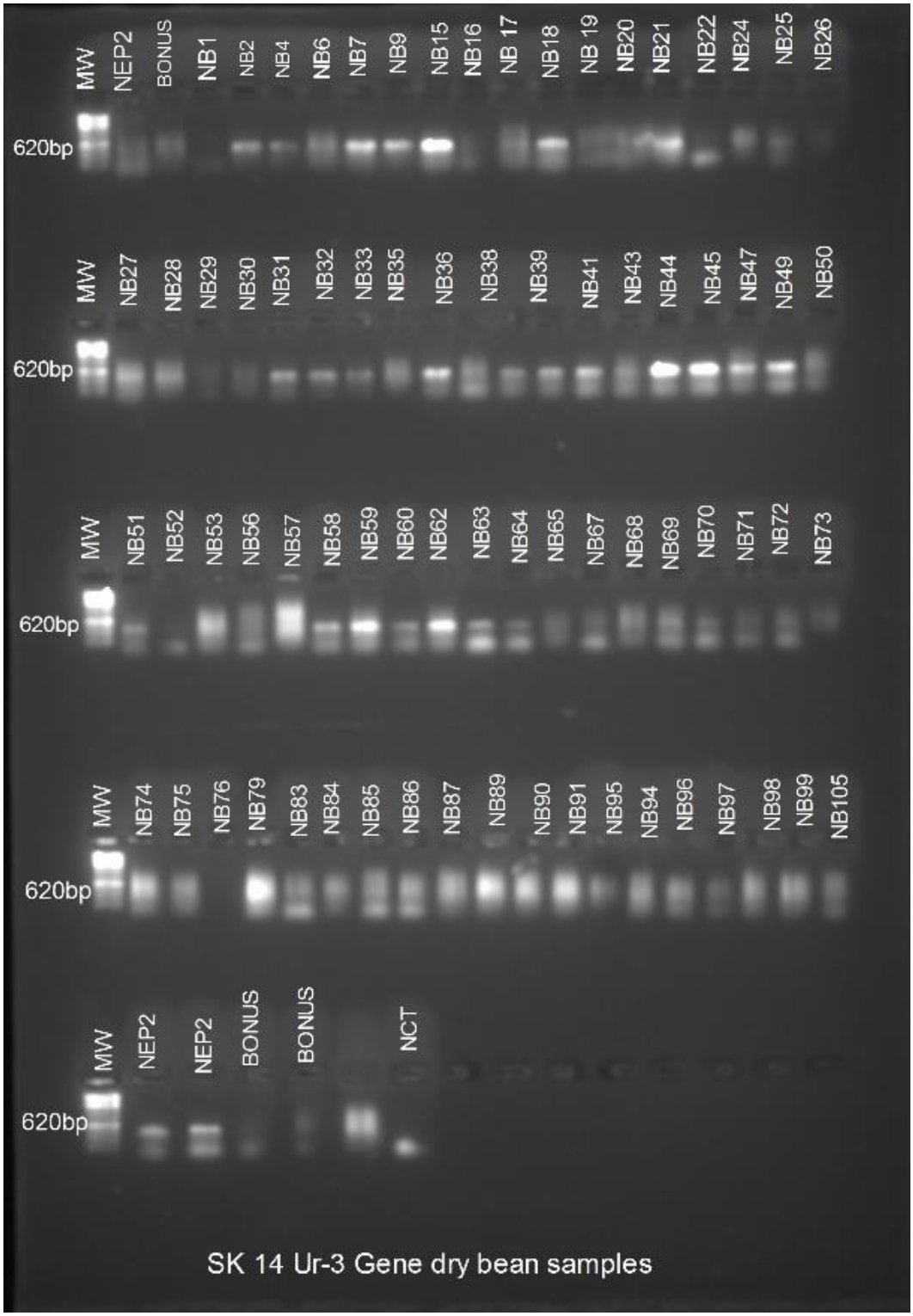

Figure 6: Agarose Gel of the parents and F_2 population from the Bonus x Nep-2 (*Ur-3)* cross using marker SK14 linked to the *Ur-3* rust resistance gene (620 bp). MW: 100 bp ladder. NCT: No template control.

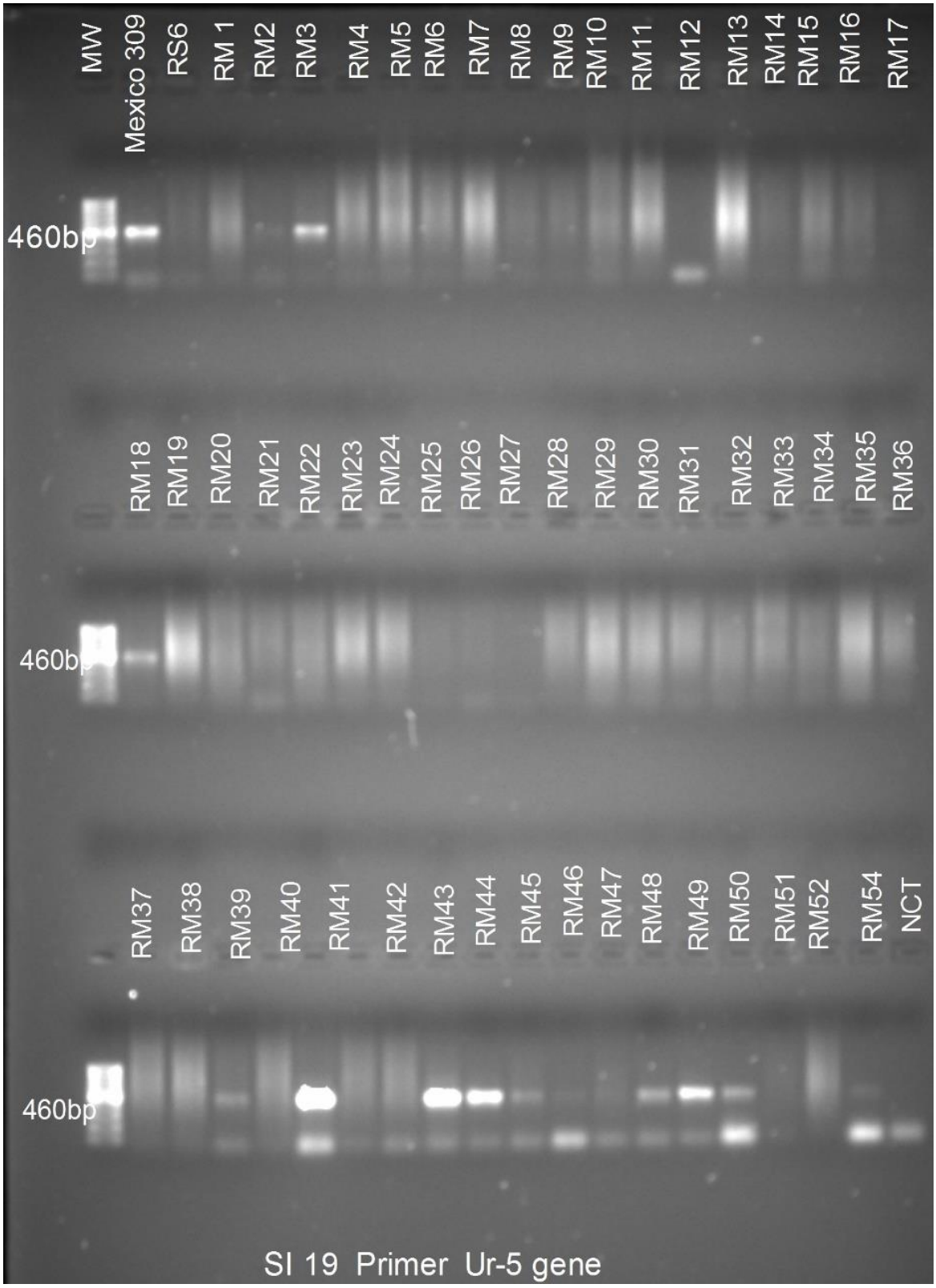

Figure 7: Agarose Gel of the parents and F_2 population from the RS6 x Mexico 309 (*Ur-5*) cross using marker SI19 linked to the *Ur-5* rust resistance gene (460 bp). MW: 100 bp ladder. NCT: No template control.

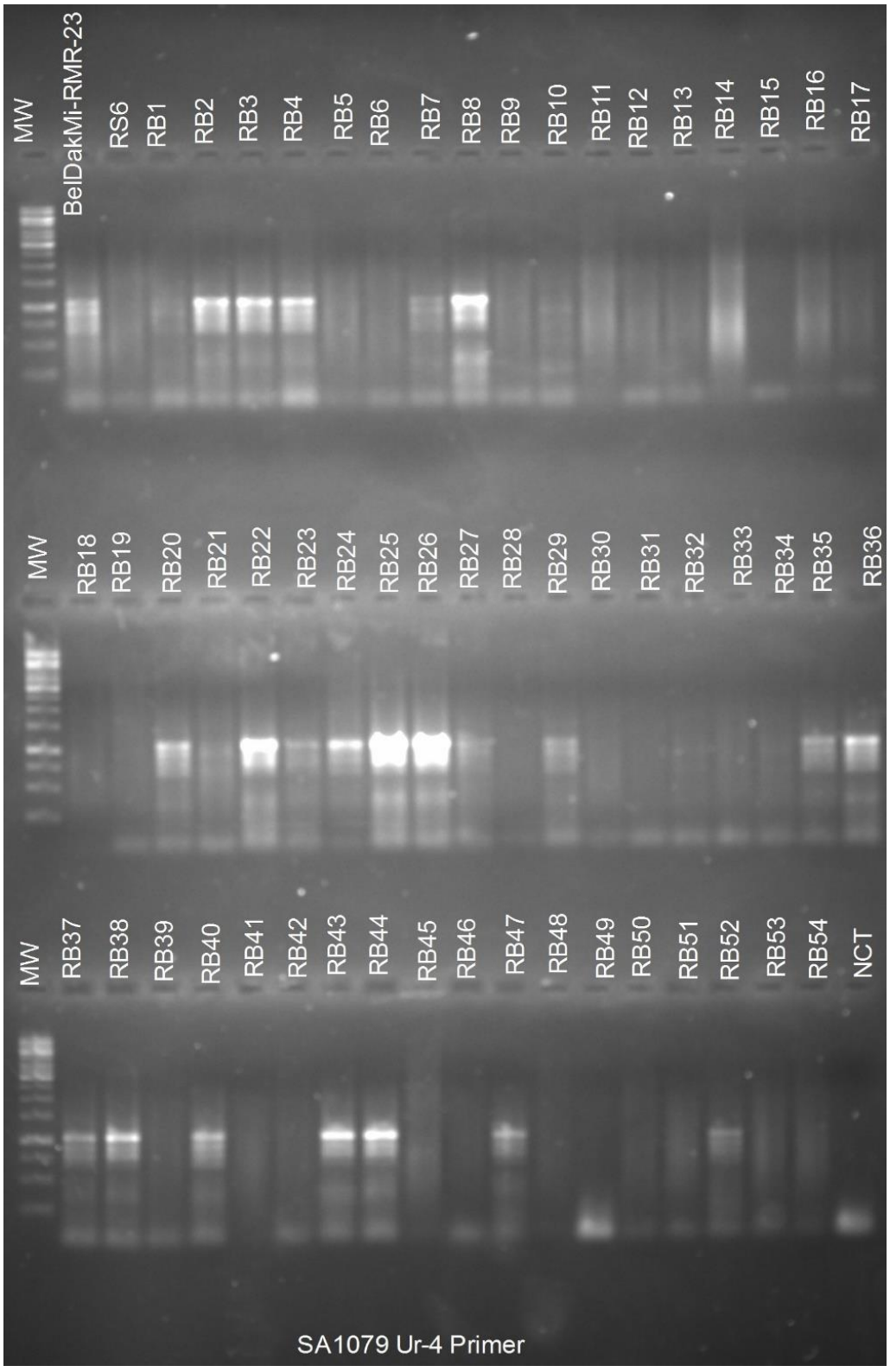

Figure 8: Agarose Gel of the parents and F_2 population from the BelDakMi-RMR-23 x RS6 cross using marker Ur4-SA1079/800 linked to the *Ur-4* rust resistance gene. MW: 1 kb ladder. NCT: No template control.

