Breeding for ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] resistance of the common bean (*Phaseolus vulgaris* L.) in Rwanda

By

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Thesis Abstract

Ascochyta blight, caused by the fungus *Phoma exigua var. diversispora*, is an important air-borne disease that reduces common bean (*Phaseolus vulgaris* L.) yields, and hence food security, in Rwanda and elsewhere in the Great Lakes Region. The key aim of this study was to explain the significance of bean ascochyta, to assess the yield loss incurred by ascochyta blight, to screen germplasm for resistance to ascochyta blight, to determine the inheritance of resistance to ascochyta blight in the common bean and to develop an advanced line resistance to ascochyta blight.

A participatory rural appraisal (PRA) was conducted in four districts of Rwanda, to ascertain the farmers' awareness of ascochyta blight and their preferred bean genotypes. Bean ascochyta is considered to be the most devastating and most recognized disease, especially in northern Rwanda. Control measures for ascochyta have been very minimal, and in some cases, non-existent. The use of resistant genotypes to control the disease has not been evident, because the most popular genotypes have been susceptible to the disease. The resistant bean genotypes that are currently available have undesirable characteristics, such as a small seed size, black seeds and late maturity. Large-seeded bean genotypes, even though cited as being more susceptible to ascochyta than the small-seeded genotypes, are still very popular. The study highlighted the need for breeding ascochyta resistance in the large-seeded bean genotypes, which are highly preferred by farmers.

Yield loss assessment studies were conducted in Rwanda to quantify the yield loss attributed to bean ascochyta blight on 64 common bean genotypes, including the bush and climbing types. Using a split plot design, trials were conducted at three locations, where the ascochyta disease is prevalent. The study showed that the market class genotypes recorded a higher disease severity and higher yield losses, compared to the controls. There was a strong positive correlation between the relative area under the disease progress curve (RAUDPC) values and yield losses. It was established that the yield of a susceptible genotype is reduced by about 75.7% by ascochyta. This loss is minimized if a resistant genotype is used, or if a fungicide is used to protect the crop against the effects of the pathogen. Unfortunately, the available resistant genotypes are not as marketable as the susceptible genotypes.

Seventy-five common bean genotypes were evaluated in three sites for resistance to ascochyta blight under natural field conditions over two seasons. The findings of the study showed that there were some local and exotic common bean genotypes that were resistant to the ascochyta blight. The study was able to show that out of the 75 genotypes, 13 gave a consistent resistant reaction to the ascochyta pathogen in Rwanda; 29 gave an intermediate resistant reaction and 23 were susceptible. Based on their adaptability, eight resistant genotypes were selected for use as parents in the study of the inheritance of resistance to ascochyta.

An 8 x 8 diallel mating design was used to develop 56 F_1 and F_2 populations, plus their reciprocal crosses, with the aim of studying the mode of inheritance of resistance to ascochyta. The F_1 and F_2 progeny evaluations showed that ascochyta resistance was mainly governed by additive gene action in most populations. However, there were a few crosses that displayed highly significant specific combining ability (SCA) effects, implying that dominant effects were important in some populations. Maternal effects were also highly significant in both the F_1 and F_2 generations, suggesting that resistance was modified by cytoplasmic genes. The non-maternal effects were significant in some populations, suggesting that the cytoplasmic genes were interacting with nuclear genes. The number of genes governing resistance to ascochyta varied from two to eight, among the eight sources of resistance. The allelism test of resistant x resistant populations, and the observation of the continuous distribution of severity scores, suggested the presence of many loci governing ascochyta resistance in beans. The broad sense heritability of disease resistance varied from 0.21-0.64, while heritability in the narrow sense was estimated as 0.30±1.04 for the bush type and 0.29±0.07 for climbers, respectively. These results suggested that recurrent selection would be the best breeding procedures for improving resistance in the popular large-seeded bean genotypes in Rwanda. However, there could be complications in breeding for resistance to ascochyta in beans, because resistance was modified by cytoplasmic gene effects and their interaction with nuclear genes, in some of the populations.

Ten bush and ten climbing advanced F_6 bean lines and two standard checks, were evaluated at five locations in Rwanda during the short rainy season, to identify ascochyta resistant, stable and high-yielding genotypes and the extent of GXE interaction. The study included farmers through the participatory variety selection method (PVS). Both the AMMI and Eberhart and Russell models revealed that bush genotype Lines 1B and 8B and climbing genotype Lines 2C and 6C were widely adaptable, stable and high yielding. The genotypes selected by farmers were those that have exhibited a high tolerance to both abiotic and biotic stresses. The study

showed that advanced bush Lines 1B and 8B and climber Line 6C are high yielding, stable and ascochyta-resistant. However, further regional trials are needed, before the release of these lines.

Declaration

I, Clement Urinzwenimana, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. The thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a) Their words have been re-written but the general information attributed to them has been referenced.
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5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference sections.

Signed: Date: 1st March, 2017.

Clement Urinzwenimana (Student)

As the candidate's supervisors we agree to the submission of this thesis

Signed: ..... Date: .....

Prof Rob Melis (Supervisor)

Signed: ..... Date: .....

Dr Julia Sibiya (Co-Supervisor)

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Dedication

In memory of my late parents, François Hakizimfura and Pascasie Mukandoli.

To my wife, Alice and our two lovely children, Talita and Verone.

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Introduction

The common bean is one of five cultivated species from the genus *Phaseolus* and is a major grain legume crop that is grown worldwide. It is third in importance, after soybean and peanut, but it is first, with regard to direct human consumption (Broughton et al., 2003; Mukamuhirwa et al., 2015). It is grown for its green leaves, green pods and soft and/or dry seeds. Green leaves, green pods, and immature and/or dry seeds may all be eaten, because they are very rich in iron and zinc (Kimani et al., 2006). Dry leaves, threshed pods, stalks and bean seeds that do not meet human food quality standards are fed to animals, or used as fuel for cooking, especially in Africa and Asia (Sperling et al., 1996; Buruchara, 2006). Per capita consumption varies within each producing and consuming country, and among the regions of each country, depending on consumer preferences, but it can be as high as 66 kg/capita/year in the Great Lakes Region (Broughton et al., 2003).

Beans provide a cheaper source of protein than that obtained from animals, making it highly competitive and important in the dietary regimes of poor people in Africa. It provides 60% of the dietary protein in Rwanda (MINAGRI, 2015) and is often the principal source of dietary protein for the urban poor. Beans also contribute as much as 30% of dietary energy in the widespread maize-based cropping systems of the mid-altitude areas of eastern and southern Africa (Pachico, 1993). Beans provide valuable sources of vitamin B complex, iron, zinc, sulphur and other essential minerals (CIAT, 1997). Bean production is associated with high human population densities (Wortmann et al., 1998) because they are easily grown, are suitable for intercropping and have short growing cycles.

Beans contribute a great deal to the improvement and sustainability of soil fertility, due to their ability, as legumes, to fix nitrogen in the soil. They are hence used in crop rotation, as mixtures with grass in leys and pastures, and as cover crops and green manures (Purseglove, 1968). Thus, beans fit well into the farming systems in Rwanda and sub-Saharan Africa, where the crop is an important source of income, especially for women, who grow it both for subsistence purposes and for sale to urban populations (CIAT, 1997).

Approximately 90% of the Rwandan bean production is consumed domestically, while some is exported to neighboring countries (Mukamuhirwa et al., 2015) by means of informal border trade (RAB, 2014). The annual global production of dry beans is estimated at 19.5 million tons. Brazil is the highest producer, with an estimated annual production of 4.1 million tons (FAOSTAT, 2014). Production in Africa is estimated at 2.8 million tons on 4.8 million ha (FAOSTAT, 2014). East Africa accounts for over 75% of the total production in Africa, and Rwanda is third, after Kenya and Uganda, with its production of 438 236 tons (FAOSTAT, 2014). However, even though Rwanda is ranked high in bean production, it is ranked among the last five countries in Africa, with regard to production per unit area (FAOSTAT, 2014). Over the past 10 years, there has been a steady increase in the area planted to beans in Rwanda, (FAOSTAT, 2014). However, production per unit area has been continuously declining. This decline has been attributed to several biotic and abiotic factors, ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] being one of the major biotic constraints to bean production in Rwanda.

Bean ascochyta blight was reported to have occurred in Rwanda in 1985 (ISAR, 1985) and it is responsible for most of the yield losses in the Great Lakes Region (RAB, 2014). In Rwanda, especially in the north-western highland regions, ascochyta is one of the most serious constraints to bean production (RAB, 2014), with significant losses occurring due to susceptible genotypes. It has also emerged as the most important constraint to bean production in western Uganda, some regions of the Republic of Burundi and the Democratic Republic of the Congo (RAB, 2014).

The pathogen is particularly severe in large-seeded bean genotypes, due to a lack of genetic resistance in these seed types (Seijas et al., 1985; Beebe et al., 1991). An overemphasis on quality traits in previous breeding programmes, and a consequent reduction in genetic variability, is likely to have contributed to the lack of resistance in the large-seeded bean genotypes (Musoni et al., 2005; Waggoner and Berger, 1987). The intensification of agriculture that has resulted from the ever-increasing human population in the highland regions of Rwanda, could also have led to higher ascochyta epidemics. Genotypes that could previously tolerate the low levels of inoculum have since succumbed to the disease (Musoni et al., 2005).

The bean improvement programme against diseases in Rwanda has been targeting root rot, anthracnose and angular leaf spot, because they were found to be the most predominant pathogens in Rwanda (ISAR, 2011; RAB, 2014). However, ascochyta has also become

predominant, it occurs often and has been found to be even more destructive (RAB, 2014). This highlights the need for research, in order to develop a strategy for its control.

Although several measures have been used to control ascochyta, none have been effective. Ascochyta management has been possible, to some extent, by using a combination of control options (cultural, chemical and biological), as a part of integrated pest management (Buruchara, 2006). However, the single most effective and practical management strategy, especially for the resource-poor farmers, is the use of bean genotypes that are resistant (Hall and Nasser, 1996). Unfortunately, the popular commercial bean genotypes that are currently grown in Rwanda are susceptible, while known resistant genotypes are associated with undesirable characteristics, such as late maturity, the black seed colour and small seed size (RAB, 2014). Large-seeded genotypes are the major market-class, or preferred, bean seed types in most parts of Rwanda.

There is hence a need to improve the resistance of ascochyta in the farmers' preferred varieties. Participatory plant breeding (PPB) has been shown to result in the wider adoption of new genotypes (Danial et al., 2007). Previous studies on resistance to ascochyta blight (Schwartz et al., 1981; Schmit and Baudoin, 1992) have not considered the mode of inheritance of this characteristic. A knowledge of the inheritance of a trait is critical when designing appropriate breeding strategies for incorporating such a trait into economically useful populations. This study will therefore help in shedding more light on the genetic basis of resistance to ascochyta blight.

Objectives of the study

The study aimed at contributing to improved food security, by improving resistance to ascochyta blight in the farmers' preferred bean genotypes. The study specifically aimed at:

1. assessing the farmers' perceptions, knowledge and management of ascochyta blight and documenting the production constraints and breeding priorities of bean production in Rwanda;
2. assessing yield losses, due to ascochyta blight resistance;
3. screening and identifying bean genotypes with resistance to ascochyta blight among Rwandan landrace germplasm and other collections;

4. studying the inheritance pattern of ascochyta blight resistance (gene action and heritability) among progenies that are developed from crosses involving resistant and susceptible parents; and
5. developing advanced lines with resistance to ascochyta blight.

Organization of thesis

This thesis is made up of eight sections that include six chapters, as shown below:

1. The Introduction;

2. Chapter One: Literature review;

3. Chapter Two: Farmers awareness and perceptions of bean ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] and evaluation of constraints of bean production in Rwanda;

4. Chapter Three: Yield loss assessment in the common bean (*Phaseolus vulgaris* L.) due to theascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] disease;

5. Chapter Four: Genotypic response of dry bean (*Phaseolus vulgaris* L.) to natural field infection of ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] under diverse environmental conditions in Rwanda;

6. Chapter Five: Genetic analysis for resistance to bean ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] among common bean genotypes in Rwanda;

7. Chapter Six: Yield performance and ascochyta blight [*Phoma exigua var. diversispora (Bubak) Boerema*] resistance in advanced common bean (*Phaseolus vulgaris* L.) lines in Rwanda; and

8. An overview of the study.

All chapters are written in the AIMRAD format, namely, the Abstract and Introduction which are followed by the materials and methods section, which is followed by the results and discussion

sections. Chapters all have a reference list, hence there may be limited repetition, as well as an overlap of content, especially in the references and the Introduction sections of these chapters.

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Chapter One: Literature review

Bean ascochyta blight, caused by *Phoma exigua var.diversispora,* is an important disease of the common bean (*Phaseolus vulgaris* L.) throughout Rwanda. It is known to cause total crop losses, especially under humid conditions and low soil fertility. This review focuses on the taxonomy and agronomy of the common bean. It discusses the origin and diversity of beans, and highlights the production constraints. The review dwells on studies on genetic improvement of the common bean for resistance against ascochyta blight attack, and it highlights further opportunities available for rapid advance. It discusses the research achievements in the field, as well as greenhouse disease screening methods in the common bean, and it highlights the breeding methods used in common beans.

1.1 Taxonomy of the common bean

Common beans are classified in the sub-phylum Dicotyledons, the division Magnoliophyta, the class Magnoliopsida, the family Leguminosae, the sub-family Papilionoideae/ Fabaceae/Lotoideae (pulse family characterized by edible seeds and pods) and the order Leguminales. It is diploid (2n = 2x = 22) and a self-pollinated crop (Rutger and Beckham, 1970; Saettler and Correa-Victoria, 1983), possessing complete, papilionaceous flowers with 10 stamens, and an ovary with a long, coiled style and a hairy intorse stigma. The crop is highly polymorphic, showing considerable variation in its growth habits, vegetative character, flower colour and size, shape and colour of its pods and seeds (Purseglove, 1968).

There are two major commercial classes of the common bean, as well as snap and dry beans (Singh, 2001). Snap beans are also known as string beans, or green beans, and are mainly grown for their pods, while dry beans are mainly grown for their seed. Erect bush bean types are the most common, with mechanical harvesting being practised in commercial agriculture. Climbing types are largely restricted to high altitude areas, especially in south-western Uganda, Rwanda, Burundi and the eastern parts of the Democratic Republic of the Congo (Baudoin et al., 2001; Mukamuhirwa et al., 2015).

The common bean is well-adapted to elevations of 1200–2200 m, with a mean temperature during the growing season of between 15–23°C. Still, 20% of common bean production takes place at a mean temperature higher than 23°C (Freytag and Debouck, 2002). The crop can withstand occasional daytime temperatures of 35°C, but this often results in flower

abortion. Growth stops below 10°C and the plant is killed by frost (Messiaen and Seif, 2004). *Phaseolus vulgaris* may be grown at low altitudes during the cooler months, generally with irrigation, and usually for an immature pod harvest (Freytag and Debouck, 2002). Common bean production occurs in regions where there is a 250 mm mean rainfall during the growing season, but 65% of the production is estimated to occur in areas with an average rainfall higher than 400 mm during the season (Freytag and Debouck, 2002). Occasional water deficits severely reduce yields. Diseases that are favoured by humid conditions are an even more important constraint than the water deficit (Kelly and Miklas, 1998). Bean genotypes vary with regard to photoperiod sensitivity, which is typically greater in genotypes of Andean origin than those of meso-American origin.

Beans prefer medium-textured, well-drained soils over 0.5 m deep and they are sensitive to soil acidity, including the associated aluminium and manganese toxicities. The optimum pH is 6.0–7.5, but the most common bean production in tropical Africa is at a soil pH of 5–6, 20% taking place on soils with a pH below 5 (Freytag and Debouck, 2002). Common bean production in Africa occurs mostly under conditions of P deficiency. Where the *Phaseolus* species have not been previously grown, symbiotic N-fixation may be inadequate to meet the N requirement of the plants (Giller, 2001).

For seed germination, the soil must be warmer than 12°C, with optimal emergence occurring at soil temperatures of 22–30°C (Qi et al., 1998). Plant growth habits are broadly grouped into those that are determinate, and those that are indeterminate. Flowering in the common bean generally starts 28–45 days after sowing. Self-fertilization is the rule, but with 1–3% outcrossing. Immature pods for vegetable use can be harvested 25–30 days after flowering (Johnson et al., 2003). The seed-filling period may take 23–50 days. The length of the crop cycle ranges from 60–90 days, for the determinate types, and may be as long as 250–300 days, for the indeterminate climbing types (Johnson et al., 2003).

Several Rhizobium species fix nitrogen with *Phaseolus vulgaris*, including *Rhizobium leguminosarum* bv. *phaseoli*, *Rhizobium etli* and *Rhizobium tropici*. The nitrogen-fixing ability of the common bean is often considered poorer than that of other pulses, such as cowpea, soya bean and groundnut, although fixation rates of up to 125 kg of N per ha have been recorded (Giller, 2001).

The common bean is normally propagated by seed, but vegetative propagation, using stem cuttings, is possible. The 1000-seed weight is 150–600 g, depending on the variety. The common bean may be sown by broadcasting and by row planting (Messiaen and Seif, 2004). Sole-crop sowing rates range from 150,000–400,000 seeds per ha. Sowing rates are less for

intercropping, than for sole cropping (Messiaen and Seif, 2004). Indeterminate climbing beans are sown 2–3 seeds per planting hole, in rows 40–60 cm apart and with a 40–50 cm spacing within each row. Seeds are normally sown 3–4 cm deep, but it can be as deep as 7 cm if the soil surface is dry and not too heavy, or if it is prone to crusting (Baudoin et al., 2001). Mixtures of different seed types are often sown in Rwanda, Tanzania and Malawi (Baudoin et al., 2001). In traditional agriculture, the land is prepared by hand, or animal traction, before sowing. Cultivation is mostly on flat land, but sowing on hills or ridges may be practised, where the soil is heavy and the groundwater table is high (Baudoin et al., 2001).

Only about 30% of the bean production area in tropical Africa is planted as a sole crop. Intercropping with maize, bananas and root or tuber crops is important, with these intercrop associations accounting for 40–50%, 10–20% and 10–20%, respectively, of the common bean production area (Johnson et al., 2003). Intercropping with sorghum, millet, pea, faba bean, coffee and other crops is less common. Climbing genotypes are more often produced in sole cropping than the bush type, but the dense foliage in sole cropping easily creates a humid environment that promotes diseases (Baudoin et al., 2001).

1.2 Origin and genetic diversity of the common bean

The common bean originated in Mexico. Small-seeded and climbing ecotypes are found in the wild in northern Argentina and Central America. The common bean was independently domesticated in both Central America (Mexico and Guatemala) and in the South American Andes (mainly Peru) (Purseglove, 1968; Harlan, 1975; Evans, 1980; Gepts and Debouck, 1991; CIAT, 1995 and Bitocchi et al., 2013). The resulting gene pools are distinct. Archaeological evidence indicates that the common bean was already a domesticated crop in 6000 BC and 5000 BC in Peru and Mexico, respectively (Gepts and Debouck, 1991). The common bean was taken to other parts of the world after the 16th century. Portuguese traders probably introduced the common bean to Africa from the 16th century onwards, through the ports of Sofala (Mozambique), Zanzibar and Mombasa, from where it was carried to the higher altitude areas of the interior by slave trading caravans and merchants (Gepts and Debouck, 1991; Chacon et al., 2005). The common bean was well-established as a pulse crop in parts of Africa before the colonial era. Linguistic evidence indicates that the common bean, with its genetic diversity and pathogens, became a major crop in the Central African highland areas (e.g. in Rwanda and Burundi) earlier than in other parts of Africa (Gepts and Debouck, 1991). Nowadays, the common bean is a crop of global importance, especially in North and South America, Europe and Africa. The crop is of

significance in many African countries and it is most intensively grown in the Great Lakes areas of Central Africa. In tropical Africa, the common bean is a major food crop in both urban and rural areas (Gepts and Debouck, 1991).

The genetic diversity of beans on farms in Africa is usually broader than it is in Latin America, where the bean was domesticated (Grisley and Mwesigwa, 1991). However, where consumer preferences are more specialised, beans are often limited to one or two seed types (Wortmann et al., 1998).

A high degree of diversity exists, in terms of growth habits, seed shape, size and colour, but the most common bean varieties grown in Africa are of the bush type, with small- to medium-sized seeds (Evans, 1980; Gepts and Debouck, 1991; CIAT, 1995). The bush type is preferred to the climbing type because of its low production costs and its convenience for market production. The climbers pre-dominate the highland areas, where population density is high and land is limited. The traditional growing areas include Burundi, Rwanda, the Democratic Republic of the Congo and, to a lesser extent, the south-western highlands of Uganda and the western highlands of Ethiopia, Kenya and Malawi (Allen and Edje, 1990; Wortmann et al., 1998). In recent years, climbers have been extended to other countries, like Tanzania, Kenya, Angola and Madagascar. Nevertheless, climbing beans still account for a small share of the land under beans, compared to the bush type. Bush types are popular in areas where commercial bean production has gained importance, because of their early maturing characteristics.

It has been reported that there is a large diversity of common bean seed types in Africa, but that it varies across the regions (Wortmann et al., 1998). It is highest in the mainly subsistence areas, such as the Great Lakes Region (Rwanda, Burundi and the Democratic Republic of the Congo) and southern Uganda. Wortmann et al. (1998) classified the common bean varieties into nine major classes, according to their colour and size, namely: pure large reds, medium and small reds and red mottled, purple, yellow and tans, cream, navy/white and black. The spatial distribution of seed types in eastern and southern Africa is a result of many factors, but market forces and agro-ecological conditions are the most important.

The red and red mottled beans are the most common types, due to market preferences. Wortmann et al. (1998) estimated an aggregate area share of about 50% for pure reds and red mottled beans in eastern Africa and about 27% in southern Africa. With the economic growth steadily increasing in most of the sub-Saharan African countries, and the high rate of non-industrial urbanization, the commercialisation of the common bean is expected to grow

rapidly in the medium term (Wortmann et al., 1998). However, the current preferred market genotypes are generally less tolerant to the important biophysical constraints (drought, poor soils and diseases) and the predicted effects of global warming on the climate in the region could alter the genotype distribution trend (Wortmann et al., 1998).

1.3 Common bean production in Rwanda

Beans are produced in all regions of Rwanda. According to the Rwandan Ministry of Agriculture and Animal Resources, dry bean production between 2013 and 2014 ranked fifth, in terms of volume, just after bananas, Irish potatoes, sweet potatoes and cassava (MINAGRI, 2011; RAB, 2014)

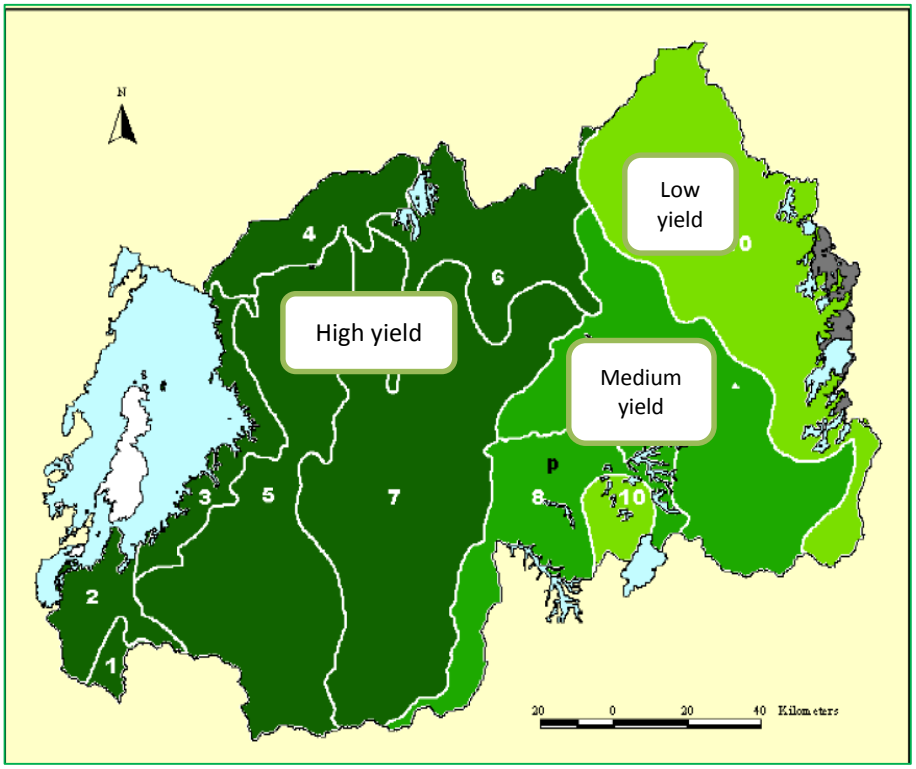

Figure 1.1: Common bean production in Rwanda

Common bean production in Rwanda is mainly influenced by the intensity and distribution of rainfall (Figure 1.1) throughout the year. The production is high in the areas where the rainfall is sufficient and well-distributed throughout the year.

Dry beans are grown by small-scale farmers in Rwanda, mainly as cash crops and food crops, and they therefore play an important role in food security (ISAR, 2011; FAOSTAT, 2013). The average plot size for these farmers ranges from 0.1 to 0.5 ha per household

(ISAR, 2011). Therefore, the greater percentage of beans is usually grown for household consumption, with a small percentage sold at a market, or through other venues (Wortmann et al., 2004). In Rwanda, beans are grown by about 500 000 farmers on 479 899 ha per year (FAOSTAT, 2011; RAB, 2014), with an annual production of about 432 857 t in the three growing seasons (FAOSTAT, 2011).

Typically, bush bean genotypes are grown in most of the low-land bean-growing areas of Rwanda and they are intercropped with various crops like maize, cassava, bananas and peas (RAB, 2014). The climbing beans, on the other hand, are mainly grown in the highlands of the northern and western regions of Rwanda and they are intercropped with maize, because of its strong agronomic compatibility and because it helps with staking (Mukamuhirwa et al., 2015). However, farmers prefer to grow climbers in pure stands, because they have a higher yield potential than when they are intercropped (ISAR, 2011).

Rwanda's total bean production increased between 2005 and 2014, as indicated by the FAO statistics in Table 1.1. These statistics correspond with the area under bean crops that were estimated by ISAR during the same period (MINAGRI, 2011). The ISAR (Beans Research Programme in Rwanda) released improved bean varieties with different physiological and genetic characteristics, as well as farmers' preferences, during the same period of time. During this period, the productivity per hectare did not increase every year. There were a series of fluctuations in bean production, resulting in a general decline in domestic food supply per capita during. The statistics of 2002 to 2005 show an upward trend in bean production; however, the country's productivity per hectare has been on the decline since 2002. It is also evident that the area under bean cultivation has been increasing each year since 2010, which could also explain the increase in production during this period.

Table 1.1: Twelve-year bean production trends in Rwanda

Years	Area (ha)	Production (T/ha)	Productivity (T/ha)
2003	356519	239394	0.67
2004	319349	198224	0.62
2005	313019	199648	0.64
2006	356381	283387	0.80
2007	358208	329000	0.92
2008	336577	308000	0.92
2009	345851	326532	0.94
2010	319252	327497	1.03
2011	341819	331166	0.97
2012	479899	492857	0.93
2013	480012	500120	1.04
2014	454250	505760	0.91

FAO, 2014

Various policy initiatives have been taken to promote sustainable agricultural development for the up-scaling of rural incomes and food security in Rwanda. One of these is the Crop Intensification Programme (CIP). The CIP was launched in September 2007, with the main goal of increasing agricultural productivity in high-potential food crops and ensuring food security and self-sufficiency (MINAGRI, 2011).

This initiative has spearheaded the agricultural development in Rwanda, by promoting land use consolidation, by using improved seeds and fertilizers and by strengthening the agro-input dealer's network. It stimulates a reliable private-sector input and output market and an agricultural product marketing system (MINAGRI, 2011).

1.4 Bean production constraints

Despite the nutritional importance of beans, production growth rates have been declining throughout Africa. In most low input systems, where the majority of beans are produced, the principal factors responsible for bean yield and quality losses are low fertility, plant nutritional deficiencies, drought, insect pests, weeds and diseases (Liebenberg and Pretorius, 1997; Wortmann et al., 1998).

Low soil fertility and drought are among the most widely-distributed abiotic stresses. Deficiencies in soil nitrogen, phosphorous (P), zinc, as well as the toxicity of aluminium and manganese, are particularly problematic for bean production (Karen et al., 2006). Low P soils are a major constraint to bean production in regions of Africa and Latin America, where farmers lack access to sufficient P fertilizer (Wortmann et al., 1998). Complete crop failure, due to drought, is very common in dry land conditions (Carlos et al., 2006). Temperatures below 15^{o}C, as well as frost at the beginning and the end of the growing season in the highlands, can also reduce yields (Wortmann et al., 1998; Singh, 2001). CIAT evaluated several insect pests as being important constraints to bean production, including the following: aphids (*Aphis fabae* Scopoli); pod borers (*Helicoverpa spp*. and *Maruca testulalis* Geyer); foliage beetles (*Ootheca spp*.); bruchids (*Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say); and thrips (*Megalurothrips sjostedti*). Pod bugs, mostly *Clavigralla spp*., are also a common pest (Wortmann et al., 1998).

Weeds are an important constraint to bean production, because they compete for light, water, space and nutrients (Alteiri and Liebman, 1986; Alemán, 2001). Good weed control may be achieved by a single weeding, three weeks after planting. However, major losses in the tropics result when farmers lack sufficient labour for timely hand-weeding (Alemán, 2001). Alemán (2001) reported an increased yield of the common bean, when using mechanical and chemical weed control, with minimum or no tillage.

In most tropical bean production regions, diseases are often the most important constraint to bean production (Wortmann et al., 1998; Pereira et al., 2016). More plant pathogens in Africa, attack beans with greater pathogenic variation, and more virulent isolates of the pathogens, than in the more temperate regions. The prevalence and importance of each disease varies considerably, depending on the locality, season, year and cultivar (Schwartz and Corrales, 1989; Pereira et al., 2014).

ISAR (2011) listed 20 bean diseases in Rwanda, out of which 10 are the most important, depending on the ecological zone. These include the common bacterial blight [*Xanthomonas campestris* pv *phaseoli (*Smith) Dye], angular leaf spot [*Phaeoisariopsis griseola (*Sacc*.)* Ferraris], bean rust [*Uromyces appendiculatus (Pers.) Unger]* and the bean common mosaic virus (bean common mosaic virus), especially in the low altitude, high temperature areas. Other diseases are halo blight [*Pseudomonas syringe* pv*. Phaseolicola (Burkholder) Young et al.]*, anthracnose [*Colletotrichum lindemuthianum (*Sacc. and Magn*.)* Lams.Scrib*.]*, ascochyta blight [*Phoma exigua var. diversispora (Bubak)* Boerema*]*, and root rots (*Pythium ultimum* Trow*, Rhizoctonia solani (*Kuhn *and* and *Fusarium solani f. sp. phaseoli (*Mart*.) Sacc. f. sp. phaseoli (*Burkholder) W. C. Snyder& H. N. Hans), which are more important in

high-altitude and low-temperature areas. In Rwanda, especially in the northern and western highland regions, ascochyta is one of the most serious constraints to bean production (RAB, 2014), with significant losses occurring to susceptible genotypes.

1.5 Bean ascochyta blight

Ascochyta blight, also known as ascochyta leaf-and-pod spot, is a fungal disease of economic importance in regions with cool humid conditions, such as those found at elevations above 1000 m (RAB, 2014). The disease is important in the high-altitude valleys of Guatemala, Colombia and Peru (Schwartz and Corrales, 1989). Furthermore, the disease has been reported in Brazil (Schwartz and Corrales, 1989), Venezuela (Wellman, 1972), Costa Rica (Schwartz and Corrales, 1989), the United States, and other regions of the world (Zaumeyer and Meiners, 1975). In Africa, ascochyta blight is common in the high-altitude, humid, cool, bean-growing regions of Burundi, Rwanda, Uganda, the DRC, Kenya and Zambia (CIAT, 1997).

The taxonomy and etiology of the causal agent of the ascochyta blight pathogen is not well understood. However, the fungus causing ascochyta blight is generally recognized as *Phoma exigua var. diversispora (Bubak) Boerema* (Boerema, 1982). *Phoma exigua var. exigua* Desmazieres (Boerema et al., 1981), formerly known as *Ascochvta phaseolorum* Saccardo, has also been reported as a less important pathogen associated with ascochyta blight. Yield losses greater than 40% were measured in Colombia under moderate disease pressure (Hughes and Madden, 1997; Schwartz and Corrales, 1989). The common names frequently used for ascochyta blight (leaf spot) in Latin America are "ascochyta" and "mancha de ascochyta".

Phoma exigua isolates produce hyaline, septate, submerged mycelium in culture. Spores are usually two-celled (Zaumeyer and Meiners, 1975). Sporulation and germination are greatest at 21ºC, while mycelial growth is greatest at 24ºC. The fungus is inactivated by temperatures above 30ºC (Schwartz and Corrales, 1989). The fungus produces pycnidia, which measure 60-150 mm in diameter (Zaumeyer and Thomas, 1975). *Phoma .exigua* var. *diversispora* pycnidia measure 160 by 120 nm and conidia measure 6.8 by 2.7 pm.

Most conidia are one-celled (Boerema et al., 1981). Infection by *Phoma exigua var*. *diversispora* is favored by high humidity, continuous rains that are accompanied by winds, as well as cool to moderate temperatures (Boerema et al., 1981).

Figure 1.2: Symptoms of ascochyta blight on leaf

The symptoms first appear on leaves. These appear as black, concentric, zonate lesions (Figure 1.1) 1-3 cm in diameter, and they may later contain small black pycnidia (Boerema et al., 1981).

Figure 1.3: Symptoms of ascochyta blight on pods

These dark to black lesions may also appear on the peduncle, the petiole (Figure 1.2), the node and the pod (Figure 1.2), and they can cause stem girdle and plant death. The fungus may also spread systemically throughout the plant. Premature leaf drop may occur during

severe epidemics (Weber, 1973) and the fungus is seed-borne (Boerema et al., 1981). The control measures that are used are crop rotation, wide plant spacing, the planting of clean seed, the chemical treatment of seed and the foliar application of fungicides (Schwartz and Corrales, 1989).

Common bean germplasm is being screened to identify sources of resistance that may contribute to disease control. Although there are genotypic differences in the reaction to the ascochyta blight pathogen, most *P. vulgaris L.* accessions that have been evaluated thus far, are either susceptible or have low levels of resistance. However, high levels of resistance and immunity are present in the accessions of *P. coccinus* L., particularly in the subspecies *polyanthus,* such as Guate 1076 (G 35182), and in interspecific hybrids obtained by crossing these two species (CIAT, 1987).

1.6 Breeding for ascochyta blight resistance in the common bean

Genotypes with improved resistance to biotic and abiotic stresses constitute the primary goal of many bean-breeding programmes throughout the world (Miklas, et al., 2006). Diseases and pests can cause significant losses in common bean production (Schwartz et al., 2005; Wortmann et al., 1998)**.**

The control of these biotic constraints, by using fungicides, can increase production costs and create the potential for the contamination of the environment (James and Juan, 2009). On the other hand, genotypes with improved stress resistance can reduce the dependence on pesticides in high-input systems, and enable more stable bean production across diverse and adverse environments and poor soil conditions (Miklas et al., 2006).

The selection of parents for a breeding programme that seeks resistance to a variable pathogen is of utmost importance. One of the best possible methods that can be used to identify resistance is to expose the potential source of resistance to all existing pathogenic variations, over different production areas and over a period of several years. This can be accomplished through the international disease nurseries (Schoonhoven and Voysest, 1991).

Very little research has focused on breeding beans for resistance to ascochyta blight. Efforts have been limited to identifying tolerant cultivars. Some of this work has been conducted by the National Bean Programme of the ICTA in Guatemala, whereby several accessions of *Phaseolus vulgaris* and *P.polyanthus* that have a tolerance to the pathogen, were identified (Schoonhoven and Voysest, 1991). Similar research has been conducted at CIAT, where

most of the work has concentrated on the identification of the sources of resistance and the transfer of resistance into commercial cultivars (Schoonhoven and Voysest, 1991). CIAT has distributed the International Bean Ascochyta Blight Nurseries (IBABN) to bean researchers in national programmes. Most of the results obtained to date, suggest that resistant, or susceptible, accessions in one location, show a similar reaction in another location (Schoonhoven and Voysest, 1991).

Hanson et al. (1993) studied the heritability and inheritance of resistance to ascochyta blight in three climbing beans with resistance. The generation means analysis of resistant parents crossed with susceptible parents, indicated that additive, dominance and epistatic effects were important in the inheritance of resistance. Baudoin et al. (2001) evaluated 200 populations of *P. coccines* L. and *P. polyanthus* for ascochyta resistance at the two highland stations of Ronegro and Popayan in Colombia. However, only low levels of resistance to *Phoma exigua var.diversispora* were found among the wild and cultivated forms of the common bean. In Rwanda, no research has been done on breeding for ascochyta disease resistance, despite the importance of the disease.

1.7 Screening methods against diseases in the common bean

A number of different screening techniques have been developed and modified over time for the resistance screening of bean genotypes against diseases in the field and in greenhouses. Details of these screening techniques are described below.

The field screening of bean genotypes for disease resistance is generally done at hot spots, where the field selection for the evaluation is extremely important (Schoonhoven and Voysest, 1991). In general, the agronomic management of bean fields for the evaluation of disease reaction should be carried out under local conditions and they should not suffer from nutritional deficiencies (Schoonhoven and Voysest, 1991). Field screening needs to be conducted over a number of seasons and locations, to account for variations in weather conditions and inoculum, as variations in races occur both within and between locations (Ryan, 1971; Stegmark, 1991; Thomas and Kenyon, 2004)

It is often necessary to inoculate artificially when the initial inoculum of the disease does not develop, or if it is present at a low level (Inglis et al., 1988)**.** For many foliar diseases, the pathogen survives on the seed and plant residues. Hence, planting-infected seed, or sowing in a field where the disease was previously present, would usually result in higher levels of the disease (Tera´n and Singh, 2009).

The use of spreaders planted in advance of the test material can contribute to adequate levels of inoculum. The spreaders are planted one to three weeks before the test material, and they should be composed of a mixture of different susceptible varieties, to attract the greatest possible variation of the pathogen population (Schoonhoven and Voysest, 1991).

Infected plant tissue, such as leaves and pods with symptoms, can be collected and placed in rows between the test plots. This has also been used to serve as a source of the initial inoculum in foliar diseases (Ragagnin et al., 2005). Infected leaves can also be washed in water, to remove the spores, and can be applied to the test material with a knapsack sprayer (Mahuku et al., 2003).

The date of planting is a critical consideration in the field screening techniques against fungal diseases, as most fungal pathogens are favoured by rain and high humidity. Therefore, the planting date should be adjusted, to ensure humidity at the infection stage (Schoonhoven and Voysest, 1991). A higher planting density creates a more favourable micro-environment for foliar fungal diseases, which can facilitate secondary disease development, from plant to plant (Ragagnin et al., 2005).

Finding a reliable, cost-effective, and rapid greenhouse screening technique that has a high resolving power to detect physiological resistance against plant diseases, has been the goal of several pathologist breeders (Kim et al., 2000; Vuong et al., 2004). Screening under controlled conditions allows responses to be evaluated rapidly and uniformly (Grzesiak et al., 1996) and the method should be non-destructive, accurate and able to handle many samples (Wery et al., 1994).

Studies under controlled conditions allow epidemiological factors to be observed in detail, which may be affected by other biotic or abiotic stresses under field conditions (Tivoli et al., 2006). This type of experiment allows for the effective control of environmental conditions and is necessary for testing the infectivity of different isolates. Controlled environmental tests are likely to be more efficient than the field for testing large-scale screening, especially during the early stages of breeding programmes (Sillero et al., 2006).

The greenhouse screening technique can be classified in the following way: (i) those using direct intact plants; (ii) those using direct detached plant organs; and (iii) those using indirect approaches. For the direct screening of live plants, researchers have used the mycelial plug inoculation of cotyledons (Grau and Bissonnette, 1974; Kim et al., 2000; Kull et al., 2003), the straw-test or cut stem (Vuong et al., 2004; Carlos et al., 2006), infected oat seed for stem inoculation (Adams et al., 1973), mycelial infested celery for limited-term stem inoculation

(Pennypacker and Hatley, 1995), ascospores to inoculate flowering plants (Cline and Jacobsen, 1983) and the mycelial inoculation of foliage (Wegulo et al., 1998). The direct inoculation of excised common bean plant parts has included detached leaves with a spore suspension or mycelium (Miklas et al., 1992) and excised stems (Miklas et al., 1992). The indirect methods use pathogen filtrates to detect physiological resistance (Miklas et al., 1992), an oxalic acid diffusion test (Tu, 1985), a modified oxalate test (Kolkman and Kelly, 2000), and soluble stem pigment production in oxalic acid (Wegulo et al., 1998).

Several inoculation techniques have been compared, with Hunter et al. (1981) comparing three of them. Whole plants were sprayed with ascospores of white mould, detached flowers were infected with ascospores, placed in the axils of leaves, and mycelial colonized celery pieces were attached to stems. The ascospore method gave variable results and escapes occurred, whereas the limited term celery inoculation method detected partial resistance and was a more rapid and consistent test.

Kull et al. (2003), using three cultivars of the common bean and soybean, tested the efficacy of three screening methods to identify isolate aggressiveness and the incidence of white mould. The screening methods used were a mycelial plug placed on cotyledons, as well as a cut stem and detached leaves inoculated with a mycelial suspension. The cut stem method was the most efficient for identifying susceptible and resistant white mould genotypes in dry bean and soybean. Cline and Jacobsen (1983) compared three screening methods to determine the white mould reaction of 17 soybean cultivars. The screening methods included spraying the whole plant, using an ascospore suspension, using colonized carrot pieces placed on the leaf surfaces, and colonized celery pieces attached to the stem nodes. Only the latter method was successful in differentiating susceptible and resistant cultivars, whereas all plants were susceptible in the other two methods.

The effectiveness of a greenhouse screening method for detecting physiological resistance depends on many factors. These include plant age, the plant organ inoculated, pathogen aggressiveness, the type of inoculum, variance in the inoculum delivered, time between inoculation and disease assessment and the environment in the greenhouse (Tera´n and Singh, 2009). Furthermore, the efficacy of a screening method is dependent upon the ability to detect differences among genotypes with varying levels of resistance and susceptibility (Tera´n and Singh, 2009). Differences in the inoculation method and the growth of fungi on the infected surface may also cause differences (Mahuku et al., 2004).

1.8 Breeding methods in common bean

A diallel cross refers to a set of all possible matings between several genotypes (Hayman, 1954). The genotypes may be individuals, clones, homozygous genotypes, etc. The diallel analysis helps to obtain information on the genetic systems governing the inheritance of attributes that are to be improved, and may hence help in predicting the performance in subsequent generations, by assessing the potential of different crosses in F_1 and F_2 (Dabholkar, 1992). Like other mating designs, diallel mating is a frequently-used design for estimating the additive and dominance genetic (polygenic) effects involved in quantitative traits observed in the half- and full-sib progenies generated in plant breeding programmes (Singh and Chaudhary, 2004). The diallel design has additional benefits, in that the analysis applies to all the crosses involved and permits the estimation of parameters for additive, dominance and environmental effects, and it allows the recognition of non-allelic interactions (Christie and Shattuck, 1992; Griffing, 1956; Hayman, 1954; Jinks, 1956; Mather and Jinks, 1982). In addition, this technique enables the breeder to combine desirable genes that are found in two or more genotypes (Dabholkar, 1992).

There are four basic designs and analyses for the diallel mating design (Christie and Shattuck, 1992), including:

1. the analysis of the general and specific combining ability, or Griffing's analysis (Griffing, 1956);
2. the analysis of array variances and covariance's, or Hayman and Jinks analysis (Jinks, 1956; Jinks and Hayman, 1953);
3. the analysis of additive and dominance effects, also referred to as the Gardner and Eberhart's analysis (Gardener and Erberhart, 1966); and
4. the partial diallel analysis (Gilbert, 1958; Kempthorne and Curnow, 1961).

The present study will use Griffing's analysis to determine the combining ability of genotypes and to characterise the nature and extent of gene action (Christie and Shattuck, 1992). This analysis requires no genetic assumptions (Wright, 1985) and has been shown to convey reliable information on the combining potential of parents (Nienhuis and Singh, 1986). This design provides breeders with useful genetic information, such as general combining ability (GCA) and specific combining ability (SCA), to help them devise appropriate breeding and selection strategies (Zhang et al., 2001). The GCA and SCA effects help to locate the parents and crosses that will be responsible for bringing about a particular type of gene action (Dabholkar, 1992). The general combining ability refers to the mean performance of a

line with all its crosses, and is expressed as a deviation from the mean of all crosses (Falconer and Mackay, 1996). It is the average value of all F_1s having this line as one parent, the value being expressed as a deviation from the overall mean of crosses. Any particular cross has an expected value, which is the sum of the general combining abilities of its two parental genotypes. However, the cross may deviate from this value, to a greater or lesser extent. This deviation is called the SCA of the two genotypes in combination (Falconer and Mackay, 1996). Differences in GCA have been attributed to the additive, additive x additive and higher order interactions of additive genetic effects in the base population, while differences in SCA have been attributed to non-additive genetic variance (Baker, 1978).

Heritability (h^2) is used to evaluate the genetic control of traits determined by many loci and can be used to effectively plan strategies for incorporating character traits into new cultivars (Falconer and Mackay, 1996). Breeders are interested in heritability for the simple reason that characters with higher values can be improved more rapidly, and with less intensive evaluation, than those with lower heritability. However, the estimated heritability is unique to the population being studied and the environmental conditions to which individuals have been subjected (Dabholkar, 1992; Falconer, 1989). Populations that are genetically uniform, such as inbred varieties, are expected to show lower heritability than genetically-diverse populations. When heritability is high, more reliance can be placed on mass selection, and when it is low, more emphasis is placed on progeny, sib or family selection.

The heritability is used to estimate the improvement due to selection. The ratio of the genotypic variance (VG) to the phenotypic variance (VP) expresses the extent to which individual phenotypes is determined by the genotypes, and is referred to as heritability, in the broad sense (H^2), or the degree of determination. Broad sense heritability estimates include additive (VA), dominance (VD) and epistatic (VI) sources of genetic variation. The ratio VA/VP expresses the extent to which the phenotypes are determined by the genes transmitted from the parents, and is termed as heritability, in the narrow sense (h^2). It determines the degree of resemblance between relatives and is therefore of greatest importance in breeding programmes (Falconer and Mackay, 1996). Heritability is a reflection of only the additive sources of variation. Environmental variance (VE) forms part of the phenotypic variance and affects the magnitude of heritability; when it is high, the heritability is low, and when it is low, the heritability is high.

1.9 Genotypes by environment interaction (G x E) in the common bean

A high and stable seed yield is one of the main objectives in most breeding programmes. To be widely accepted, a genotype must show good performance across a range of environments (Acikgoz et al., 2009). However, it is often difficult to find such genotypes. Genotypes respond to changes in environmental conditions, such as temperature, rainfall, soil type and moisture (Robertson, 1959; Cockerham, 1963; Falconer and Mackay, 1995). Genotypes selected in a breeding programme should be tested at various locations for several years, and analysed appropriately, to determine the extent of the genotype by environment interaction (G x E), before being released as varieties.

Several characteristics should be considered in the selective process, to obtain a common bean genotype that meets the requirements producers and consumers (Mendes et al., 2011). Common beans are grown in several regions and under varied environmental conditions (Pereira et al., 2011). In view of this, the genotype x environment interaction is highly relevant (Cochran, 1954; Pereira et al., 2011). Factors that affect the G x E interaction for the common bean are the year, location and growing season. Among these, the growing season is particularly important, since there are three different seasons per year in some bean-growing regions (Pereira et al., 2011).

Ramalho et al. (1998) studied the importance of some factors of the G x E interaction in Brazil. The study revealed that the most significant interactions in the dry and winter seasons were the genotype and growing season (G x S) and the genotype and year (G x Y), ahead of the interaction between the genotype and location (G x L). It showed that the evaluation of genotypes over several years and different seasons is more important than the evaluation at various locations. Matos et al. (2007), however, reported that the G x L interaction is very important. In Rwanda, beans are grown under diverse environments, and this may result in significant G X E interactions for both yield and ascochyta blight. This, therefore, needs to be investigated, in order to recommend appropriate genotypes to the different agro-ecological zones and to identify stable cultivars.

The genotype x environment interaction for yield and its components can be analysed, using the additive main effect and multiplicative interaction (AMMI) and the genotype plus genotype-by-environment interaction (GGE) biplot analyses (Gauch, 1992; Yan and Tinker, 2006; Hogos-Vollegas et al., 2016). The mathematical models are used to study the performance of the genotypes across different environments. The AMMI model is widely

used for comparing the effects of G x E interaction (Romagosa and Fox, 1994). This model extracts the main effects of genotype and environment and uses the principal component analysis (PCA) to explain patterns in G x E (Romagosa and Fox, 1994; Hogos-Vollegas et al., 2016). It is more effective in partitioning the effects of genotypes, the environment and their interaction, and can be used to determine the effect of G x E for the traits studied. The GGE biplot is a methodology used for the graphical analysis of Multi-Environmental Trials (MET) data (Yan et al., 2001). The GGE refers to the genotype main effect (G) plus the genotype x environment interaction (GE). The model for a GGE biplot (Yan, 2002) is based on the singular value decomposition (SVD) of the first two principal components. For this study, AMMI analysis and biplots will be used.

1.10 Conclusion

The literature reviewed has shown that ascochyta, which is caused by the fungus *Phoma exigua var.diversispora*, has the capacity of affecting both the quantity and quality of the beans produced. The review has also highlighted that the fungus has the potential of genetically modifying itself in different pathotypes. Scientists have suggested that resistance to ascochyta is a quantitative trait. The diallel method was hence proposed as a mating design for this study, to improve resistance to ascochyta. The diallel analysis is able to estimate several genetic parameters, such as additive, dominance and environmental effects, and allows the recognition of non-allelic interactions. The GCA and SCA effects obtained will help to identify the parents and crosses that are responsible for bringing about a particular type of gene action, and it is these crosses that will be advanced in the next generations.

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Chapter Two: Farmers awareness and perceptions of bean ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] and evaluation of constraints of bean production in Rwanda.

Abstract

The awareness and perception of ascochyta blight by farmers is an important factor that affects the type of bean genotype adopted. Although farmers in Rwanda prefer large-seeded bean genotypes, both for consumption and for market, these genotypes are susceptible to ascochyta blight. It has been observed that farmers are abandoning large-seeded bean genotypes, in preference for smaller seeded genotypes, which seem to be more resistant. Therefore, the objective of this study was to assess the awareness and perceptions of bean farmers regarding the influence of ascochyta on the type of bean genotypes being grown. A participatory study was conducted in the districts of Burera and Musanze in northern Rwanda, Kamonyi in southern Rwanda and Rwamagana in eastern Rwanda, during February-November, 2014. In addition, the severity and incidence of ascochyta in beans was assessed over two seasons (2014B and 2015A). The study revealed that diseases were the most important bean production constraints. Based on the visual symptoms in the bean fields visited, as a whole, and on the leaves and pods of plants sampled per field, there were more ascochyta infections during Season B than during Season A. The incidence of ascochyta was generally highest in northern Rwanda where, in villages such as Busogo and Rwerere, all the bean fields visited had ascochyta symptoms. Bean growers were able to identify bean ascochyta, but the control measures taken were insignificant, probably due to the lack of knowledge and resources. The disease was associated with excessive rainfall and many other environmental factors, as well as poor crop management practices. Varietal preferences were based on yielding ability, early maturity, marketability, diseases and drought tolerance. Other factors that were considered important, included taste, climbing growth habit, cooking time, large seed size and seed colour. Generally, large-seeded bean varieties were the most preferred in both regions. Farmers that preferred the small-seeded bean genotypes based their preferences on the ability to resist pests and diseases and on their ability to thrive under harsh environments, such as excessive rainfall, drought and mist. However, the large-seeded climbing and red mottled kidney bean genotypes, though susceptible to ascochyta, were the most popular bean varieties grown both for consumption and for sale in the northern and eastern regions, respectively. This therefore, indicated the need to

develop bean genotypes that have the qualities of the large-seeded genotypes, but which are resistant or tolerant to, diseases such as ascochyta blight.

2.1 Introduction

Beans are produced in all regions of Rwanda. According to the Ministry of Agriculture and Animal Resources, dry bean production between 2013 and 2014 ranked fifth in terms of volume, just after bananas, Irish potatoes, sweet potatoes and cassava (MINAGRI, 2014). It is grown mainly by small-scale farmers at a subsistence level (FAOSTAT, 2013), and these farmers experience a wide range of biotic, abiotic and socio-economic constraints.

Among the biotic constraints, ascochyta blight has been cited as one of the important foliar diseases of the common bean grown in the high altitude regions of Rwanda. The disease is particularly favoured by cool temperatures and high relative humidity (ISAR, 2011). It infects all major bean parts, like leaves, stems and pods, and is seed transmitted, resulting in total crop losses, especially when infected seed is planted (Schwartz and Corrales, 1989).

Several measures directed at controlling bean ascochyta have been developed and applied, but none have provided adequate control. An integrated control strategy, employing cultural practices and fungicides, is useful but not always feasible for a smallholder farmer, due to varying growing conditions and inadequate monetary resources. The use of resistant genotypes is therefore the most viable option because it is cost-effective for the poor rural farmer in Rwanda.

The most popular and preferred bean genotypes (red and red mottled large-seeded genotypes) by both consumers and traders are susceptible to bean ascochyta (Tusiime, 2003; RAB, 2013); hence, the acreage grown to these genotypes is fast declining. Moreover, although the ascochyta disease is amongst the major diseases in Rwanda, its prevalence and impact on the yield has not been documented. Therefore, a participatory plant breeding approach would be useful to collect information on the presence of the disease and its impact on the bean crop of the farmers.

Several studies have been carried out to assess the impact of farmer involvement in breeding programmes. Conventional and centralized plant breeding programmes have been shown to have a significant impact in high input areas, but they have a low impact in the marginal and

small-scale farming sector (Morris and Bellon, 2004). Ceccarelli and Grando (2012) showed that decentralized and demand-driven research is essential, especially for the poor farmers in low input farming systems, where farmers choose the genotypes that do well under local environmental conditions. Fufa et al. (2010) emphasised the importance of decentralized participatory plant breeding for increasing and stabilizing productivity and maintaining genetic diversity. Research in the Andean region of South America has shown that certain genotypes (potatoes, maize, wheat and barley) were not accepted by farmers, due to the poor quality traits of grain or its susceptibility to disease (Danial et al., 2007). Hence, it is important to involve farmers in the early stages of the breeding process, during selection of advanced lines, rather than at the end (Danial et al., 2007).

Surveys, interviews and participatory rural appraisal (PRA) have been used to determine the farmers' preferred traits in crops. The information has successfully been used in the breeding process to develop resistance to bean fly (Ojwang et al., 2009), resistance to fusarium root rot (Mukankusi et al., 2008) and resistance to angular leaf spot (Ng'ayu-Wanjau et al., 2013) in the common beans.

The pioneering work on the participatory selection of improved bean genotypes in Rwanda showed that the faster identification of superior genotypes and their rapid adoption could be achieved through this approach (Sperling and Berkowitz, 1994; Isaac et al., 2016). Results showed that participatory selections produced up to 38% more than local mixtures (ISAR, 2011). Twenty-one varieties that are suited to a wide range of growing niches, were identified over a period of nine years (ISAR, 2011). Adoption rates of40 % for improved climbing beans were reported after eight years. Since then, the participatory approach has been embraced in other project activities (RAB, 2014), for example, in programmes to improve soil fertility (Farley, 1998). In PRA, farmers/respondents are able to conduct the analysis and make presentations, and to plan and own their outcomes (Chambers, 1993; Scoones and Thompson, 1994). The PRA also allows for direct contact between the investigator and the local people in the field.

Although there has been some success of formal breeding in alleviating the challenges of bean farmers in small-scale farming areas, the bean programmes have yet to come up with technologies that are able to meet the diverse needs. It is, therefore, imperative to orientate the research strategy, in order to come up with possible solutions and to develop sustainable bean production systems under the prevailing circumstances.

Therefore, the objectives of this study were:

1. to assess the farmers' awareness of ascochyta blight as a constraint to bean production in Rwanda;
2. to determine the factors that influence the farmers' preferences of the genotypes and criteria for selection;

3. to assess the farmers' perceptions on factors affecting the bean yield;

4. to evaluate the incidence and severity of bean ascochyta blight in the farmers' fields; and,

5. to identify the farmers' practices that are used to combat bean ascochyta blight.

2.2 Material and methods

2.2.1 Study area

The PRA was carried out in four bean-producing districts of Rwanda, namely, Rwamagana (in the Eastern Agriculture Zone Division), Kamonyi (in the Southern Agriculture Zone Division), Burera and Musanze (in the Northern Agriculture Zone Division) (Figure 2.1). Agricultural productivity in the northern highlands is the highest in the country, due to its endowment with fertile volcanic soil and a cool moist temperate climate (RAB, 2013) (Table 2.1).

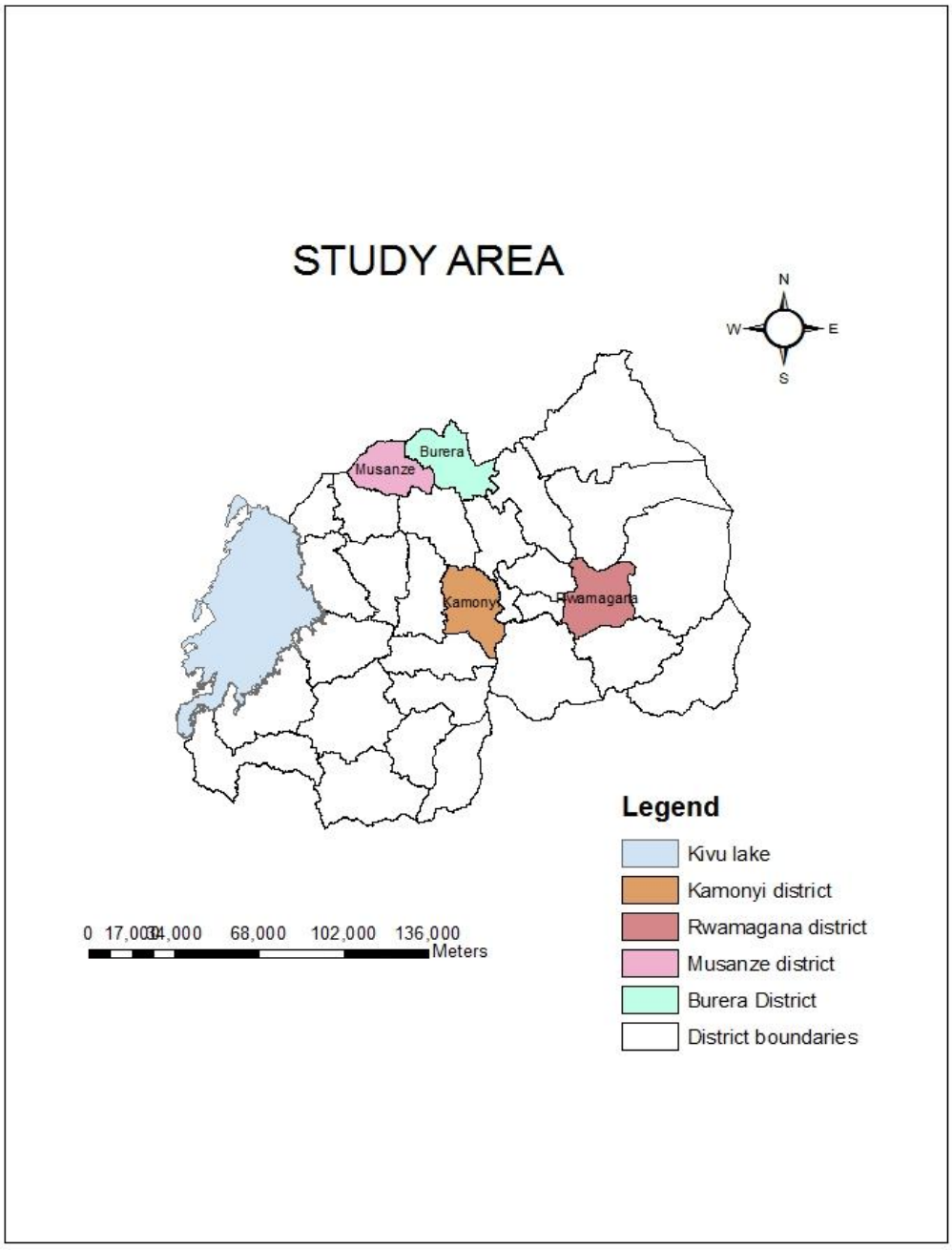

Figure 2.1: Four districts where the survey was undertaken

Table 2.1: Description of sites

District	size (km^2)	Latitudes	Longitude	Altitude (m)	Mean Temp(^oC)	Rainfall (mm/year)	Soil type
Burera	664	-1° 25' and 20.748" south of equator	29° 40' and 3.288" east	2401	18	1500	Volcanic
Musanze	530	-1°30' and 27.47" south of equator	29°36' and 23.84" east	2200	20	1400	Volcanic
Kamonyi	655	-2°0' and 18.72" south of equator	29°53' and 53.41‴ east	1500	23	900	Sandy
Rwamagana	691	-1°58' and 22.12" south of equator	30°21' and 15.41" east	1700	22	1000	Loam

Climbing beans are mainly produced in the high-altitude areas and bush beans in the lower-altitude areas (RAB, 2014)**.**

2.2.2 Data collection

Data was collected, using a structured questionnaire. Questions included information on the background of respondents, bean genotype preferences, the farmers' perceptions of ascochyta and its management and the characteristics of good bean genotypes. Fifteen questionnaires per sector were pre-tested in the Musanze District and changes were made accordingly, before conducting the survey in the four districts. Visits were organized with the help of district and sector agronomist and government extension workers in the different districts. Secondary data on bean production and district data (climate, administration, etc.) were obtained from the district agronomist offices, the Ministry of Agriculture, the Rwanda Agriculture Board, non-governmental organisations (NGOs), as well as from literature.

Two enumerators were selected from each district, to help gather information, using the questionnaires. These enumerators were government agricultural extension workers. Before

conducting the survey, all enumerators were trained on the objective of the survey and on how to conduct an effective interview. Thirty-one bean farmers per district were interviewed, giving a total of 124 respondents for the whole survey. The respondents were selected by using a random systematic technique and an accidental sampling technique, that is, the fourth household on a particular selected footpath, or the owner of a bean field with ascochyta symptoms, were selected. Interviews were carried out if the respondent was a regular bean grower and had a bean field at the time. The questionnaire involved open-ended questions that allowed the farmers to express themselves, in order to gain as much information as possible.

To assess the incidence and severity of ascochyta blight, observations were made in the farmer's fields in the four districts, over two seasons. Fifteen bean fields per district per season were assessed, resulting in a total of 120 fields for the whole assessment. The bean fields were selected by using a non-random systematic technique, that is, the household on a particular selected footpath, or the owner of a bean field with symptoms of ascochyta, was selected. The ascochyta ratings in the field were made during the podding stage, using a one-square metre frame. This means that the plant population was determined over a one square metre area at three locations in the field, and observations were made on the leaves and pods. In addition, the general appearance of the bean field was noted. The incidence of ascochyta was scored as the average percentage in bean fields that had plants infected with ascochyta. The severity of ascochyta was scored as the average percentage of the ratings at the three locations per visited field.

2.2.3 Data analysis

Data was analysed, using the Statistical Package for the Social Sciences (SPSS) Version 16.0 statistical software. Disease data were analysed, using the Genstat computer package (Trust, 2007).

2.3 Results

2.3.1 Bean production constraints and cropping system

Several factors in this survey were considered by farmers as major constraints to bean production and are listed, by district, in Table 2.2. In general, farmers had similar ($P \leq 0.05$)

perceptions about the importance of diseases, the high cost of input, the lack of improved seed and poor soil, with respect to bean production. However, they had different ($P \leq 0.01$) perceptions about the importance of other factors on bean production across the four districts. Diseases were the most important constraint to bean production in Burera and Musanze with means of 88.1%, and ranging between 79.9-95.8%; while in Rwamagana, pests such as the beanfly, cutworms, bruchids/bean weevils, and aphids, were said to be most prevalent. The high cost of input and the lack of improved bean seed were considered as major constraints in the Kamonyi and Rwamagana Districts. Soil erosion was considered a problem in Burera and Musanze, due to the heavy rains on steep mountain slopes, which leads to shallow soils. Infertile soil was most mentioned in Kamonyi and Rwamagana, compared to Burera and Musanze.

The lack of stakes was the main problem in Musanze and Burera, where climbing beans are more popular than in the eastern and southern regions. Bean price fluctuation was considered a problem in the Burera District, probably because this district is near to Uganda and the price of beans is also affected also by the Ugandan market.

Excessive rainfall was considered to be a major constraint for bean production in Musanze and Burera, and was said to escalate the ascochyta problem, while drought was a major constraint in Kamonyi, compared to the other districts. This could well have been because Kamonyi had received less rainfall in the previous seasons, compared to the other districts.