Table 9: Rust resistance score according to phenotypic and marker analyses of F_2 population of RS6 X BelDakMi-RMR-23

Plant	**Rust score**	**SAE19 (*Ur-11)***	**$SA_{1079/800}$ (*Ur-4)***
RS 6 (Female parent)	6	-	-
BelDakMi-RMR-23 (Male Parent)	1	+	+
RB-1	1	+	-
RB-2	4	-	+
RB-3	1	+	+
RB-4	5	-	+
RB-5	1	+	-
RB-6	6	+	-
RB-7	5	+	+
RB-8	1	+	+
RB-9	1	+	-
RB-10	1	-	+
RB-11	4	-	-
RB-12	1	+	-
RB-13	2	+	-
RB-14	5	-	-
RB-15	6	-	-
RB-16	5	-	-
RB-17	5	-	-
RB-18	1	+	-
RB-19	1	+	-
RB-20	1	+	+
RB-21	3	+	+
RB-22	4	-	+
RB-23	3	+	+
RB-24	1	+	+
RB-25	1	+	+
RB-26	1	+	+
RB-27	1	+	+
RB-28	4	-	-
RB-29	1	+	+
RB-30	1	+	-
RB-31	1	+	-
RB-32	1	+	-
RB-33	3	+	-

Plant	Rust score	SAE19 (*Ur-11)*	$SA_{1079/800}$ (*Ur-4)*
RB-34	1	+	-
RB-35	1	+	+
RB-36	3	+	+
RB-37	1	+	+
RB-38	1	+	+
RB-39	1	+	-
RB-40	1	+	+
RB-41	1	+	-
RB-42	1	+	-
RB-43	1	+	+
RB-44	1	+	+
RB-45	5	-	-
RB-46	5	-	-
RB-47	1	-	+
RB-48	1	+	-
RB-49	5	-	-
RB-50	5	-	-
RB-51	1	+	-
RB-52	6	-	+
RB-53	6	-	-
RB-54	5	-	-
RB-55	5	-	-
RB-56	1	+	-
RB-57	1	+	-
RB-58	4	-	-
RB-59	1	+	-
RB-60	1	+	-
RB-61	1	+	-
RB-62	1	+	-
RB-63	1	+	-
RB-64	1	+	-
RB-65	1	+	-
RB-66	1	+	-
RB-67	1	+	-
RB-68	1	+	-
RB-69	4	-	-
RB-70	4	-	-
RB-71	1	+	-

Plant	Rust score	SAE19 (*Ur-11)*	$SA_{1079/800}$ (*Ur-4)*
RB-72	1	+	-
RB-73	1	+	-
RB-74	1	+	-
RB-75	1	+	-
RB-76	5	-	-
RB-77	1	+	-
RB-78	4	-	-
RB-79	1	+	-
RB-80	5	-	-
RB-81	6	-	-
RB-82	1	+	-
RB-83	1	+	-
RB-84	1	+	-
RB-85	1	-	-
RB-86	1	+	-
RB-87	1	+	-
RB-88	1	+	-
RB-89	1	+	-

+ Positive, -Negative

Table 10: Rust resistance score according to phenotypic and marker analysis of F_2 population of RS6 X Mexico 309

Plant	Rust score	SI19 *(Ur-5)*
RS 6 (Female parent)	6	-
Mexico 309 (Male)	1	+
RM-1	6	-
RM-2	1	+
RM-3	1	+
RM-4	5	-
RM-5	5	-
RM-6	4	-
RM-7	4	-
RM-8	5	-
RM-9	6	-
RM-10	4	-
RM-11	5	-
RM-12	6	-

Plant	**Rust score**	**SI19 *(Ur-5)***
RM-13	7	-
RM-14	6	-
RM-15	7	-
RM-16	8	-
RM-17	5	-
RM-18	1	+
RM-19	4	-
RM-20	4	-
RM-21	4	-
RM-22	4	-
RM-23	5	-
RM-24	5	-
RM-25	4	-
RM-26	5	-
RM-27	5	-
RM-28	5	-
RM-29	6	-
RM-30	7	-
RM-31	6	-
RM-32	5	-
RM-33	5	-
RM-34	6	-
RM-35	5	-
RM-36	5	-
RM-37	4	-
RM-38	5	-
RM-39	1	+
RM-40	4	-
RM-41	4	-
RM-42	5	-
RM-43	1	+
RM-44	1	+
RM-45	1	+
RM-46	1	+
RM-47	1	+
RM-48	1	+
RM-49	1	+
RM-50	1	+

Plant	Rust score	SI19 *(Ur-5)*
RM-51	5	-
RM-52	6	-
RM-54	1	+
RM-55	4	-

Table 11: Rust resistance score according to phenotypic and marker analysis of F_2 population of Bonus X Nep-2

Plant	Rust score	SK14 (*Ur-3)*
Bonus (Female parent)	7	_
Nep2 (male parent)	1	+
NB-1	6	-
NB-2	1	+
NB-4	1	+
NB-6	1	+
NB-7	1	+
NB-9	1	+
NB-15	1	+
NB-16	6	-
NB-17	2	+
NB-18	1	+
NB-19	4	-
NB-20	5	-
NB-21	1	+
NB-22	5	-
NB-24	1	+
NB-25	5	-
NB-26	5	-
NB-27	1	+
NB-28	1	+
NB-29	4	-
NB-30	5	-
NB-31	1	+
NB-32	1	+
NB-33	1	+
NB-35	6	-
NB-36	1	+

Plant	Rust score	SK14 (*Ur-3)*
NB-38	6	-
NB-39	1	+
NB-41	1	+
NB-43	1	+
NB-44	1	+
NB-45	1	+
NB-47	1	+
NB-49	1	+
NB-50	5	-
NB-51	1	+
NB-52	6	-
NB-53	6	-
NB-56	4	-
NB-57	5	-
NB-58	1	+
NB-59	1	+
NB-60	1	+
NB-62	1	+
NB-63	1	+
NB-64	1	+
NB-65	7	-
NB-67	1	+
NB-68	7	-
NB-69	1	+
NB-70	1	+
NB-71	4	-
NB-72	5	-
NB-73	4	-
NB-74	1	+
NB-75	6	-
NB-76	5	-
NB-79	4	-
NB-80	4	-
NB-83	5	-
NB-84	5	-
NB-85	4	-
NB-86	5	-
NB-87	5	-

Plant	Rust score	SK14 (*Ur-3)*
NB-88	5	-
NB-89	5	-
NB-91	4	-
NB-92	6	-
NB-93	5	-
NB-94	6	-
NB-96	6	-
NB-97	5	-
NB-98	5	-
NB-99	5	-
NB-105	4	-

4.4 Discussion

The resistance to four South African rust races (1, 3, 5 and 11) by the donor parents Nep-2 with *Ur-3* (Miklas *et al.,* 2002), Mexico 309 with *Ur-5*, and BelDakMi-RMR-23 with *Ur-11 and Ur-4* (Pastor-Corrales, 2006) and the susceptibility of the South African dry bean cultivars Bonus and RS6 were verified in a greenhouse trial (Table 7). The results of the resistant parents used in hybridization in this study confirm that the four targeted rust resistance genes are still important for South African bean production since they are not overcome by the local rust races. The four targeted rust resistance genes were confirmed by the results and they indicated to be important for South African bean production since they are not overcome by the local rust races. The three sources for the *Ur-3, Ur-5* and *Ur-11* rust resistance genes are from the Mesoamerican gene pool (Park *et al*., 2004) while *Ur-4* is from the Andean gene pool (Stavely, 2000; Pastor-Corrales, 2001). Both the South African cultivars are large seeded types and therefore belong to the Andean gene pool (Miklas *et al*., 2006; Wright *et al.,* 2008). It has been demonstrated previously that the Andean and Mesoamerican rust isolates show virulence specificity to landraces belonging to their respective gene pools due to co-evolution (Araya *et al*., 2004). The general preference for large seeded Andean-type beans in Africa as a whole (Sprecher and Isleib, 1989), and particularly in South Africa has undoubtedly contributed to the predominance of the Andean-type rust races in Africa (Liebenberg, 2003).

The rust races (1, 3, 5 and 11) used in this study are part of the pathogen collection of the ARC-GC in South Africa. The rust resistance donor parents Mexico 309 (*Ur-5*) (De Souza *et al*., 2007a, 2007b), BelDakMi-RMR-23 (*Ur-11*, *Ur-4*) (Stavely *et al*., 1994) and Nep-2 (*Ur-3*)

(Miklas *et al*., 2002) have been reported to be very resistant across the African continent including the Southern African countries. The four targeted rust resistance genes (*Ur-3, Ur-4, Ur-5* and *Ur-11*) were therefore identified and considered highly important for the South African dry bean industry (Liebenberg *et al*., 2005). Identification of new RR genes from the Mesoamerican and Andean gene pools is important for the future of the common bean breeding program regarding rust resistance as it will broaden the spectra of the RR genes (Liebenberg *et al*., 2006; Pastor-Corrales *et al*., 2008) and as such be a great asset to the bean industry.

Resistance to rust was also observed among all the F_1 individual plants of the three populations developed in this study, suggesting dominant resistance. The χ^2 values of two of the populations (Bonus x Nep-2 and RS6 x Mexico 309) indicated that the RR trait did not segregate according to a 3:1 ratio as expected. This could not confirm numerous previous findings that resistance to bean rust is controlled by single dominant genes (Alzate-Martin *et al*., 2004; De Souza *et al*., 2007a; 2007b; De Souza *et al*., 2011), but this observation is most probably due to the low number of plants used in the study or deviations in environmental conditions in the greenhouse. The F_2 population of the RS6 x BelDakMi-RMR-23 cross segregated close to a 3:1 ratio as expected (3.9:1, $P>0.05$).

SCAR markers were tested (Figure 5-Figure 8) and the markers produced polymorphic fragments in the positive parent indicating successful mutation. The fragments that amplified in the resistant parents were 890bp (SAE19, *Ur-11* BelDakMi-RMR-23), 620bp (SK14, *Ur-3* Nep2) and Ur-4SA1079/800, *Ur-4* BelDakMi-RMR-23). These markers can be used when more detailed information is needed about the genetics of the specific crosses that where done in this study and have been useful in locating the genes. The markers showed to be useful in tracing the sources of the RR that are present in BelDakMi-RMR-23 and Nep2, as well as the as the identification of other cultivars with the same resistance such as PI 181996 with *Ur-11* and Aurora with *Ur-3* (Pastor-Corrales,2006).

SCAR markers linked to major resistance genes are developed using near-isogenic lines (NILs) that have been developed from resistant and susceptible individual varieties or breeding lines (Haley *et al*., 1993; Miklas *et al*., 1993). SCAR markers linked to the target genes were previously identified (Stavely *et al*., 1994). The advantage of using SCAR markers is that it is highly reproducible and can also be used in a multiplex PCR analysis since they are able to identify specific loci (De Souza *et al*., 2011). They also have an added application that is useful in tracing the sources of the RR that are present in the South African cultivars (Liebenberg, 2003), as well as other cultivars, which may have the same resistance materials (Liebenberg

and Pretorius, 2004a). Furthermore, all four markers have previously been used in identifying the *Ur-3, Ur-4, Ur-5* and *Ur-11* rust resistance genes in local bean varieties (Liebenberg, 2003; Liebenberg and Pretorius, 2004; Mienie *et al*., 2004). The four genes are reportedly also effective against most of the rust races that occur in the dry bean production areas of South Africa (Liebenberg, 2003). The *Ur-4* gene is reportedly not of particular value in Africa, but can however be of importance if the Beltsville race 22-52 enters the continent since this race overcomes the *Ur-11* gene (Liebenberg *et al.,* 2004). Molecular markers linked to major genes could be useful when several resistance genes are transferred into a susceptible bean cultivar for rust resistance (Liebenberg *et al*., 2005).