Table 2.2: Percentage (%) of farmers/respondents mentioning different constraints to bean production in four districts of Rwanda (2014)

Constraints	District				Overall mean	P value
	Burera	Kamonyi	Musanze	Rwamagana		
Diseases	91.2	85.4	95.8	79.9	88.1	0.12
Pest	68.3	79.7	71.2	80.2	74.8	0.00
Drought	34.6	98.0	28.0	93.2	63.4	0.00
High cost of input	56.2	63.2	62.3	61.6	60.8	0.09
Lack of improved seed	57.8	61.3	57.9	60.1	59.2	0.08
Soil erosion	65.2	31.8	61.1	34.2	48.1	0.00
Poor / Infertile soil	22.5	34.1	18.5	29.1	26.1	0.11
Lack of stakes	26.7	10.4	34.3	15.0	21.6	0.00
Market price fluctuation	22.1	18.0	14.6	19.6	18.5	0.00
Excessive rainfall	17.0	3.0	13.1	5.6	9.6	0.00

The farmers produce beans during both the long and short rainy seasons. The short rains occur from February to May, while the short rains occur from September to December. The best yield is obtained during the short rainy season, rather than during the long rains. The main activity during the bean season is weeding, which is carried out twice, just before flowering and after pod set. Only 4.3% of farmers reported that they use agro-chemicals to control insect-pests and diseases. Most of these farmers are commercial seed multipliers and bean production cooperatives. The remaining 95.7% of farmers depended on good agronomic practices, such as weeding and the clearing of plant debris, during the production season. A total of 37.9% of farmers reported that they did not use any fertilizers. However, 42.8% used organic manure, while 19.3% reported that they used both organic and mineral fertilizers.

The farmers grow beans on small land holdings and 53.2% of the interviewed farmers planted beans on less than 0.5 ha.

Figure 2.2: Bean intercropping with maize and bananas

The beans were planted as an intercrop by 81.2% of the farmers and as a pure stand by 18.8% of the farmers. The farmers intercropped beans with several other crops, including maize (35.5%), bananas (25.6%), cassava (11.3%), potatoes (4.1%), sorghum (2.4%) and coffee (1.3%) (Figure 2.2 and 2.3).

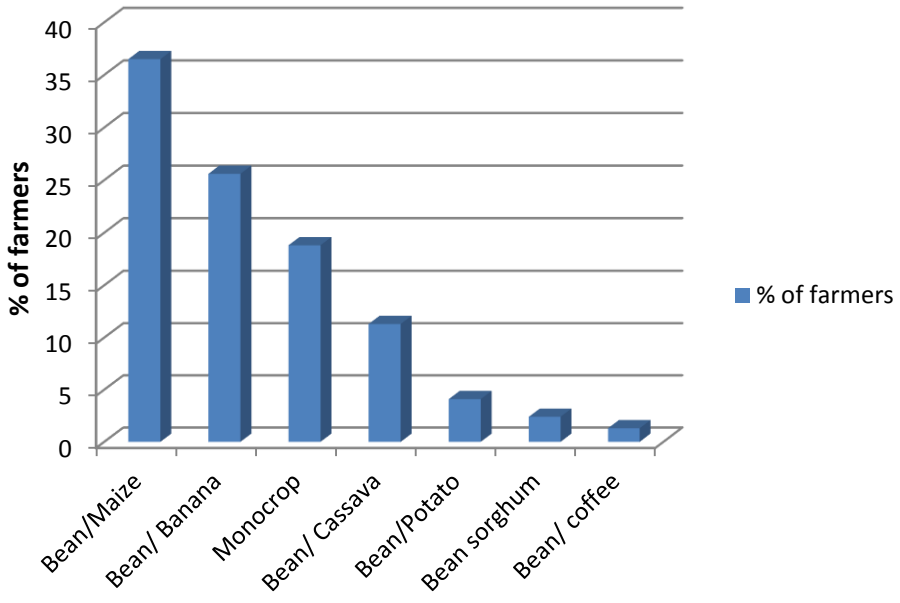

Figure 2.3: Bean cropping systems

2.3.2 Farmers' knowledge and perceptions of bean diseases

Farmers in Rwanda found it difficult to differentiate between diseases and pests. During the survey, they mentioned moles, rats, aphids and beanfly, and even weeds, as the diseases of beans. Farmers described diseases, based on their effects on the plant (symptoms) and associated them with environmental factors. The disease symptoms that were mentioned included Kabore/Imvura (ascochyta) *Kurisuka* (root rot), *Kirabiranya* (wilting or drying up), Imfunyarazi (probably viruses), *Ibenja* (probably angular leaf spot), *Kubabuka kw'amababi/ Kuboza imiteja* (probably anthracnose) and Muhondo/Umugese (rust). They associated ascochyta with poor soil, the over-use of land, over-cultivation and too much rainfall (Table 2.3). Similarly, all other diseases were mainly associated with heavy rainfall, drought and poor soil (Table 2.3).

Table 2.3: Farmers' perceptions of bean diseases and their influencing factors in Rwanda.

	Diseases	Influencing factors
1	Ascochyta blight (Kabore/ Imvura	Poor soil, over cultivation, severe drought, excessive rainfall, and late planting
2	Root rot (Kurisuka)	Poor soil, over-cultivation, severe drought, and excessive rainfall
3	Wilting or drying up (Kirabiranya)	Poor soil and drought
4	Viruses (Imfunyarazi)	Excessive rainfall, mist, poor soil, and weeds
5	Angular leave spot (Ibenja)	Poor soil, over cultivation, severe drought, excessive rainfall, and late planting
6	Anthracnose (Kubabuka kw'amababi kuboza imiteja)	Poor soil, over cultivation, excessive rainfall, and late planting
7	Rouille (Muhondo/ Umugese)	Poor soil, severe drought, excessive rainfall, and late planting

2.3.3 Farmers' awareness of bean ascochyta blight

Bean ascochyta was recognized by all the interviewed farmers in the Burera and Musanze Districts, while 51.8% and 61.4%, respectively, recognized the disease in Rwamagana and Kamonyi (Figure 2.4).

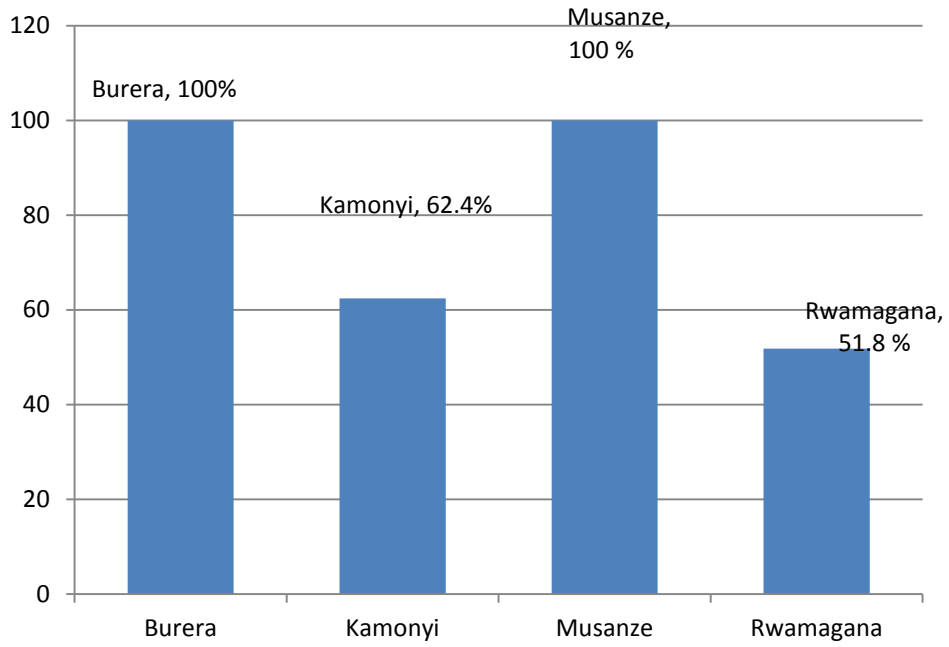

Figure 2.4: Percentage of bean farmers who could recognize bean ascochyta in Burera, Kamonyi, Musanze and Rwamagana

Bean ascochyta blight was not considered to be as important in eastern and southern Rwanda, compared to northern Rwanda, that is, 37% of the respondents in Rwamagana and 54% in Kamonyi considered ascochyta to be important, compared to 94% in Musanze and 92% in Burera (Figure 2.5). In Musanze and Burera, it was ranked as the highest cause of bean yield losses.

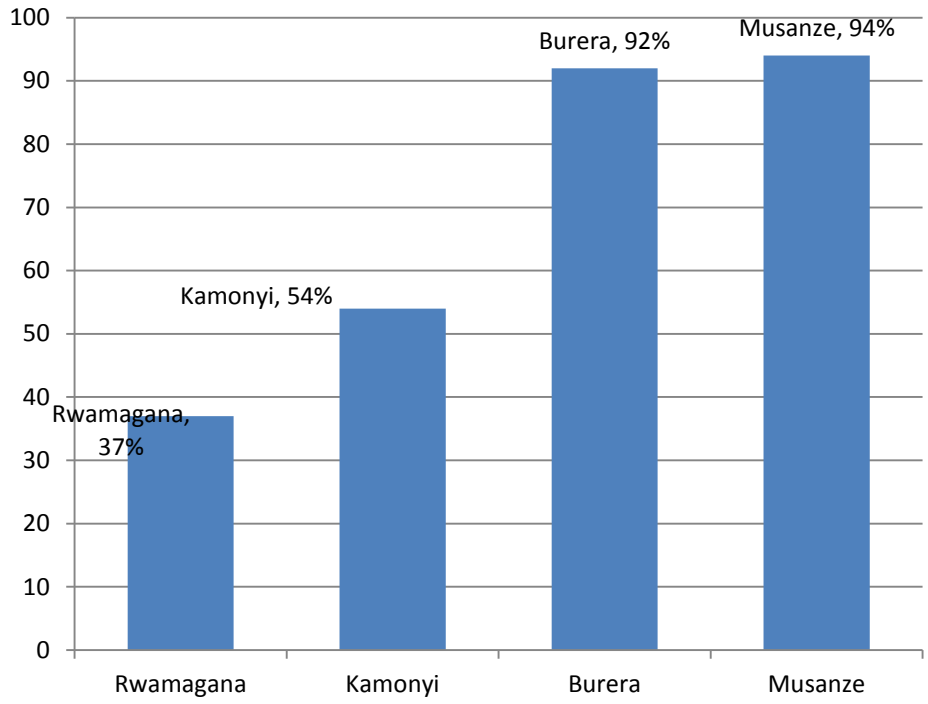

Figure 2.5: Percentage of farmers who considered bean ascochyta to be important to bean production in Burera, Musanze, Kamonyi and Rwamagana districts

2.3.4 Farmers' perceptions of the symptoms of bean ascochyta blight

Bean ascochyta was mainly observed before flowering, that is, at the 3-4 leaf stage. Farmers recognized bean ascochyta blight, based on several symptoms, including black spots on leaves, petiole drop, the drop of stem nodes, the drop of peduncles and pods.

Of the symptoms mentioned, the blackening of the nodes and premature defoliation were the main symptoms that farmers associated with ascochyta, followed by the drying-up of the whole plant (Table 2.4). Bean ascochyta symptoms were said to be most severe during the rainy season and in places where the soil was considered infertile.

Table 2.4: Different symptoms of bean ascochyta blight mentioned by respondents over four districts (Burera, Kamonyi, Musanze and Rwamagana) in Rwanda

Symptom	% Respondents
Plant blackening of nodes	78.3
Premature defoliation	64.1
Drying-up of the whole plant	46.3
Black spots on leave	44.6
Petiole drop	12.3
Drop of stem nodes	8.8
Drop of peduncle	3.4
Drop of nodes	1.2

2.3.5 Farmers' perceptions of the factors causing bean ascochyta blight

The factors that farmers associated with the cause of ascochyta blight were similar to the ones mentioned for bean diseases as a whole. However, in the case of bean ascochyta, excessive rainfall was considered the major predisposing factor, while poor soils were considered most important for all diseases. In addition, lack of crop rotation was considered to be a major factor in predisposing beans to ascochyta, especially in northern Rwanda (Table 2.5), while poor soil was ranked as the second and third most important factor that predisposes beans to ascochyta in southern and eastern Rwanda, respectively. A few farmers said ascochyta in beans is caused by factors like weeds, intercropping, the lack of a resistant variety and debris.

The other factors mentioned included poor soil drainage and shallow soils caused by soil erosion, because most bean fields are on slopes in northern Rwanda and these fields are generally over-cultivated as result, due to overpopulation.

Table 2.5: Mean percentage of farmers in the four districts of Rwanda (Burera, Kamonyi, Musanze and Rwamagana) mentioning different factors that influence the occurrence and severity of bean ascochyta

Cause	% Respondents				
	Burera	**Kamonyi**	**Musanze**	**Rwamagana**	**Mean**
Excessive rain	92.2	47.6	93.8	56.4	72.5
Poor soil	14.3	32.4	12.2	20.5	19.9
Lack of crop rotation	37.9	10.5	26.1	3.6	19.5
Weeds	3.2	4.2	5.0	4.0	4.1
Intercropping	4.0	3.1	3.0	3.0	3.3
Lack of resistant varieties	6.1	3.0	4.4	3.0	4.1
Bean debris	3.9	0.0	4.1	0.0	2.0
Water stagnation	3.4	0.0	3.0	0.0	1.6

2.3.6 Farmers' practices in combating bean ascochyta blight

Most farmers, especially those in Kamonyi and Rwamagana, did nothing once the disease manifested itself. However, crop rotation was the main control practice used in all of the surveyed districts. Roguing was the main control practice in southern and eastern Rwanda. Other control measures included planting mixed bean varieties, and planting mature seed, mainly in northern Rwanda, while weeding was mentioned in Kamonyi and Rwamagana only (Table 2.6). Spraying with chemicals (only mentioned in Burera and Musanze), timely planting, good quality seed, soil conservation using drainage trenches, fallowing, intercropping, planting improved genotypes and burying infected plants, were other control measures that were mentioned.

Table 2.6; Percentage of farmers mentioning different control measures for bean ascochyta in four districts of Rwanda

Control measure	% Respondents				
	Burera	**Kamonyi**	**Musanze**	**Rwamagana**	**Mean**
Nothing	8.7	54.2	12.1	39.4	28.6
Farmyard manure	5.3	3.2	4.6	3.5	4.2
Roguing	13.4	30.4	7.6	18.5	17.5
Crop rotation	70.3	25.0	64.3	19.4	44.8
Mixtures of varieties	20.1	37.9	17.5	29.5	26.3
Improved varieties	33.1	8.4	40.8	10.6	23.2
Chemicals	29.2	0.0	23.7	0.0	13.2
Fallowing	5.4	0.0	3.4	0.0	2.2
Weeding	0.0	4.2	0.0	3.2	1.9
Timely planting	0.0	3.2	0.0	3.7	1.7

2.3.7 Sources of seed and varietal preferences by farmers

During the interview, the farmers stressed the importance of good quality seed and their contribution to the yield. The farmers had different ways of sourcing their common bean seeds for planting. About 68.1% of the farmers mentioned that their main source of seed is retained seed. Those who are not self-sufficient in supplying their own seed, source it from their neighbouring farmers (3.2%), the local market (11.2%), seed traders (seed merchants) (1.2%) and non-government organizations (7.6%). Almost 8.7% of the farmers have a direct link with the research stations, where researchers use their fields for multi-location trials (Figure 2:6).

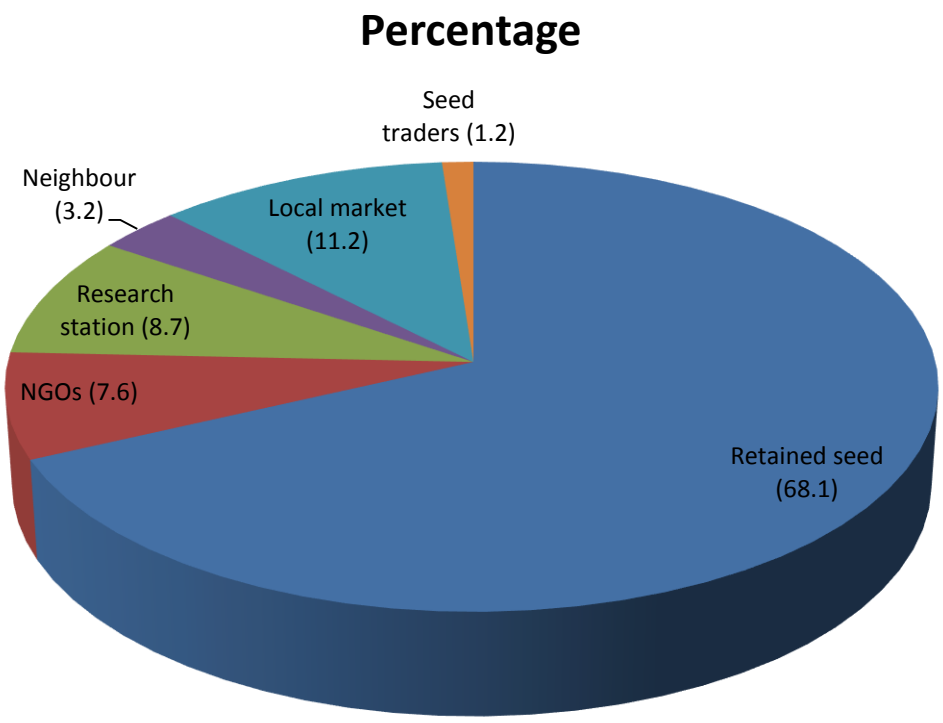

Figure 2.6: Sources of the common bean seed (%)

Farmers considered several factors when choosing bean genotypes, with the yield being the most important factor, followed by early maturity, marketability, disease resistance, taste and drought tolerance (Figure 2.7). Other factors that were considered included climbing growth habits, short cooking duration, seed size, especially large seed-sized genotypes, seed color (brown, red or white) storability and resistance to excessive rainfall.

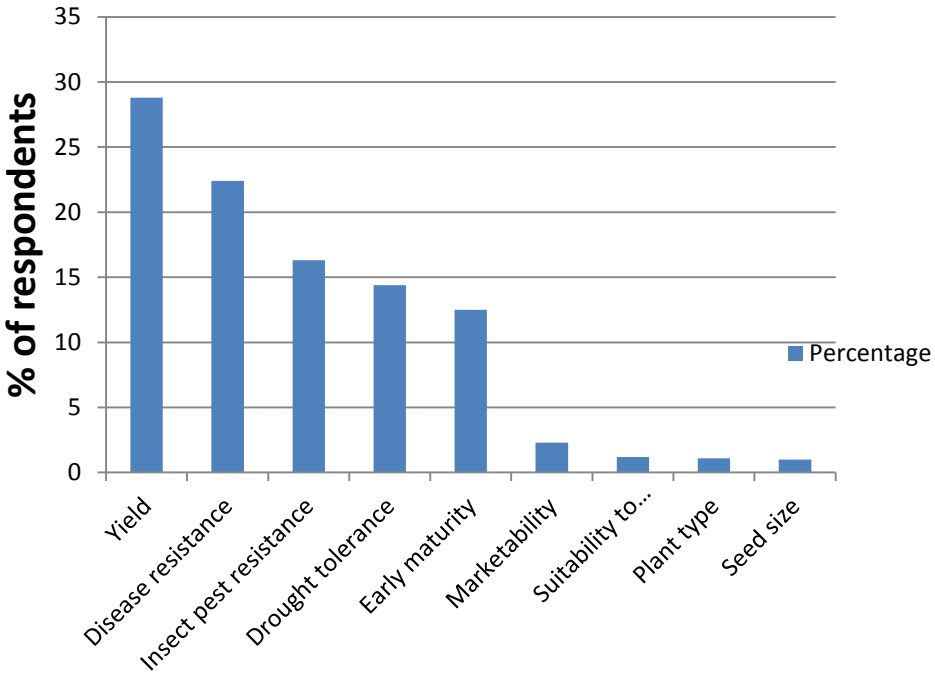

Figure 2.7: Criteria used by farmers for variety choice

Generally, large-seeded climbing bean varieties were the most preferred in eastern and northern Rwanda. The percentage of farmers preferring small-seed genotypes was greater in southern Rwanda. This could probably be related to the reports of farmers, stating that the small-seeded varieties are resistant to pests, diseases and drought.

Farmers mentioned various reasons as to why they preferred the large-seeded genotypes. Their reasons ranged from the ability of large-seeded beans to give higher yields, their preference on the market, a better taste/texture when eaten and a good appearance, especially for farmers who market the beans.

The farmers who preferred the small-seeded bean v genotypes, based their preference on their ability to resist pests and diseases and to thrive under harsh environments, such as excessive rainfall, drought and mist, when compared to the large-seeded genotypes.

Yield was also mentioned because most of the small-seeded genotypes are very high-yielding and hence ensured food security. Most farmers grew small-seeded varieties for consumption and they rarely, if at all, marketed them.

2.3.1 Incidence and severity of bean ascochyta blight in the farmers' fields

Based on the visual symptoms in those bean fields that were visited, as a whole, there were significant differences (P≤0.01) (Table 2.7) between the districts and seasons, regarding the incidence and severity of bean ascochyta blight.

Figure 2.8: A farmers' bean field in Musanze, showing symptoms of ascochyta blight

Generally, the incidence of bean ascochyta blight was highest in Musanze and Burera where all the bean fields in some villages had ascochyta symptoms (Figure 2.8). The bean fields visited in Rwamagana and Kamonyi did not have such a high incidence of ascochyta.

Table 2.7: Incidence and severity of bean ascochyta in bean fields in the Burera, Kamonyi, Musanze and Rwamagana Districts of Rwanda.

District	Season	(% Incidence	% Severity
Burera	2014B	88	29.3
	2015 A	79	21.7
Kamonyi	2014B	45	19.0
	2015 A	32	14.4
Musanze	2014B	98	32.1
	2015 A	92	30.1
Rwamagana	2014B	56	20.8
	2015 A	54	18.4
Mean		68	23.2
s.e.d ($P \leq 0.001$)			6.44

S.e.d=standards error deviation

Ascochyta severity ranged between 14.4% and 32.1%, based on the observations of the leaf and pod symptoms on the plants. Bean fields were more infected during Season B than during Season A. Although the ascochyta infection was high in the highlands of northern Rwanda, this disease has also been observed in the mid- and lowlands (Rwamagana and Kamonyi).

2.4 Discussion

The participatory study helped in elucidating the farmers' perceptions of various issues related to bean ascochyta blight that will guide future breeding programmes, by solving real problems. This study was carried out mainly to determine the need for new varieties, with improved resistance to bean ascochyta, which is one of a complex of fungal pathogens that causes foliar diseases in beans. The study established the key factors limiting bean production in Rwanda and assessed the farmers' knowledge, management and perceptions of ascochyta blight and their preferences in bean genotypes. It also evaluated the level of bean ascochyta infection on the farmers' fields. The major characteristics of beans that farmers considered, when adopting a new bean genotypes, were also identified.

The study showed that bean ascochyta was recognized by farmers as a constraint to bean production, especially in northern Rwanda. Resistance to diseases, as well as seed quality traits, especially the large seed size and the light seed color, were seen as the major traits that need intervention by breeders.

The study revealed that 45%-100% of the visited bean fields were infected with bean ascochyta. Generally, the incidence and severity of bean ascochyta blight was highest in the highlands (Musanze and Burera), where all the bean fields in some villages had ascochyta symptoms. Bean fields are more infected during Season B than during Season A. Although the ascochyta infection is said to be present in the cool highland region, this disease has also been observed in the warm mid- and low-land (Rwamagana and Kamonyi) regions. This shows how important it is for breeders to focus their attention on this disease.

The disease was easily recognized by farmers in Burera and Musanze Districts in the northern highlands, where it was associated with low bean production. In these districts it is referred to as "Kabore". The factors which farmers associated with the causes of bean ascochyta were similar to those mentioned for all other bean diseases. They tended to consider the general appearance of the whole bean plant and not the specific disease that is attacking a particular plant part. This is important for breeders to consider, as they usually target specific diseases and may be misled by the farmers' responses. Excessive rain, poor soil fertility, as well as a lack of crop rotation, were the major factors predisposing beans to ascochyta, while other factors, such as water stagnation, weeds, the lack of intercropping, the lack of fertilizer and farmyard manure and over cultivation, were all soil-related. This indicates that poor soil fertility and soil

sanitation were the major causes. However, even though farmers were able to observe the causes of ascochyta, they were not able to explain the reasons for it. For instance, farmers who associated bean ascochyta with excessive rain could not explain why it was also observed during periods of drought. Bean ascochyta is associated with the intensification of agriculture, which has resulted from the increasing human population. The high population density of the highland regions of Rwanda has led to land fragmentation, and hence a lack of crop rotation, resulting in a decline in soil fertility (ISAR, 2011). This has created a situation where there is an imbalance between the beneficial and disease, causing organisms in the debris, and hence an increase in ascochyta inoculum levels (RAB, 2013). It was evident that farmers did not have a clear understanding of the organism that causes bean ascochyta. Even though some mentioned excessive rainfall, it was probable that they were referring to other fungal diseases. The idea of an airborne pathogen was poorly understood.

Most of the control measures that farmers used to manage bean ascochyta were directed at cultural management. Crop rotation was a major control measure for the disease. Roguing was a routine measure for any damaged plants and was the main disease control measure, especially in southern Rwanda. Farmers mentioned the use of improved genotypes to control bean ascochyta in the Burera and Musanze Districts; however, their use was not very evident, as most farmers still grew the old bean genotypes which were susceptible to bean ascochyta.

The bean genotype preference was generally based on high yield, early maturity period, resistance to pests and diseases, drought tolerance, seed size, taste, cooking time and seed colour. Farmers associated susceptibility to ascochyta with the large seed size and the bush growth habit. Even though the large-seeded bean genotypes were the most preferred bean seed types, farmers were slowly abandoning them, in preference of the small-seeded types, due to their susceptibility to many diseases. This was most evident in southern Rwanda, where a good percentage of farmers said that they preferred growing a mixture of small-seeded genotypes, rather than the large-seeded genotypes. Small-seeded genotypes were said to be resistant to excessive rainfall, drought and diseases. The lack of resistance to ascochyta over the years may well be due to the breeding efforts and management practices concentrating on other factors, such as seed size and growth habits, rather than pest and disease resistance. With regard to the growth habits, climbing beans are said to be more resistant to ascochyta, compared to the bush type beans (Opio et al., 2001). In addition, they are generally higher-yielding (2500-4000 kg ha^{-1}) than bush beans (RAB, 2014). In some bean production areas (for example, the highlands), bush beans have been completely abandoned. However, the

production of climbing beans is hindered by the need for stakes, which are difficult to obtain. Wooden stakes are the common type of stake used by all farmers, but the disadvantage of these is that they are damaged by termites over time, hence complicating the situation and increasing the expenses of growing climbing beans. This, therefore, indicates a need for non-wooden stakes for beans, as well as an opportunity for their production.

This study was able to obtain important information to help guide interventions aimed at controlling bean ascochyta, or other bean diseases, on farmers' fields. The need to involve farmers in all the steps of developing new genotypes, was highlighted. Such genotypes would be met with less rejection, than are the unfamiliar genotypes that are bred elsewhere and then introduced, without considering the needs and preferences of the farming community. The importance of bean ascochyta as a major constraint to bean production was highlighted; hence there is an urgency to provide these farmers with a bean genotype that is resistant to this disease, as well as one that can easily be adopted, to control it.

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Chapter Three: Yield loss assessment in the common bean (*Phaseolus vulgaris* L.), due to ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema].

Abstract

Ascochyta blight (*Phoma exigua var. diversispora (Bubak)* Boerema causes a severe, rapidly-developing disease in common beans *(Phaseolus vulgaris* L.), that can bring about complete plant defoliation and extensive yield loss. Studies were conducted in Rwanda on 64 common bean genotypes including bush and climbing types, to quantify the yield loss attributed to bean ascochyta blight. Using a split plot design, trials were conducted at three locations, where ascochyta blight is prevalent. The different genotypes used had variable levels of susceptibility and were compared with resistant genotypes ICTA Hunapu and ASC 87, for the bush type, and G 35034 G 35306, for climber type. The results obtained showed that most market class genotypes recorded higher disease severity and greater yield losses than the controls. There was a strong positive correlation between the Relative Area Under Disease Progress Curve (RAUDPC) values and yield losses. It was also established that the yield of a susceptible genotype is reduced by about 75.7%, as a result of ascochyta infection. Pod infection had a direct effect on seed yield for both the bush and climber genotypes. However, the bush genotypes showed a larger reduction in yield, compared to climbers. The study suggests that the use of desirable resistant genotypes are the best way of reducing yield losses caused by ascochyta blight.

3.1 Introduction

Ascochyta blight, caused by *Phoma exigua var. diversispora (Bubak) Boerema*, is a highly destructive disease of the common bean in cool and wet bean production regions (Allen et al., 1996). It causes a severe, rapidly-developing disease that can bring about complete plant defoliation and extensive yield loss, if plants become diseased prior to, and/or during, pod filling (Campbell and Madden, 1990).

The disease can easily be identified by its symptoms, which initially appear on the leaves as circular dark gray lesions, and when they enlarge, they become like a set of concentric rings 1-3 cm in diameter on both the leaves and pods, which can lead to collapsed and black nodes, petioles and stems (Boerema et al., 1981). The disease is spread in cool and humid bean-growing regions and often causes severe damage, which affects the yield, the seed quality, and the marketability of beans. It is one of the most important bean diseases in Rwanda and yield losses are extensive, especially when infected seeds are used (ISAR, 1985).

Ascochyta blight occurs mostly in the high altitude, low temperature areas of Rwanda (ISAR, 2011), where the disease causes severe losses because of the favourable climatic conditions that enhance its development.

Being a seed-borne disease, ascochyta blight is easily spread, as farmers depend highly on farm-saved seed and the exchange of seed is common (ISAR, 2011). With the exchange of farm-saved seed between farmers, there is an increased chance of disease transmission between the farmers' fields, and between different agro-ecological zones where the beans are grown. It has been observed that ascochyta blight develops and spreads rapidly during the rainy season and when the temperatures are low (Allen et al., 1996; Bailey, 2011). Significant disease development and yield losses occur if these weather conditions are prolonged during the pod formation and pod filling stages of the crop (Schwartz et al., 1981; Corrales and van Schoonhoven, 1987; Beebe et al., 1991). Yield loss is due to premature defoliation and plant death, shrunken seed and an increase in the number of seeds that have diseased lesions on the seed coat (Seijas et al., 1985; Da Silva et al., 2003). Such beans have a poor appearance and are not popular with consumers, which results in both reduced marketability and income. It has been observed that, for many fungal diseases, a high lesion density is associated with the premature yellowing and defoliation of leaves (Hall, 1991; Wortmann et al., 1998), resulting in lower yields. The extent of the yield loss depends on the climatic conditions and the crop growth

stage at which the disease starts (van Schoonhoven, 1991; Hartung and Piepho, 2007;). In areas where the disease is endemic, yield losses have been known to be very high, especially in susceptible genotypes (van Schoonhoven, 1991). A study conducted by Schwartz et al (1993) showed that the yield loss due to ascochyta can approach to 43%. The extent of the economic losses caused to the different Rwandan market class genotypes, is not known. However, before developing and/or implementing any research interventions against any pathogen or disease, it is necessary to quantify the extent and nature of damage attributed to that disease. It is for this reason, that a study was conducted to quantify yield loss, due to ascochyta blight, for common beans in Rwanda.

3.2 Materials and methods

3.2.1 Study area

The study was conducted at three research stations, namely, Rwerere, Musanze and Kitabi. Rwerere is in the Burera District, located in north-eastern Rwanda at 2060–2312 m above sea level (masl), at a longitude of 29° 19’ East and a latitude of 1° 36’ South, with an annual rainfall and temperature of 1200 mm and 20ºC, respectively. Musanze represents the highlands and has volcanic soils. It is located at an altitude of 2200 (masl), with a longitude of 29° 38’ East and a latitude 1° 30’ South (RAB, 2014). The annual temperature and rainfall averages are 16ºC and 1480 mm, respectively. The Kitabi Research Station is in the Nyamagabe District, with an altitude of 1600–2800 masl, a longitude of 29° 33’ East and a latitude of 1° 33’ South, and with an annual rainfall and temperature of 1600 mm and 19ºC, respectively. Climatically, all research stations are in bean-growing areas and ascochyta blight epidemics are common because of the moderate temperature and high moisture conditions (RAB, 2013), both of which favour the development of ascochyta blight. Rainfall is bimodally distributed, with the short rains from March-May and the long heavy rains from September-December. The main occupation of the people are agriculture-related activities and it is estimated that over 90% of the population is engaged in agriculture (ISAR, 2011). Most of the crops produced are consumed at household level and only the surplus is marketed (RAB, 2013). Of the crops produced, common beans rank among the most important food and cash crops within these regions (MINAGRI, 2014).

3.2.2 Field experimental design and treatments

The study used 64 bean genotypes. These genotypes have been selected based on their level of susceptibility as revealed in the previous ascochyta screening trials. The details of the germplasm are described in Table 3.1.

Table 3.1: List of 64 genotypes used in the study

Genotypes	Origin	Growth habit	Seed size	Genotypes	Origin	Growth habit	Seed size
Agronome	Rwanda	Climber	Large	ALB 102	Rwanda	Bush	Large
CAB 2	Rwanda	Climber	Large	ALB 155	Rwanda	Bush	Medium
Claudine	Rwanda	Climber	Medium	ALB 58	Rwanda	Bush	Large
G 2331	Rwanda	Climber	Large	ASC 87	CIAT Col	Bush	Small
G 2333	Rwanda	Climber	small	CAL 96	Rwanda	Bush	Large
G 35034	CIAT Col	Climber	Medium	CMS 17	Rwanda	Bush	Medium
G 35306	CIAT Col	Climber	Medium	ECAB 026	Rwanda	Bush	Large
Garukurare	Rwanda	Climber	Small	ICTA Hunapu	CIAT Col	Bush	Small
Gasilida	Rwanda	Climber	Large	Maharagesoja	Rwanda	Bush	Small
Gitanga	Rwanda	Climber	Medium	NUA 377	Rwanda	Bush	Medium
Ibanga 2	Rwanda	Climber	Medium	NUA 379	Rwanda	Bush	Medium
Kenyerumpure	Rwanda	Climber	Medium	NUA 397	Rwanda	Bush	Medium
Kigondo	Rwanda	Climber	Medium	NUA 566	Rwanda	Bush	Medium
Kivuzo	Rwanda	Climber	Large	RWR 1180	Rwanda	Bush	Large
MAC 44	Rwanda	Climber	Large	RWR 1668	Rwanda	Bush	Large
MAC 49	Rwanda	Climber	Large	RWR 2154	Rwanda	Bush	Large
MBC 12	Rwanda	Climber	Medium	RWR 2245	Rwanda	Bush	Large
Nyamanza	Rwanda	Climber	Small	RWR 229	Rwanda	Bush	Large
Nyaragikoti	Rwanda	Climber	Large	RWR 278	Rwanda	Bush	Large
Nyirabukara	Rwanda	Climber	Small	RWR 281	Rwanda	Bush	Large
Nyiramagorori	Rwanda	Climber	Small	RWR 3033	Rwanda	Bush	Large
Rwibarura 2	Rwanda	Climber	Large	RWR 310	Rwanda	Bush	Large
RWV 1129	Rwanda	Climber	Large	RWR 3194	Rwanda	Bush	Large
RWV 1348	Rwanda	Climber	Large	RWR 3228	Rwanda	Bush	Large
RWV 2070	Rwanda	Climber	Large	RWR 3332	Rwanda	Bush	Large
RWV 2872	Rwanda	Climber	Large	RWR 3338	Rwanda	Bush	Large
RWV 2887	Rwanda	Climber	Large	RWR 390	Rwanda	Bush	Large

Genotypes	Origin	Growth habit	Seed size	Genotypes	Origin	Growth habit	Seed size
RWV 3006	Rwanda	Climber	Large	SER 16	Rwanda	Bush	Small
RWV 3316	Rwanda	Climber	Large	SER 83	Rwanda	Bush	Medium
RWV 3317	Rwanda	Climber	Large	SER 96	Rwanda	Bush	Large
UCB 82013	Rwanda	Climber	Medium	SMC 18	Rwanda	Bush	Medium
Vuninkingi	Rwanda	Climber	Small	SMC 21	Rwanda	Bush	Medium

Large: weight of 100 seeds > 40g; Medium: weight of 100 seeds 25-40g; small: weight of 100 <25g.

Two trials were conducted, one for bush (Types I, II and III), and another for climbers (Type IV). The experiments were planted in March 2014 at three sites. Each main plot was subjected to either a Benlate fungicide spray, at a rate of 2.5 g per l of water, or it was inoculated with ascochyta infected debris.

Figure 3.1: Plot of beans treated against ascochyta infection at the Kitabi site

The experiment was arranged in a split plot design, with three replicates. The main plots were those with either fungicide application or ascochyta debris inoculation, while the subplots were the genotypes. Each subplot measured 2 x 2 m and consisted of four rows of beans planted at a spacing of 0.5 m between the rows and 0.2 m within the rows for bush, and 0.6 m between row and 0.3 m within row for climbers.

The inoculation was done 15 days after planting by spreading the infected debris uniformly in the bean field. The debris was collected from the same region in the previous season. In the fungicide-protected plots, fungicide application started one week after planting and continued at two-week intervals, until physiological maturity.

Figure 3.2: Plot of beans inoculated with ascochyta debris at the Rwerere site

Before planting, 0.24 kg of NPK (17 % 1:1:1) fertilizer was applied to each plot. The plots were kept weed-free by regular hand-hoeing.

Data were collected from the two inner rows 15 days after planting. Disease severity, defined as the percentage of the plants showing necrosis of the surface area (leaves and pods), caused by ascochyta, was recorded at two-week intervals.

Plots were harvested by hand after physiological maturity and total grain weight, moisture percentage and weight of the two inner rows were recorded. Plot grain yield was converted to kg ha^{-1} at a 14.5% grain moisture basis. The yield loss was determined by using the equation below:

Yield loss (%) is calculated as {[1-(yield of infected plot/yield of protected plot] x 100} (Abadio et al., 2012).

Equation…… 1

Data were analysed using the analysis of variance and the the least significance difference (P≤ 0.05) statistic, using the correct error term in split plot analysis. The least significance difference was used to compare the mean grain yield of inoculated versus non-inoculated plots of each genotype, as well as the ascochyta severity ratings of the inoculated plots of each genotype.

The severity was calculated as a mean percentage of the blighted foliar area per plot at each rating. Evaluation continued until susceptible genotypes reached 90-100% of the leaf blight assessments. The area under the disease progress curve AUDPC (Campbell and Madden 1990) was calculated within a single experiment (Bradshaw, 2007), following this formula:

$$AUDPC = \sum_{i=1}^{n} [(X_{i+1} + X_i)/2] * \ [t_{i+1} - t_i\ ]$$

Equation ……..2

Where X_i is the percentage necrosis at each at i^{th} the evaluation day, t_{i+1} - t_i is Times (in days) between two diseases scores and n is the total number of observations. Means were separated by the least significant difference at P≤ 0.05.

The estimate of AUDPC was normalized and RAUDPC (%) was used in the analysis of variance. The RAUDPC was calculated, using the following formula:

$$RAUDPC = \frac{\sum(T_{i+1}) * \left(\frac{D_{i+1} + D_i}{2}\right)}{T_{total} * 100}$$

Equation…….3

In Equation 3, Ti is the i^{th} day when an estimation of percentage foliar late blight is made and Di is the estimated percentage of area with blighted foliage at Ti. T_{total} is the number of days at which the final assessment was recorded. The correlations between RAUDPC, yield, mean RAUDPC and mean yield for Seasons A and B were determined. The RAUDPC was used to evaluate and select genotypes with low ascochyta infection, which can then be used in the breeding experiment.

Linear regression was used to determine the relationship between the percentage loss in grain and ascochyta severity.

3.3 Results

3.3.1 Ascochyta severity and RAUDPC on bean genotypes

Results of ascochyta severity for pod filling, to the end of seed development stage (R6), are presented in Table 3.2. Significant differences in ascochyta severity are indicated between the sites (P≤0.01) and the bean genotypes (P≤0.01) for both bush and climbers.

A significance difference (P≤0.01) is also seen for site and the genotype for the relative area under the disease progress curve (RAUDPC) for both the climber and the bush type (Table 3.2). The two-way interaction between the site and the genotype was also significant for the climbers (P≤0.05).

Table 3.2: Mean square on ascochyta severity and RAUDPC of bean genotypes tested at three locations in Rwanda

		Mean square			
Source	**DF**	**Severity**		**RAUDPC**	
		Bush	**Climber**	**Bush**	**Climber**
Site	2	1816.89**	200.83**	597.89**	100.27**
Rep*Site	4	403.73	12.05	193.35	10.96
Genotype	31	1506.15**	82.12**	629.37**	60.63**
Site*Genotype	62	160.63	21.79	61.54	15.48*
Residual	92	157.39	12.68	70.45	9.51
Total	191				
CV (%)		19.4	15.1	17.8	13.7

*, ** = significant at 0.05 and 0.01 respectively; CV= Coefficient of variation; DF=Degrees of freedom

Table 3.3: Mean severity of ascochyta disease on bean genotypes tested at three locations in Rwanda

Genotypes	**Type**	**(%)Mean severity**	**Genotypes**	**Types**	**(%)Mean severity**
ALB102	Bush	40.0	Agronome	Climber	8.8
ALB155	Bush	33.3	CAB2	Climber	8.2
ALB58	Bush	14.2	Claudine	Climber	6.2
ASC87	Bush	3.0	G2331	Climber	10.3
CAL96	Bush	25.0	G2333	Climber	5.2
CMS17	Bush	4.3	G35034	Climber	1.7
ECAB026	Bush	31.5	G35306	Climber	1.3
ICTAHunapu	Bush	2.2	Garukura	Climber	7.8
Maharagesoja	Bush	32.8	Gasilida	Climber	8.3
NUA377	Bush	19.7	Gitanga	Climber	11.5
NUA379	Bush	16.2	Ibanga2	Climber	17.7
NUA397	Bush	22.5	Kenyerum	Climber	16.4
NUA566	Bush	51.5	Kigondo	Climber	14.3
RWK10	Bush	60.8	Kivuzo	Climber	15.8
RWR1180	Bush	12.3	MAC44	Climber	25.0

Genotypes	Type	(%)Mean severity	Genotypes	Types	(%)Mean severity
RWR1668	Bush	50.0	MAC49	Climber	33.0
RWR2154	Bush	14.3	MBC12	Climber	16.5
RWR2245	Bush	68.4	Nyamanza	Climber	12.8
RWR229	Bush	17.8	Nyaragik	Climber	9.0
RWR278	Bush	55.0	Nyirabuk	Climber	10.4
RWR281	Bush	12.3	Nyiramag	Climber	7.2
RWR3033	Bush	29.5	RWV1129	Climber	13.2
RWR310	Bush	23.2	RWV1348	Climber	9.0
RWR3194	Bush	32.0	RWV2070	Climber	8.2
RWR3228	Bush	23.3	RWV2872	Climber	9.0
RWR3332	Bush	19.5	RWV2887	Climber	8.8
RWR3338	Bush	38.8	RWV3006	Climber	8.7
RWR390	Bush	14.3	RWV3316	Climber	10.3
SER16	Bush	24.0	RWV3317	Climber	9.7
SER83	Bush	40.0	Rwibarur	Climber	15.7
SMC18	Bush	16.3	UCB82013	Climber	15.7
SMC21	Bush	10.0	Vuninkin	Climber	4.3
Grand Mean		24.2	**Grand Mean**		8.9
LSD= 12.6			LSD= 6.3		

LSD=least significant difference

Among the local bush genotypes, CMS 17 and CMS 21 had the lowest ascochyta severity levels across the three sites, while genotypes RWK 10 and RWR 2245 consistently had the highest severity levels. Climbing genotypes G 2333 and Vuninkingi had the lowest ascochyta severity; while MAC 44 and MAC 49 had the highest severity level of all climbing genotypes at all sites (Table 3.3). In general, bush genotypes had a higher severity (24.2%), compared to climbers (8.9%).

3.3.2 Effect of ascochyta on bean yield

The results presented in Table 3.4 show the analysis of variance of yields obtained from the protected and inoculated bean genotypes that were tested. The data obtained showed that there were significant differences between the genotypes and between the sites for both

protected and inoculated (P≤0.01) genotypes. The combination between genotype and sites was significantly different from those that were both inoculated and treated (P≤0.05).

Table 3.4: Mean square yield of ascochyta treated and inoculated field of bean genotypes tested at three locations in Rwanda

		Mean square			
Source	**DF**	**Treated**		**Inoculated**	
		Bush	**Climber**	**Bush**	**Climber**
Site	2	5500412.91**	9547307.94**	3275576.98**	6241472.76**
Rep*Site	4	18739.97	157975.26	65417.5	95166.43
Variety	31	107887.26**	260584.26**	141525.09**	182947.54**
Site*Variety	62	68449.65*	258756.20*	97042.39*	133875.16*
Residual	92	46350.91	156933.59	66652.4	121823.12
Total	191				
CV		16.4	10.1	9.4	12.2

*, ** = significant at 0.05 and 0.01 respectively; CV= Coefficient of variation; DF=Degrees of freedom

Table 3.5: Comparison of yield (kg ha^{-1}) of bush bean genotypes grown under ascochyta inoculated and protected conditions

Genotypes	Kitabi site		Rwerere site		Musanze site			
	Protected	Inoculated	Protected	Inoculated	Protected	Inoculated	M P	M I
ALB 102	760	125	1250	625	625	120	878.3	290.0
ALB 155	760	125	775	500	375	120	636.7	248.3
ALB 58	760	375	625	375	1125	120	836.7	290.0
ASC 87	1020	1025	1450	1500	1000	1150	1156.7	1225.0
CAL 96	760	125	1250	875	875	719	961.7	573.0
CMS 17	630	250	1100	875	525	240	751.7	455.0
ECAB 026	1020	250	750	625	750	120	840.0	331.7
ICTA Hunapu	1280	1240	1000	985	1125	1100	1135.0	1108.3
Maharagesoja	760	375	1250	1000	375	24	795.0	466.3
NUA 377	760	113	1375	1000	375	120	836.7	410.8
NUA 379	630	125	1250	375	625	48	835.0	182.7
NUA 397	604	250	875	875	875	240	784.7	455.0
NUA 566	630	250	750	625	850	719	743.3	531.3
RWK 10	1150	250	1125	375	1125	24	1133.3	216.3
RWR 1180	760	125	1250	1000	750	120	920.0	415.0
RWR 1668	760	375	875	750	1125	839	920.0	654.7
RWR 2154	760	625	1250	750	625	479	878.3	618.0
RWR 2245	1150	750	875	750	1375	479	1133.3	659.7
RWR 229	630	125	1250	875	875	240	918.3	413.3
RWR 278	890	125	975	750	750	719	871.7	531.3
RWR 281	1260	875	1250	1125	500	359	1003.3	786.3
RWR 3033	1150	250	1750	1375	875	359	1258.3	661.3

Genotypes	Kitabi site		Rwerere site		Musanze site			
	Protected	Inoculated	Protected	Inoculated	Protected	Inoculated	M P	M I
RWR 310	604	375	875	750	1125	240	868.0	455.0
RWR 3194	630	375	925	875	1375	599	976.7	616.3
RWR 3228	760	125	1250	625	1000	359	1003.3	369.7
RWR 3332	1280	375	1125	1000	875	240	1093.3	538.3
RWR 3338	1150	875	1400	1250	1500	240	1350.0	788.3
RWR 390	1020	500	1000	875	1250	120	1090.0	498.3
SER 16	630	375	750	625	375	240	585.0	413.3
SER 83	1150	500	1375	1000	1250	168	1258.3	556.0
SMC 18	890	125	1375	500	875	24	1046.7	216.3
SMC 21	604	125	875	750	625	120	701.3	331.7
Mean	862.6	371.2	1101.6	819.8	867.2	337.8	943.8	509.6
lsd (0.05)a	242.1							
lsd (0.05)b	562.7							
lsd (0.05)c	864.0							

[a] Treatment; [b] Genotype; [c] Treatment x Genotype; MP= Mean protected genotype; MI= Mean of inoculated genotype

Table 3.5 show the yields obtained from the protected and inoculated bush bean genotypes that were tested at the three sites. The protected bean at Rwerere yielded the highest (1101.5 kg ha^{-1}), followed by Musanze (867.2 kg ha^{-1}) and Kitabi (862.5 kg ha^{-1}). For the inoculated plots, the bean yield at Rwerere was the highest (819.8 kg ha^{-1}), followed by Kitabi (371.2 kg ha^{-1}) and Musanze (337.7 kg ha^{-1})

In the protected plots, genotype RWR 3338 (1350 kg ha^{-1}) yielded the highest, followed by RWR 3033 (1258.3 kg ha^{-1}) and SER 83 (1253.2 kg ha^{-1}). Genotype SER 16 yielded the lowest (585.0 kg ha^{-1}). In the inoculated plots, the checks ICTA Hunapu (1108.3 kg ha^{-1}) and ASC 87 (1225.0 kg ha^{-1}) had higher yields, compared to other genotypes.

Table 3.6: Comparison of yield (kg ha^{-1}) of climbing bean genotypes grown under ascochyta inoculated and protected conditions

Genotypes	Kitabi site		Rwerere site		Musanze site			
	Protected	Inoculated	Protected	Inoculated	Protected	Inoculated	MP	MI
Agronome	587	363	1775	1025	1900	1063	1420.7	817.0
CAB 2	838	250	1775	975	1588	375	1400.3	533.3
Claudine	1025	813	2150	1125	2212	750	1795.7	896.0
G2331	1525	1562	2462	1125	1650	938	1879.0	1208.3
G2333	1462	995	2150	1400	1462	1000	1691.3	1131.7
Garukurare	1025	650	2088	1350	1900	1375	1671.0	1125.0
Gasilida	1150	570	2462	1175	1650	613	1754.0	786.0
Gitanga	1588	938	1338	1025	2025	938	1650.3	967.0
Ibanga 2	1612	950	2087	1250	2212	1188	1970.3	1129.3
Kenyerumpure	1213	438	2212	925	1525	938	1650.0	767.0
Kigondo	1525	1000	2025	875	1712	563	1754.0	812.7
Kivuzo	1275	525	1650	1200	1462	388	1462.3	704.3
MAC 49	837	438	1462	900	1588	1063	1295.7	800.3
MAC44	1087	375	1900	950	1462	1188	1483.0	837.7
MBC 12	1400	313	1088	600	1588	750	1358.7	554.3
G 35306	1062	1005	1775	1725	1712	1735	1516.3	1488.3
Nyamanza	1088	500	1463	825	1400	750	1317.0	691.7
Nyaragikoti	1212	250	1775	1450	1275	813	1420.7	837.7
Nyirabukara	1362	1188	1900	1150	1275	635	1512.3	991.0
Nyiramagorori	1900	938	2212	1400	1962	875	2024.7	1071.0

Genotypes	Kitabi site		Rwerere site		Musanze site			
	Protected	**Inoculated**	**Protected**	**Inoculated**	**Protected**	**Inoculated**	**MP**	**MI**
Rwibarura 2	637	875	1525	725	1462	500	1208.0	700.0
RWV 1129	400	375	1775	800	1962	1125	1379.0	766.7
RWV 1348	900	363	1963	1550	2400	875	1754.3	929.3
RWV 2070	1588	375	1838	1375	1275	750	1567.0	833.3
G 35034	1900	1870	2338	2250	1963	2000	2067.0	2040.0
RWV 2887	1025	688	1650	1250	2138	1125	1604.3	1021.0
RWV 3006	1150	438	1338	1000	1462	813	1316.7	750.3
RWV 3316	712	363	1588	975	1275	313	1191.7	550.3
RWV 3317	838	375	1525	700	1775	688	1379.3	587.7
RWV2872	588	500	2150	1050	1525	1188	1421.0	912.7
UCB 82013	962	688	1462	950	1738	1000	1254.0	879.3
Vuninkingi	1150	313	1550	825	1588	875	1429.3	671.0
Mean	1144.5	665.1	1826.6	1021.9	1978.8	912.2	1550.0	899.7
lsd (0.05)a	120.9							
lsd (0.05)b	421.1							
lsd (0.05)c	774							

[a] Treatment; [b] Genotype; [c] Treatment x Genotype; MP= Mean protected genotype; MI= Mean of inoculated genotype; lsd= least significant difference.

Table 3.6 shows the yields obtained from the treated and inoculated climbing bean genotypes tested at the three sites. It is observed from the above table that yields at Musanze were highest (1978.8 kg ha^{-1}), followed by the Rwerere site (1826.6 kg ha^{-1}), whereas the Kitabi site yielded the lowest (1144.5 kg ha^{-1}). The yield of the inoculated plots at Rwerere were highest (1021.9 kg ha^{-1}), followed by Musanze (912.2 kg ha^{-1}) and Kitabi (665.1 kg ha^{-1})

In the treated plots, genotype G35034 (2067.0 kg ha^{-1}) had the highest yield, compare to other genotypes including checks, followed by Nyiramagorori (2024.7 kg ha^{-1}) and Ibanga2 (1970.3 kg ha^{-1}). Genotype RWV 3316 gave the lowest yield (1191.7 kg ha^{-1}). The inoculated plots checks G 35034 (2040.0 kg ha^{-1}) and G35306 (1488.3 kg ha^{-1}) yielded the highest, compared to all other genotypes. The results further show that, for all genotypes, protected plots had significantly higher yields than inoculated plots (see Tables 3.5 and 3.6).

3.3.3 Yield loss

The analysis of variance showed a significant difference ($P \leq 0.01$) in the yield decrease between both sites and for both climber and bush varieties (Table 3.7). The two-way interaction between site x genotype was also significant at ($P \leq 0.05$).

Table 3.7: Analysis of variance on % yield decrease caused by ascochyta on bean genotypes, tested at three locations in Rwanda

Source	DF	Mean square	
		Bush	**Climber**
Site	2	2191.59**	2228.58**
Rep*Site	4	422.92	368.33
Genotype	31	1910.75**	1802.76**
Site*Genotype	62	557.99*	592.89*
Residual	93	393.72	185.52
Total	191		
CV (%)		7.8	16.1

*, ** = significant at $P \leq 0.05$ and $P \leq 0.01$ respectively; CV= Coefficient of variation; DF=Degrees of freedom

Table 3.8: Yield loss obtained per bush genotype and the disease assessment

	Kitabi		Rwerere		Musanze			
Genotype	**Yield loss (%)**	**RAUDPC**	**Yield loss (%)**	**RAUDPC**	**Yield loss (%)**	**RAUDPC**	**MYL**	**M RAUDPC**
ALB 102	50.6	107.8	36.7	5.8	55.4	4.5	47.6	39.4
ALB 155	54.0	16.2	43.6	9.2	46.4	109.7	48.0	45.0
ALB 58	6.3	4.0	54.9	9.2	42.7	141.7	34.6	51.6
ASC 87	72.8	2.2	46.8	2.7	44.9	2.3	54.8	2.4
CAL 96	36.6	4.0	35.3	55.7	27.6	87.5	33.2	49.1
CMS 17	76.4	14.2	25.2	6.3	41.2	5.5	47.6	8.7
ECAB 026	55.3	1.5	54.1	8.3	61.2	7.8	56.9	5.9
ICTA Hunapu	28.5	0.7	35.0	2.3	25.3	2.0	29.6	1.7
Maharagesoja	65.5	17.7	50.0	6.2	18.7	88.3	44.7	37.4
NUA 377	20.7	36.3	47.7	8.3	66.1	6.3	44.8	17.0
NUA 379	37.4	5.8	52.5	9.8	65.8	8.7	51.9	8.1
NUA 397	47.7	14.8	38.4	46.8	33.1	11.7	39.7	24.4
NUA 566	24.0	108.0	54.3	90.3	43.2	5.8	40.5	68.1
RWK 10	15.0	1.5	51.2	9.2	22.1	7.8	29.4	6.2
RWR 1180	50.4	4.0	52.3	60.8	62.8	7.5	55.2	24.1
RWR 1668	70.2	35.7	45.1	6.8	76.4	6.5	63.9	16.3
RWR 2154	5.4	105.7	2.8	9.2	0.0	8.0	2.7	40.9
RWR 2245	77.6	31.7	44.9	10.5	52.8	8.0	58.4	16.7
RWR 229	79.4	5.8	18.3	30.8	36.2	11.0	44.6	15.9
RWR 278	31.9	21.5	34.9	57.5	31.6	7.0	32.8	28.7
RWR 281	38.2	36.5	42.3	78.3	44.1	127.2	41.5	80.7
RWR 3033	34.4	21.3	56.8	15.2	67.1	12.7	52.8	16.4

	Kitabi		Rwerere		Musanze			
Genotype	**Yield loss (%)**	**RAUDPC**	**Yield loss (%)**	**RAUDPC**	**Yield loss (%)**	**RAUDPC**	**MYL**	**M RAUDPC**
RWR 310	49.0	2.7	38.6	13.2	75.5	10.5	54.4	8.8
RWR 3194	41.1	4.5	40.1	16.2	46.3	15.0	42.5	11.9
RWR 3228	1.6	5.0	3.8	90.3	0.0	6.3	1.8	33.9
RWR 3332	12.8	17.3	39.5	7.3	50.2	6.2	34.1	10.3
RWR 3338	61.9	105.2	25.3	90.7	44.4	55.8	43.9	83.9
RWR 390	58.8	118.3	27.3	14.3	73.5	14.3	53.2	49.0
SER 16	40.9	74.3	23.4	8.7	53.7	24.3	39.3	35.8
SER 83	63.9	7.0	58.2	7.5	38.5	6.0	53.5	6.8
SMC 18	59.7	5.7	21.0	11.5	63.5	9.8	48.1	9.0
SMC 21	32.9	5.0	24.2	11.2	47.4	126.7	34.8	47.6
Mean	43.8	29.4	38.3	25.3	45.5	29.8	42.5	28.2
Isd (0.05)1	32.6							
R (P<0.05)	0.68							

MYL= Genotype mean yield loss; MAUDPC= Genotype mean relative area under disease progress curve

Table 3.8 shows that Musanze had highest yield decrease (45.5%) due to ascochyta blight, followed by Kitabi (43.8%), whereas Rwerere showed the lowest yield decrease. Genotype RWR 1668 had the greatest yield loss (63.9%) among the bush genotypes, while checks ICTA Hunapu and ASC 87 had the lowest yield losses of 9.6% and 4.8%, respectively. When all sites are considered, the Musanze site had the highest RAUDPC and Rwerere had the lowest. Genotype RWV 3338 (83.89) had the highest RAUDPC, followed by RWR 281 (80.67). Checks (ICTA Hunapu, ASC 87) had the lowest RAUDPC.

Table 3.9: Yield loss obtained per climber genotype and the disease assessment

	Kitabi		Rwerere		Musanze			
Genotype	**Yield loss (%)**	**RAUDPC**	**Yield loss (%)**	**RAUDPC**	**Yield loss (%)**	**RAUDPC**	**MYL**	**M RAUDPC**
Agronome	80.2	10.3	77.0	20.9	70.0	15.4	75.7	15.5
CAB 2	50.7	2.8	28.0	4.6	20.0	15.6	32.9	7.7
Claudine	78.3	17.6	76.7	7.8	66.7	16.3	73.9	13.9
G 2331	56.5	9.1	25.3	6.0	27.3	14.4	36.4	9.8
G 2333	50.7	6.4	16.3	8.3	14.3	4.2	27.1	6.3
G 35034	3.1	0.4	1.5	0.2	1.5	0.4	2.0	0.3
G 35306	0.0	0.4	0.0	0.1	0.0	0.0	0.0	0.2
Garukurare	86.0	11.2	25.1	7.0	23.1	0.7	44.7	6.3
Gasilida	30.6	3.6	11.0	1.4	10.0	4.7	17.2	3.2
Gitanga	40.5	2.7	12.7	3.5	16.7	6.0	23.3	4.1
Ibanga 2	75.5	10.8	18.7	8.0	16.7	14.0	36.9	10.9
Kenyerumpure	23.9	6.6	18.7	3.0	10.7	14.0	17.8	7.9
Kigondo	83.6	9.8	52.0	2.9	50.0	10.7	61.9	7.8
Kivuzo	85.2	9.6	37.3	1.4	27.3	11.3	49.9	7.4
MAC 44	78.3	22.2	31.4	8.0	21.4	9.8	43.7	13.3
MAC 49	37.9	5.1	18.3	23.5	14.3	13.1	23.5	13.9
MBC 12	51.0	4.3	84.5	2.7	12.5	15.1	49.3	7.3
Nyamanza	80.2	9.7	32.0	24.0	30.0	12.1	47.4	15.2
Nyaragikoti	86.0	13.2	64.6	0.7	63.6	16.2	71.4	10.1
Nyirabukara	83.6	9.7	33.0	10.6	30.0	3.0	48.9	7.8
Nyiramagorori	60.3	6.6	26.7	5.4	16.7	2.6	34.6	4.9
Rwibarura 2	40.5	3.1	7.4	3.5	5.4	9.4	17.8	5.3

	Kitabi		Rwerere		Musanze			
Genotype	**Yield loss (%)**	**RAUDPC**	**Yield loss (%)**	**RAUDPC**	**Yield loss (%)**	**RAUDPC**	**MYL**	**M RAUDPC**
RWV 1129	58.6	4.9	10.0	18.7	0.0	12.1	22.9	11.9
RWV 1348	83.6	10.8	34.5	5.9	35.5	11.3	51.2	9.4
RWV 2070	83.6	8.8	56.0	3.9	50.0	13.5	63.2	8.7
RWV 2872	83.6	10.9	21.0	10.2	20.0	14.0	41.5	11.7
RWV 2887	50.7	4.2	60.0	2.0	40.0	14.9	50.2	7.0
RWV 3006	17.8	1.2	46.0	1.8	40.0	3.9	34.6	2.3
RWV 3316	70.7	19.8	73.1	3.4	11.1	12.1	51.6	11.8
RWV 3317	79.3	6.5	16.3	0.5	14.3	13.5	36.6	6.8
UCB 82013	34.8	3.9	24.3	2.2	14.3	10.9	24.5	5.7
Vuninkingi	60.3	3.5	28.5	0.4	20.5	9.0	36.4	4.3
Average	58.9	7.8	33.4	6.3	24.8	10.1	39.0	11.1
Isd (0.05)1	47.3							
R (P<0.05)	0.72							

MYL= Genotype mean yield loss; MAUDPC= Genotype mean relative area under disease progress curve

Table 3:9 shows the yield loss per climber genotype and disease progress for all sites. The data shows that the yield decrease, due to ascochyta blight, was highest at Kitabi (58.9%), followed by Rwerere (33.4%) and Musanze had (24.8%). Musanze had the highest RAUDPC (10.1) and Rwerere had the lowest (6.3).

The climbing genotype Agronome had the greatest yield loss (75.7%), followed by Claudine (73.9%), while checks G35034 and G35306 had the lowest yield losses of 2.1% and 0.9%, respectively. This trend was the same for the RAUDPC values. In addition, there was a strong positive correlation (r =0.68 and 0.72) between the RAUDPC values and the yield loss for bush and climbers, respectively.

3.3.4 Relationship between yield loss and ascochyta severity

Disease severity varied greatly in, and between, experimental sites and was generally the highest during the 9^{th} and 10^{th} weeks after germination. Disease inoculations successfully initiated infection. Abundant rainfall and low to moderate temperatures persisted throughout the growing season and were conducive to infection by ascochyta. The application of Benomyl effectively controlled ascochyta blight in the treated plots.