The F_2 populations were tested for the presence of the markers developed for the resistance genes and the linkage to the phenotypic trait determined to validate the use of the marker in the subsequent generations of the specific crosses. Significant linkages were observed between markers and selected genes for all the crosses. The SAE19 marker explains 64% of variation in the segregation (p=0.000) of the *Ur-11* gene in the RS6 x BelDakMi-RMR-23 population, with the $SA_{1079/800}$ marker explaining only 4% of variation of the *Ur-4* gene (p=0.540). Thus, only the *Ur-11* marker can be used in the progeny from this population, with the *Ur-4* marker having a high probability for false positive and negative plants. The *Ur-3* marker SK14 could not be used in this population. In the Bonus x Nep2 population the SK14 marker was linked 92% to the resistance gene *Ur-3* (p=0.000), making it excellent to be used in this population in the subsequent generations. In the RS6 x Mexico 309 population the SI19 marker was linked 80% to the *Ur-5* gene (p=0.000) and can be used reliably in the progeny to get an indication of the resistant plants to be selected.

It was previously reported that existing molecular markers tracing *Ur-3* for use in marker-assisted selection could produce false positive and negative results (Hurtado-Gonzales *et al*., 2017), however, the SK14 marker was successfully used to trace the *Ur-3* gene in the population used in this study. The close proximity of *Ur-3–Ur-11* may be one of the main reasons why it has been difficult to find DNA markers that are specific for the *Ur-3* gene (Hurtado-Gonzales *et al*., 2017). Hurtado-Gonzales *et al*. (2017) further recommended the option of the SS68 marker for the *Ur-3* gene, which was reportedly highly accurate and produced no false results. This marker will be important to consider for future studies of this nature. In validating the use of SCAR marker SK14, Melmaiee *et al.* (2013) reported that both the positive and negative control bacterial artificial chromosomes showed corresponding PCR products on the gel, thus showing indiscriminate results. This was attributed to the fact that SK14 is derived from a repetitive sequence region of the genome. The *Ur-3* gene confers resistance to 55 of 94 races of the bean rust pathogen maintained at Beltsville, MD, including

races 84, 101 and 108 (Pastor-Corrales *et al*., 2001) where the latter (currently known as race 22-52) is the only race known to overcome the broad spectrum resistance of the *Ur-11* gene present in PI 181996 and PI 190078, and of the *Ur-14* gene present in Ouro Negro (Alzate-Marin *et al*., 2004; Stavely, 1998). The *Ur- 3* gene in Nep 2 overcomes race 3 and race 11 and is overcome by race 1 while offering a moderately susceptible reaction to race 5. All four races included in this study overcame the *Ur-4* gene.

The SCAR marker SI19 has previously been validated as linked to the rust resistance gene *Ur-5* from cultivar Mexico 309 (De Souza *et al*., 2007a, 2007b) and has been confirmed in this study. It was also verified that this marker can be used for the indirect selection of gene *Ur-5* in the presence of genes *Ur-11* and *Ur-14*. The *Ur-5* gene in Mexico 309 overcomes all rust races (1, 3, 5 and 11) evaluated in this study. Liebenberg *et al*. (2011) reported that Mexico 309 was resistant to ten different rust races, including race 11, and that the genotype was also resistant in the field under natural infection in South Africa.

The resistance in BelDakMi-RMR-23 is governed by four dominant genes (*Ur-3*, *Ur-11, Ur-6* and *Ur-4*), each effective against different rust races. The repulsion phase molecular marker SAE19 (*Ur-11*) was originally developed by Stavely (1998). However, it was found that it was linked in parallel in the BelDakMi-RMR-23 line (Pastor-Corrales, 2003), where the *Ur-(3+11)* are found in coupling as resistance source (Liebenberg *et al*., 2004). The marker could thus be used successfully to trace the *Ur-11* rust resistance gene present in BelDakMi-RMR-23 as confirmed using the F_2 population where this genotype was used as a donor parent in this study (Figure 5). The SAE19 marker is reportedly located at 1.0 cM from the resistance gene *Ur-11* (Queiroz *et al*., 2004). The *Ur-11* overcomes a significantly large number of rust races occurring in South Africa and this includes races 1, 3 and 5, however, Liebenberg *et al*. (2005) indicated that the gene (contained in BelDakMi-RMR-1) is overcome by race 11, an observation inconsistent with the findings of this study where the gene is contained in BelDakMi-RMR-23. This study also demonstrated that the *Ur-11* gene can be traced in the presence of *Ur-3* and *Ur-4* in BelDakMiRMR-23.

The F_2 population from a BelDakMi-RMR-23 and RS6 cross showed that out of 90 plants screened, 18 amplified for both the *Ur-11* and the *Ur-4* rust resistance genes, indicating that these genes were successfully stacked together. There was a variation with the remaining 72 plants where some amplified for the *Ur-11* gene alone or the *Ur-4* gene alone or did not amplify at all, indicating that one or both genes were lost during segregation. Although the linkage values indicated that the marker was not significantly linked in this population, it should not be totally disregarded in the plants that had both the markers and the resistance trait and should

be tested again when these plants are used in subsequent crosses. Germplasm variety BelDakMi-RMR-23 already possess a number of stacked rust resistance genes (*Ur-3*, *Ur-4*, *Ur-6* and *Ur-11)* conferring resistance to several races (De Souza *et al*., 2011).

Bean rust is a hyper-variable pathogen that can rapidly overcome deployed resistance genes. It is therefore highly imperative to stack important and useful rust resistance genes in one cultivar or breeding line. Three of the tested markers yielded informative results when used in analysing the F_2 populations developed for the *Ur-3*, *Ur-4*, *Ur-5* and *Ur-11* rust resistance genes. To be effective, the gene pyramiding strategy must be a continuous effort that seeks to combine resistance genes from different gene pools since this would widen the resistance spectra (De Souza *et al*., 2014). It is well-documented that the use of gene pyramiding is an effective strategy for disease control (Thrall and Burdon, 2003) and that a gene pyramid of more than two genes is extremely difficult to be overcome by a pathogen (Schafer and Roelfs, 1985).

Further studies and research need to be done with a clear hybridization plan that will seek to stack all four RR genes (*Ur-3*, *Ur-4*, *Ur-5* and *Ur-11*) as efficiently as possible. A four-way crossing system, where the F_1 progenies of the two different crosses ((Bonus x Nep-2; RS6 x Mexico 309) and (RS6 x Mexico 309; RS6 x BelDakMi-RMR-23)) will be considered in achieving this goal. Crosses of the F_1 progenies of the parents Bonus x Nep-2 and RS6 x Mexico 309 will stack the *Ur-3* and *Ur-5* genes in one cultivar while crosses of the F_1 progenies of the parents RS6 x Mexico 309; RS6 x BelDakMi-RMR-23 will stack the *Ur-3, Ur-5* and *Ur-11* genes in one or more cultivars. Molecular markers SK14, SI19 and SAE19, which were successfully validated in this study, will be of great value in demonstrating successful transfer of the *Ur-3*, *Ur-5* and *Ur-11* RR genes and will be used for selection of putative resistant plants during the breeding process.

4.5 Conclusions

There was a clear distinction between the donor (resistant) and the receptive (susceptible) parent as far as the phenotypic reaction to the rust disease is concerned. The molecular markers used to screen individual F_2 plants for *Ur-3, Ur-5* and *Ur-11* genes showed a good correlation between the presence of the target gene and the phenotypic expression. This study demonstrated the phenotypic effectiveness of *Ur-3, Ur-4, Ur-5* and *Ur-11* rust resistance gene combination against important rust races (1, 3, 5 and 11) of common bean in South Africa. The SK14, SI19 and SAE19 SCAR markers were validated to be used successfully to select

the rust resistance genes (*Ur-3* (Nep 2), *Ur-5* (Mexico 309) and *Ur-11* (BelDakMi-RMR-23)), respectively. The SK14 marker can be used to follow the *Ur-3* gene in the Bonus x Nep-2 cross. However, the SK14 marker did not amplify for the *Ur-3* gene on the BelDakMi-RMR-23 genotype. The $SA_{1079/800}$ marker can also not be used for selection of *Ur-4* in this population. The *Ur-5* gene is closely linked to marker SI19 in the RS6 x Mexico 309 population and can be used for selection in future crosses with the hybrids. The SCAR SAE19 can be used for marker assisted selection in future generations and crosses for gene stacking using the RS6 x BelDakMi-RMR-23 hybrids. Rust resistance in BelDakMi-RMR-23, Nep-2, and Mexico 309 is reported to be governed by single dominant genes, however this could not be observed in this study, as the marker was unreliable and was giving false negative/positive results of the case tested. Further pyramiding of rust resistance genes under study and marker assisted selections will continue in order to achieve homozygosity among progenies.

Working on several major there is value in using pyramided genotypes as parents in disease resistance breeding programs. However, the marker for the *Ur-3* gene could not be used in the BelDakMi-RMR-23 population to track the gene. The BelDakMi-RMR-23 genotype has also been highly resistant to rust in South Africa and this could be attributed to the nature of the stacked genes. Nep-2 can also be used as a resistant source for *Ur-3* and can be tracked by the marker (SK14). A broad spectra of rust resistance genes is important in combating the high virulence diversity and variability present in the rust pathogen populations. A future study could also explore the use of other *Ur-5* sources.

CHAPTER 5 - DISCUSSION

Common bean (*Phaseolus vulgaris* L.) is an important legume crop for human consumption throughout the world including South Africa (Liebenberg *et al*., 2005; Petry *et al*., 2015). The crop is considered as staple food and a major source of calories and protein in a number of developing countries including South Africa. The crop acts as a health addition to any diet since it is a rich source of zinc and iron (Beebe *et al*. 2013; Petry *et al.,* 2012; Katuuramu *et al*., 2018). Common bean also has high carbohydrate content and provides minerals, vitamins and phenolic compounds with antioxidant properties that help prevent certain chronic diseases (Machado *et al*., 2008).

Production of dry bean encounters numerous challenges ranging from abiotic to biotic. Rust, caused by an airborne fungal pathogen *Uromyces appendiculatus,* is one of the major production challenges around the world wherever dry beans are produced (Kelly *et al*., 2003) with significant yield losses on every dry bean growing season (Miklas *et al*., 2006; De Souza *et al*., 2007a, 2007b). Rust is considered an economically important disease of dry beans in South Africa owing to high yield losses and wide distribution in major production areas.

The *U. appendiculatus* spores travel long distances as they are wind-borne. This phenomenon, exacerbated by the pathogen's nature of changing its population composition within and between growing seasons and production areas (McMillan *et al*., 2003), make control of the pathogen a daunting task. Through the past decades, a number of control methods for the rust disease have been developed and applied with varying success rate. These rust control methods include sanitation (keeping the fields as clean as possible), deep ploughing of rust-infected debris, crop rotation with non-host plants, effective weed control, biological control, planting beans early, application of fungicides and planting rust resistant cultivars (De Souza *at el*., 2008). Of the above-mentioned rust control methods, the most effective, long lasting, economical and environmentally-friendly method is considered to be the use of rust resistant cultivars (Stavely and Pastor-Corrales, 1989). Genetic disease resistance has been favourable amongst dry bean producers across the world. The strategy can however, be complicated by great diversity that continuously exist and change within the rust pathogen (Stavely and Pastor-Corrales, 1989). Rust control can be complicated further by the fact that the disease spreads easily through various means including wind, rain splash and human activities and it is common that multiple races occur in the same area or different races occurring in different localities (Mienie *et al*., 2005).

Differential pathogenic (or aggressiveness) behaviour towards different rust resistance genes indicates the existence of different rust races within *U. appendiculatus*. This behaviour therefore requires the existence of multiple rust resistance genes in a cultivar in order to broaden and ensure sustainability of the resistance. The main objectives of the study were to phenotypically and genetically screen selected common bean varieties for their reaction to the rust pathogen, to develop segregating breeding lines containing pyramided rust resistant genes, to validate the application of the SCAR markers SK14, $SA_{1079/\ 800}$, SI19 and SAE19 linked to the *Ur-3, Ur-4, Ur-5* and *Ur-11* genes, respectively, in the F_2 breeding populations of interest. Findings of the study provided information on the usability of the SCAR markers for selection purpose in further generations of these crosses.