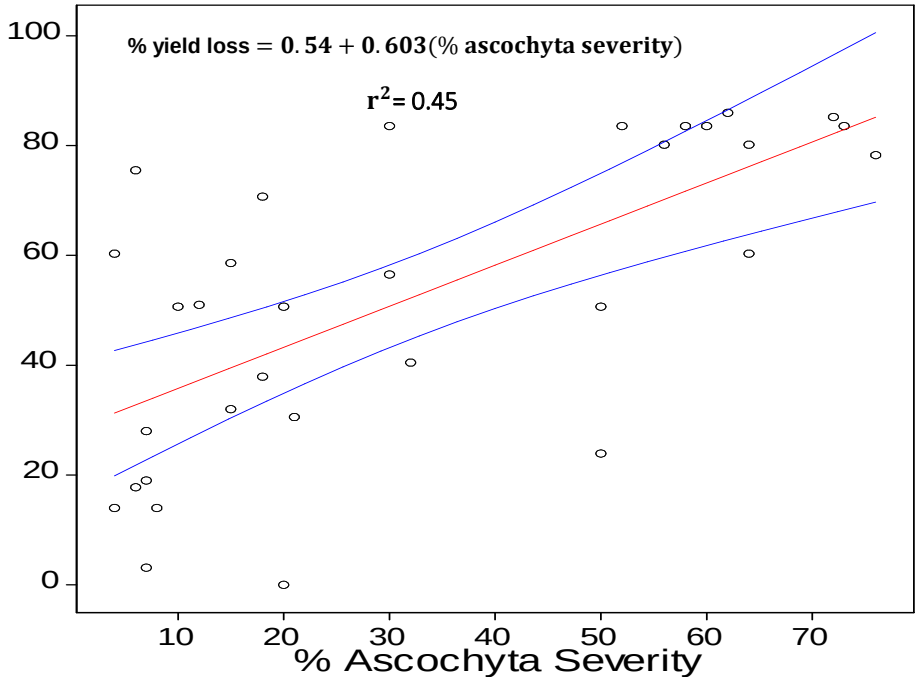

Figure 3.3: Relationship between the severity of bean ascochyta blight (%) and the percentage yield loss for bush. Data points are the means of three replications at the Kitabi Research Station

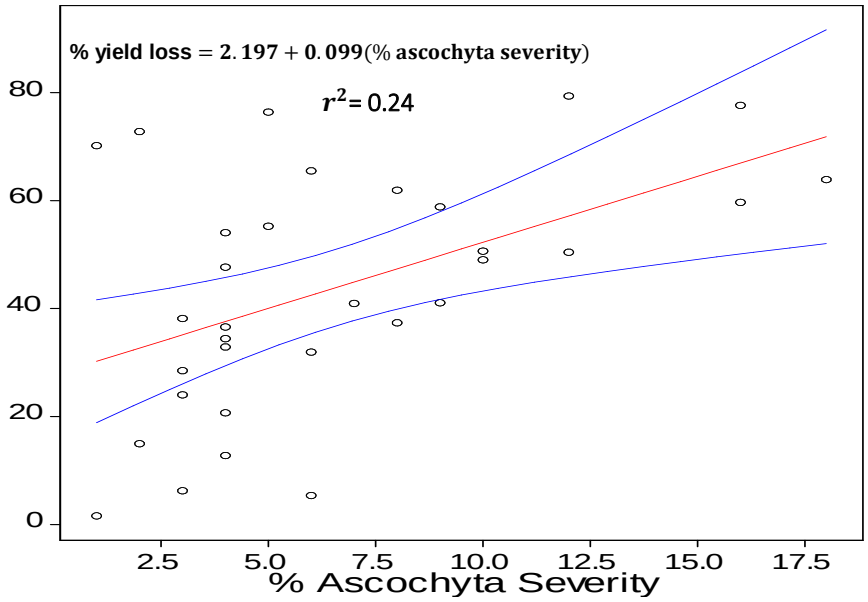

Figure 3.4: Relationship between the severity of bean ascochyta blight (%) and the percentage yield loss for climbers. Data points are the means of three replications at the Kitabi Research Station

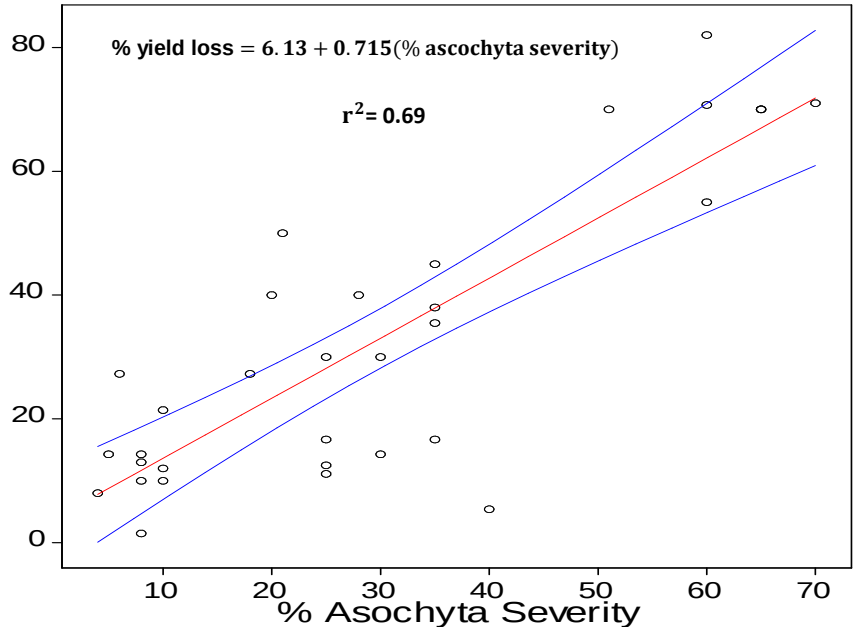

Figure 3.5: Relationship between the severity of bean ascochyta blight (%) and the percentage yield loss for bush. Data points are the means of three replications at the Musanze Research Station

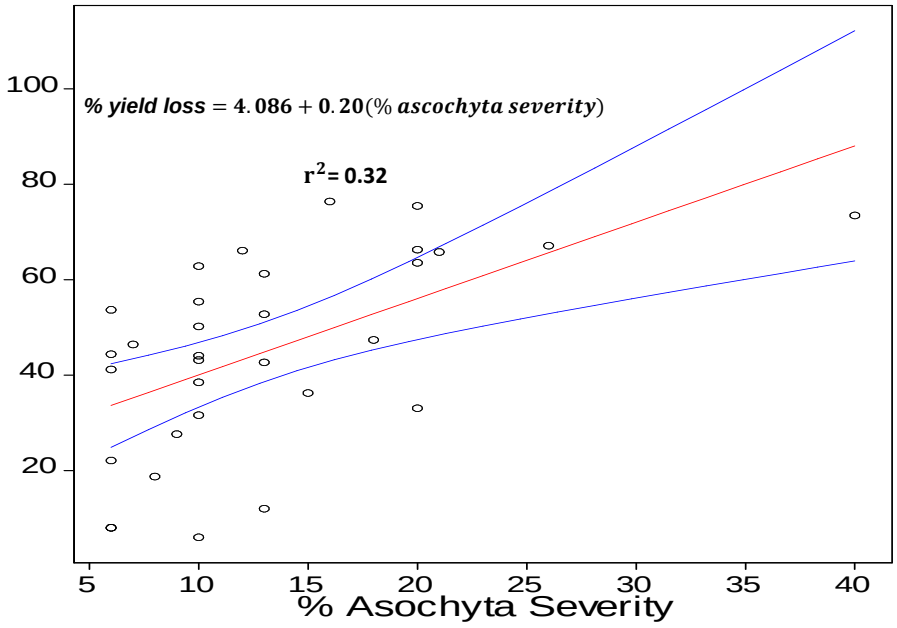

Figure 3.6: Relationship between the severity of bean ascochyta blight (%) and the percentage yield loss for climbers. Data point are the means of three replications at the Musanze Research Station

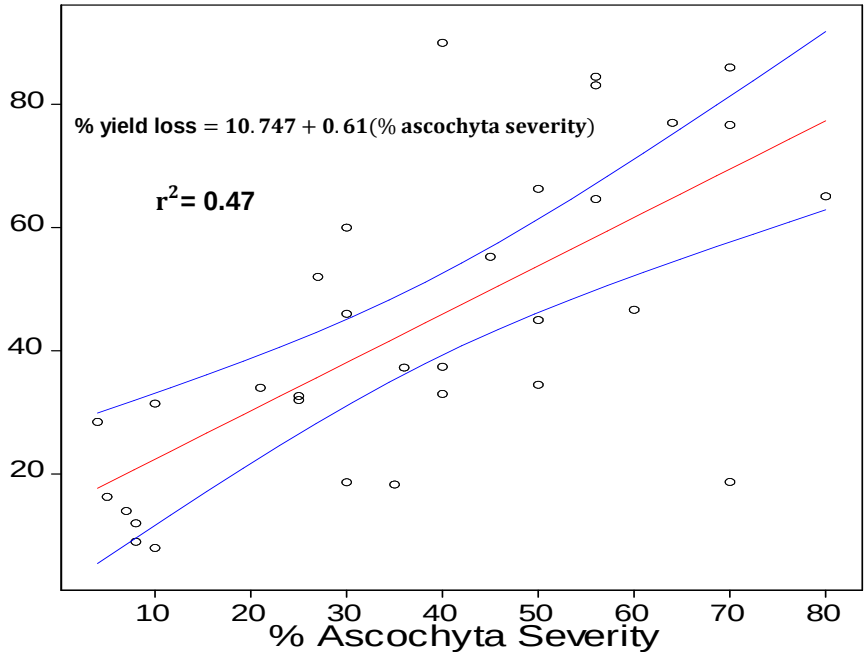

Figure 3.7: Relationship between the severity of bean ascochyta blight (%) and the percentage yield loss for bush. Data points are the means of three replications at the Rwerere Research Station

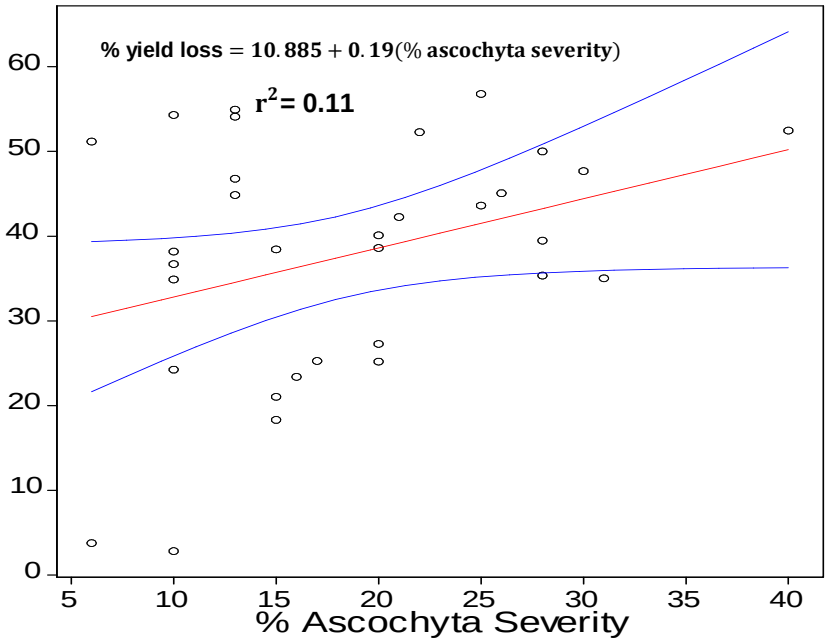

Figure 3.8: Relationship between the severity of bean ascochyta blight (%) and the percentage yield loss for climbers. Data points are the means of three replications at the Rwerere Research Station.

3.4 Discussion

Ascochyta blight has been reported on beans in Rwanda since the late 1980s (ISAR, 1985), but the yield loss caused by the disease has not been quantified. This study was conducted to establish the effect of ascochyta blight on the yield of 32 bush common bean and 32 climbing common bean across three sites.

As an aggressive foliar disease, ascochyta destroys plant photosynthetic tissue (Bailey, 2011), causing premature defoliation and early maturation, thus lowering yields (Beebe et al., 1991). Although susceptible bean genotypes can be infected at all growth stages, yield loss will depend on the crop stage when infection occurs (Corrales and van Schoonhoven, 1987). The most important crop stages determining yield loss are between the early flower stage (R1), through to pod fill and to the end of seed development stage (R6) (Da Silva et al., 2003). The significant differences in ascochyta severity between genotypes in this study could be indicative of differences in susceptibility levels. Significantly, different RAUDPC values between genotypes were found, indicating that ascochyta progressed differently among genotypes. The study showed that *Phoma exigua* grows faster on the more susceptible genotypes. These findings are in agreement with earlier studies, which showed that susceptible common bean genotypes succumbed to fungal diseases earlier than the resistant varieties (Gaunt, 1995; Hughes and Madden, 1997). The visual estimation of leaf, foliar or pods damage was generally reliable and consistent for the disease.

The analysis of variance showed significant differences ($P \leq 0.01$) in the yield decrease between both sites and genotypes for both climber and bush types. Musanze showed the highest yield decrease (45.5%) due to ascochyta blight, followed by Kitabi (43.8%), whereas Rwerere showed the least yield decrease. Genotype RWR 1668 had the greatest yield loss (63.9%) among the bush genotypes, while checks ICTA Hunapu and ASC 87 had the lowest yield losses of 9.6% and 4.8%, respectively. For climbers, the yield decrease due to ascochyta blight was highest at Kitabi (58.9%), followed by Rwerere (33.4%) and Musanze (24.8%). The climbing genotype Agronome had the greatest yield loss (75.7%), followed by Claudine (73.9%), while checks G 35034 and G 35306 had the lowest yield losses of 2.1% and 0.9%, respectively. This trend was the same for the RAUDPC values for both bean types. The data obtained show a high positive correlation ($r = 0.68$ and 0.72) between the RAUDPC and the genotype yield losses. In comparison to the resistant genotypes, severe yield losses were observed in all

susceptible genotypes for the three locations. In general, the bush genotypes were more susceptible than the climbers. The relatively low losses are consistent with those expected from a delay in the development of ascochyta to the pod filling growth stage.

When the percentage yield losses of bush genotype and climbers from different locations were regressed against the severity of ascochyta, differences in variation were observed. For bush genotypes, grain yield losses were reduced by 0.60%, 0.71% and 0.61% for each percentage of ascochyta severity at the pod filling growth stage, for Kitabi, Musanze and Rwerere, respectively. Climber grain yields were reduced by an average of 0.09%, 0.2% and 0.19% for each percentage of ascochyta severity at the pod filling stage, at Kitabi, Musanze and Rwerere, respectively (Figure 3.3, 3.4, 3.5, 3.6, 3.7 and 3.8). This study demonstrates that the most susceptible genotypes, which were artificially inoculated and grown under conditions favorable for disease, sustain a significant grain yield loss to ascochyta.

It has been reported that a total yield loss of 45% may occur when ascochyta contaminated seed is used and the climatic conditions are conducive for disease development (Schmit and Baudoin, 1992; Schwartz et al., 1981; Waggoner and Berger, 1987; Wortmann et al., 1998). This loss in marketable yield can be a big hindrance to the farmers, in terms of household income, considering that over 85% of the common bean producers in Rwanda have very limited resources for diseases control (FAOSTAT, 2013). The significant low percentage yield losses observed for the resistant genotypes highlights the importance of using plant resistance to manage ascochyta. Unfortunately, the resistant genotypes used in this study have poor marketability in Rwanda (RAB, 2014) and they cannot, therefore, easily replace the marketable susceptible genotypes. The use of suitable ascochyta resistant or tolerant genotypes would be the best option in the management of this disease. Therefore, it is recommended that a breeding programme should be established to know the genetic basis of ascochyta resistance and facilitate the introgression of ascochyta resistance genes into the susceptible preferred market class genotypes.

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Chapter Four: Genotypic response of dry bean (*Phaseolus vulgaris* L.) to natural field infection of ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] under diverse environmental conditions in Rwanda

Abstract

Ascochyta, which is caused by *Phoma exigua var. diversispora (Bubak)* Boerema, is a serious constraint in the cultivation of the common bean *(Phaseolus vulgaris* L.) in Rwanda, particularly in the cool and wet highland production areas. In order to identify resistant genotypes, a germplasm evaluation was conducted to quantify the impact of the disease on phenotypic and agronomic traits under natural conditions. Field screening trials of 39 bush (Types I, II and III) and 36 climbing (Type IV) genotypes from different accessions within and outside the country were conducted at three sites, namely, Rwerere, Nyamagabe and Musanze Research Stations, for two seasons. The relative area under the disease progress curve (RAUDPC) based on evaluations of the disease severity (percentage leaf area infected), was used to screen the genotypes. Thirteen genotypes were identified with some level of ascochyta resistance. Additional results showed a negative relationship (r=-0.42 and -0.51 for Seasons A and B, respectively) between ascochyta severity and yield. Further relationships were identified between the plant flower colour, seed size and growth habit, as well as ascochyta resistance. Some of the identified resistant genotypes can be used to introgress ascochyta resistance into susceptible Rwandan market class common bean genotypes.

4.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume in Rwanda (Gethi et al., 1997; RAB, 2015). Although there has been an increase in bean production, due to different policies aimed at the expansion of beans into marginal agricultural lands, the productivity per unit area of land has continued to decline (Nderitu et al., 2007; RAB, 2015). Typical bean yields obtained on the farmers' fields are only 20% to 30% of the genetic potential of improved genotypes (Wortmann and Kaizzi, 1998). These low yields are attributed to a number of constraints, the most important of which are diseases, insect pests, low soil fertility and periodic water stress (Allen et al., 1996; Otsyula et al., 2005). Ascochyta blight of the common bean, caused by *Phoma exigua var. diversispora (Bubak)* Boerema, is one of the most damaging diseases of the common bean (Liebenberg and Pretorius, 1997).

The disease particularly favours cool temperatures and a high relative humidity (ISAR, 2011). It infects all major bean parts, such as the leaves, stems and pods, and is seed transmitted, and can cause total crop losses, especially when infected seed is planted (Schwartz and Corrales, 1989). In Africa, particularly in Rwanda, Uganda, Burundi and the Eastern DRC, where beans constitute the most important source of dietary protein, ascochyta blight is a significant constraint to bean production (; Schwartz and Corrales, 1989; Beebe and Pastor-Corrales, 1991 and ISAR, 2012).

In Rwanda, most market-class bean genotypes, including the recently-released ones and landraces, are susceptible to ascochyta blight. In severe situations, this disease reduces expected harvests significantly, causing food shortages and a loss of income for Rwandan farmers. Ascochyta blight is prevalent in the highland region of Rwanda where beans are extensively grown (ISAR, 2011). Although bean ascochyta is amongst the major diseases in Rwanda, its prevalence and impact on the yield has not been well documented in Rwanda. In addition, no screening has been done for resistance to the disease in Rwanda.

Since the fungus is seed-borne and can be spread through debris and the air, the carry-over of the disease from season to season has always been possible, due to the small-scale farming systems and seed recycling that is practiced by the small-holder farmers in Rwanda. Small-scale farmers in the highland regions of Rwanda, like the other parts of sub-Saharan Africa, are compelled to rely upon traditional disease management practices (Allen et al., 1996; Otsyula et al., 2005), mainly due to financial constraints.

Poor crop management practices, as well as the adverse biophysical environment, lead to a build-up of field inoculum (Letourneau et al., 2006). A range of ascochyta blight management methods have been suggested for beans, including biological control, agronomic or cultural practices and the use of genetic diversity (local landraces) and chemicals (Ampofo and; Byabagambi et al., 1999; Letourneau et al., 2006; Massomo, 2014). However, many of these methods are not feasible in Rwanda, due to the varying growing conditions and limited resources that characterize the small-scale farming system. The use of resistant genotypes, combined with other disease management practices, is regarded as the most practical approach to disease control at farm level.

Farmers exploiting the diversity available in landraces and genotypes reduce the risk of ascochyta blight infection (Letourneau et al., 2006). The identification of useful sources of resistance to the most important diseases is valuable, in that such sources could be used to confer resistance to locally-adapted materials. However, breeding programmes should place more emphasis on the development of genotypes with durable mechanisms of resistance. Resistance genes may be found within the landrace populations, due to long-term co-evolution between crops and disease, natural selection and intentional selection by farmers.

Very little research has been conducted to identify sources of resistance to ascochyta blight in the common bean germplasm. Schmit and Baudoin (1992) evaluated 200 populations of *P. coccineus L*. and *P. polyanthus* L. for ascochyta resistance, in the two highland stations of Rionegro and Popayan in Colombia. However, only low levels of resistance to *Phoma exigua var.diversispora* have been found among the cultivated forms of the common bean.

Similarly, research conducted at CIAT and the National Bean Programme in Guatemala showed some differences in reaction to the ascochyta pathogen. Most evaluated genotypes were either susceptible, or had low levels of resistance. A high level of resistance and immunity were present in genotypes of *P. coccineus L.*, particularly in the sub-species *polyanthus* and in interspecific hybrids that were obtained by crossing these two species (Wortmann, 1993).

Apart from the yield reduction caused by diseases such ascochyta blight, unstable climatic conditions are a persistent problem in Rwanda. Therefore, the adaptation of bean genotypes to such environments requires a genotype with a wide spectrum of abiotic tolerance, in addition to disease resistance. The severity and distribution of bean ascochyta varies, depending on the location and the season.

According to Ceccarelli and Grando (2007), when different genotypes of a given crop are evaluated in a range of environments, genotype (G) x environment (E) (GE) interactions of cross-over types appear to be quite common. Significant GE interactions cannot be disregarded. The options are to manage them by selecting genotypes that are broadly adapted to a whole range of target environments, or to basically carry out selection for an array of genotypes, whereby each is adapted to a specific environment (Ceccarelli and Grando, 2012). Such selection requires separate GE analyses, namely, genotype (G) x year (Y) (GY), which is highly unpredictable and genotype x location (L) (GL), which identifies a distinct target environment (Ceccarelli and Grando, 2012). Selecting for specific adaptation is important, predominantly for crops grown under unfavorable conditions, as unfavorable environments can be very different from each other (Ceccarelli and Grando, 2007). Therefore, a breeding strategy to identify genotypes suitable for unfavorable environmental and variable seasonal conditions should exploit the analysis of GE components (Ceccarelli and Grando, 2012). This is because seasonal variation of bean ascochyta infection, rainfall patterns, and a negative or low correlation between farmer field and research stations, may complicate the breeder's selection process. This may hamper the positive identification of superior materials for the intended specific target environment or wide range of environments.

The objectives of this study were therefore, (1) to identify sources of resistance to bean ascochyta blight available in landraces and other collections, and (2) to determine the effect of seasonal variation on common bean genotypes in relation to bean ascochyta attack in Rwanda.

4.2 Materials and methods

The reaction to ascochyta blight caused by *Phoma exigua var. diversispora (Bubak) Boerema* was evaluated in the field, using 75 bush and climber genotypes. The 75 genotypes were collected from different collections within and outside Rwanda. The genotypes were then screened in the field for ascochyta resistance for two growing seasons in the years 2014 and 2015 at the Nyamagabe, Musanze and Rwerere Research Stations, where conditions are favorable for disease development and establishment.

4.2.1 Experimental site

The field experiments were carried out across three selected locations in Rwanda. The locations are the major research sites of the Rwanda Agriculture Board (RAB) in areas known for their bean production and ascochyta epidemics. The Musanze site is in the highlands of the northern agricultural zone and is located at coordinates 01°14'59.7" South and 036°44'28.8" East at an altitude of 1820 m above sea level (masl). The area receives an average rainfall of 1746 mm annually, and has a mean maximum temperature of 23°C and mean minimum temperature of 12°C. The soils are dark red or brown friable clay. Rwerere is located at an altitude of 2312 masl on a longitude of 29° 19' East and a latitude of 1° 36' South, with an annual rainfall and temperature of 1200 mm and 20ºC, respectively. It represents the highlands of Buberuka. Nyamagabe is located at an altitude of 2080 masl on a longitude of 29° 33' East and altitude of 1° 33' South, with the annual rainfall and temperature being 1600 mm and 19ºC, respectively. It represents of the highlands of the Congo/Nile Divide (ISAR, 1985).

In warm and moist regions, such as the tropical highlands of Rwanda, inoculum is always present due to the continuous cropping of beans, combined with the right conditions for ascochyta occurrence and its spread. In most bean-growing areas in Rwanda, the average annual precipitation ranges from 1200 mm to over 1800 mm. In general, rainfall is bimodal, with a minor peak occurring in October and a major peak in April. High elevations and low latitudes combine to form an isothermal temperature regime, with an average annual temperature of about 16ºC (ISAR, 2011).

4.2.2 Planting material

The 75 bush and climbing genotype lines (Table 4.1) were tested for resistance to ascochyta. Screening was done in the field, using ascochyta-infected debris as the inoculum, which had been previously collected from the same region.

The 75 genotypes comprised of 39 bush (Types I, II and III) bean genotypes and 36 climbers (Type IV). The entries include ascochyta-resistant lines acquired from the Centro Internacional de Agricultura Tropical (CIAT) in Colombia, landraces from the National Gene Bank of Rwanda and improved genotypes, mainly released from the Rwanda Agriculture Board (RAB), as well as checks.

Table 4.1: List of bean genotypes used in the study

Bush	Source	Species	Seed size	Climber	Source	Species	Seed size
ALB 102	Rwanda	*P. phaseolus*	Small	Agronome	Rwanda	*P.phaseolus*	Medium
ALB 155	Rwanda	*P. phaseolus*	Medium	CAB 2	Rwanda	*P.phaseolus*	Large
ALB 58	Rwanda	*P. phaseolus*	Small	Claudine	Rwanda	*P.phaseolus*	Medium
ASC 107	CIAT Col	*P. phaseolus*	small	G 10747	CIAT Col	*P.Coccineus*	Small
ASC 87	CIAT Col	*P. phaseolus*	small	G 2331	Rwanda	*P.phaseolus*	Large
ASC 92	CIAT Col	*P. phaseolus*	small	G 35034	CIAT Col	*P.Coccineus*	Large
ASC 94	CIAT Col	*P. phaseolus*	small	G 35084	CIAT Col	*P.coccineus*	Large
CAL 96	Rwanda	*P. phaseolus*	Medium	G 35182	CIAT Col	*P.Coccineus*	Small
CMS 17	Rwanda	*P. phaseolus*	Medium	G 35306	CIAT Col	*P.Coccineus*	Small
ECAB 026	Rwanda	*P. phaseolus*	Small	G2333	Rwanda	*P.phaseolus*	Large
ICTA Hunapu	CIAT Col	*P. phaseolus*	small	Garukurare	Rwanda	*P.phaseolus*	Medium
LSA142	CIAT Col	*P. phaseolus*	small	Gasilida	Rwanda	*P.phaseolus*	Large
Maharagesoja	Rwanda	*P. phaseolus*	Small	Gitanga	Rwanda	*P.phaseolus*	Medium
MIB 755	CIAT Col	*P. phaseolus*	Small	Ibanga 2	Rwanda	*P.phaseolus*	Medium
Mixture(Check)	Rwanda	*P. phaseolus*		Kenyerumpure	Rwanda	*P.phaseolus*	Small
NUA 377	Rwanda	*P. phaseolus*	Medium	Kigondo	Rwanda	*P.phaseolus*	Medium
NUA 379	Rwanda	*P. phaseolus*	Medium	Kivuzo	Rwanda	*P.phaseolus*	Medium
NUA 397	Rwanda	*P. phaseolus*	Medium	MAC 44	Rwanda	*P.phaseolus*	Medium
NUA 566	Rwanda	*P. phaseolus*	Medium	MAC 49	Rwanda	*P.phaseolus*	Medium
RWK 10	Rwanda	*P. phaseolus*	Large	MBC 12	Rwanda	*P.phaseolus*	Medium
RWR 1180	Rwanda	*P. phaseolus*	Large	Mixture(check)	Rwanda	*P.phaseolus*	
RWR 1668	Rwanda	*P. phaseolus*	Large	Nyamanza	Rwanda	*P.phaseolus*	Medium
RWR 2154	Rwanda	*P. phaseolus*	Medium	Nyirabukara	Rwanda	*P.phaseolus*	Small

Bush	Source	Species	Seed size	Climber	Source	Species	Seed size
RWR 2245	Rwanda	*P. phaseolus*	Large	Nyiramagorori	Rwanda	*P.phaseolus*	Small
RWR 229	Rwanda	*P. phaseolus*	Large	Rwibarura 2	Rwanda	*P.phaseolus*	Large
RWR 278	Rwanda	*P. phaseolus*	Large	RWV 1129	Rwanda	*P.phaseolus*	Large
RWR 281	Rwanda	*P. phaseolus*	Large	RWV 1348	Rwanda	*P.phaseolus*	Medium
RWR 3033	Rwanda	*P. phaseolus*	Large	RWV 2070	Rwanda	*P.phaseolus*	Large
RWR 310	Rwanda	*P. phaseolus*	Large	RWV2269(Check)	Rwanda	*P.phaseolus*	Large
RWR 3194	Rwanda	*P. phaseolus*	Large	RWV 2872	Rwanda	*P.phaseolus*	Large
RWR 3228	Rwanda	*P. phaseolus*	Large	RWV 2887	Rwanda	*P.phaseolus*	Large
RWR 3332	Rwanda	*P. phaseolus*	Large	RWV 3006	Rwanda	*P.phaseolus*	Large
RWR 3338	Rwanda	*P. phaseolus*	Large	RWV 3316	Rwanda	*P.phaseolus*	Large
RWR 390	Rwanda	*P. phaseolus*	Large	RWV 3317	Rwanda	*P.phaseolus*	Medium
SER 16	Rwanda	*P. phaseolus*	Medium	UBC 82013	Rwanda	*P.phaseolus*	Medium
SER 83	Rwanda	*P. phaseolus*	Medium	Vuninkingi	Rwanda	*P.phaseolus*	Small
SER 96(Check)	Rwanda	*P. phaseolus*	Small				
SMC 18	Rwanda	*P. phaseolus*	Medium				
SMC 21	Rwanda	*P. phaseolus*	Medium				

Large: weight of 100 seeds > 40g; Medium: weight of 100 seeds 25-40g; small: weight of 100 <25g.

In order to identify the adapted genotypes to be used for the experiments from their introduction, a preliminary screening trial was first conducted during Season 2014A (from September 2013 to January 2014) before the main trials. A complete set of 75 genotypes was then assembled for the main trials.

4.2.3 Experimental design

Trials were conducted using an 10 x 4 row by column lattice design for bush and a 9 x 4 row by column lattice design for climbers, with 10 blocks of four plots for bush and nine blocks and four plots for climbers each, with two replications. All genotypes were established in four-row plots with an inter-row spacing of 0.6 m and an intra-row spacing of 0.4 m. Experiments were established under rain-fed conditions. The susceptible genotype Colta was planted as a border of spreader rows around each trial, to serve as a source of inoculum.

Fertilizer was applied in the form of N_{17}-P_{17}-K_{17} at a rate of 100 kg ha^{-1} in split applications at planting and ridging. Neither pesticides nor fungicides were applied. Trials were maintained with the conventional cultural practices. Weeds were controlled by hand.

The genotypes were inoculated with the bean field debris collected in the previous season from plants showing symptoms that were characteristic of ascochyta. The inoculation was done 14 days after planting by the uniform spreading of ascochyta-infected debris in the field trials.

4.2.4 Data collection

The data collected included ascochyta disease severity and plot yield weight. Ascochyta symptoms were assessed from 21 days after planting. The two inner rows in each plot were visually rated at 14-day intervals for percentage of the leaf stem and pod area with ascochyta. The percentage of the diseased foliage of individual plants was estimated. The plant assessments were converted to a single value for each plot and the mean percentage diseased foliar area per plot was calculated (Madden, 1990; Beebe et al., 1991and Bryson et al., 1997). This was done visually by comparing the green and non-green leaf portions affected by the disease and by using a percentage scale, with severity scores ranging between 0 and 100, 1-25 being resistant, 26-50 being intermediate resistant and 51-100 being susceptible. The evaluations continued up to physiological maturity. At the end of each growing season, the plants were harvested and the dry seed yields for each genotype (kg ha^{-1}) were recorded.

4.2.5 Data analysis

For all plots and assessment dates, the area under the disease progress curve AUDPC was calculated for each genotype, using the midpoint rule method as per the equation suggested by Madden et al. (2007) in Equation 1 below:

$$AUDPC = \sum_{i=1}^{n} [(X_{i+1} + X_i)/2] * \ [t_{i+1} - t_i \ ]$$

Equation……. 1

Where X_i = the disease percentage representing the affected foliage at each at i^{th} evaluation day; t_{i+1} - t_i = times (in days) between two diseases scores and n = the total number of observations. Means were separated by the least significant difference at P≤0.05.

The estimates of AUDPC were normalized by dividing with the total area of the graph (i.e. the number of days from first appearance of the disease till the end of the observation period), in order to facilitate a better visual comparison among host genotypes, over the seasons and the sites tested (Fry, 1978). The normalized AUDPC was referred to as the relative area under the disease progress curve (RAUDPC).

$$RAUDPC = \frac{\sum(T_{i+1}) * \left(\frac{D_{i+1} + \ D_i}{2}\right)}{T_{total} \ * 100}$$

Equation…….2

In Equation 2 above, Ti is the i^{th} day when an estimation of percentage foliar blight is made and Di is the estimated percentage of area with diseased foliage at Ti. T_{total} is the number of days at which the final assessment was recorded.

In addition, the correlations between mean RAUDPC and the mean yield for Seasons A and B were determined. The RAUDPC was used to evaluate and select the parents to be used in a breeding programme. Total plot weight (based on the middle inner row) was measured and expressed in tons per hectare.

All the collected quantitative data were subjected to residual (or restricted) maximum likelihood (REML) spatial model analysis to fit the variance-components, using a computer software programme GENSTAT Version 17. Data from environments and cropping seasons (years) were combined.

Genotypes, environments and cropping seasons were considered fixed terms, while replications, rows and columns were considered random terms, as shown on the model below:

$$y_{ijklm} = M + Rep_i + Row(Rep)_{ij} + Col(Rep)_{ik} + V_i + L_m + VL_{lm} + VL_{jk} + eijklm$$

Equation…….3

Where Y_{ijklm} = observed value;

M = general mean;

Rep_i = effect of the i^{th} replication (where i=1, 2,3);

$Row(Rep)_{ij}$ = row effect nested within rep (where j= 1, 2…n);

$Col(Rep)_{ik}$ = column effect nested within rep (where k= 1, 2…n);

V_i = effect of the l^{th} genotype (where l= 1, 2….n);

L_m = effect of the m^{th} location (m=1,2);

VL_{lm} = interaction effect of the l^{th} genotype and m^{th} location;

VL_{jk} = interaction genotype x location; and

eijklm = random error.

Means were separated by the least significance difference (LSD) test, using a suitable error term.

4.3 Results

4.3.1 Weather data

Weather conditions were conducive to the development of ascochyta. There was regular rainfall and mean temperatures were around 18ºC (Table 4.3) throughout the two growing seasons, which promoted the development of ascochyta.

Table 4.3: Rainfall and mean temperatures of Nyamagabe, Musanze and Rwerere during the experimental period

		Location					
		Nyamagabe		Musanze		Rwerere	
Season	Month	Rainfall (mm)	Mean Temp (ºC)	Rainfall (mm)	Mean Temp (ºC)	Rainfall (mm)	Mean Temp (ºC)
Season 2014A	February	1520	19.0	1495	20.5	1060	20.0
	March	1530	19.2	1505	20.7	1200	20.1
	April	1870	16.8	1845	18.3	1565	19.0
	May	1600	17.8	1575	19.3	1240	20.0
	June	1470	19.6	1445	21.1	1020	20.3
Season 2015B	September	1460	18.0	1435	19.5	1300	19.0
	October	1290	19.2	1265	20.7	1390	19.9
	November	1000	18.5	975	20.0	1466	19.6
	December	1240	18.6	1215	20.1	1150	19.9
	January	1470	18.9	1445	20.4	1020	18.9

4.3.2 Disease severity

The significant main effects for ascochyta severity at growth stage R6 were obtained for genotypes, sites and year for this trait, for both bush and climbers (Table 4.4). The two-way interaction between genotypes (G) and years (Y) (GY) were significant ($P \leq 0.01$) for climbers. The three-way interaction GYL was not significant for both bean types.

Table 4.4: Analysis of variance on the disease severity of bean genotypes tested at R6 stage in Rwanda

Source	Bush		Climber	
	DF	**MS**	**DF**	**MS**
Location	2	1289.78**	2	764.36**
Year	1	707.51*	1	1112.76**
Genotype	38	2274.21**	35	4227.20**
Year*location	2	699.5*	2	96.83
Year*Genotype	37	141.25	34	1923.31**
Location*Genotype	74	234.29*	68	6.25
Year*Location*Genotype	74	141.25	68	7.35
Error	220	151.18	214	551.54
Corrected Total	450		437	
CV %		20.20		19.80

*, **= significant at 0.05 and 0.01 respectively; DF= Degree of freedom; MS= Mean square; and CV= Coefficient of variation.

Table 4.5: Mean severity of ascochyta disease (%) for bush and climbing bean genotypes at the R6 growth stage across two sites and two seasons

Bush	Nyamb	Rwere	Musze	Mean (%)	Climber	Nyamb	Rwere	Musze	Mean (%)
ALB 102	20.0	31.0	9.0	20.0	Agronome	8.3	5.5	6.5	6.8
ALB 155	20.0	26.0	50.0	32.0	CAB 2	8.0	4.0	5.8	5.9
ALB 58	8.5	5.8	7.0	7.1	Claudine	6.5	4.8	17.0	9.4
ASC 107	4.0	45.0	50.0	33.0	G 10747	1.8	1.8	1.8	1.8
ASC 87	2.0	4.0	5.5	3.8	G 35034	1.3	0.8	0.5	0.8
ASC 92	2.0	19.0	22.5	14.5	G 35084	1.5	0.8	1.0	1.1
ASC 94	1.0	1.6	1.0	1.2	G 35182	2.8	0.8	3.0	2.2
CAL 96	25.0	24.0	27.5	25.5	G 35306	1.8	0.8	3.0	1.8
CMS 17	4.5	40.0	35.0	26.5	G2331	14.3	9.3	12.3	11.9
ECAB 026	7.0	28.0	46.5	27.2	G2333	6.5	4.0	6.0	5.5
ICTA Hunapu	2.0	3.0	3.5	2.8	Garukurare	9.0	5.5	6.8	7.1
LSA142	4.0	12.0	16.5	10.8	Gasilida	6.3	6.3	9.0	7.2
Maharagesoja	16.0	28.2	22.5	22.2	Gitanga	12.3	4.3	6.3	7.6
MIB 755	3.0	7.4	7.5	6.3	Ibanga 2	9.7	6.0	8.3	8.0
Mixture (Check)	14.5	9.4	10.0	11.3	Kenyerumpure	7.0	3.5	7.0	5.8
NUA 377	15.0	17.6	17.5	16.7	Kigondo	4.8	3.5	5.0	4.4
NUA 379	17.5	54.0	30.0	33.8	Kivuzo	7.3	4.3	6.3	5.9
NUA 397	12.5	16.6	17.0	15.4	MAC 49	22.3	18.8	23.8	21.6
NUA 566	52.0	15.8	20.0	29.3	MAC44	23.0	14.8	16.5	18.1
RWK 10	57.5	38.0	45.0	46.8	MBC 12	13.8	9.8	13.0	12.2
RWR 1180	18.0	12.6	6.5	12.4	Mixture (Check)	6.5	4.3	6.5	5.8
RWR 1668	45.0	32.2	14.0	30.4	Nyamanza	8.8	8.0	9.5	8.8

Bush	Nyamb	Rwere	Musze	Mean (%)	Climber	Nyamb	Rwere	Musze	Mean (%)
RWR 2154	16.5	15.6	14.5	15.5	Nyaragikoti	11.3	4.5	8.0	7.9
RWR 2245	48.0	15.0	3.0	22.0	Nyirabukara	6.8	4.3	5.5	5.5
RWR 229	20.0	16.2	18.0	18.1	Nyiramagorori	8.5	4.5	6.3	6.4
RWR 278	37.5	8.2	3.5	16.4	Rwibarura 2	14.8	11.3	13.5	13.2
RWR 281	20.5	29.0	40.0	29.8	RWV 1129	15.8	8.0	11.3	11.7
RWR 3033	7.5	22.4	25.0	18.3	RWV 1348	7.5	4.5	6.8	6.3
RWR 310	12.0	25.0	37.5	24.8	RWV 2070	14.0	4.5	6.8	8.4
RWR 3194	23.5	37.8	52.5	37.9	RWV2269 (Check)	7.0	6.5	8.5	7.3
RWR 3228	26.0	37.6	9.0	24.2	RWV 2887	6.8	6.3	9.0	7.3
RWR 3332	11.0	27.0	9.0	15.7	RWV 3006	9.8	5.5	7.5	7.6
RWR 3338	29.0	5.6	2.5	12.4	RWV 3316	7.5	5.5	7.8	6.9
RWR 390	10.0	3.2	3.0	5.4	RWV 3317	7.3	7.0	9.0	7.8
SER 16	14.5	14.0	15.0	14.5	RWV2872	10.0	5.3	9.0	8.1
SER 83	18.5	4.6	5.0	9.4	UCB 82013	15.0	6.3	8.8	10.0
SER 96 (Check)	12.5	5.6	4.0	7.4	Vuninkingi	7.8	3.5	6.3	5.8
SMC 18	10.0	3.8	3.5	5.8					
SMC 21	23.5	4.2	6.0	11.2					
Mean (%)	17.5	22.1	21.2		Mean (%)	9.0	9.0	5.6	8.1
lsd (0.05)a	11.8				lsd (0.05)a	5.8			
lsd (0.05)b	9.7				lsd (0.05)b	3.7			
lsd (0.05)c	16.6				lsd (0.05)c	2.6			

[a] genotypes; [b] location; [c] genotype x location, [d] genotype x year, lsd=least significant difference

For the climbing beans (Table 4.5), a moderate to high level of severity was achieved, with the Rwerere site having the lowest average severity (5.6%), while the Nyamagabe had the highest ascochyta severity (9.0%).

MAC 49 had the highest ascochyta severity across sites (21.6%), compared to the other climbing genotypes, followed by MAC 44 (18.1%). The lowest severity for climbing beans was achieved for genotype G 35034 (0.8 %), followed by G 35084 (1.1%). The latter two are entries from the CIAT gene bank. For bush beans, the Rwerere site had the highest mean percentage severity (22.0%), followed by Musanze (21.1%), while Nyamagabe had the lowest severity (17.5%).

The genotype RWK 10 had the highest mean ascochyta severity across all sites (46.8%), followed by RWR 3194 (37.9%). The lowest percentage ascochyta severity is seen for genotype ASC 94 (1.2%), followed by ICTA Hunapu (2.8%), both from the CIAT.

Table 4.6: Analysis of variance on Relative Area Under Disease Progress Curve (RAUDPC) of bean genotypes tested in Rwanda

Source	**Bush**		**Climber**	
	DF	**MS**	**DF**	**MS**
Location	2	1985.91**	2	66718475.70**
Year	1	137.25**	1	634677.50**
Genotype	38	1258.29**	35	225287.20**
Year*Location	2	137.25	2	277414.20*
Year*Genotype	37	104.06	34	137057.10**
Location*Genotype	74	164.71**	68	168562.10
Year*Location*Genotype	74	104.06	68	102938.40
Error	220	90.049	214	78059.90
Corrected Total	450		437	
CV %		15.5		17.6

*, **= significant at 0.05 and 0.01 respectively; DF= Degree of freedom; MS= Mean square; and CV= Coefficient of variation.

Genotypes, location and cropping seasons (year) were significantly different for RAUDPC (Table 4.6). The two-way interaction between the genotypes and the year was significant

(P≤001) for this trait. The interaction between genotypes and location was also significant, while the three-way interaction genotype, year and location were not significant for the RAUDPC.

The mean across all sites for the severity of ascochyta measured as RAUDPC (Table 4.7 and 4.8) was significantly higher in Season 2014B for both bush (149.0) and climber (50.5) types, than in the Season 2014A. The reaction of the genotypes to the pathogen was different and there was a high positive correlation between the RAUDPC of Seasons A and B (r = 0.86).

Table 4.7: Average RAUDPC for ascochyta of 39 bush bean genotypes tested in six environments in Rwanda

Genotypes	Nyamb A	Nyamb B	MusanzeA	MusanzeB	RwereA	RwereB	M A	M B	M(A&B)	Class
ALB 102	94.5	90.9	186.5	189.0	411.0	300.0	230.7	193.3	212.0	S
ALB 155	94.0	90.4	140.5	143.0	435.5	289.3	223.3	174.2	198.8	S
ALB 58	45.5	41.9	64.0	66.5	52.0	59.3	53.8	55.9	54.9	I
ASC 107	34.0	30.4	29.5	32.0	231.5	131.8	98.3	64.7	81.5	I
ASC 87	12.0	8.4	16.5	19.0	32.0	25.5	20.2	17.6	18.9	R
ASC 92	19.0	15.4	27.5	30.0	26.5	28.3	24.3	24.5	24.4	R
ASC 94	10.0	6.4	17.5	20.0	83.0	51.5	36.8	26.0	31.4	R
CAL 96	117.0	113.4	119.0	121.5	90.5	106.0	108.8	113.6	111.2	S
CMS 17	28.0	24.4	17.5	20.0	150.5	85.3	65.3	43.2	54.3	I
ECAB 026	46.0	42.4	163.5	166.0	56.0	111.0	88.5	106.5	97.5	I
ICTA Hunapu	16.0	12.4	16.5	19.0	32.0	25.5	21.5	19.0	20.2	R
LSA142	27.0	23.4	27.5	30.0	29.5	29.8	28.0	27.7	27.9	R
Maharagesoja	74.5	70.9	151.5	154.0	188.0	171.0	138.0	132.0	135.0	S
MIB 755	27.0	23.4	38.5	41.0	23.0	32.0	29.5	32.1	30.8	R
Mixture(Check)	81.0	77.4	56.0	58.5	56.0	57.3	64.3	64.4	64.4	I
NUA 377	77.0	73.4	45.5	48.0	53.0	50.5	58.5	57.3	57.9	I
NUA 379	79.5	75.9	42.5	45.0	166.0	105.5	96.0	75.5	85.7	I
NUA 397	66.0	62.4	114.0	116.5	217.5	167.0	132.5	115.3	123.9	S
NUA 566	213.0	209.4	208.0	210.5	173.0	191.8	198.0	203.9	200.9	S
RWK 10	258.0	254.4	212.5	215.0	137.5	176.3	202.7	215.2	208.9	S
RWR 1180	86.5	82.9	60.5	63.0	172.5	117.8	106.5	87.9	97.2	I
RWR 1668	216.5	212.9	212.0	214.5	69.0	141.8	165.8	189.7	177.8	S
RWR 2154	41.0	37.4	71.5	74.0	91.5	82.8	68.0	64.7	66.4	I

Genotypes	Nyamb A	Nyamb B	MusanzeA	MusanzeB	RwereA	RwereB	M A	M B	M(A&B)	Class
RWR 2245	40.0	36.4	52.5	55.0	60.5	68.0	51.0	53.1	52.1	I
RWR 229	104.0	100.4	81.0	83.5	143.5	113.5	109.5	99.1	104.3	S
RWR 278	209.5	205.9	261.5	264.0	230.0	247.0	233.7	239.0	236.3	S
RWR 281	117.0	113.4	52.0	54.5	384.0	219.3	184.3	129.0	156.7	S
RWR 3033	39.0	35.4	168.0	170.5	219.0	194.8	142.0	133.5	137.8	S
RWR 310	56.0	52.4	112.0	114.5	106.0	110.3	91.3	92.4	91.9	I
RWR 3194	127.5	123.9	150.5	153.0	271.0	212.0	183.0	163.0	173.0	S
RWR 3228	138.5	134.9	95.0	97.5	88.5	93.0	107.3	108.5	107.9	S
RWR 3332	59.5	55.9	84.0	86.5	75.5	81.0	73.0	74.5	73.7	I
RWR 3338	141.0	137.4	164.0	166.5	130.0	148.3	145.0	150.7	147.9	S
RWR 390	51.5	47.9	86.5	89.0	65.0	77.0	67.7	71.3	69.5	I
SER 16	70.0	66.4	107.5	110.0	181.0	145.5	119.5	107.3	113.4	S
SER 83	86.5	82.9	174.0	176.5	86.0	131.3	115.5	130.2	122.9	S
SER.96(Check)	63.0	59.4	49.0	51.5	372.5	212.0	161.5	107.6	134.6	S
SMC 18	52.5	48.9	30.5	33.0	51.5	42.3	44.8	41.4	43.1	R
SMC 21	100.0	96.4	27.0	29.5	95.5	62.5	74.2	62.8	68.5	I
Mean	82.5	78.9	95.7	98.2	142.0	120.4	106.7	99.2	102.9	
lsd (0.05)a	24.5									
Lsd (0.05)b	19.6									
lsd (0.05)c	11.8									

.[a] genotype; [b] season; [c] genotype x site; [d] genotype x season

MA = Mean season A; MB = Mean season B; M (A&B) = General mean of season A and B; RAUDPC= Relative area under disease progress curve; R= Resistant; S=Susceptible; and I= Intermediate resistance

The mean RAUDPC value across two seasons for bush genotypes ranged from 31 to 266, whereby genotypes with the RAUDPC value <50 were considered resistant, 50-100 as having intermediate resistance and those having RAUDPC >100 being susceptible.

For climbing genotypes, the mean RAUDPC values across two seasons ranged from 16 to 98, with RAUDPC value of <40 being considered resistant, 40-80 as having intermediate resistance and RAUDPC > 80 being considered susceptible.

The majority of bush genotypes (46.1%) showed a susceptible reaction to the pathogen, with severe symptoms on leaves, stems and pods. A total of 36.0% of the genotypes showed an intermediate reaction, with disease symptoms limited to small lesions, and only seven genotypes (17.9%) showed resistance to the pathogen. In some resistant plants, a few symptoms were identified, mainly on the primary leaves.

Table 4.8: Average RAUDPC of 36 climbing bean genotypes tested in six environments in Rwanda

Genotypes	NyambA	NyambB	MusanzeA	MusanzeB	Rwerere A	Rwerere B	M A	M B	M(A&B)	Class
Agronome	25.0	59.5	31.5	55.5	59.5	63.5	38.7	59.5	49.1	I
CAB 2	75.5	45.0	23.0	31.5	48.5	41.0	49.0	39.2	44.1	I
Claudine	11.5	58.0	27.5	41.0	26.0	48.0	21.7	49.0	35.3	R
G 10747	13.0	9.0	30.0	10.0	16.5	21.0	19.8	13.3	16.6	R
G 35034	13.5	7.0	39.0	9.0	17.5	9.0	23.3	8.3	15.8	R
G 35084	11.5	14.0	39.5	11.0	41.5	15.0	30.8	13.3	22.1	R
G 35182	38.5	7.0	35.0	7.0	14.5	7.0	29.3	7.0	18.2	R
G 35306	28.5	7.0	42.0	7.0	16.5	7.0	29.0	7.0	18.0	R
G2331	30.0	56.5	47.5	43.0	67.5	46.5	48.3	48.7	48.5	I
G2333	36.0	49.5	29.0	33.0	23.0	37.0	29.3	39.8	34.6	R
Garukurare	26.0	55.5	32.5	46.0	19.5	55.5	26.0	52.3	39.2	R
Gasilida	16.5	49.0	21.5	55.0	28.5	68.0	22.2	57.3	39.8	I
Gitanga	80.5	54.0	44.5	40.5	27.0	48.0	50.7	47.5	49.1	I
Ibanga 2	7.0	60.0	33.5	55.0	23.0	56.5	21.2	57.2	39.2	R
Kenyerumpure	46.0	41.0	37.0	34.0	38.5	39.5	40.5	38.2	39.3	R
Kigondo	22.0	44.0	70.5	37.5	34.5	41.0	42.3	40.8	41.6	I
Kivuzo	21.0	51.5	31.0	35.0	29.5	49.0	27.2	45.2	36.2	R
MAC 49	43.0	71.0	38.5	74.0	21.0	90.5	34.2	78.5	56.3	I
MAC44	76.0	112.0	28.5	90.5	68.0	94.5	57.5	99.0	78.3	I
MBC 12	30.5	103.5	34.5	92.5	33.5	101.0	32.8	99.0	65.9	I
MixtureCheck	16.0	55.5	17.0	39.5	29.5	49.0	20.8	48.0	34.4	R
Nyamanza	54.5	43.0	41.5	62.5	27.0	82.5	41.0	62.7	51.8	I
Nyaragikoti	23.0	57.5	41.0	48.0	36.0	59.5	33.3	55.0	44.2	I

Genotypes	NyambA	NyambB	MusanzeA	MusanzeB	Rwerere A	Rwerere B	M A	M B	M(A&B)	Class
Nyirabukara	90.0	36.5	69.0	34.5	51.0	43.5	70.0	38.2	54.1	I
Nyiramagorori	36.0	57.5	42.0	44.0	21.0	52.0	33.0	51.2	42.1	I
Rwibarura 2	147.0	86.5	35.5	94.5	47.0	105.0	76.5	95.3	85.9	I
RWV 1129	72.0	98.0	27.5	79.0	50.5	80.5	50.0	85.8	67.9	I
RWV 1348	52.0	53.0	25.5	44.0	41.0	45.5	39.5	47.5	43.5	I
RWV 2070	37.0	71.5	32.0	38.0	39.0	44.0	36.0	51.2	43.6	I
RWV 2887	52.0	51.5	30.0	60.5	26.5	69.0	36.2	60.3	48.3	I
RWV 3006	24.0	60.5	30.0	48.5	24.5	58.0	26.2	55.7	40.9	I
RWV 3316	43.0	42.0	38.5	40.5	40.0	50.0	40.5	44.2	42.3	I
RWV 3317	40.0	65.5	51.5	59.5	30.0	64.0	40.5	63.0	51.8	I
RWV2269Check	9.0	49.0	36.0	51.5	30.5	64.5	25.2	55.0	40.1	I
RWV2872	68.0	54.5	76.5	48.0	28.5	69.5	57.7	57.3	57.5	I
UCB 82013	36.5	57.0	33.0	53.0	49.5	65.0	39.7	58.3	49.0	I
Vuninkingi	9.0	51.0	54.5	28.0	37.5	37.5	33.7	38.8	36.3	R
Mean	39.5	52.5	37.8	45.4	34.1	53.4	37.1	50.5	43.8	
lsd (0.05)a	25.5									
Lsd (0.05)b	18.9									
lsd (0.05)c	22.4									

[a] genotype; [b] season; [c] genotype X season; MA = Mean season A; MB = Mean season B; M (A&B) = General mean of season A and B; RAUDPC= Relative area under disease progress curve; R= Resistant; S=Susceptible; and I= Intermediate resistance.

Most of the climbers had intermediate resistance (69.4%). Only six genotypes (16.6%) showed a resistant reaction and five genotypes showed a susceptible reaction (14%) (Table 4.8). The ascochyta was more severe at the Rwerere and Nyamagabe sites, than at the other sites, for both bush and climber.

Using the RAUDPC values obtained in the trials, the climbing beans had a lower number of resistant genotypes, compared to the bush types. The obtained data show that all the genotypes that gave a resistant reaction to ascochyta, possessed light pink or dark pink flowers. Findings further revealed that all resistant genotypes were small-seeded, but with varying seed colours.

On resistant genotype, the lesions caused by the ascochyta pathogen were dark concentric spots with defined borders and, in most cases; the symptoms were limited to the primary leaves. In susceptible bush genotypes, symptoms were scattered throughout the canopy leaves, stems and pod. Lesions caused by the pathogen gave a black coloration to the leaf veins, with brown margins, while the pods had sunken cankerous centres. These symptoms varied in intensity, depending on the resistance resident in the different genotypes.

4.3.3 Relationship between ascochyta and yield

The analysis of variance shows that significant differences were observed in the yield obtained from the different genotypes, locations and two-way interactions of location x genotype and genotype x year, for both bush and climber type (Table 4.9).

Table 4.9: Analysis of variance on the yield of bean genotypes tested in Rwanda

Source	Bush		Climber	
	DF	**MS**	**DF**	**MS**
Location	2	9558998.61**	2	66718475.7**
Year	1	405282.30*	1	634677.50*
Genotype	38	173477.56**	35	225287.20**
Year*location	2	385939.36*	2	277414.20*
Year*Genotype	37	23347.38	34	137057.10**
Location*Genotype	74	106748.51*	68	168562.10**
Year*Location*Genotype	74	23002.07	68	102938.40
Error	220	62878.88	214	78059.90
Corrected Total	450		437	
CV %		11.60		13.20

*, **= significant at 0.05 and 0.01 respectively; DF= Degree of freedom; MS= Mean square; and CV= Coefficient of variation.

Significant GY interaction for seed yield indicates that seasonal variation affected the relative yield performance of genotypes under the natural infection of bean ascochyta. The severity of bean ascochyta was found to depend on seasonal variation, and genotypic variation was observed among tested genotypes (Figure 4.1 and 4.2).

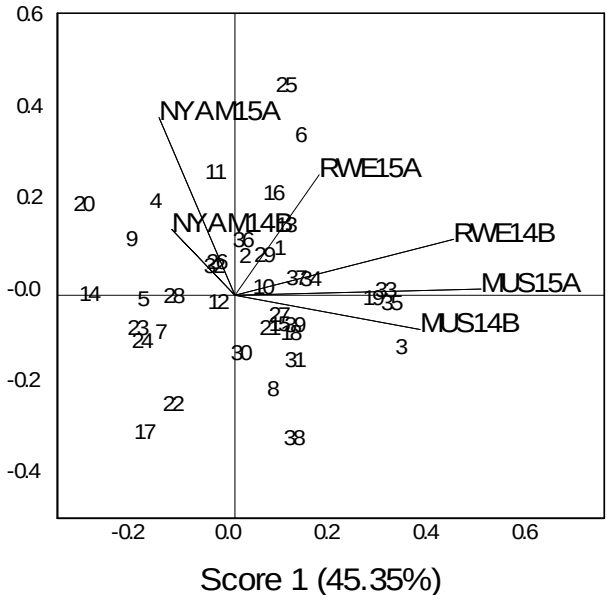

Figure 4.1: GG Biplot of seed yield for 39 bush bean genotypes for two cropping seasons in three environments subjected to natural ascochyta infection in Rwanda. Genotypes are indicated by numbers and environments by vectors

The differences of mean grain yield across geographic locations imply that not only the genotypes and locations, but also variations in seasons or environmental conditions during different seasons, greatly influence the grain yield performance. Similarly, grain yield obtained in different locations in the first season was different from that obtained during second.

The biplots show that the bush genotypes LSA 142 (12), RWR 3194 (30), CAL 96 (8) and the climber genotypes CAB 2 (2), Nyiragikoti (23) and Kivuzo (17) attained values relatively close to zero and hence are more stable and widely adaptable genotypes across all locations (Figure 4.1 and 4.2).

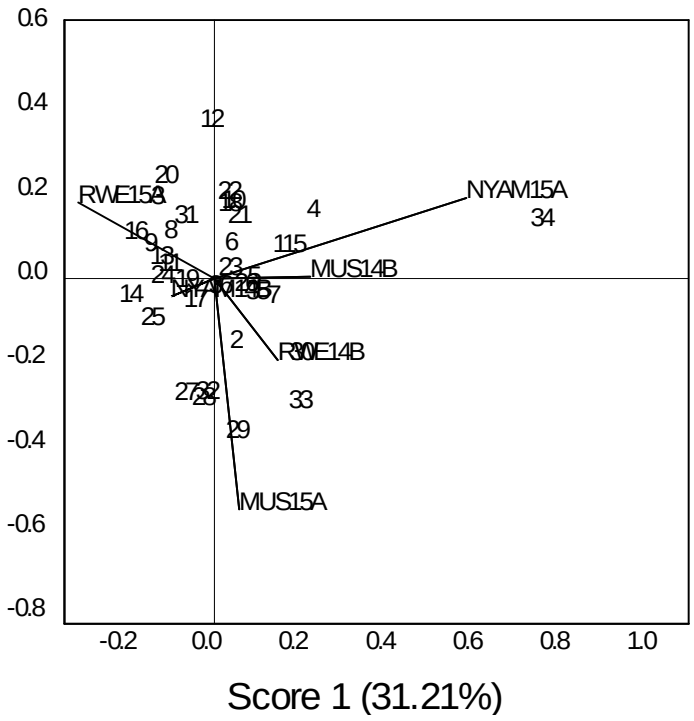

Figure 4.2: GG Biplot of seed yield for 36 climbing bean genotypes for two cropping seasons in three environments subjected to natural ascochyta infection in Rwanda. Genotypes are indicated by numbers and environments by vectors

Table 4.10: Average yield (kg ha^{-1}) of bush bean genotypes evaluated in six environments

Genotypes	Nyamb A	Nyamb B	MusanzeA	MusanzeB	RwereA	RwereB	M A	M B	M(A&B)	Class
ALB 102	187.5	195.0	250.0	239.6	562.5	312.5	333.3	249.0	291.2	LY
ALB 155	250.0	260.0	187.5	179.7	375.0	375.0	270.8	271.6	271.2	LY
ALB 58	312.5	325.0	187.5	179.7	437.5	562.5	312.5	355.7	334.1	MY
ASC 107	500.0	520.0	250.0	239.6	875.0	1070.0	541.7	609.9	575.8	HL
ASC 87	562.5	585.0	187.5	179.7	1062.5	950.0	604.2	571.6	587.9	HL
ASC 92	937.5	975.0	250.0	239.6	875.0	975.0	687.5	729.9	708.7	HL
ASC 94	750.0	780.0	187.5	179.7	250.0	750.0	395.8	569.9	482.9	MY
CAL 96	187.5	195.0	437.5	419.3	625.0	1125.0	416.7	579.8	498.2	MY
CMS 17	187.5	195.0	250.0	239.6	687.5	500.0	375.0	311.5	343.3	MY
ECAB 026	312.5	325.0	437.5	419.3	687.5	812.5	479.2	518.9	499.0	MY
ICTA Hunapu	562.5	585.0	250.0	239.6	750.0	1162.5	520.8	662.4	591.6	HL
LSA142	300.0	312.0	312.5	299.5	937.5	1050.0	516.7	553.8	535.2	HL
Maharagesoja	175.0	182.0	137.5	131.8	437.5	375.0	250.0	229.6	239.8	LY
MIB 755	625.0	650.0	75.0	71.9	687.5	880.0	462.5	534.0	498.2	MY
MixturCheck)	162.5	169.0	275.0	263.5	875.0	687.5	437.5	373.3	405.4	MY
NUA 377	875.0	910.0	125.0	119.8	750.0	1187.5	583.3	739.1	661.2	HY
NUA 379	125.0	130.0	87.5	83.9	375.0	1125.0	195.8	446.3	321.1	LY
NUA 397	425.0	442.0	187.5	179.7	750.0	937.5	454.2	519.7	486.9	MY
NUA 566	250.0	260.0	562.5	539.1	625.0	687.5	479.2	495.5	487.3	MY
RWK 10	375.0	390.0	75.0	71.9	500.0	1000.0	316.7	487.3	402.0	MY
RWR 1180	250.0	260.0	93.8	89.8	437.5	750.0	260.4	366.6	313.5	LY
RWR 1668	625.0	650.0	562.5	539.1	562.5	750.0	583.3	646.4	614.8	HY
RWR 2154	437.5	455.0	500.0	479.2	812.5	1187.5	583.3	707.2	645.3	HL

Genotypes	Nyamb A	Nyamb B	MusanzeA	MusanzeB	RwereA	RwereB	M A	M B	M(A&B)	Class
RWR 2245	625.0	650.0	312.5	299.5	625.0	812.5	520.8	587.3	554.1	HY
RWR 229	125.0	130.0	187.5	179.7	562.5	1125.0	291.7	478.2	384.9	MY
RWR 278	312.5	325.0	437.5	419.3	625.0	437.5	458.3	393.9	426.1	MY
RWR 281	562.5	585.0	312.5	299.5	1062.5	812.5	645.8	565.7	605.7	HY
RWR 3033	437.5	455.0	250.0	239.6	812.5	750.0	500.0	481.5	490.8	MY
RWR 310	175.0	182.0	375.0	359.4	500.0	937.5	350.0	493.0	421.5	MY
RWR 3194	125.0	130.0	375.0	359.4	625.0	750.0	375.0	413.1	394.1	MY
RWR 3228	187.5	195.0	312.5	299.5	437.5	1125.0	312.5	539.8	426.2	MY
RWR 3332	562.5	585.0	250.0	239.6	750.0	1062.5	520.8	629.0	574.9	HY
RWR 3338	500.0	520.0	250.0	239.6	687.5	1000.0	479.2	586.5	532.8	HY
RWR 390	312.5	325.0	312.5	299.5	625.0	937.5	416.7	520.7	468.7	MY
SER 16	125.0	130.0	187.5	179.7	562.5	687.5	291.7	332.4	312.0	LY
SER 83	562.5	585.0	337.5	323.4	812.5	750.0	570.8	552.8	561.8	HL
SER96Check)	562.5	585.0	250.0	239.6	375.0	1125.0	395.8	649.9	522.8	HL
SMC 18	237.5	247.0	137.5	131.8	500.0	500.0	291.7	292.9	292.3	LY
SMC 21	100.0	104.0	312.5	299.5	875.0	750.0	429.2	384.5	406.8	MY
Mean	381.7	397.0	268.4	257.2	650.6	840.4	433.6	498.2	465.9	
lsd (0.05)a	13.8									
Lsd (0.05)b	20.1									
lsd (0.05)c	19.5									

[a] genotype; [b] season; [c] genotype x site; genotype x season; MA = Mean season A; MB = Mean season B; M (A&B) = General mean of season A and B; HY= High yielding genotype; LY=Low yielding genotype; and MY=Medium yielding genotype.

The dry seed yield of the different genotypes across seasons and locations ranged from 241.0–709.0 kg ha^{-1} and from 723.0 – 1402.0 kg ha^{-1}, for bush and climbers, respectively (Table 4.10 and 4.11). Considering the reaction to ascochyta, the mean yield varied widely. For the resistance genotype, the yield range was between 292.0 – 709.0 kg ha^{-1} and 870.2-1322.9 kg ha^{-1} for bush and climber resistant genotypes, respectively.

The genotypes with intermediate resistance showed a yield range of 271.19 – 645.2 kg ha^{-1} and 723.1 – 1401.5 kg ha^{-1} for bush and climber types, respectively, whereas for susceptible genotypes the yield range was from 239.7 – 661.1 kg ha^{-1} and 723.0 – 943.0 kg ha^{-1} for bush and climbing genotypes, respectively (Tables 4.7, 4.8, 4.10 and 4.11).