Twenty-five South African commercial dry bean cultivars (including one rust spreader) and thirty-one selected international germplasm varieties from the ARC-GC, Potchefstroom, South Africa were evaluated for their reaction to rust under field conditions at three localities (Potchefstroom, Cedara and Makhathini) for two seasons (2015/2016 and 2016/2017). South African cultivars Mkuzi, Kamiesberg, Teebus-RCR2, OPS-KW1 (*Ur-3*), Tygerberg, PAN 9292, CAR 2008, Teebus-RR1 (possibly containing rust resistance gene *Ur-3*) showed rating scores between 1 to 3, which is considered to be resistant in this study. Cultivars DBS 360, OPS-RS4, PAN 9249, RS6, Bonus, Sederberg, Kranskop-HR1, DBS 310, DBS 830 and PAN 116 showed intermediate reactions with ratings ranging from 4 to 6. Cultivar Teebus and Pinto 114 (spreader) showed high susceptibility to rust in this study with ratings of 7 and 8, respectively.

Germplasm varieties (Nep-2 (*Ur-3*), Mexico 235 (*Ur-3*), Mexico 309 (*Ur-5*), BelDakMi-RMR-22 (*Ur-3, Ur-4, Ur-6* and *Ur-11*), BelDakMi-RMR-23 (*Ur-3,Ur-4, Ur-6* and *Ur-11*) and BelMiNeb-RMR-7(*Ur-3,Ur-4* and *Ur-11*) showed good rust resistance with a rating of 1 to 3 while varieties Pinto 114 (rust spreader) and GN 1140 were most susceptible to rust with highest severity rating of 8 across different seasons and localities. Generally, the majority of the rust differential cultivars were resistant to rust in this study. The varieties that were evaluated in the field were also screened in the greenhouse for reaction to rust through an artificial inoculation method. Artificial inoculations were also done in field trials in Potchefstroom. Rust occurs naturally in Makhathini and Cedara and no artificial inoculation was necessary. The same rust resistance trend from field results was observed in the greenhouse results, allowing for a more decisive identification of resistant materials for use in crosses as donor parents. Varieties BelDakMi-RMR-23, Mexico 305 and Nep-2 contain the targeted rust resistance genes (*Ur-3, Ur-4*, *Ur-5* and *Ur-11*) and were therefore selected as donor parents to improve resistance of the local commercial cultivars.

The South African dry bean commercial cultivars (Bonus and RS6) as well as the selected rust resistant germplasm varieties (BelDakMi-RMR-23, Mexico 305 and Nep-2) were used in crosses for the development of breeding populations. Three F_1 breeding populations were developed and allowed to self-pollinate, resulting into three segregating F_2 populations. The F_2 populations were phenotyped with a mixture of rust races, as well as genotyped with the selected molecular markers linked to the target genes. The markers were successfully optimised and validated on five parental genotypes and 230 plants of the segregating F_2 populations. Five parental genotypes RS6 (susceptible), Bonus (susceptible), Nep-2 (*Ur-3*), Mexico 309 (*Ur-5*) and BelDakMi-RMR-23 (*Ur-11*) were used for the confirmation of rust resistance using the SCAR markers mentioned previously. Marker SK14 (*Ur-3*) showed the positive resistance banding pattern at 620 bp, SAE19 (*Ur-11*) amplified at 890 bp, SI19 (*Ur-5*) amplified at 460 bp and marker $SA_{1079/\ 800}$ (*Ur-4*) amplified at 1079 bp indicating the presence of the targeted genes. In this study the SCAR markers SK14 (*Ur-3*) and SAE19 (*Ur-11*) showed single dominant polymorphism amongst the resistant and the susceptible F_2 plants. This observation confirmed the successful transfer of the desired rust resistant genes from the donor parents to the susceptible South African dry bean commercial parents. The three rust resistance genes in this study (*Ur-3*, *Ur-5* and *Ur-11*) have shown resistance in this study and is in line with the findings by Liebenberg, (2003). The genes are reportedly very important for South Africa. The dry bean lines developed in this study are now part of germplam at ARC-GCI and can be explored further in order to eventually enhance rust resistance in dry bean production in the country. Hybridization of a SK14-derived overgo probe showed erratic (weak to normal signal intensities) results by identifying bacterial artificial chromosomes (BACs) in and outside of the *Ur-3* region (Melmaiee *et al*., 2013). In a study done by Melmaiee *et al*., (2013), it was demonstrated that markers SK14 and SAE19 successfully amplified the 600 bp and 800 bp regions, respectively, in a common bean variety Sierra. The sequences that were identified subjected on SAE19 molecular marker showed 99 percent sequence similarity with previously reported sequence (Melmaiee *et al*., 2013).

The diagnostic application of SCAR markers helps the plant breeder to be able to eliminate susceptible genotypes with a high degree of probability that leaves only the resistant plant for further evaluations. The availability of the SCAR markers (Blair *et al*., 2007; Miklas *et al*., 2000) has complemented direct disease screening in the presence of the rust fungus, indicating the effectiveness of these markers. It has been reported that presently thirteen dominant rust resistance genes have been identified (*Ur-1* to *Ur-13*) and in addition, there are other unnamed genes that have been identified in the common bean cultivars. Plant breeding and crop improvement is achieved by known and unknown selections of desirable rust resistance

genes. One of the problems facing plant breeders is uncertainty of whole plant assessment as an indicator of genetic potential. The process of dry bean selection can be improved if the whole plant assessment could be backed by direct analysis of the genetic composition of the plant through use of molecular markers. Marker validation is an important aspect of marker assisted selection. After this initial validation, the markers can be used for selection of the resistant genes in subsequent generations. Valuable information was gained on which SCAR markers were closely linked with rust resistance genes and suitable for use as foreground markers in the marker-assisted introgression process. The improvement of integrated genetics using multiple parent crosses combined with the use of laboratory (marker assisted selection) and greenhouse (direct disease screening) is suggested for the development of high yielding and well-adapted dry bean cultivars.

CHAPTER 6 – CONCLUSION

Field resistance to rust largely exists amongst the selected international germplasm varieties and to a lesser extent amongst the South African commercial dry bean cultivars. The germplasm varieties, containing known rust resistance genes of interest (*Ur-3, Ur-5* and *Ur-11*) for the South African dry bean industry were resistant across different seasons and localities. There was a clear distinction between the donor (resistant) and the receptive (susceptible) parents as far as the phenotypic reaction to the rust disease are concerned. Greenhouse screenings of the parental materials showed a similar trend of reaction to rust as that observed from the field studies.

The findings of this study demonstrated great prospects to adequately improve rust resistance and the general genetic base of both the large seeded and the small seeded dry bean cultivars in the breeding program of the ARC-GC. It further demonstrated the phenotypic effectiveness of the *Ur-3, Ur-4, Ur-5* and *Ur-11* rust resistance gene combination against important rust races (1, 3, 5 and 11) of common bean in South Africa. The SK14, SI19 and SAE19 SCAR markers were validated to be used successfully to select the rust resistance genes (*Ur-3* (Nep-2), *Ur-5* (Mexico 309) and *Ur-11* (BelDakMi-RMR-23)), respectively. However, the SK14 marker did not amplify the *Ur-3* gene on the BelDakMi-RMR-23 genotype and could not be used in the F_2 population where this genotype was used as a donor parent. The $SA_{1079/800}$ marker explained only 4% of the variation in the *Ur-4* gene and can thus also not be used for marker selection in this population. Rust resistance in BelDakMi-RMR-23, Nep-2, and Mexico 309 is reported to be governed by single dominant genes, however this could not be observed in this study. It is clear from the findings of this study that the accuracy of the SCAR molecular markers used in this study is variable from marker to marker. It is therefore advisable to only consider SCAR molecular makers that have demonstrated repeatability of the desired results in a MAS breeding program in order to carry out reliable selections of the targeted rust resistance genes.

It is therefore recommended that more diverse rust resistance genes should be introduced, using a genetic pyramiding approach, into the South African commercial dry bean cultivars of the dominant market classes. Further pyramiding of rust resistance genes under study and marker assisted selections will continue in order to achieve homozygosity among progenies. This will directly benefit farmers and all other stakeholders involved in the production and consumption chain. The new South African breeding lines obtained thus far indicated that

breeding for rust resistance is important to local cultivars since there is a considerably clear improvement in resistance, without loss of adaptability and yield potential amongst cultivars. It also appears that full resistance of target genes has been retained, both for intra- and inter-gene pool transfer of resistance.

Stability, effectiveness and usefulness of pyramided resistance remain to be determined across the greenhouse and different field environments against pathogen races of varying aggressiveness and their deployment in cultivar development. Efforts need to continuously be made in order to stack rust resistance genes that are specifically important for South Africa so that the disease is more effectively controlled.

REFERENCES

Adam-Blondon, A.F., Sevignac, M., Bannerot, H. & Dron, M. 1994. SCAR, RAPD, and RFLP markers linked to dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theoretical and Applied Genetics 88:865–870.

Adjebeng-Danquah, J., Manu-Aduening, J., Gracen, V.E., Asante, I.K. & Offei, S.K. 2017. AMMI stability analysis and estimation of genetic parameters for growth and yield components in cassava in the forest and Guinea Savannah ecologies of Ghana. International Journal of Agronomy. 8075846. https://doi.org/10.1155/2017/8075846

Allen, D.J. 1982. *Verticillium lecanii* on the bean rust fungus *Uromyces appendiculatus*. Transactions of the British Mycological Society 79:362-364.

Allen, D.J. 1995. An annotated list of diseases, pathogens and associated fungi of the common bean (*Phaseolus vulgaris*) in eastern and southern Africa. Psychopathological Papers No 34, CAB International, Wallingford, UK, 23 pp.

Alvarez-Ayala, G. & Schwartz, H.F. 1979. Preliminary investigations of pathogenic variability expressed by *Isariopsis griseola*. Annual Report of the Bean Improvement Cooperative 22:86-88.

Alzate-Marin, A.L., Cervigni, G.D.L., Moreira, M.A. & Barros, E.G. 2005. Selection assisted by molecular markers aiming at the development of disease resistant plants, with emphasis on common bean and soybean. Brazilian phytopathology 30:333-342.

Alzate-Marin, A.L., De Souza, T.L.P.O., Sartorato, A., Moreira, M.A., de Barros E.G. 2004. Analyses of the pathogenic variability of *Uromyces appendiculatus* in some regions of Brazil, Annual Report of the Bean Improvement Cooperative, 47:251-252.

Andersen, J.R. & Lubberstedt, T. 2003. Functional markers in plants. Trends Plant Science 8:554–560.

Anderson, J.W., Smith, B.M. & Washnock, C.S. 1999. Cardiovascular and renal benefits of dry bean and soya bean intake. The American Journal of Clinical Nutrition 70: 464S-474S.

Araya, C.M., Alleyne, A.T., Steadman, J.R., Eskridge, K.M. & Coyne, D.P. 2004. Phenotypic and genotypic characterization of *Uromyces appendiculatus* from *Phaseolus vulgaris* in the Americas. Plant Disease 88:830-836.

Arumuganathan, K. & Earle, E. 1991. Nuclear DNA content of some important plant species, Plant Molecular Biology Reporter 9:208-218.

Asfaw, A., Blair, M.W. & Almekinders, C. 2009. Genetic diversity and population Structure of common bean (*Phaseolus vulgaris* L.) landraces from the East African highlands. Theoretical and Applied Genetics 120:1-12.

Aust, H.J., Filho, A.B. & Menton, J.O.M. 1984. Resistance of three bean cultivars to *Uromyces phaseoli* expressed through sporulation of the fungus. Phytopathology 110: 30-36.

Awori, E., Kiryowa, M., Souza, T.L.P.O., Vieira, A.F., Nkalubo, S.T., Kassim, S.& Tusiime, G. 2017. Resistance Sources to Bean Anthracnose Disease in Uganda and Brazil. Journal of Agricultural Science and Food Research 9(2): 225.

Baker, C.J., Stavely, J.R., Thomas, C.A., Sasser, M. & MacFall, J.S. 1983. Inhibitor effect of *Bacillus subtilis* on *Uromyces phaseoli* and development of rust pustules on bean leaves. Phytopathology 73:1148-1152.

Bassi ,D., Briñez, B., Rosa, J.S., Oblessuc, P.R., de Almeida, C.P, Nucci, S.M., Domingos da Silva, L.C., Chiorato, A.F., Vianello, R.P., Camargo, L .E.A., Blair, M.W. & Benchimol-Reis, L.L. 2017. Linkage and mapping of quantitative trait loci associated with angular leaf spot and powdery mildew resistance in common beans. Genetics and Molecular Biology. 40(1)

Beebe, S.E. & Pastor-Corrales, M. 1991. Breeding for disease resistance. In: A. van Schoonhoven & O. Voysest (Eds.), Common Beans: Research for Crop Improvement, p. 561–617. CAB International, Wallingford, UK/CIAT, Cali, Colombia.

Beebe, E.S.; Rao, I.M. Blair, M.W. & Acosta-Gallegos, J.A. 2013. Phenotyping common beans for Adaptation to drought. Frontiers in Physiology 4:1–20.

Beebe, S.E., Rao, I.M., Devi, M.J. & Polania, J. 2014. “Common Beans, Biodiversity, and Multiple Stresses: Challenges of Drought Resistance in Tropical Soils”, Crop and Pasture Science, 65:667–75.

Beebe, S., Rengifo, J., Gaitan, E., Duque, M.C. & Tohme, J. 2001. Diversity and Origin of Andean Landraces of Common Bean. Crop Science 41:854-862.

Beebe, S., Skroch, P.W., Thome, J., Duque, M.C., Pedroza, F. & Nienhuis, J. 2000. Structure of genetic Diversity among common bean landraces of Middle American origin based on respondence analysis of RAPD. Crop Science 40:264-273.

Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Science 48:1649–1664,

Bitocchi, E., Bellucchi, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E. 2013. Molecular analysis of the parallel domestication of the common bean (Phaseolus vulgaris) in Mesoamerican and the Andes. New Phytologist Journals 197:300-313.

Bitocchi, E. & Nanni, L. 2012. Mesoamerican origin of the common bean (*Phaseouls vulgaris* L.) is revealed by sequence data. Proceedings of the National Academy of Sciences 109:788-796.

Blair, M.W., Cortes, A.J., Farmer, A.D., Haung, W., Ambachew, D., Penmetsa, R.V., Carrasquilla-Garcia, N., Assefa, T. & Cannon, S.B. 2018. Uneven recombination rate and linkage disequilibrium across a reference SNP map for common bean (*Phaseolus vulgaris* L.) PLoS ONE 13 (3): E 0189597.http://doi.org/10.1371/journal.pone.0189597

Blair, M.W., Díaz, L.M., Buendía, H.F. & Duque, M.C. 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., Díaz, J.M., Hidalgo, R., Díaz, L.M. & Duque, M.C. 2007. Microsatellite characterization of Andean races of common bean (*Phaseolus vulgaris* L). Theoretical and Applied Genetics 116:29-43.

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

Blair, M.W., Soler, A. & Cortes, A. J. 2012. Diversification and population structure in common beans (*Phaseolus vulgaris* L.) PLoS ONE 7 (11): e 49488. Doi: 10.1371/journal. Pone 0049488.

Boone, W.E., Stavely, J.R., & Weeden N.F. 1999. Development of a sequence tagged site (STS) marker for *Ur-11*, a gene conferring resistance to the bean rust fungus, *Uromyces appendiculatus*. Annual Report of the Bean Improvement Cooperative 42:33-34.

Borrelli, G.M., Mazzucotelli , E., Marone , D., Crosatti , C., Michelotti , V., Valè G., Anna , M. & Mastrangelo , A.M. 2018. Regulation and Evolution of NLR Genes: A Close Interconnection for Plant Immunity International Journal of Molecular Sciences 19:1662.

Botstein, D., White, R.L., Skolnick, M. & Davis, R.W. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. The American Journal of Human Genetics 32:314-331.

Brick, M.A. & K.F. Grafton. 1999. Improvement of medium-seeded race Durango cultivars. In Singh S.P. (Ed.). Common bean improvement in the twenty-first century. Kluwer Academic Publication.The Netherlands.

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

Carrijo, I.V., Chaves, G.M. & Pereira, A.A.1980. Reaction of twenty-five variants of *Phaseolus vulgaris* at the beginning and new physiological races if *Uromyces Phaseoli* var.typica Arth. Under conditions of vegetation. [Behaviour of 25 varieties of *Phaseolus vulgaris* L., 39 races of *Uromyces Phaseoli* var. Ty. Arth. Under greenhouse conditions]. Fitopatologis Brasileria 5:245-255.

Chacon, M.I., Pickersgill, B., Debouck, D.G., & Arias, J.S. 2007. Phylogeographic analysis of the chloroplast DNA variation in wild common bean (*Phaseolus vulgaris* L. in the Americas. Plant Systematics and Evolution 266:175-195.

Choudhary. N. Bawa, V., Paliwal, R., Singh B., Ashraf Bhat, M., Mir, J.I., Gupta M., Sof, P.A., Thudi, M., Varshney, R.K. & Mir, R.R. 2018. Gene/QTL discovery for Anthracnose in common bean (*Phaseolus vulgaris* L.) from North-western Himalayas. PLos ONE 13(2):e0191700.

Collard, B.C.Y., Jahufer. M.Z.Z., Brouwer, J.B. & Pang, E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142:169-196.

Collard, B.C.Y. & Mackill, D.J. 2008. Marker assisted selection: an approach for precision plant breeding in the twenty- first century. Philosophical Transactions of the Royal Society of Britain 363:557-572.

Correa, R.X., Costa, M.R., Good-God, P.I., Ragagnin, V.A., Faleriro, F.G., Moreira M.A., & De Barros, E.G., 2000. Sequence characterized amplified regions linked to rust resistance genes in the common bean. Crop Science 40:804-807.

Correa-Victoria, F.J. 1987. Pathogenic variation, production of toxic metabolites and isoenzyme analysis in *Phaseoisariospsis griseola* (Sacc) Ferr. (PhD, Thesis). Michigan State University.

Coyne, D.P. 1965. A genetic study of crippled morphology resembling virus symptoms in *Phaseolus vulgaris* L. Journal of Heredity 56:162-163.

Coyne, D.P. & Schuster, M.L. 1975. Genetic and breeding strategy for resistance to rust (*Uromyces phaseoli* (Reben) Wint.) in bean (*Phaseolus vulgaris* L.). Euphytica 24:795-803.

De Jesus Jr, W.C., do Vale, F.X.R., Coelho, R.R., Hau, B., Zambolim, L., Costa, L.C. & Bergamin A. 2001. Effects of Angular leaf spot and rust on yield loss of *Phaseolus vulgaris*. Phytopathology 91:1045-1053.

Department of Agriculture, Forestry and Fisheries, (DAFF). 2015. A profile of the South African dry bean market value chain. Food pricing monitoring committee report online www.daff.gov.za access date 20/10/2018.

Department of Agriculture, Forestry and Fisheries (DAFF) 2017. A profile of dry bean Trends in the Agricultural Sector. Economic statistical analysis information committee report online www.daff.gov.za access date 20/10/2018.

De Souza, T.L.P.O., Alzate-Marin, A.L., Dessaune, S.N., Nunes, E.S., De Quiroz, V.T.,Moreira, M.A. & De Barros, E.G. 2007b. Inheritance study and validation of SCAR molecular marker for rust resistance in common bean. Crop Breeding and Applied Biotechnology 7:11-15.

De Souza, T.L.P.O., Alzate-Marin, A.L., Faleiro, F.G. & Barros., E.G. 2008. Pathosystem common bean- *Uromyces appendiculatus*: Host resistance, pathogen specialization, and breeding for rust resistance. Pest Technology 2:56-69.

De Souza, T.L.P.O., Alzate-Marin, A.L., Moreira, M.A. & De Barros, E.G. 2002. Use of BelMiDak-RR-3 as a source for rust resistance in Central Brazil. Annual Report of the Bean Improvement Cooperative 45:140-141.

De Souza, T.L.P.O, Alzate-Marin, A.L., Moreira, M.A. & De Barros, E.G. 2005a. Analyse of the pathogenic variability of *Uromyces appendiculatus* in some region. Brazilian Phytopathology Brasilia 30:143-149.

De Souza, T. L. P. O., De Barros, E.G, Bellato , C.M. ; Hwang, E.Y., Cregan, P.B. & Pastor-Corrales, M.A. 2009. Single nucleotide polymorphisms (SNPs) in common bean. Annual Report of the Bean Improvement Cooperative. 52:6-7.

De Souza, T.L.P.O., Dessaune, S.N., Sangalard, D.A., Moreira, M.A. & De Barros, E.G. 2007c .Rust resistance gene present in common bean cultivar Ouro Negro (Ur-ON) does not respond to Ur-3. Annual Report of the Bean Improvement Cooperative 50:119-120.

De Souza, T.L.P.O., Dessaune, S.N., Sanglard, D.A., Moreira, M.A. & De Barros, E.G. 2011. Characterization of the rust resistance gene present in the common bean cultivar Ouro Negro, the main rust resistance source in Brazil. Plant Pathology 60:839-845.

De Souza, T.L.P.O., Faleiro, F.G., Dessaune, S.N.,de Paula-Junior, T.J., Maurilio A. Moreira, M.A. & de Barros, E.G. 2013. Breeding for common bean (Phaseolus vulgaris L.) rust resistance in Brazil. Tropical Plant Pathology 38:361-374.

De Souza, T.L.P.O, Ragagnin, V.A., Alzate-Marin, A.L., Faleiro, F.G, Moreira, M.A. & Barros, E.G. 2003. Backcross assisted by RAPD markers to develop common bean lines with carioca type grains containing the *Ur-11* rust resistance gene. Annual Report of the Bean Improvement Cooperative 46:195-196.

De Souza, T.L.P.O., Ragagnin, V.A., Dessaune, S.N., Sanglard, D.A., Carneiro, J.E.S., Moreira, M.A. & Barros, E.G. 2014. DNA marker-assisted selection to pyramid rust resistance genes in "carioca" seeded common bean lines. Euphytica 199:303-316.

De Souza, T.L.P.O., Ragagnin, V.A., Sanglard, A.D., Moreira, M.A. & De Barros., E.G. 2007a. Identification of races of selected isolates of Uromyces appendiculatus from Minas Gerais (Brazil) based on the new International Classification System. Fitopatologis Brasília 32:104-109.

De Souza, T.L.P.O., Sanglard, D., Ragagnin, V.A., Alzate-Marin, A.L., Moreira, M.A., & Barros, E.G. 2005b. Development of carioca-type common bean lines resistant to rust with the aid of molecular markers. Annual Report of the Bean Improvement Cooperative 48:138-139

Doveri,S., Maheswaran, M. & Powell, W. 2008. Molecular markers-history, features and application .In: Kole. C., Abbott, A.G (eds) Principles of Plant Genomics. Vol 1: Genome Mapping. Science Publishers, Enfield, Jersey, Plymouth.

Eathington, S.R., Crosbie, T.M., Edwards M.D., Reiter, R.S. & Bull, J.K. 2007. Molecular Markers in a commercial breeding program. Crop Science Society of America 47(S3):S154–S163

Faleiro, F.G. Ragagnin, V.A., Correa, R.X., Vinhadelli, W.S., Moreira M. & De Barros, E.G., 2000. Genetic connection of rust resistance and anthracnose in variety of black Oura bean. Revista Ceres 47:375-382

Faleiro, F.G, Vinhadelli, W.S. & Ragagnin, V.A. 1999. Identification of physiological races of *Uromyces appendiculatus*in the state of Minas Gerais, Brazil. Brazilian Phytopathology 24:166-169.

Faleiro, F, G., Vinhadelli, W.S., Ragagnin, V.A., Correa, R.X., Moreira, M.A. & De Barros, E.G. 2000. RAPD markers liked to a block of genes conferring rust resistance to the common bean. Genetics and Molecular Biology 23:399-402.

Food and Agriculture Organization of the United Nations (FAO) 2018. FAOSTAT DATABASE [http:appsl.fao.serlet] site visited on 2018/10/02.

Food and Agriculture Organization of the United Nations (FAO), 2018. Global and Regional Trends in Production Trade and Consumption of Food Legume Crops. [https://www.researchgate.net/publication/266353209].

Finke, M.L., Coyne, D.P. & Steadman, J.R. 1986. The inheritance and association of resistance to rust, common bacterial blight, plant habit and folia abnormalities in *Phaseouls vulgaris L*. Euphytica 35:969-982.

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

Fourie, D. 2015. Dry bean cultivar recommendations 2015/2016. Agricultural Research Council – Grain Crops Institute, Potchefstroom, South Africa. 5-6.