Table 4.11: Average yield (kg ha^{-1}) of 36 climbing bean genotypes tested in six environments in Rwanda

Genotypes	NyambA	NyambB	MusanzeA	MusanzeB	Rwerere A	Rwerere B	M A	M B	M(A&B)	Class
Agronome	750.0	551.3	787.5	784.0	1687.5	1500.0	1075.0	945.1	1010.0	MY
CAB 2	625.0	110.3	575.0	673.8	1000.0	1500.0	733.3	761.3	747.3	LY
Claudine	375.0	520.0	687.5	851.5	1250.0	1875.0	770.8	1082.2	926.5	LY
G 10747	333.8	204.6	750.0	977.5	937.5	2250.0	673.8	1144.0	908.9	LY
G 35034	1003.0	775.0	975.0	1077.8	1375.0	1075.0	1117.7	975.9	1046.8	MY
G 35084	671.0	635.0	987.5	882.0	1437.5	1425.0	1032.0	980.7	1006.3	MY
G 35182	473.8	691.8	875.0	1183.8	1250.0	1275.0	866.3	1050.2	958.2	LY
G 35306	437.5	850.0	1050.0	1184.5	1500.0	1280.0	995.8	1104.8	1050.3	MY
G2331	312.5	1163.8	1187.5	869.8	625.0	2187.5	708.3	1407.0	1057.7	MY
G2333	1062.5	857.5	725.0	526.8	1312.5	1875.0	1033.3	1086.4	1059.9	MY
Garukurare	1312.5	1408.8	812.5	1029.0	1562.5	1812.5	1229.2	1416.8	1323.0	HY
Gasilida	1000.0	490.0	537.5	600.3	1687.5	2187.5	1075.0	1092.6	1083.8	MY
Gitanga	562.5	367.5	1112.5	1874.3	1437.5	1062.5	1037.5	1101.4	1069.5	MY
Ibanga 2	500.0	367.5	837.5	673.8	1562.5	1812.5	966.7	951.3	959.0	LY
Kenyerumpure	375.0	490.0	925.0	906.5	1187.5	1937.5	829.2	1111.3	970.3	LY
Kigondo	500.0	1041.3	1762.5	1335.3	1312.5	1750.0	1191.7	1375.5	1283.6	HY
Kivuzo	1375.0	918.8	775.0	710.5	1500.0	1375.0	1216.7	1001.4	1109.0	MY
MAC 49	375.0	245.0	962.5	943.3	1312.5	1187.5	883.3	791.9	837.6	LY
MAC44	562.5	183.8	712.5	1163.8	1250.0	1625.0	841.7	990.8	916.3	LY
MBC 12	625.0	428.8	862.5	698.3	1250.0	812.5	912.5	646.5	779.5	LY
Mixture (Check)	562.5	673.8	765.0	468.5	1375.0	1500.0	900.8	880.8	890.8	LY
Nyamanza	562.5	367.5	1037.5	624.8	1875.0	1187.5	1158.3	726.6	942.5	LY
Nyaragikoti	937.5	428.8	1025.0	735.0	1250.0	1500.0	1070.8	887.9	979.4	LY

Genotypes	NyambA	NyambB	MusanzeA	MusanzeB	Rwerere A	Rwerere B	M A	M B	M(A&B)	Class
Nyirabukara	500.0	1163.8	1725.0	796.3	1625.0	1625.0	1283.3	1195.0	1239.2	MY
Nyiramagorori	1562.5	1225.0	1050.0	759.5	1500.0	1937.5	1370.8	1307.3	1339.1	HY
Rwibarura 2	1312.5	428.8	887.5	1090.3	687.5	1250.0	962.5	923.0	942.8	LY
RWV 1129	237.5	490.0	687.5	845.3	1250.0	1500.0	725.0	945.1	835.0	LY
RWV 1348	500.0	171.5	637.5	735.0	1250.0	1687.5	795.8	864.7	830.3	LY
RWV 2070	687.5	612.5	800.0	943.3	1312.5	1562.5	933.3	1039.4	986.4	LY
RWV2269(Check)	375.0	1041.3	900.0	2593.0	1437.5	2062.5	904.2	1898.9	1401.5	HY
RWV 2887	562.5	612.5	750.0	771.8	1375.0	1375.0	895.8	919.8	907.8	LY
RWV 3006	300.0	355.3	750.0	808.5	1062.5	1062.5	704.2	742.1	723.1	LY
RWV 3316	750.0	428.8	962.5	1016.8	1125.0	1312.5	945.8	919.3	932.6	LY
RWV 3317	625.0	367.5	1287.5	1261.8	1625.0	1250.0	1179.2	959.8	1069.5	MY
RWV2872	500.0	367.5	1912.5	1690.5	1500.0	1875.0	1304.2	1311.0	1307.6	HY
UCB 82013	487.5	490.0	825.0	563.5	1062.5	1187.5	791.7	747.0	769.3	LY
Vuninkingi	1187.5	796.3	1362.5	1004.5	1437.5	1000.0	1329.2	933.6	1131.4	MY
Mean	672.5	603.3	953.1	963.6	1329.4	1531.9	985.0	1032.9	1008.9	
lsd (0.05)a	28.6									
Lsd (0.05)b	31.1									
lsd (0.05)c	23.4									

[a] genotype; [b] season; [c] genotype x site; genotype x season; MA = Mean season A; MB = Mean season B; M (A&B) = General mean of season A and B; HY= High yielding genotype; LY=Low yielding genotype; and MY=Medium yielding genotype

There were genotypes with an intermediate resistant reaction that yielded significantly higher than genotypes with a resistant reaction. This observation was also true for the susceptible, versus the intermediate resistant, genotypes (Tables 4.7, 4.8, 4.10 and 4.11). Although there was a strong correlation ($r = 0.62$; $P \leq 0.05$) between the yields of the two seasons, mean yields obtained in the second season (Season 2014B) were significantly higher than those obtained in the first season for bush and climbers types. The results also indicate a significant negative correlation between the RAUDPC and the yield ($r = -0.51$).

Bush genotypes NUA 377, RWR 2154 and ASC 92, and climbing genotypes Garukurare, G2331 and RWV 2269, were adapted to the short rainy season (Season B). Bush genotypes, RWR 281, ASC 87 and ICTA Hunapu, and climbing genotypes Vuninkingi, Nyiramagorori and RWV 2872, were best adapted to the long rainy season (Season A)

Considering the genotypic performance based on the geometric mean (M A&B), which is associated with yield performance in different sites, a number of bush genotypes, both landraces and bean ascochyta resistant genotypes (introductions from CIAT), consistently outperformed the local checks, indicating their broad adaptation under a varied environment (Tables 4.10 and 4.11). For climbers, the best check from Rwandan germplasm (RWV 2269) was outstanding in its yield. A range of seed sizes, from small to large, existed among the genotypes. The top two performing lines had relatively small seed sizes.

4.4 Discussion

Sources of good resistance are an important tool to pursue as the principal element in a breeding programme. The best possible method for identifying resistant sources is to expose the potential sources of resistance to all dominant pathogens over different production areas, in order to eliminate the highly susceptible genotypes (Beebe et al., 1991; Beebe et al., 2000). In this study, a germplasm collection of 75 dry bean genotypes was screened to establish whether there were any genotypes which could be used as effective sources of ascochyta resistance. The use of severity and the relative area under the disease progress curve (RAUDPC), as tools for the plant resistance evaluation, help to reflect on the progress of the disease throughout the growing season (Campbell and Madden, 1990). In this study, the highest severity and RAUDPC values represented genotypes with the highest disease infection. There were differences in the severity and RAUDPC values between genotypes, within the seasons and between the seasons. The differences that were observed suggest differences in the resistance of the individual genotypes. On the other hand, the difference observed between seasons could be explained by the differences in the climatic conditions. Related studies conducted by Hanson et al. (1993) on bean ascochyta, show that climatic conditions have a strong influence on the severity.

According to Evans (1993), disease is one of the major factors affecting crop yield, as it disrupts the balance between the sources and sink activities of the plant. In this study, ascochyta had a negative effect on yield, especially with the most susceptible genotypes. The season with the highest disease severity, was also observed to have a better yield performance. The inconsistency in results could be explained by the Gaunt (1995) theory, which states that the green leaf area and the green area duration is directly correlated to yield, in both the healthy and diseased crop species. The observations from the study show that Season B, which had higher disease severity, also had better climatic conditions, resulting in longer green leaf area duration, which culminated in higher yields. In addition, halo blight disease was observed in the field during the first season trials, and which caused some plant deaths. This could also have contributed to the lower yield observed in Season A.

Furthermore, a strong negative correlation between disease and yield would be expected but, as reported in this study, the correlation between these two factors, although negative, was only moderately strong and cannot fully explain all the yield variations. A partial explanation could be

offered with regard to the tolerance observed in some genotypes, which resulted in high yields despite high disease severity. According to Gaunt and Bryson (2013), the absence of a strong negative relationship between yield and RAUDPC is more common when data from different seasons are used, as was the case in this study. The analysis of data for the individual seasons showed a moderate correlation (r =-0.42 and r = 0.51 for Seasons A and B, respectively). This moderate correlation between yield and RAUDPC could partially be explained by the variation in defoliation, the variation in growth habits and the differences in yield potential that was exhibited by the different genotypes. In addition, measuring disease by visual rating lacked precision and accuracy. As revealed by O'Brien and Van Bruggen (1992), the inaccuracies made while measuring disease in the field are a major constraint, when relating disease to yield, and in some cases, there may be no relationship between these two variables. Similar studies by Waggoner and Berger (1987), Gaunt (1995) and Filho et al. (1997) have indicated that the measurement of disease severity, based on lesion number or leaf area, may be less related to yield.

It can thus be noted that the measurement of disease may not have a direct relationship to yield, but it may give an indication of the amount of yield that may be lost if the plant is susceptible to the pathogen. Ascochyta attacks plant leaves, stems and pods, not only interrupting the plant's ability to take in photosynthetic materials, but also utilizing the plant's substrates and damaging the host's functions, thus reducing its ability to yield effectively. According to Gaunt (1995), disease severity implies that when the host is damaged, the yield obtained will not be based on the level of pathogen development, but rather on the host reaction.

The study further indicates the relationship between crop resistance and some phenotypic traits. Results showed that a number of resistant genotypes had either pink or red flowers and were small-seeded. A large amount of bush germplasm that was collected for use in this study, was susceptible. It was also observed that most of the genotypes showing resistance, yielded far better than those showing an intermediate and susceptible disease reaction. However, some genotypes gave intermediate reactions, but yielded better than some resistant genotypes. These genotypes could be described as being tolerant to *Phoma exigua*. With the exception of the thirteen genotypes obtained from outside Rwanda, the remaining two resistant genotypes originated from Rwanda. It is possible that resistant genotypes may have been selected by farmers as a result of the high disease pressure, but this is a hypothesis which needs further investigation.

Finally, the use of RAUDPC as the measure of resistance was very useful in this study, as it was able to show that out of the 75 germplasm genotypes, 13 gave a consistent resistant reaction to the ascochyta pathogen in Rwanda, 29 gave an intermediate resistance reaction and 23 were susceptible. It is therefore suggested that use should be made of the identified resistant lines in the development of an ascochyta breeding programme for the Rwandan common bean genotypes. There is also a need for further studies, to determine the quality of resistance exhibited by the resistant genotypes.

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Chapter Five: Genetic analysis of resistance to bean ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] among common bean genotypes in Rwanda

Abstract

Suitable genotypes resistant to bean ascochyta are currently not available and there is limited information on the inheritance of bean ascochyta resistance traits in the common bean. Understanding the mode of inheritance of the disease would facilitate the development of an appropriate breeding strategy. Therefore, this study was conducted to determine the mode of inheritance of resistance to ascochyta in two trials involving bush (Types I, II, III) and climber parents (Type IV). An 8 x 8 diallel mating design, including reciprocals, was used to develop 112 F_1 crosses for both bush and climbers. Resistance to ascochyta was found to be additive in nature, because the GCA effects were highly significant in F_1 generations. Even though overall SCA effects were not significant, two bush crosses (RWR 2245 x ASC 87 and RWR 275 x MIB 755) and two climbers crosses (MAC 44 x G 10747 and MBC 12 x G 35084) had negative and significant SCA effects. Maternal effects were highly significant, suggesting the importance of cytoplasmic genes for resistance to ascochyta. Non-maternal effects were also significant in some populations, suggesting that the cytoplasmic genes were interacting with nuclear genes. The evaluation of F_1 and F_2 generations showed that ascochyta resistance was governed by recessive genes in most of the resistant parents. However, there was evidence of a larger number of resistant genes in the bean line ICTA Hunapu, than in the other resistant parents. Broad sense heritability (H^2) varied from 0.21-0.64 among the crosses (both types), while narrow sense heritability (h_2) 0.30±1.04 for bush type and 0.29±0.07 for climbers. The number of genes governing resistance to ascochyta varied from two to eight among the eight sources of resistance. The allelism test of resistant x resistant populations suggested the presence of many loci governing ascochyta resistance in beans. Therefore, selection should develop improved populations for resistance to ascochyta. Selection with the recurrent parent would be the best breeding procedure for improving resistance to ascochyta. However, there could be complications, because the resistance is modified by cytoplasmic gene effects, as well as their interaction with nuclear genes in some of the populations.

5.1 Introduction

The common bean (*Phaseolus vulgaris* L.) is the most important grain legume (pulse) consumed by Rwandans (Mukamuhirwa et al., 2015; RAB, 2014). Around 300,000 hectares of beans are cultivated annually in Rwanda (FAO, 2015). Both bush and climbing beans constitute an important economic income for farmers, and they are a staple food for millions of Rwandan families (RAB, 2014). However, production is limited by a number of constraints, including diseases.

Bean ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] is among the most important diseases of the common bean in Rwanda (ISAR, 2011). At higher elevations, ascochyta is prevalent early in the season (ISAR, 2011). Ascochyta damage is most critical at the pod filling stage (R6), when it can result in total plant death (Schwartz et al., 1981). Bean crop damage reports from on-station and on-farm studies are variable, but they both conclude that, in general, early season infections can result in considerable yield losses approaching 75.7% (C.Urinzwenimana unpublished).

Chemical control to combat bean ascochyta damage can be effective under high disease pressure, but most beans in the semi-arid regions of eastern Africa are produced by small-scale farmers with limited financial capacity to purchase chemical pesticides. On the contrary, subsistence farmers rely upon traditional disease control approaches that are less effective on bean ascochyta. Host plant resistance is a promising approach for an integrated disease management system in the common bean (Miklas et al., 2006). The development of genotypes with some level of genetic resistance to bean ascochyta would greatly benefit small- and large-scale farmers, as a cost-effective and sustainable measure. Such genotypes could be deployed as an important component of integrated disease management. In addition, a combination of multiple traits, for instance yield improvement and tolerance to insect pest, drought or low soil fertility, are requisites for adaptability to a range of bean production agro-ecologies (Hillocks et al., 2006). Furthermore, such attributes ought to be combined with others, such as seed size, seed colour, suitable taste and good cooking qualities, so as to make the genotype appealing to small-scale farmers.

In addition, a precise understanding of the gene action involved in resistance and the available resistance genes in the germplasm, are pre-requisites for the achievement of the desirable resistance breeding goal.

A combining ability analysis of the parents gives an indication of which type of gene action is important for the optimization of a breeding strategy. Nevertheless gene action tends to vary, depending on the genetic background used, and results obtained elsewhere may not necessarily give an indication of the behavior of the genes in a different environment. Falconer and Mackay (1996) reported that combining ability and heritability information is pertinent to the set of genotypes and the environment in which it has been tested.

Estimates of additive genetic variance in a population are important for the accurate selection and prediction of genetic gain. However, these estimates may be confused with other sources of environmental or genetic variance, such as dominance, epistasis or maternal effects. Maternal effects are one of the factors that may lead to an over-estimation or under-estimation of the additive genetic variance (Roach and Wulff, 1987; Shaw and Byers, 1998 and Gustavo et al., 2003). Variations in seed, seedling, and adult traits that are caused by maternal effects can have important consequences for the biological behavior of an individual (Roach and Wulff, 1987). Maternal effects refer to the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Roach and Wulff, 1987). Maternal effects are most common in the early stages of the lifecycle of a plant and may influence the selection for resistance done at an early stage. It is therefore important to estimate the maternal effects in the parents that were used in this study, by estimating the reciprocal cross-effects of the populations developed.

In addition, an understanding of allelic relationships between the resistance genes, in different sources of resistance, may help to refine the selection of resistance genes for use in a breeding programme and avoid the over-deployment of a single locus. Therefore, allelism tests are crucial for the identification of the resistance genes to be used in the improvement of resistance to ascochyta in the common bean.

A diallel mating design is used for estimating the combining ability of lines and characterizing the nature and extent of gene action (additive and dominance effects) (Dabholkar, 1999). Even though the diallel analysis largely involves the use of F_1 progeny means from a set of crosses, F_2 progeny means and, in some cases, a combination of F_1 and F_2 generations means, have been used (Fan, 2009). The use of F_2, rather than F_1, in the implementation of a diallel experiment could arise from the cost implications involved, resulting from the difficulty in obtaining adequate F_1 seed. However, the genetic expectations for the diallel of F_2 is the same as that for an F_1 generation (Eberhart and Russell, 1966), but decreased heterozygosity occurs

due to selfing, and as a result, the dominance contribution to SCA is halved (Falconer and Mackay, 1996).

Therefore, based on the importance of understanding the inheritance of the ascochyta disease, F_1 progenies and F_2 populations were developed, using a diallel mating design, and their performance was analyzed, using Griffing's (1956) analysis of diallel designs. The main objectives of the study were: (1) to study the gene action governing resistance to ascochyta in beans; (2) to estimate the number of genes governing resistance to ascochyta in common bean crosses; (3) to estimate the role of maternal effects controlling resistance to ascochyta in beans; (4) to estimate narrow sense heritability (h^2) for resistance to ascochyta in common bean populations; and (5) to determine the allelic relationship between the resistance genes in the common bean.

5.2 Materials and methods

5.2.1 Germplasm

Eight genotypes were selected as sources of resistance to ascochyta after having been screened for resistance. The screening for resistance to ascochyta was conducted during the 2014B and 2015A cropping seasons at three sites, Musanze, Nyamagabe and Rwerere. The evaluation was done for both bush and climbers under natural field infection. The eight genotypes included SMC 17, ASC 87, MIB 755 and ICTA Hunapu for the bush type, and G 10747, G 35084, G 35034 and RWV 1348 for the climbing type (Table 5.1). Varying levels of resistance to ascochyta were obtained, with climbers being the more resistant than the bush types. Bush and climbers that were large-seeded, popular, commercial, but susceptible, bean genotypes included RWR 2245, RWK 10, RWR 275, RWR 1668, MAC 44, MAC 49, MBC 12 and Rwibarura (Table 5.1). These genotypes also had varying levels of susceptibility to ascochyta, with bush, in general, being more susceptible. The detailed descriptions of the germplasm are presented in Table 5.1.

Table 5.1: Characteristics of bean parents used in the inheritance study

Genotypes	Ascochyta resistance reaction	Agronomic characteristics	Origin
RWR 2245	Very Susceptible	Large and red-mottled seed with bush growth habit; Yield potential: 1200-2000 kg ha^{-1}; Marketable; Tolerant to angular leaf spot disease, bean fly and drought diseases but susceptible to bean ascochyta	Rwanda
RWK 10	Very Susceptible	Large and white-mottled seed with bush growth habit; Yield potential: 1200-2200 kg ha^{-1}; Marketable; Tolerant to anthracnose disease, drought diseases but susceptible to bean ascochyta	Rwanda
RWR 275	Very Susceptible	Large and red-mottled seed with bush growth habit; Yield potential: 1500-2500 kg ha^{-1}; Marketable; Tolerant to angular leaf spot disease, bean fly and drought diseases but susceptible to bean ascochyta	Rwanda
RWR 1668	Very Susceptible	Large and red seed with bush growth habit; Yield potential: 1400-2300 kg ha^{-1}; Marketable; Tolerant to angular leaf spot disease, drought diseases but susceptible to bean ascochyta	Rwanda
ASC 87	Moderately tolerant	Black and small seed with bush growth habit	CIAT
MIB 755	Moderately tolerant	Black and small seed with bush growth habit	CIAT
CMS 17	Moderately tolerant	Red and small seed with bush growth habit	Rwanda
ICTAHunapu	Moderately tolerant	Black and small seed with bush growth habit	CIAT
MAC 44	Very Susceptible	Large and red-mottled seed with climbing growth habit; Yield potential: 4000 kg ha^{-1}; Marketable; Tolerant to angular leaf spot disease, bean fly and drought diseases but susceptible to bean ascochyta and antrachnose	Rwanda
MAC 49	Very Susceptible	Large and red-mottled seed with climbin growth habit; Yield potential: 3500 kg ha^{-1}; Marketable; bean fly and drought diseases but susceptible to bean ascochyta	Rwanda
MBC 12	Very Susceptible	Large and red-mottled seed with climbing growth habit; Yield potential: 3000 kg ha^{-1}; Low Marketable; Tolerant to drought diseases but susceptible to bean ascochyta	Rwanda
Rwibarura	Very Susceptible	Large and Kaki seed with climbing growth habit; Yield potential: 1200-2000 kg ha^{-1}; Marketable; Tolerant to angular leaf spot disease, bean fly and drought diseases but susceptible to bean ascochyta	Rwanda
G 10747	Moderately tolerant	Black and small seed with climbing growth habit	CIAT
G 35084	Moderately tolerant	Black and small seed with climbing growth habit	CIAT
G 35034	Moderately tolerant	Black and small seed with climbing growth habit	CIAT
RWV 1348	Moderately tolerant	Small and red-seeded with climbing growth habit, Yield potential: 2500-4000 kg ha^{-1}, Low marketability	Rwanda

5.2.2 Diallel cross and field evaluation of progenies

The eight bean parents, with varying levels of resistance to ascochyta blight, were crossed in a full diallel mating design with reciprocals in a screenhouse at the Rubona Research Station in Rwanda. Due to the different flowering dates of the parents, planting was staggered, so as to synchronize flowering. To ensure adequate seed for advancement and evaluation, 12 crossing blocks were planted. Crossing was done by hand pollination, using the emasculation and hooking methods (Buishand, 1956). Care was taken to avoid the contamination of the new crosses with pollen from the previous parental bean genotypes, by sterilizing the forceps used to tease open the flowers in 70% alcohol. Seed of 112 full sib populations (F_1) were advanced to F_2 populations by selfing.

The resulting 112 F_1 progenies and their reciprocals were evaluated in the field at the Kinigi Research Station (1° 30' S; 29° 38' E, 2200 m.a.s.l) and at the Rwerere Research Station (1° 36' S; 29° 19' E, 2060 m.a.s.l.) during the long rainy season (Season 2016A). The two sites have an annual long-term mean rainfall of about 1800 mm and maximum temperatures of about 15-21^{0}C. They represent the high-altitude environments of Rwanda.

The experiments were laid out as row by column designs, with two replications at each site. Seeds of each entry were planted by hand in two-row plots of 2 m lengths, at a 0.40 m inter-row and a 0.20 m intra-row spacing. The trials were supplied with organic manure 10 t ha^{-1} and 100 kg ha^{-1} mineral fertiliser (17:17:17, N: P: K). The fields were kept weed-free by hand weeding. All parents and the progenies were inoculated in the field by ascochyta infected debris that was collected from the previous season, in the region where the trial was being conducted.

Ascochyta severity was assessed 21 days after planting by making observations of the leaf and pod tissue, using percentage disease severity rating scales, that is, it was based on percentage leaf and pod tissue affected/extent of infection,

where:

- 0% = no visible symptoms;

- 25% = approximately a quarter of the leaf and pod tissue have lesions, but tissue is still firm;

- 50% = approximately half of the leaf and pod tissues have lesions; and

- 75%-100% = the whole of the leaf and pod tissues have lesions of ascochyta and the infection is in an advanced stage, to complete plant defoliation.

F_2 and reciprocal seed were also planted in separate field and considered as separate crosses, as for the F_1 trial. This trial was planted together with susceptible and resistant checks. Ascochyta severity was assessed and disease severity scores were taken, as described for the F_1 population above. For ease of interpretation, the segregation of resistant (R) x susceptible (S) populations at F_2 resistance was classified into the main divisions below:

1. Tolerant/resistant reaction = 0-15%;

2. Moderately resistant = 15.1-25%;

3. Moderately susceptible = 25.1-40%;

4. Susceptible = 40.1-50%; and

5. Very susceptible = >50%

5.2.3 Data analysis

Several analyses were done to estimate the combining abilities of the parents, heritability, gene action, number of genes and loci governing resistance to ascochyta, as discussed below.

5.2.3.1 Combining ability analysis

The bush and climbing F_1 data were analyzed separately, using the Diallel SAS 9.3 computer programme developed by Zhang et al. (2005), using Model I (fixed effects) and Method III (crosses and reciprocals) of Griffing (1956), to determine the general combining ability (GCA) and specific combining ability (SCA) effects of the different genotypes and crosses. The statistical model for this analysis was as follows:

$$Yijk= \mu + g_i + g_j + s_{ij} + r_{ij} + b_k + (bv)_{ijk} + e_{ijkl}; \quad \text{.....(1)}$$

where μ is the population mean effect, gi is the GCA effect of the i^{th} parent, gj is the GCA effect of the j^{th} parent, s_{ij} is the SCA effect of the ij^{th} genotype, r_{ij} is the reciprocal effect of the ij^{th} genotype, b_k is the effect of k^{th} block, $(bv)_{ijk}$ is the interaction of ij^{th} genotype with the k^{th} block,

and e_{ijkl} is the environmental effect of the ijklth observation. Components of the reciprocal effects were also estimated, that is, the maternal and non-maternal effects.

5.2.3.2 Estimation of narrow sense heritability (h^2) for resistance to ascochyta blight

A parent-offspring regression model (Vogel et al., 1980) was used to estimate h^2 as follows:

$Yi = a + b*X_i + E_i$……...(2)

Where: Y_i = performance of offspring of ith parent; a = mean performance of all parents evaluated; b = linear regression coefficient; X_i = performance of the ith parent; and E_i = experimental error associated with the measurement of X_j.

The means for the parents and offspring were plotted against each other and the regression coefficient “b” calculated, the heritability was estimated, using the relationship between relatives as follows:

$h^2 = 4VA/VP$ and “b” = h^2. .....(3)

Where, h^2 = narrow sense heritability; VA = variance due to additive gene effects; VP = total phenotypic variance; and “b” = regression coefficient.

5.2.3.3 Estimation of number of loci and genes governing ascochyta resistance

The number of loci and genes governing ascochyta resistance were determined, using the original Castle Wright method (Kcw) (Equation 4) and modifications by Bjarco and Line (Equation 5) (Bjarko and Line, 1988; Das and Griffey, 1994; Zeng et al., 1990).

At F_2 generation:

$n = (GR)^2 [1.5\text{-}2 h(1 - h)]/ 8 [VF_2 - (V_{PS} + V_{PR} + 2V_{F1})4]$ .....(4)

$Kcw=D^2/8VG= D^2/8[V_{F2} - (V_{PS} + V_{PR} + V_{F1})4]$ .....(5)

Where: n = the estimated number of segregating genes estimated by the Bjarco and Line Formula; K_{cw} = number of loci estimated by the original Castle – Wright formula; GR = genotype range; P_R = mean of resistant parent; P_S = mean of susceptible parent; $F1_M$ = mean of F_1 progenies; V_{PR}, V_{PS} = variance of resistant and susceptible parents, respectively; VF_1, VF_2 =

variance of F_1 and F_2 generations, respectively; h = $(F1_M-P_R)/(Ps-P_R)$; D = difference in parental mean (P2 - P1); and VG = genotypic variance.

The above formulae were based on the assumptions as per Lande (1981) and Zeng et al. (1990):

1. One parent contains all the trait-increasing alleles and the other contains all the trait-decreasing alleles;
2. All crosses are obtained by mating individuals chosen at random in appropriate populations; and

3. The segregating genes are not linked and are in random combinations.

The presence of linkage, dominance or unequal effects at different loci will result in an underestimation of the actual number of segregating genes present, while the presence of epistasis may cause either an overestimation or an underestimation of the actual number of segregating genes (Lande, 1981; Zeng et al., 1990).

In this study, the genotypic range (GR) was estimated, using the phenotypic range of the segregating population, which does not assume that segregating genes come from a single parent and can hence be applied to resistant x resistant crosses, as well as resistant x susceptible crosses (Zhang et al., 2001), while the D is the difference between the parents. Genotypic variance was estimated by subtracting the environmental variance from the phenotypic variance of segregating populations.

5.2.3.4 Allelism test for ascochyta resistance genes from several potential sources of resistance

Segregation ratios for each of the 16 R x R crosses were computed, as shown in Table 5.2. Using the percentage scale data, disease score ratings of 0-19.9% were considered resistant, 20-39.9% as moderately resistant, 40-59.9% as moderately susceptible, 60-79.9% as susceptible, and 80-100% as highly susceptible.

Table 5.2: The R x R crosses developed for testing the allelic interaction of resistance genes to ascochyta

	Bush		Climbing
1	ASC 87 X MIB 755	1	G 10747 X G35084
2	ASC 87 X CMS 17	2	G 10747 X G35034
3	ASC 87 X ICTA Hunapu	3	G 10747 X RWV 1348
4	MIB 755 X CMS 17	4	G35084 X G35034
5	MIB 755 X ICTA Hunapu	5	G35084 X RWV 1348
6	CMS 17 X ICTA Hunapu	6	G35034 X RWV 1348

Several different genetic hypotheses were tested for the significance of each population, using the chi-square goodness of fit test in the Genstat 14 Release (Payne et al., 2010). The chi-square goodness of fit test was used to determine the departure of the observed frequencies from the hypothesized frequencies, based on a critical value of 5.991 for two degrees of freedom at the 0.05 probability level. Eleven phenotypic classes were tested (Singh and Chaudhary, 2004; Caixeta et al., 2005), namely: 1:0 (alleles on same locus); 15:1 (two independent dominant genes); 9:7 (two complementary dominant genes); 13:3 (two epistatic genes, one dominant and one recessive); 63:1 (three independent dominant genes); 57:7 (one dominant and two complementary genes); 27:37 (three complementary dominant genes); 61:3 (two dominant and one recessive gene), 49:15 (one dominant and two recessive genes); and 249:7 (two dominant and two complementary genes).

5.3 Results

5.3.1 Gene action determining ascochyta blight resistance

The analysis of variance of the bush and climbing beans showed that the F_1 crosses were highly and significantly ($P \leq 0.01$) different from each other (Table 5.3). The GCA effects were highly significant ($P \leq 0.01$), while the SCA effects were not significant. GCA effects accounted for 68% of the phenotypic variance observed for bush and 76% for climbers, while the SCA effects accounted for 5% and 6% of the total variance for bush and climbers, respectively.

Table 5.3: Mean squares for ascochyta severity of bean F_1 crosses tested in Rwanda

Source	df	Bush	Climber
Crosses	54	187654.08*	123486.96**
GCA	9	176246.77**	151003.73**
SCA	35	ns	ns
Reciprocals	56	28103.72*	25653.23*
Maternal effect	9	12163.83*	16103.78*
Non maternal effect	47	ns	ns
R^2		60.91	60.57
CV (%)		18.96	24.70

Ns = not significant, *, **, = significant at P≤ 0.05 and P≤ 0.01, respectively

Reciprocal effects and maternal effect were significant at P≤ 0.05. The non-maternal effects were not significant for both the growth types (Table 5.3).

5.3.2 Estimation of combining ability effects for developed crosses

Negative GCA effects for disease resistance are desirable in this study, based on the disease rating scale used, as they indicated the bean line's contribution to resistance to ascochyta, while positive GCA effects are not desirable, because they indicated the bean line's contribution to susceptibility. In the F_1 generation, RWR 2245, RWR 1668 had significant (P≤ 0.01) positive GCA effects for bush, and MAC 44 for climber, genotypes (Table 5.4. Bush genotypes ASC 87, ICTA Hunapu and MIB 755 displayed the highest significant negative (P≤ 0.05) GCA value. For climbers, genotype G 35084 had the highest GCA value, followed by G 10747 and G 35034. Crosses involving these genotypes also had low ascochyta severities (Table 5.4). The genotype CMS 17 had an insignificant negative (P≤ 0.05) GCA effect and RWV 1348 had a low positive GCA (Table 5.4.

Table 5.4: General combining ability effects of bean parents for resistance to ascochyta

Bush Parents	GCA	Climbing parents	GCA
RWR 2245	5.64**	MAC 44	4.76**
RWK 10	3.36*	MAC 49	3.22*
RWR 275	1.14	MBC 12	1.14
RWR 1668	7.86**	Rwibarura	2.26
ASC 87	-11.43**	G10747	-13.74**
MIB 755	-4.42*	G35084	-22.14**
CMS 17	-1.12	G35034	-8.56**
ICTA Hunapu	-5.68**	RWV 1348	0.64
S.e.d (P= 0.05)	0.21		1.44

*, **, = significant at P≤ 0.05 and P≤0.01 respectively.

Even though the SCA effects were not significant in most crosses of bush parents, three crosses displayed significant SCA effects at P≤ 0.05 (Table 5.5). The SCA effects for the bush crosses RWR 2245 x ASC 87 and RWR 275 x MIB 755 were negative and significant at P≤0.05. The SCA effects for crosses ASC 87 x CMS 17 were also positive and significant at P≤0.05.

Table 5.5: Specific combining ability effects of the F1 bush bean genotypes for resistance to ascochyta blight

Parents	RWR 2245	RWK 10	RWR 275	RWR 1668	ASC 87	MIB 755	CMS 17	ICTA Hunapu
RWR 2245		0.68	0.35	0.01	-1.17*	-0.45	0.19	-0.34
RWK 10			0.44	0.85	-0.53	0.17	-0.89	-0.65
RWR 275				0.72	0.18	-1.20*	0.11	-0.50
RWR 1668					0.35	0.25	0.18	0.09
ASC 87				.		0.67	1.08*	-0.47
MIB 755							-0.32	-0.43
CMS 17								-0.36
ICTA Hunapu								
S.e.d (P= 0.05)					0.34			

s.e.d = standards error deviation; *, **, = significant at P≤ 0.05, P≤ 0.01, respectively.

Two combinations of climber parents, namely, MAC 44 x G 10747 and MBC 12 x G 35084, had a significant negative SCA effect, while the cross G 10747 x G 35034 had a significant positive SCA effect (Table 5.6).

Table 5.6: Specific combining ability effects of F_1 climbing bean crosses for resistance to ascochyta blight

Parents	**MAC 44**	**MAC 49**	**MBC 12**	**Rwibarura**	**G10747**	**G35084**	**G35034**	**RWV1348**
MAC 44		0.72	0.38	0.21	-1.24*	-0.45	0.18	-0.44
MAC 49			0.14	0.65	-0.43	0.11	-0.82	-0.62
MBC 12				0.78	0.12	-1.28*	0.19	-0.52
Rwibarura					0.24	0.32	0.28	0.07
G10747				.		0.66	1.48*	-0.54
G35084							-0.39	-0.42
G35034								-0.32
RWV 1348								
S.e.d (P=0.05)					0.46			

s.e.d = standards error deviation; *, **, = significant at P≤ 0.05, P≤0.01 respectively.