Fourie, D., Herselman, L., & Mienie C.M.S. 2011. Improvement of common bacterial blight resistance in South African dry bean cultivar Teebus. African Crop Science Journal 19:377-386

Freyre, R., Skroch, P.W., Greffroy, V., Adam-Blondon, A.F., Shirmohamadali, A., Johnson, W.C., Llaca, V., Nodari, R.O., Pereira P.A., Tsai, S.M., Tohme,J., Dron, M., Nienhuis, J., Vallejos, C.E., & Gepts, P. 1998.Towards an integrated linkage map of common bean.4. Development of a core linkage map and alignment of RFLP maps. Theoretical and Applied Genetics 97:847-856.

Garzon, L. N. & Blair, M. W. 2014. Development and mapping of SSR markers linked to resistance-gene homologue clusters in common bean. The Crop Journal, 2 (4), 183-194.Gentry, H.S. 1969. Origin of the common bean, *Phaseolus vulgaris*. Economic Botany 23: 55-69.

Gentry, H.S. 1969. Origin of the common bean*, Phaseolus vulgaris*. Economic Botany 23:55-69.

Gepts, P. 1993. The use of molecular and biochemical markers in crop evolution studies. Evolutionary Biology 27:51-94.

Gepts, P. & Bliss, F. 1985. F_1 hybrid weakness in the common bean Differential geographic origin suggest two gene pools in cultivated bean germplasm. Journal of Heredity 76: 447-450.

Gepts, P. & Debouck, D. 1991. Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris L*.). In: A.V. Schoonhoven and V.O. (ed). Common beans: Research for crop improvement. CIAT. p7-53.

Gioia, T. Loggozzo, G, Attene, G. Bellucci, E. Benedettelli,S.,& Negri, V. 2013. Evidence for introduction Bottle neck and Extensive Inter gene pool (Mesoamerican x Andes) Hybridization in the European common bean (Phaseolus vulgaris L.) PLoS One. 8:1-14.

Goncalves-Vidigal, M.C., Meirelles, A.C., Poletine, J.P., De Souza, L.L., Cruz A.S. Nunes. M.P., Lazanallo. G.F. & Vidigal, P.S. 2011. Genetics analysis of Anthracnose resistance in Pitanga dry bean cultivar. Heredity 75:234-242.

Grabski, G. C. & Mendgen, K. 1986. The parasitization of the bean crumb *Uromyces appendiculatus* var. appendiculam by the hyper parasite *verncilhuni lecanii*: Studies on the detection, penetration and degradation of the rust fungus pores. Phytopathological Journal 115:116-123.

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

Groth, J.V., McCain, J.W. & Roelfs, A.P. 1995 .Virulence and isozyme diversity of sexual versus asexual collections of *Uromyces appendiculatus* (bean rust fungus) Heredity volume 75:234–242.

Groth, J.V. & Roelfs, A.P. 1982. Genetic diversity for virulence in bean rust collections. Phytopathology 72:982-983.

Haley, S.D., Miklal, P.N., Stavely, J.R., Byrum, J., & Kelly, J.D. 1993. Identification of RAPD markers linked to a major rust resistance gene blocked in common bean. Theoretical and Applied Genetics 85:505-512.

Hanai, L.R, Campos, T., Camargo, L.E.A., Benchimol, L.L., De Souza, T.L.P.O., Melotto, M., Carbonell, S.A.M., Chioratto, A.F., Consoli, L. & Formighieri, E.F. 2007. Development characterization and comparative analysis of polymorphism at common bean SSR loci isolated from genic and genomic sources. Genome 50:266–277.

Harter, L.L., Andrus, C.F., & Zaumeyer, W.J. 1935. Studies on bean rust caused by *Uromyces phaseoli* typical. Journal for Agricultural Research 50:737-759.

Hnatowich, G. 2000. Pulse Production Manual. Saskatchewan Pulse Growers. Saskatoon, Saskatchewan, Canada.

Holden, J. & Haytowitz, D. 1998. The nutrient value of beans. Annual Report of the Bean Improvement Cooperative 41:41-42

Huang, H. C., Hutcheson, S. W. & Collmer, A. 1991. Characterization of the *hrp* cluster from *Pseudomonas syringae pv. syringae* 61 and TnphoA tagging of genes encoding exported or membrane-spanning Hrp proteins. Molecular Plant Microbe Interactions 4:469-476.

Hutchins, A.M., Winham D.M.& Thompson S.V. 2012.Phaseolus beans: impact on glycaemic response and chronic disease risk in human subjects. British Journal of Nutrition108(S1):S52-S65.

Hurtado-Gonzales, O.P, Valentini, G., Gilio T.A.S., Martins, A.M., Song, Q. & Pastor-Corrales, M.A. 2017. Fine Mapping of *Ur-3*, a Historically Important Rust Resistance Locus in Common Bean. Annual Report of the Bean Improvement Cooperative 41:41-42.

Jochua, C., Amane, M.I.A., Steadman, J.R., Xue, X. & Eskridge, K.M. 2008. Virulence diversity of the common bean rust pathogen within and among individual bean fields and development of sampling strategies. Plant Disease 92:401-402.

Jochua, C., Steadman, J.R., Amane, M., & Fenton, J. 2004. Pathotype variation and sources of resistance to the common bean rust pathogen in Southern Mozambique. Annual Report of the Bean Improvement Cooperative 47:113-114.

Johnson, E., Miklas, P.N., Stavely, J.R. & Marinez-Cruzado, J.C. 1995. Coupling and repulsion-phase RAPDs for marker assisted selection of PI 181996 rust resistance in common bean. Theoretical and Applied Genetics 90:659-664.

Jung, G., D.P. Coyne, J.M. Bokosi, J.R. Steadman & Nienhuis, J. 1998. Mapping genes for specific and adult plant resistance to rust and abaxial leaf pubescence and their genetic relationship using Random Amplified Polymorphic DNA (RAPD) markers in common bean. Journal of the American Society for Horticultural Science 123: 859–863.

Kalavacharla, V., Stavely, J., Myers, J. & McClean, P. 2000. *Crg*, a gene required for *Ur-3* mediated rust resistance in common bean, maps to a resistance gene analog cluster. Molecular Plant-Microbe Interactions: 1237-1242.

Katungi, E., Farrow, A., Chianu, J., Sperling, L. & Beebe, S. 2009. Common bean in Eastern and Southern Africa: a situation and outlook analysis.

Katuuramu, D.N., Hart, J.P., Porch, T.G., Grusak, M.A., Glahn, R.P. & Cichy, K.A. 2018. Genome-wide association analysis of nutritional composition-related traits and iron bioavailability in cooked dry beans (*Phaseolus vulgaris* L.). Molecular Breeding 38:44

Kelly, J.D. 1995. Use of random amplified polymorphic DNA markers in breeding for major gene resistance to plant pathogens. Horticultural Science 30:461-465.

Kelly, J.D., Gepts, P., Miklas, P.N. & Coyne, D.P. 2003. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. Field Crops Research 82:135–154

Kelly, J.D. & Miklas P.N. 1998. The role of RAPD markers in breeding for disease resistance in common bean. Molecular Breeding 4:1-4.

Kelly, J.D. & Miklas, P.N. 1999. Marker-assisted selection. In: Singh, S.P. (Ed.). Common Bean Improvement in the Twenty-First Century. Kluwer Academic Publishers, London, UK. pp. 93-123.

Kelly, J.D., J.R. Stavely, and P.N. Miklas. 1996. Proposed symbols for rust resistance genes. Annual. Report. Bean Improvement. Cooperative 39:25–31.

Kimani, P., Buruchara, R., Ampofo, K., Pyndji, M. Chirwa, R & Kirkby, R. 2001. Breeding beans for smallholder farmers in Eastern, Central, and Southern Africa: Constraints, achievements, and potential. In: R. Kirkby, editor PABRA Millennium Workshop. International Centre for Tropical Agriculture (CIAT), PO Box 6247, Kampala, Uganda, Novotel Mount Meru, Arusha, Tanzania. P.13-15

Koening, R. & Gepts, P. 1989. Allozyme diversity in wild Phaseolus vulgaris: further evidence for two mayor centres of genetic diversity. Theoretical and Applied Genetics 78:809-817.

Koinange, E.M.K, & Gepts, P. 1992. Hybrid weakness in wild *Phaselous vulgaris* Journal of Heredity 83:135-139.

Kordrostami, M. & Rahimi, M. 2015. Molecular markers in plants: Concepts and applications. Genetics in the Third Millennium. VL - 13: 4024- 4031.

Kwak, M. & Gepts, P. 2009. Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* .L. Fabaceace).Theoretical and Applied Genetics 118: 979-992.

Langridge, P. Lgudah, E., Holton, T., Appels, R. Sharp, P. & Chalmers, K. 2001. Trends in genetic and genome analysis in wheat: A review. Australian Journal of Agricultural Research 52:1043-1077.

Liebenberg, M.M. 2003. Breeding for resistance to rust of dry bean (*Phaseolus vulgaris*) in South Africa. University of Free State, South Africa. (Thesis-PhD).

Liebenberg, A.J., Heenop, H.W & Fourie, M. 2002. Report of the national Dry Bean Cultivar Trial. 2001/2002 ARC-Grain Crop Institute, Private Bag x1251, Potchefstroom, South Africa.

Liebenberg, A.J., Heenop, H.W. & Fourie, M. 2003. Report of the National Dry Bean Cultivar Trial. 2002/2003. ARC – Grain Crops Institute, P/Bag X1251, Potchefstroom, South Africa.

Liebenberg, M.M. & Liebenberg, A.J. 1998. Rust susceptibility of important South African commercial dry bean cultivars, and possible sources of resistance. Second Plant Breeding Symposium of the South African Plant Breeders Association, Golden Gate, South Africa, 17-19 March.

Liebenberg, M.M. & Liebenberg, A.J. 2000a. Breeding Rust Resistant Dry Beans for South Africa: A Tribute to Dr. J.R. Stavely. Annual Report of the Bean Improvement. Cooperative.43:80-81.

Liebenberg, M.M. & Liebenberg, A.J. 2000b. Characterization of bean rust (*Uromyces appendiculatus*) isolates from Southern Africa. Annual Report of the Bean Improvement. Cooperative. 43:78-79.

Liebenberg, M.M., Liebenberg, A.J. & Pretorius, Z.A. 2005. Breeding for rust resistance increases dry bean productivity in South Africa. South African Journal of Plant and Soil 222:129-139.

Liebenberg, M.M, Mienie, C.M.S. & Pretorius, Z.A. 2004. Some applications of co-dominant SCAR marker $SA14_{1079/800}$ for *Ur-4*. Annual Report of the Bean Improvement Cooperative. 47:259-260.

Liebenberg, M.M., Mienie, C.M.S. & Pretorius, Z.A. 2006. The occurrence of rust resistance gene *Ur-13* in common bean cultivars and lines. Euphytica 150:365–386.

Liebenberg, M.M. & Pretorius, Z.A. 2004a. Inheritance to *Uromyces appendiculatus* in the South African dry bean cultivar Kranskop. South African Journal of Plant and Soil,21:245-250.

Liebenberg, M.M. & Pretorius, Z.A. 2004b. Proposal for designation of a rust resistance gene in large seeded cultivar Kranskop. Annual Report of the Bean Improvement Cooperative 47: 255-256.

Liebenberg, M. M. & Pretorius, Z.A. 2010. Common bean rust: pathology and control. Horticultural reviews. American Society for Horticultural Science 37:1–99.

Liebenberg, M. & Pretorius, Z.A. 2011. Pathogenic diversity in and sources of resistance to *Uromyces appendiculatus* in Southern Africa. Journal of Phytopathology 159: 287-297.

Lindgren, D.T., Eskridge, K.M., Steadman, J.R. & Schaaf, D.M. 1995. A model for dry bean yield loss due to rust. Horticultural Technology. 5:35-37.

Luann-Finke, M., Coyne, D.P. & Steadman J.R. 1986. The inheritance of resistance to rust, common bacterial blight, plant habit and foliar abnormalities in *Phaseolus vulgaris* L. Euphytica 35:969-982.

Machado, C.M., Ferruzi, M.G. & Nieslsen, S.S. 2008. Impact of the hard to cook phenomenon on phenolic antioxidant in dry beans (*Phaseolus vulgaris*). Journal for Agricultural and Food Chemistry 56:3102-3110.

Marechal, R., Mascherpa, J.M. & Stainer, F. 1978. Taxonomic study of a group species complex of the genera Phaseolus and Vigna (Papilionaceae) on the basis of morphological data. Boissiera, 28:1-273.

McClean, D.J, Braithwaite, K.S., Irwin, J.A.G., Manners, J.M. & Groth, J.V. 1995. Random Amplified Polymorphic DNA reveals relationships among diverse genotypes in Australian and American collections of *Uromyces appendiculatus*. Phytopathology 85:757-765.

McGrath M.T 2014. When is the Best time to Apply fungicides for foliar Diseases.Department of Plant pathology and Plant Microbe Biology, Cornell University LongIsaland Horticultural Research and Extension Center

McMillan, M.S. & Schwartz, H.F. 1994. Progress report: Overwintering of bean rust. Annual Report of the Bean Improvement Cooperative 37:27-28.

McMillan, M.S., Schwartz, H.F. & Otto, K.L. 2003. Sexual stage development of *Uromyces appendiculatu*s and its potential use for disease resistance screening of *Phaseolus vulgaris*. Plant Disease Journal 87:1133-1138.

Melloto, M. & Kelly, J.D. 1998. SCAR markers linked to major disease resistance genes in common bean. Annual Report of the Bean Improvement Cooperative 41:64-65.

Melmaiee, K., Todd, A., McClean, P., Lee, R., Schlueter, J., Jackson, S., & Kalavacharla, V. 2013. Identification of molecular markers associated with the deleted region in common bean (*Phaseolus vulgaris*) *Ur-3* mutants. Australian Journal of Crop Science 7:354-360.

Mendes B. M. J., & Filho, B. A. 2008. Influence of Temperature, Wetness Duration, and Leaf Type on the Quantification of Monocyclic Parameters of Bean Rust. Journal of Phytopathology. 126: 183 - 189.

Mendgen, K. 1978. The course of infiltration of *Uromyces* phaseoli in susceptible and resistant bean varieties. Phytopathology. Z. 93:295-313.

Mensack, M.M., Fitzgerald, V.K., Ryan E.P., Lewis, M.R., Thompson, H.J. & Brick, M.A. 2010. Evaluation of diversity among common beans (*Phaseolus vulgaris* L.) from two centres of domestication using 'omics' technologies. Genomics. 11:686.

Mienie, C.M.S., Liebenberg, M.M., Pretorius, Z.A., & Miklas, P.N. 2005. SCAR markers linked to the common bean rust resistance gene *Ur-13*. Theoretical and Applied Genetics 111:972-979.

Mienie, C.M.S., Naidoo, R. & Liebenberg, M.M. 2004. Conversion of the RAPD marker for *Ur-4* to a co-dominant SCAR marker $SA14_{1079/800}$.Annual Report of the Bean Improvement Cooperative 47:261-262.

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

Miklas, P.N., Pastor-Corrales, M.A., Jung, G., Coyne, D.P., Kelly, J.D., McLean, P.E. & Gepts, P. 2002. Comprehensive linkage MAP of bean rust resistance genes. Annual Report of the Bean Improvement Cooperative, 45:125-129.

Miklas, P.N., & Singh, S.P. 2007. Common bean. In Kole C, editor. Genome mapping and molecular breeding in plants Vol 3. Berlin: Pulses, sugar and tuber crops. Springer Verlag. 1-31.

Miklas, P.N., Stavely, J.R & Kelly J.D. 1993. Identification and potential use of molecular markers for rust resistance in common bean. Theoretical and Applied Genetics 85:745-749.

Miklas, P.N., Stone, V., Daly, M.J., Stavely, J.R., Steadman, J.R., Bassett, M.J., Delorme, R. & Beaver, J.S. 2000. Bacterial, fungal, and viral disease resistance loci mapped in a recombinant inbred common bean population Journal of the American Society for Horticultural Science 125:476–481.

Mmbaga, M.T., Steadman, J.R. 1992. Nonspecific resistance to rust in pubescent and globous common bean genotypes. Phytopathology 82:1283-1287.

Mmbaga, M.T., Steadman, J.R. & Stavely, J.R. 1996. The use of host resistance in disease management of rust in common bean. Integrated Pest Management Reviews 1:191-200.

Monda, E.O., Munene, S. & Ndwgua. A. 2003. French beans production constraints in Kenya. African Crop Science Conference. Proceedings 6:683-687.

Mukankusi, C., Nkalubo, S., Katungi, E., Bruno, A., Luyima, G., Maren, R. & James, K. 2015. Participatory Evaluation of Common Bean for Drought and Disease Resilience Traits in Uganda. 10.13140/RG.2.1.3815.4646.

Mukherjee, A.K., Mohapatra, N.K., Bose, L.K., Jambhulkar, N.N. & Nayak, P. 2013. Additive main effects and multiplicative interaction (AMMI) analysis of GxE interactions in rice-blast pathosystem to identify stable resistant genotypes. Academic Journals 8:5492-5507.

Naidoo, R., Fourie, D., Mienie, C.M.S., Du Plessis, S., & Van Rensburg, L. 2003. Identification and development of molecular markers linked to common bacterial blight resistance genes from Wilk 2. Annual Report of the Bean Improvement Cooperative, 46:221-221.

Nau, R. 2005. Stat graphics version 5: Overview & tutorial guide. Fuqua School of Business Duke University

Nemchinova, Y.P. & Stavely, J.R. 1998. Development of SCAR primers of the Ur-3 rust resistance gene in common bean. Phytopathology 88, S67.

Nietzsche, S. 1997. Identification of roots of *Phaeoisariopsis griseola* determination of resistance sources in *Phaselous vulgaris*. Vicosa. Federal University of Viçosa. (Master's thesis).

Ochoa, J., Cruz, E., Murillo, A. & Danial, D.L. 2007. Variation in virulence and resistance in the bean-bean rust pathosystem in Ecuador. Euphytica 153:313-319.

Ogbannaya, F.C., Subrahmayam, N.C., Moullet, O, De Majnik, J, Eagles, H.A., Brown J.S., Eastwood, R.F., Kollmorgen, J., Appels, R. & Lagudah, E.S. 2000. Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. Australian Journal of Agricultural Research 52:1367-1374.

Pachico, D. 1993. The demand for bean technology. Trends in CIAT commodities 1993. Working Document number 128. Pp 60-74. Henry, G, ed. Centro International de Agricultural Tropical (CIAT), Cali, Colombia.

Panigrahi, J. 2011. Molecular mapping and map based cloning of genes in plants. In Thangadurai D. (Ed) Plant Biotechnology and Transgenic. Bentham Science Publisher, USA

Park, S. O., Coyne, D. P., Steadman, J. R. & Brick, M. A. 2004. Survey of RAPD and SCAR markers linked to the *Ur-6* gene in pinto beans. Annual Report of the Bean Improvement Cooperative, 47:267-268

Park, S. O., Coyne, D. P., Steadman, J. R., Crosby. K. M. & Brick, M. A. 2004. Rust Resistance in Common Bean. Crop Science, 44:1799-1807.

Pastor-Corrales, M. A. 2001. The reaction of 19 bean rust differential cultivars to 94 races of *Uromyces appendiculatus* and the implication for the development of rust resistance cultivars. Annual Report of the Bean Improvement Cooperative, 44:103-104.

Pastor-Corrales, M.A. 2003. Sources, genes for resistance and pedigrees of 52 rust and mosiac resistant dry bean germplasm lines released by the USDA bean project in collaboration with the Michigan, Nebraska and North Dakota Agricultural Experimental Stations, Annual Report of Bean Improvement.Coorperative.,46: 252-257.

Pastor-Corrales, M.A. 2004. Sources, genes for resistance and pedigrees of 52 rust and mosaic resistant dry bean germplasm lines released by the USDA Beltsville bean project in collaboration with the Michigan, Nebraska and North Dakota Agricultural Research Stations. USDA-ARS, Plant Sciences Institute, Vegetable laboratory, Beltsville, MD 20705-2350. 235-241.

Pastor-Corrales, M.A. 2005. Inheritance of resistance in PI 260418 an Andean bean resistant to most races of the bean rust pathogen. Annual Report of the Bean Improvement Cooperative 48:134-135.

Pastor-Corrales, M.A. 2006. Diversity of the rust pathogen and common bean guides gene deployment for development of bean cultivars with durable rust resistance. Annual Report of the Bean Improvement Cooperative 49:51-52.

Pastor-Corrales, M. A., Kelly, J. D., Steadman, J. R., Lindgren, D. T., Stavely, J. R. 2007. Registration of six great northern bean germplasm lines with enhanced resistance to rust and bean common mosaic and necrosis poty viruses. Journal of Plant Registrations 1: 77–79.

Pastor-Corrales, M.A., Pereira, A.P.A., Lewers, K., Brondani,R.V, Buso,G.C., Fereira, M.A. & Martins, W. S. 2008. Identification of SSR markers linked to rust resistance in Andean common bean PI260418. Annual Report of the Bean Improvement Cooperative 51:46-47.

Pastor-Corrales, M.A, & Stavely, J.R. 2002. Using races of the common bean rust pathogen to detect resistance genes in Phaseolus vulgaris. Annual Report of the Bean Improvement Cooperative 45:78-79.

Petry. N., Boy, E., James P. Wirth J.P. & Hurrell, R.F. 2015. Review: The Potential of the Common Bean (*Phaseolus vulgaris*) as a Vehicle for Iron Bio fortification. Nutrition Journal 7:1144-1173.

Petry, N., Egli, I., Gahutu JB, Tugirimana, P.L., Boy, E.,& Hurrell, R. 2012. Stable iron isotope studies in Rwandese women indicate that the common bean has limited potential as a vehicle for iron bio fortification. Nutrition Journal 142:492-497.

Queiroz, V.T., Sousa, C.S, De Souza, T.L.P.O., Costa, M.R., Sanglard, D.A., Ragagnin, V.A., De Barros, E.G. & Moreira, M.A. 2004. SCAR marker linked to the common bean rust resistance gene *Ur-11*. Annual Report of the Bean Improvement Cooperative, 47:271-272.

Ragimekula, N., Naidu, N. Varadarajula, S., Mallapuram, S. P. Gangimeni, G., Reddy, R. K. & Kondreddy, H.R. 2012. Marker assisted selection in disease resistance breeding. Journal. Plant Breeding Genetics 1:90-109.

Rasmussen, J.B., Graton, K.F., Gross, P.L. & Donohue, C.M. 2002. Genetics of resistance in Compuesto Negro Chilmaltenango (CNC). Annual Report of the Bean Improvement Cooperative 45:94-95.

Rodriguez, B.B., Blair, M.W., Kilian, A., Carbonell, S.A.M., Chiorato, A.F. & Rubiano, L.B. 2011. A whole genome DArT assay to assess germplasm collection diversity in common beans. Molecular Breeding 30:181-193.

Rondon MA, Lehmann J, Ramirez J. & Hurtado M. 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biology and Fertility of Soils. 43: 699-708.

Rossi, M., Bitocchi, E., Bellucci, E., Nanni, L. & Rau, D. 2009. Linkage disequilibruim and population structure in wild and domesticated population of *Phaseolus vulgaris* L. Evolutionary Application 2:504-522.

Sambrook, J., Fritsch, E, F. & Maniatis, T. 1989. Molecular cloning: a laboratory manual.2edn. Cold Spring Harbor Laboratory Press, New York.

Sanchez, A.C., Brar, D.S., Huang, N., Li, Z.& Khush, G.S. 2000. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. Crop Science 40:792-797.

Sandlin, C.M., Steadman, J.R., Araya, C.M. & Coyne, D.P. 1999. Isolates of *Uromyces appendiculatus* with specific virulence to landraces of *Phaseolus vulgaris* of Andean origin. Plant Disease 83:108-113.

Sanglard, D.A., Ribeiro, C.A.G., Balbi, B.P., Barros, E.G. & Moreira, M.A. 2009. Reaction of common bean cultivars and elite lines to isolates of *Pseudocercospora griseola.* Annual Report of the Bean Improvement Cooperative 52:64-65.

Santalla, M., Monteagudo, A.B., Gonzalez, A.M. & De Ron, A.M. 2004. Agronomical and quality traits of runner bean germplasm and implications for breeding. Euphytica 135:205–215.

Sartorato, A. & Rava, C.A.1984. Physiological specialization of *Isariopsis griseola* Sacc Me *Phaseolus vulgaris* L. In: Anais, 6 ºCongresso Paulista de Fitopatologia, Botucatu. Sum Phytopathologica 10:58-59.

Sartorato, A., Rava, C.A., Menten, J.O.M. & Bergamin Filho, A. 1991. Resistance vertical of the common beech (*Phaseolus vulgaris*) to *Isariopsis griseola*. Fitopatologis Brasileria 16: 43-46.

Schafer, J.F. & Roelfs, A.P. 1985. Estimated relation between numbers of Urediniospores of *Puccinia graminis* f. sp. *tritici* and rates of occurrence of virulence. Phytopathology 75:749-750.

Schein, R.D. 1961. Some effects of effects temperature during the colonization of bean rust. Phytopathology 51:674-680

Schneider, K.A., Brothers, M.E. & Kelly, J.D. 1997. Marker assisted selection to improve drought resistance in common bean. Journal of Crop Science. 37:51-60

Schwartz, H. F., Gent, D. H., Franc, G. D. & Harvison, R. M. 2005. Bacterial brown spot. Colorado State University Co-operative Extension. http://highplainsipm.org/HpIPMSearch/Docs/BacterialBrownSpot-DryBean.htm [Date of access: 06-12-2007].

Schwartz, H.F., McMillian, M.S. & Lienert K. 1994. Evaluations of Foliar fungicides and their timing for white mold control in dry beans, 1992. Fungi. Nematicide Tests 49:85

Schwartz, H.F. & Pastor-Corrales, M.A, 1989. Bean production problems in the tropics, 2nd edn. Center International de Agriculture Tropical (CIAT), Cali, Colombia.726 p.

Schwartz, H.F., Steadman, J.R. & Harveson, R.M. 2011. Rust of dry bean Crop series. Disease fact sheet No2:936.

Semagn, K., Bjornstad, A. & Ndjiondjop, M.N. 2006. An overview of molecular marker methods for plant. African Journal Biotechnology 25:2540-2569.

Shands, W. A. Jn. & Schein, R. S. 1962. Time-temperature interactions of *Uromyces phaseoli* during spore germination and tube elongation. Phytopathology 52: 27 (Abstract.).

Sharp, P.J, Johnston, S., Brown, G., McIntosh, R.A., Pallotta, M., Carter M., Bariana, H.S., Lagudah, E.S. Singh, S.P, Khairallah, M., Potter, R. & Jones, M.G.K .2001. Validation of molecular markers for wheat breeding. Australian Journal of Agricultural Research 5:1357-1366.

SHOLIHIN. 2011. AMMI model for interpreting clone-environment interaction in starch yield in cassava. Hayati Journal of Biosciences 18:21-26.

Singh, S.P. 1999. Integrated genetic improvement. In: S.P Singh, (Ed) Common bean improvement in the twenty-first century. Kluwer Academic Publication. Dordrecht, the Netherlands. Page 133-165.

Singh, S. P. 2001. Broadening the genetic base of common bean cultivars: A review. Journal of Crop Science 41:1659-1675.

Singh, S.P., Gepts P. & Debouck, D.G. 1991a. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Economic Botany 45:379-396.

Singh, S.P. & Munoz, C.G 1999. Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. Journal of Crop Science 39:80-89.

Singh, S.P., Nodari, R. & Gepts, P .1991b. Genetic diversity in cultivated common bean: I. Allozymes. Crop Science 31:19-23.

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

Singh, S.P., Teran, H., Molina, A. & Gutierrez, J.A. 1991. Genetics of Seed Yield and its Components in Common Beans (*Phaseolus vulgaris* L.) of Andean Origin. Plant Breeding, 107:254-257.

Singh, S.P., Urrea, C.A., Molina, A. & Gutiérrez, J.A. 1992. Performance of small-seeded common bean from the second selection cycle and multiple-cross intra- and interracial populations. Canadian Journal of Plant Science 72:735-741.

Spielmeyer W., Sharp, P.J. & Lagudah, E.S. 2003. Identification and validation of markers linked to broad-spectrum stem rust resistance genes *Sr2* in wheat (*Triticum aestivum* L). Crop Science 43:333-336.

Sprecher, S.L. & Isleib, T.G. 1989. Morphological and phenological diversity between gene pools. Annual Report of the Bean Improvement Cooperative 32:54-55.

Sprecher, S.L. & Khairallah, M. 1989. Association of male sterility with gene pool recombinants in bean. Annual Report of the Bean Improvement Cooperative 32:56-57.

Stavely, J.R. 1984. Genetics of resistance to *Uromyces phaseoli* in a *Phaseolus vulgaris* line resistance to most of the races of the pathogen. Phytopathology 74:339-334.

Stavely, J.R.1990. Bean rust of additional plants introductions. Annual Report of the Bean Improvement Cooperative 33:185-186.

Stavely, J.R. 1991. Rust. In: R. Hall (ed.). Compendium of bean Diseases. APS Press. St. Paul, USA. 24-25.

Stavely, J. R., 1998. Recombination of two major dominant rust resistance genes that are linked in repulsion. Annual Report of the Bean Improvement Cooperative 41:17-18.

Stavely, J.R. 2000. Pyramiding rust and viral resistance genes using traditional and marker techniques in common bean. Annual Report Bean Improvement Cooperative 43:1-4.

Stavely, J.R. & Kelly, J.D. 1996. Evidence for the independence of the *Ur-4* and *Ur-6* rust resistance genes. Annual Report of the Bean Improvement Cooperative 39:302-393.

Stavely, J.R., Kelly, J.D. & Grafton, K.F. 1994. BelMiDak-rust resistant navy dry bean germplasm lines. Horticultural Science 29:709-711.

Stavely, J.R. & Pastor-Corrales, M.A. 1989. Rust. Pages 159-194 in: H.F. Schwartz and M.A. Pastor-Corrales (eds.). Bean Production Problems in the Tropics. CIAT, Cali, Colombia.

Steadman, J.R. 1995. Progress reported at the 2nd. International Bean Rust Workshop. Annual Report of the Bean Improvement Cooperative, 38:1-10.

Steadman, J.R., Pastor-Corrales, M.A. & Beaver, J.S. 2002. An Overview of the 3rd Bean Rust and 2nd Bean Common Bacterial Blight International Workshops, March 4-8, 2002 Pietermaritzburg, South Africa. Annual Report of the Bean Improvement Cooperative, 45:120-124.

Stoskopf, N.C., Tomes, D.T. & Christie, B.R. 1993. Plant breeding theory and practise. San Francisco, CA; Oxford: Westview Press Inc.

Sunnucks, P. 2000. Efficient genetic markers for population biology. Trends in Evolution and Ecology 15:199–203.

Tanksley, S.D. 1983. Molecular markers in plant breeding. Plant Molecular Biology Reporter 1: 3-8.

Tanksley, S.D., Young, N.D., Paterson, A.H., & Bonierbale, M.W. 1989. RFLP mapping in plant breeding; new tools for an old science. Bio/Technology 7:257-238.

Tarakanovas, P. & Ruzgas, V. 2006. Additive main effect and multiplicative interaction analysis of grain yield of wheat varieties in Lithuania. Agronomy Research 4:91-98.

Taramino, G. & Tingey, S. 1996. Simple sequence repeats for germplasm analysis and mapping in maize. Genome 39:277-287.

Terán, H., Jara C., Mahuku, G., Beebe, S. & Singh, S.P. 2013. Simultaneous selection for resistance to five bacterial, fungal, and viral diseases in three Andean × Middle American inter-gene pool common bean populations. Euphytica 189:283-292.

Thrall, P.H., & Burdon, J.J. 2003. Evolution of virulence in a plant host-pathogen met population. Science 299:1735-1737.

Thudi, M., Li,Y., Jackson, S.A., May, G.D. & Varshney, R.K. 2012. Current state-of-art of sequencing technologies for plant genomics research. Brief Function. Genomics, 11:3–11.

Thung, M.D.T., & Rao, I. 1999. Integrated management of abiotic stresses. In: Singh, S.P. (ed). Common bean improvement in the twenty-first century. Kluwer, Dordrecht, Netherlands. 331–370.

Todd, A. 2008. Development of molecular tools for isolation of the common bean (*Phaseolus vulgaris*) rust resistance gene *Ur-3*. Development of Agriculture & Natural Resources Delaware State University, Dover, D.E. (Master's thesis).

Tryphone, G.M., Chilagane, L.A., Deogracious, P.M., Kusolwa, P.M. & Nchimbi-Msolla, S. 2013. Marker Assisted Selection for Common Bean Diseases Improvements in Tanzania: Prospects and Future Needs. In book: Plant Breeding from Laboratories to Fields.

Vallejos, C.E., Sakiyama, N.S. & Chase, C.D. 1992. A Molecular Marker-Based Linkage Map of (*Phaseolus vulgaris L*.). Genetics 131:733-740.

Van Schoonhoven, A. & Pastor-Corrales, M.A. 1987. Rust. In: Standard System for the evaluation of Bean Germplasm. CIAT, Cali, Colombia. 24-27

Varshney, R.K., Terauchi, R. & McCouch, S.R. 2014. Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. PLoS Biology, 12:e1001883.

Vieira, R.F., Paula Junior, T.J., Teixeira H., & Carnerio, J.E.D.S. 2010. White mold management in common bean by increasing within-row distance between plants. Plant Disease 94:361- 367.

Villegas, J.M. 1959. Variability of Isoriopsis griseola Sacc.La causal agent. Angular spot of the bean (*Phaseolus vulgaris* L.). Calda. University dad of Caldas. (BS Thesis)

Vorster, H. H. & Venter, C. S. 1994. Health benefits of dry beans *(Phaseolus vulgaris*): a review. South African Journal of Food Science and Nutrition 6:72-76.

Vos, P., Hogers, R., BleekeR, M., Reijans, M., Van der Lee, T., Hornes, M., Frijters A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. 1995. AFLP; a new technique for DNA finger printing. Nucleic Acids Research 23:4407-4414.

Wahome, S.W, Kimani, P.M, Muthomi, J.W., Narla, R.D. & Bucuchara, R. 2011. Multiple disease resistance in snap bean genotype in Kenya African Crop Science Journal. 28:9309.

Walker, D., Boerma, H.R., All, J., Parrott, W. 2002. Combining *cry1Ac* with QTL alleles from PI 229358 to improve soybean resistance to lepidopteran pests. Molecular Breeding 1:43-51.

Wasonga, C.J., Pastor-Corrales, M.A., Porch, T.G., Griffiths, P.D. 2010. Targeting gene combinations for broad-spectrum rust resistance in heat-tolerant snap beans developed for tropical environments. Journal of the American Society for Horticultural Science 135:521-532.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalksi, J.A. & Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535.

Winter, P. & Kahl, G. 1995. Molecular marker technologies for plant improvement. World Journal of Microbiology & Biotechnology 11:438-448.

Wortmann, C. S., Kirkby, R. A., Eledu, C. A. & Allen, D. J. 1998. Atlas of common bean (*Phaseolus vulgaris L*.) production in Africa. CIAT, Cali, Colombia.

Wright, E.M., Awele, H.E. & Kelly, J.D. 2008. Use of TRAP markers to map resistance to a new race of common bean rust in Michigan. Annual Report of the Bean Improvement Cooperative 51:210-211.

Yan, W. & Kang, M.S. 2003. GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists, and Agronomists. CRC Press.

Young, R.A. & Kelly, J.D. 1996. RAPD markers flanking the are gene for anthracnose resistance in common bean. Journal of the American Society for Horticultural Science 21:37-41.

Yu K., Park, S. & Poysa, V. 2000. Marker-assisted selection of common beans for resistances to common bacterial blight: Efficacy and economics. Plant Breeding 119:411-415.

Zaiter, H.Z., Coyne, D.P. & Steadman, J.R. 1989. Inheritance of resistance to a rust isolate in beans. Annual Report of the Bean Improvement Cooperative 32:126-127.

Zaumeyer, W. J. & Thomas, H. R. 1957. A monographic study of bean disease and methods for their control. USDA, Agricultural Technology Bulletin number 868. 74-84.