5.3.3 Reciprocal cross effects on ascochyta resistance

The crosses MIB 755 x RWR 2245 and SMS 17 x ASC 87 had a significant positive reciprocal effect, as shown in Table 5.7. This implies that ascochyta severity was higher when RWR 2245 and ASC 87 were the maternal parents in these crosses, and lower when MIB 755 and SMS 17 were the maternal parents. This suggested that the cytoplasmic genes of RWR 2245 and ASC 87 contributed to the susceptibility to ascochyta in these crosses. The reciprocal effect for the crosses RWR 275 x ICTA Hunapu was significant and negative, indicating that ascochyta severity was lower, when ICTA Hunapu was the maternal parent in these crosses. This implies that the cytoplasmic genes in ICTA Hunapu contributed to the resistance to ascochyta in these crosses.

Table 5.7: Reciprocal effects of F_1 bush bean crosses for resistance to ascochyta blight

Parents	RWR 2245	RWK 10	RWR 275	RWR 1668	ASC 87	MIB755	CMS17	ICTA Hunapu
RWR 2245								
RWK 10	-0.58							
RWR 275	0.37	0.44						
RWR 1668	0.21	0.85	0.72		.			
ASC 87	0.17	0.53	-0.18	0.35				
MIB 755	1.45*	0.17	0.20*	-0.25	0.67			
CMS 17	-0.19	0.89	0.11	0.18	1.08*	0.32		
ICTA Hunapu	0.34	0.65	-1.05*	0.09	0.47	0.43	0.36	
S.e.d (P= 0.05)					0.28			

s.e.d = standards error deviation; *, **, = significant at P≤ 0.05 and P≤ 0.01, respectively

Amongst the climbing genotypes, reciprocal effects were observed, with the cross G 10747 x MAC 44 having the highest significant (P≤ 0.05) negative reciprocal effect. Similarly the crosses G 35084 x MBC 12 and G 35034 x G 10747 had the highest significant (P≤ 0.05) positive reciprocal effects (Table 5.8).

Table 5.8: Reciprocal effects of F_1 climbing bean crosses for resistance to ascochyta blight

Parents	MAC 44	MAC 49	MBC 12	Rwibarura	G10747	G35084	G35034	RWV 1348
MAC 44								
MAC 49	0.72							
MBC 12	0.38	0.14						
Rwibarura	0.21	0.65	0.78		.			
G10747	-1.64*	0.46	0.12	0.44				
G35084	0.48	0.31	1.21*	0.36	0.68			
G35034	0.18	0.86	0.14	0.38	1.36*	0.42		
RWV 1348	0.48	0.66	0.38	0.17	0.62	0.98	0.62	
S.e.d (P= 0.05)					0.38			

s.e.d = standards error deviation; *, ** = significant at P≤ 0.05 and P≤ 0.01, respectively.

Amongst the bush types, maternal effects were significant and negative for ICTA Hunapu, which had the highest negative and significant (P≤ 0.01) maternal effects, followed by CMS 17 (P≤ 0.01), RWR 1668 (P≤ 0.01) and ASC 87 (P≤ 0.05) (Table 5.9). Genotypes RWR 275 and MIB 755 had significant positive maternal effects.

The crosses RWR 2245 x ASC 87 and RWK 10 x SMS 17 had significant (P≤ 0.05) negative non-maternal effects (Table 5.9). Similarly, negative non-maternal effects were observed in the cross RWK 10 x SMS 17.

Table 5.9: Maternal and non-maternal effects of 8 bush bean parents for resistance to ascochyta blight

Parents	**RWR 2245**	**RWK 10**	**RWR 275**	**RWR 1668**	**ASC 87**	**MIB 755**	**CMS 17**	**ICTAHunapu**
RWR 2245	**0.08**	0.68	0.35	0.01	-1.17*	-0.45	0.19	-0.34
RWK 10		**-0.02**	0.44	0.85	-0.53	0.17	-0.89*	-0.65
RWR 275			**0.42****	0.72	0.18	-1.22	0.11	-0.51
RWR 1668				**-0.32****	0.35	0.25	0.18	0.09
ASC 87				.	**-0.28***	0.67	1.08	-0.47
MIB 755						**0.14***	-0.32	-0.43
CMS 17							**-0.36****	-0.36
ICTA Hunapu								**-0.47****
S.e.d Maternal Effect (P= 0.05)					0.11			
S.e.d No Maternal effect (P= 0.05)					0.42			

s.e.d = standards error deviation; *, ** = significant at P≤ 0.05 and P≤ 0.01, respectively.

Amongst the climbers, the trend of maternal effects was not different from that observed in the bush type, with G 10747 having the highest (P≤ 0.01) negative maternal effects, followed by G35084 (P≤ 0.01), and lastly, G 35034 (P≤ 0.05) (Table 5.10). The bean line MBC 12 had the highest (P≤ 0.01) positive maternal effect, followed by Rwibarura (P≤ 0.05). The negative non-significant reciprocal effects were observed in the crosses MAC 44 x G 10747 and MBC 12 x G 35084 (Table 5.10).

Table 5.10: Maternal and non-maternal effects of 8 climbing bean parents for resistance to ascochyta blight

Parents	MAC44	MAC49	MBC 12	Rwibarura	G10747	G35084	G35034	RWV 1348
MAC 44	**0.05**	0.32	0.28	0.11	-1.34	-0.42	0.28	-0.44
MAC 49		**0.08**	0.24	0.61	-0.48	0.18	-0.42	0.62
MBC 12			**0.72****	0.38	0.42	-1.38	0.29	-0.22
Rwibarura				**0.33***	0.34	0.50	0.18	0.07
G10747				.	**-0.6****	0.46	0.48	0.54
G35084						**-0.52***	1.35*	-0.42
G35034							**-0.31***	-0.14
RWV 1348								**-0.03**
S.e.d Maternal Effect (P= 0.05)					0.13			
S.e.d No Maternal effect (P= 0.05)					0.42			

s.e.d = standards error deviation;*, ** = significant at P≤ 0.05 and P≤ 0.01 respectively.

5.3.4 Estimation of narrow sense heritability of resistance to ascochyta

The mid-parent offspring regression analysis was significant (P≤ 0.01), with a regression coefficient “b” of 0.30±1.04 for the bush type and 0.29±0.07 for the climbers (Table 5.12).

Table 5.11: Regression analysis of F_2 crosses on parental F_1 scores

Source of Variance	df	Mean square	
		Bush	**Climbers**
Regression	1	3648.86**	2756.94**
Residual	54	346.92	562.10
Total	55	436.81	702.56
"b"		0.308±0.08	0.292±0.04
R^2		21.20	17.41

The regression coefficient “b” is an estimate of the narrow sense heritability, according to Vogel et al. (1980) and Falconer and Mackay (1996). F_2 data indicated that 17.4% of the total variation

in the mean scores of the F_2 climber population, was accounted for by the parental F_1 scores, whereas 21.2% of the bush population was accounted for by the parental F_1 scores (Table 5.11). This is very low, suggesting that the environmental effects impacting on the severity of ascochyta, were very high. Therefore, resistance expression in the F_2 generation could not be consistently predicted, based on the F_1 performance.

5.3.5 Frequency distribution of severity scores in R x S crosses and segregation ratios

The segregation ratios of the 16 bush and 16 climbing populations and their reciprocals, involving the different sources of resistant and susceptible genotypes, gave a continuous distribution, but could not be fitted into definite genetic ratios (Tables 5.12 and 5.13). All populations (bush and climbers) gave nearly continuous distributions, with the exception of RWR 1668 x ASC 87, RWK 10 x ICTA Hunapu and RWR 2245 x CMS 17, where there were no resistant plants (Table 5.12).

In addition, crosses that involved MIB 755 and ASC 87 for bush and G 35084, G 35034 and G 10747 for climbers, resulted in the lowest ascochyta severity scores (Tables 5.12 and 5.13). Similarly, the lowest disease severity scores were obtained when these genotypes were crossed with each other. The F_1 mean severity was higher than the F_2 mean severity for only four bush crosses and three climbing crosses (Tables 5.12 and 5.13).

Table 5.12: Segregation of resistance to ascochyta blight resistance in (S x R) F_2 bush genotypes and their reciprocal (R x S) crosses involving susceptible genotypes

		Mean severity (%)		No of plants assessed in each class			
	Cross	**F1**	**F2**	**S**	**MR**	**R**	**Total**
1	RWR 1668 x ICTA Hunapu	17.00	18.00	22.00	12.00	4.00	40.00
	ICTA Hunapu x RWR 1668	15.10	16.50	13.00	17.00	8.00	38.00
2	RWR 1668 x ASC 87	15.00	17.00	21.00	18.00	0.00	39.00
	ASC 87 X RWR 1668	22.00	25.80	19.00	15.00	3.00	37.00
3	RWR 1668 x CMS 17	16.40	17.50	27.00	11.00	2.00	40.00
	CMS 17 X RWR 1668	20.00	23.30	23.00	13.00	4.00	40.00
4	RWR 1668 x MIB 755	22.10	25.50	16.00	21.00	3.00	40.00
	MIB 755 X RWR 1668	21.00	22.00	8.00	27.00	5.00	40.00
5	RWR 278 x ICTA Hunapu	16.00	16.50	28.00	10.00	1.00	39.00
	ICTA Hunapu x RWR 278	21.80	24.00	23.00	14.00	3.00	40.00
6	RWR 278 x ASC 87	17.00	20.50	20.00	15.00	5.00	40.00
	ASC 87 X RWR 278	18.10	21.20	24.00	15.00	1.00	40.00
7	RWR 278 x CMS 17	19.00	20.10	21.00	17.00	2.00	40.00
	CMS 17 X RWR 278	15.00	16.00	24.00	25.00	1.00	40.00
8	RWR 278 x MIB 755	15.50	15.00	19.00	18.00	3.00	40.00
	MIB 755 X RWR 278	20.00	22.50	20.00	18.00	2.00	40.00
9	RWK 10 x ICTA Hunapu	14.00	13.00	28.00	7.00	5.00	40.00
	ICTA Hunapu x RWK 10	19.00	22.50	21.00	18.00	1.00	40.00
10	RWK 10 x ASC 87	17.00	20.30	17.00	19.00	4.00	40.00
	ASC 87 X RWK 10	19.00	20.50	24.00	15.00	1.00	40.00
11	RWK 10 x CMS 17	14.90	15.00	19.00	17.00	4.00	40.00
	CMS 17 X RWK 10	15.00	18.50	24.00	15.00	1.00	40.00
12	RWK 10 x MIB 755	13.00	14.00	21.00	17.00	2.00	40.00
	MIB 755 X RWK 10	15.00	14.50	24.00	13.00	3.00	40.00
13	RWR 2245 x ICTA Hunapu	28.50	31.60	23.00	12.00	5.00	40.00
	ICTA Hunapu x RWR 2245	25.00	26.20	19.00	18.00	3.00	40.00
14	RWR 2245 x ASC 87	24.50	28.50	21.00	17.00	2.00	40.00
	ASC 87 X RWR 2245	29.00	31.00	23.00	15.00	2.00	40.00
15	RWR 2245 x CMS 17	27.60	30.70	28.00	12.00	0.00	40.00
	CMS 17 X RWR 2245	22.00	25.50	26.00	13.00	1.00	40.00
16	RWR 2245 x MIB 755	29.70	32.20	27.00	11.00	2.00	40.00
	MIB 755 x RWR 2245	22.00	24.50	30.00	8.00	2.00	40.00

R = Resistant, MR = Moderately Resistant, S = Susceptible.

Table 5.13: Segregation of resistance to ascochyta blight resistance in (S x R) F_2 climbing genotypes and their reciprocal (R x S) crosses involving susceptible genotypes

		Mean severity (%)		No of plants assessed in each class			Total
	Cross	**F1**	**F2**	**S**	**MR**	**R**	
1	Rwibarura x G 10747	7.50	11.25	10.00	16.00	4.00	39.00
	G 10747 x Rwibarura	4.50	6.70	16.00	21.00	4.00	40.00
2	MBC 12B x G 10747	4.50	5.50	13.00	22.00	5.00	40.00
	G 10747 x MBC 12B	15.00	21.20	19.00	19.00	2.00	40.00
3	MAC 49 x G 10747	6.00	7.00	20.00	15.00	5.00	40.00
	G10747 X MAC 49	18.00	26.00	16.00	17.00	5.00	40.00
4	MAC 44 x G 10747	15.00	22.00	8.00	25.00	7.00	40.00
	G 10747 X MAC 44	18.00	26.20	6.00	31.00	3.00	40.00
5	Rwibarura x G 35084	6.00	9.10	20.00	14.00	6.00	40.00
	G 35084 X Rwibarura	15.00	20.30	12.00	18.00	10.00	40.00
6	MBC 12B x G 35084	22.50	31.50	20.00	19.00	1.00	40.00
	G 35084 X MBC 12B	19.50	28.20	12.00	21.00	7.00	40.00
7	MAC 49 x G 35084	10.50	11.50	17.00	21.00	2.00	40.00
	G 35084 x MAC 49	4.50	5.70	7.00	29.00	4.00	40.00
8	MAC 44 x G 35084	4.50	4.90	12.00	22.00	6.00	40.00
	G 35084 x MAC 44	15.00	17.50	7.00	22.00	11.00	40.00
9	Rwibarura x G 35034	18.00	25.00	6.00	27.00	7.00	40.00
	G 35034 x Rwibarura	25.50	38.50	9.00	22.00	9.00	40.00
10	MBC 12 x G 35034	22.50	32.75	12.00	23.00	5.00	40.00
	G 35034 x MBC 12B	25.50	33.25	17.00	19.00	4.00	40.00
11	MAC 49 x G 35034	3.00	3.50	7.00	21.00	12.00	40.00
	G 35034 x MAC 49	4.50	5.75	5.00	29.00	6.00	40.00
12	MAC 44 x G 35034	1.50	2.20	2.00	31.00	7.00	40.00
	G 35034 x MAC 44	4.50	6.70	11.00	17.00	12.00	40.00
13	Rwibarurax RWV1348	9.00	11.80	10.00	16.00	14.00	40.00
	RWV1348 xRwibarura	4.50	5.75	12.00	22.00	6.00	40.00
14	MBC 12 x RWV 1348	4.50	4.70	15.00	21.00	4.00	40.00
	RWV1348 x MBC 12B	10.50	12.60	17.00	19.00	4.00	40.00
15	MAC 49 x RWV 1348	7.50	11.90	8.00	26.00	6.00	40.00
	RWV 1348 x MAC 49	15.00	15.50	18.00	17.00	5.00	40.00
16	MAC 44 x RWV 1348	10.50	10.75	19.00	15.00	6.00	40.00

	Mean severity (%)		No of plants assessed in each class			Total
Cross	**F1**	**F2**	**S**	**MR**	**R**	
RWV 1348 x MAC 44	15.00	17.50	23.00	12.00	5.00	40.00

R = Resistant, MR = Moderately Resistant, S = Susceptible.

5.3.6 Estimation of the number genes governing ascochyta blight and broad sense heritability (H^2) in F_2 S X R crosses

Based on the original Castle-Wright analysis (Zeng et al., 1990) and methods used by Bjarko and Line (1988) and Das and Griffey (1994) for estimating the number of genetic factors governing a trait, different numbers of genes are important for resistance to ascochyta blight, depending on the cross. The two methods used in estimating the number of genes did not differ greatly, indicating that either method could be used. The mean of the two formulae was used to explain the results below. The number of genetic factors in ICTA Hunapu was estimated to be as follows: 2-6 genes; ASC 87, 2-3 genes; SMC 17, 3-5 genes; MIB 755, 3-5 genes; G 10747, 2-3 genes; G 35084, 2 genes; G 35034, 2-8 genes; and RWV 1348, 1-5 genes (Table 5.14). In addition, estimates of VF_2 and VE (see Equation 4) were used to estimate the heritability of the different crosses. Broad sense heritability was low (0.21-0.64), with the highest being recorded for the cross RWR 2154 x MIB 755 (H^2=0.64).

Table 5.14: Estimation of broad sense heritability (H) and number of genes controlling resistance to ascochyta in F_2 populations

Susceptible parent	Resistance parent	n	Kcw	Mean	Heritability (H)
RWR 1668	ICTA Hunapu	6.50	6.00	6.25	0.22
RWR 278	ICTA Hunapu	3.16	3.15	3.16	0.42
RWR 2154	ICTA Hunapu	-5.29	-5.24	-5.27	-0.82
RWK 10	ICTA Hunapu	3.18	2.71	2.95	0.24
RwR 1668	ASC 87	3.27	3.27	3.27	0.32
RWR 278	ASC 87	1.48	1.44	1.46	0.45
RWR 2154	ASC 87	-2.58	-2.57	-2.58	-0.51
RWK 10	ASC 87	3.03	2.69	2.86	0.32
RwR 1668	SMC 17	5.19	4.65	4.92	0.35
RWR 278	SMC 17	3.84	3.70	3.77	0.28
RWR 2154	SMC 17	-2.39	-2.25	-2.32	-0.94

Susceptible parent	Resistance parent	n	Kcw	Mean	Heritability (H)
RWK 10	SMC 17	5.41	5.18	5.30	0.24
RwR 1668	MIB 755	2.67	2.78	2.73	0.38
RWR 278	MIB 755	3.18	3.08	3.13	0.36
RWR 2154	MIB 755	2.23	2.10	2.17	0.64
RWK 10	MIB 755	1.82	1.68	1.75	0.52
MAC 44	G 10747	6.48	5.98	6.23	0.21
MAC 49	G 10747	3.14	3.13	3.13	0.41
Rwibarura 2	G 10747	-5.32	-5.26	-5.29	-0.84
MBC 12B	G 10747	3.16	2.69	2.92	0.23
MAC 44	G 35084	3.25	3.25	3.25	0.31
MAC 49	G 35084	1.46	1.42	1.44	0.44
Rwibarura 2	G 35084	-2.61	-2.59	-2.60	-0.53
MBC 12B	G 35084	3.01	2.67	2.84	0.31
MAC 44	G 35034	5.17	4.63	4.90	0.34
MAC 49	G 35034	3.82	3.68	3.75	0.27
Rwibarura 2	G 35034	-2.42	-2.27	-2.34	-0.96
MBC 12B	G 35034	5.39	5.16	5.27	0.23
MAC 44	RWV 1348	2.65	2.76	2.70	0.37
MAC 49	RWV 1348	3.16	3.06	3.11	0.35
Rwibarura 2	RWV 1348	2.21	2.08	2.14	0.51
MBC 12B	RWV 1348	1.80	1.66	1.73	0.51

n = number of genes according to Bjarco and line formula;

Kcw = number of genes according to the original Wright formula.

$H= (VF_2-VE) / VF_2$

5.3.7 Allelism test for ascochyta blight resistance genes from several potential sources of resistance

The chi-square test (X^2) results for the goodness of fit of the phenotypic classes of F_2 segregants, is presented in Table 5.15. Four out of the 11 ratios were fitted. The test indicated the presence of one dominant and two recessive genes in the cross ASC87 x ICTA Hunapu, and two complementary dominant genes in the cross ASC 87 x SMC 17, and one dominant and two complementary genes for crosses ASC 87 x MIB 755 and G 35084 x G 35034. Three

complementary dominant genes were suggested by the chi square test in the crosses RWV 1348 x G 10747 and MIB 755 x SMC 17. All the other crosses had more than three genes involved and did not fit into any of the ratios tested.

Table 5.15: Chi square testing for goodness of fit of phenotypic classes in F_2

Cross	Hypothesis	X^2 value	Df	P value	Implication
ASC87 x ICTA Hunapu	49:15	0.91	1	0.326	One dominant and two recessive genes
ASC 87 x SMC 17	9:7	0.47	1	0.482	two complementary dominant genes
ASC 87 x MIB 755	57:7	0.01	1	0.92	one dominant and two complementary genes
G35084 x G35034	57:7	0.61	1	0.44	one dominant and two complementary genes
RWV1348 x G10747	27:37	0.14	1	0.71	three complementary dominant genes
MIB 755 x SMC 17	27:37	0.28	1	0.59	three complementary dominant genes

Df= degree of freedom

5.4 Discussion

This study used an 8 x 8 diallel mating design to develop 56 F_1 and F_2 populations, plus their reciprocal crosses, as a means of designing an appropriate breeding strategy for incorporating ascochyta blight resistance into commercial and popular bean genotypes in Rwanda. In addition, the developed populations were used to obtain information on the inheritance of resistance to bean ascochyta blight. F_1 and F_2 data indicated that resistance to ascochyta was a recessive trait, with the resistant parents having varying numbers of resistance genes. The results indicated the presence of additive genes, with small effects for most of the crosses, implying that resistance to ascochyta was additive in nature. Other scientists, using different populations, have also found that resistance to ascochyta was additive (Hanson et al., 1993). The GCA effects were highly significant ($P \leq 0.01$), indicating the significance of additive gene effects. The genotypes ASC 87, ICTA Hunapu, MIB 755, G 35084, G 10747 and G35034 had desirable negative GCA effects. This implied that they were effective sources of resistance in these populations and could be recommended as sources of resistance for ascochyta in the bean improvement programme in Rwanda. The genotypes SMS 17 and RWV 1348 were not effective sources of resistance, because they had positive GCA effects, but they may still be considered to be sources of resistance, as the GCA values were better than those of the susceptible parents. The susceptible parents, RWR 2245, RWR 1668 and MAC 44, had high positive GCA effects in the F_1 generation, which indicated that they have susceptibility genes.

Two crosses had high negative and significant ($P \leq 0.05$) SCA effects, namely, RWR 2245 x ASC 87 and RWR 275 x MIB 755, which indicated the presence of non-additive gene effects for ascochyta resistance in these crosses. It is probable that either one of the parents in these crosses possesses some dominant resistance genes. Reciprocal effects were significant in these populations, which indicated the role of maternal and non-maternal effects in modifying resistance to ascochyta. Reciprocal effects are associated with cytoplasmic inheritance from the female parent. However, accidental self-pollination may be one of the reasons for the significant reciprocal and maternal effects (Dudley, 1963). The maternal effects were highly significant, compared to the non-maternal effects, indicating that for some genotypes, the cytoplasmic genes contributed to the resistance observed. Negative maternal effects were observed for ICTA Hunapu, CMS 17, RWR 1668 and ASC 87. This implied that the cytoplasm of these genotypes contributed to the resistance that was observed in the crosses involving these genotypes. It suggests that populations involving these genotypes as maternal parents should

be advanced further, over their reciprocal crosses, to enhance the levels of resistance to ascochyta.

Past studies on the inheritance of resistance to ascochyta did not consider maternal effects as a component of the additive variance (Hanson et al., 1993), which may indicate that the heritability estimated from those studies was escalated by the maternal effects, and could have been even much lower than those estimated.

Broad sense heritability (H^2), which indicates the proportion of the F_2 variance attributable to the genetic segregation, was estimated for all the populations that involved the susceptible parents, and it varied from 0.21-0.64. Heritability, in the narrow sense (h^2), was estimated by the components of the analysis of variance as 0.42 and 0.38 for bush and climbers, respectively. The heritability estimated by the regression coefficient was 0.30±1.04 for the bush type and 0.29±0.07 for climbers. The heritability estimated by the regression in the F_1 and F_2 generations, could be regarded in the broad sense, because the combining ability values in the F_2 may be inflated by heterosis, and linkage disequilibrium is greatest in these generations. Linkages can be broken by random mating in the later generations (beyond F_4). Simmonds (2011), Boss (1993) and Falconer and Mackay (1996) suggested that heritability determined by the regression coefficient, in the case of random mating, offered a more secure approach to h^2 than the partitioning of variance.

The F_2 data indicated that 17.2% and 21.2% of the total variation in the mean scores of F_2 crosses was accounted for by the parental F_1 scores for bush and climbers, respectively, indicating that there was a high environmental variance in the F_1 generation, and hence, a low heritability. However, the estimate of the heritability from the ANOVA could be assumed to be accurate because the error variance, due to the environmental and maternal effects that could have led to an overestimation of the additive variance, were estimated and included as components of the phenotypic variance. Generally, the low heritability (h^2) estimates obtained suggest that the heritability pattern of resistance to ascochyta observed was influenced by the environment, the sources of resistance used, as well as the evaluation procedures. In addition, the inclusion of reciprocal cross-effects in the estimation of heritability of resistance to ascochyta in this study helped to explain the lower heritability estimates obtained. As mentioned above, maternal effects reduce the precision of genetic studies, because they inflate the amount of genetic variance, but they slow the response to selection (Roach and Wulff, 1987). Hanson et al. (1993) reported the broad sense heritability of resistance to ascochyta varying from low to

moderate (0.19-0.64), depending on the cross and character measured. The heritability estimates obtained in this study are low, but adequate for effective selection, but they indicate the need for progeny testing and the evaluation of ascochyta as a quantitative character, if successful breeding progress is to be achieved.

The number of genes governing resistance to ascochyta was estimated using the original Castle-Wright method and a modified version of this method that estimates environmental variance. These formulae have been used by several scientists in estimating the number of genes governing traits (Zhang et al., 2001; Han et al., 2006; Santos and Simon, 2006). The number of genes varied from 2-8, among the resistant parents. Allelism tests highlighted the likelihood of many loci governing resistance to ascochyta resistance. Ratios testing the presence of up to three resistance genes were fitted in the chi square test of goodness of fit. Only four out of the 11 populations tested fitted some of these ratios. All the F_2 R x R populations exhibited continuous distributions, indicating the role of many loci governing resistance to ascochyta. The complexity of the distributions also highlighted the complexity of the nature of resistance to ascochyta in these populations. These results suggest that accumulation of the resistance genes from different loci of the eight parents would result in increased disease resistance levels and result in genotypes with durable resistance.

In conclusion, the resistance to ascochyta was governed by additive gene action, with a degree of dominance in a few crosses. Resistance was shown to be governed by 2-8 additive genes, with some genotypes probably having dominant genes, with recessive minor genes, while the other sources of resistance had mainly recessive resistance genes.

Resistance genes in these populations were also shown to be located on more than one locus. Heritability estimates obtained for ascochyta resistance, further indicate the quantitative nature of this trait. The influence of maternal effects on the trait were also highlighted and care must be taken, when selecting populations for improving resistance to ascochyta, to avoid delays in achieving progress, due to complications posed by maternal and non-maternal effects.

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Chapter Six: Yield performance and ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] resistance in the advanced common bean (*Phaseolus vulgaris* L.) lines in Rwanda

Abstract

The objectives of this study were: (1) to determine ascochyta resistant stable high yielding genotypes and the extent of genotype by environment (GXE) interaction; and (2) to evaluate and select bean genotypes through farmers' participation. Ten advanced bush lines, ten climbing bean lines and two standard checks, were evaluated at five locations in Rwanda during the short rainy season in 2016. These advanced genotypes were developed by crossing popular, but ascochyta-susceptible genotypes, with resistant genotypes from CIAT and Rwandan germplasm. Bulk selection was applied in the breeding process up to F_6. The selection criteria were mainly ascochyta resistance and seed yield, together with other important farmer-preferred traits. Using the additive main effect and multiplicative interaction (AMMI) model, significant variations among environments and GxE interactions were observed. The interaction principal component analysis (IPCA) showed significant variations for IPCA1, but insignificant variation among genotypes and the remaining IPCAs. The first IPCA1 contributed to 43.9% and 64.6% of the total GxE interaction for bush and climbers, respectively. The AMMI stability value (ASV) for yield was lowest for the bush types RWR 2154 (0.09), Line 2B (0.11), Line 8B (0.12), Line 5B (0.33) and Line 6B (0.48). Amongst the climbing genotypes, Line 6C (0.28), Line 1C (0.30), Line 7C (0.34), RWV 1129 (0.35) and Line 2C (0.36), had the lowest ASV values. The genotype selection index (GSI) revealed that Lines 3B and 1B were the top-ranking bush genotypes, integrating both stability and grain yield performance, whereas climbing Lines 6C and 5C were ranked the best genotypes, based on the GSI. Based on the Eberhart and Russell (1996) analysis model, bush genotypes Lines 1B, 2B and 8B were the most acceptable candidates, with better grain yield, regression coefficients approaching one and acceptable deviation from regression. This implied that they are stable and more widely adaptable than the other genotypes. Lines 6C, 3C and 2C were the most acceptable candidates among other climbers. Both the AMMI and Eberhart and Russell models revealed that bush genotypes Lines 1B and 8B, and climbing genotypes Lines 2C and 6C, were widely adaptable, stable and high yielding. The genotypes selected by farmers were those that exhibited high yields and tolerance to both abiotic and biotic stresses. The highest yield and low ascochyta severity were obtained

from the bush Lines 1B and 8B, and climber Line 6C. Further regional trials, however, are needed, before the release of these genotypes.

6.1 Introduction

Dry bean (*Phaseolus vulgaris* L.) consumption in Rwanda is estimated at 50 kg to 60 kg per capita annually, making it one of highest in the world (Musoni et al., 2005; RAB, 2014). Beans contribute 84% of the pulse legume and 65% of all plant and animal protein in Rwandan diets (Mukamuhirwa et al., 2015). However, in Rwanda, bean production is constrained by several environmental stresses, both biotic and abiotic, which cause significant reductions in the yield (Wortmann, 1998; RAB, 2014). Diseases represent the major hazards confronting farmers. At higher altitudes (>1500 m), ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema] is an important limiting factor. It affects the foliage and pods of beans throughout the growing season. Ascochyta blight is particularly destructive in areas where warm, humid conditions are accompanied by abundant inoculum from infested plant residues and contaminated seed (Schwartz et al., 1981; Schwartz and Corrales, 1989; Saettler and Hall, 1991).

The pathogen causing ascochyta is seed-transmitted and survives for long periods on plant residues. Therefore, the use of disease resistant cultivars and clean seed, in combination with appropriate cultural practices, is essential for the management of ascochyta blight (Schwartz and Corrales, 1989). Yield reductions caused by ascochyta can reach high proportions (20-75%) (Schwartz et al., 1981; Hanson et al., 1993). The authors suggest that differences in the magnitude of the losses may be related to weather factors, the timing of the experiments, the bean genotypes used and the prevalent strains of the pathogen. These last two parameters are of paramount importance, and refer to the genetic diversity of the pathogen and host plant species (Hanson et al., 1993). Studies have demonstrated the existence of a wide range of resistance levels (Schwartz et al., 1981; Pan et al., 2001; Pereira et al., 2011)

The exploitation of genetic variability is the most important tool in plant breeding, as it helps to assess the level of diversity of available germplasm, which has to be inferred by phenotypic expression (Pereira et al., 2014). The consequences of phenotypic variation depend largely on the environment (González et al., 2009). This variation is further complicated by the fact that not all genotypes react in the same way to changes in the environment, and no two environments are exactly the same. If the relative performance of genotypes grown in different environments is different, then genotype by environment (GxE) interaction becomes a challenging factor in crop breeding programmes. Numerous studies have shown the presence of such interactions for dry beans, mainly for grain yield (Pereira et al., 2011).

A combined analysis of variance can quantify the interactions and describe the main effects, but it does not explain the interaction effect (Kaya et al., 2002; Negash et al., 2013). An appropriate analytical model, such as the additive main effects and multiplicative interaction (AMMI), can treat both the AMMI component employing the analysis of variance (ANOVA) and the interaction principal components analysis (IPCA) (Gauch and Zobel, 1996). Furthermore, the AMMI biplot analysis is considered as an effective tool to diagnose GxE interaction patterns graphically (Gauch and Zobel, 1996; Thillainathan and Fernandez, 2001; Kaya et al., 2002). Grain yield performance is not the only parameter for selection of a genotype, as it would not necessarily be stable and adaptable across locations and years. Therefore, the Eberhart and Russell (1966) model and the AMMI stability analysis can be useful tools to identify stable, high-yielding and adaptable genotypes for wider or specific environments. It is crucial for plant breeders to identify adaptable and stable high-yielding genotypes with other desirable traits, like disease resistance under varying environmental conditions, prior to their release as a genotype (Flores et al., 1998; ; Showemimo et al., 2000; Mustapha et al., 2001). Adaptability is the result of GXE interaction and generally falls into two classes: (1) the ability to perform at an acceptable level in a range of environments, or general adaptability; and (2) the ability to perform well only in certain environments, or specific adaptability (Farshadfar and Sutka, 2008).

Beans grown in Rwanda are of varying colours, sizes and shapes and also have different agronomic and culinary characteristics (RAB, 2014). Most farmers grow a mixture that is composed of a selection of local and landraces and, occasionally, released genotypes. Farmers are very careful about which type of bean they plant, in order to maximize their production, minimize their risk and thus improve the livelihood of their families (Sperling et al., 1996; RAB, 2014). The pioneering work on participatory variety selection (PVS) of improved bean genotypes in Rwanda has shown that the faster identification of superior genotypes and their rapid adoption could be achieved through this approach (Sperling et al., 1993; Buruchara et al., 2002). The PVS can assist in the selection of new genotypes for a range of target environments and user preferences (Sperling et al., 1996; Ceccarelli and Grando, 2012). There are sound scientific and practical reasons why farmer involvement can increase the efficiency and the effectiveness of a breeding programme (Sperling et al., 1993; Ceccarelli and Grando, 2007). During this study, farmers participated in the genotype selection of tested lines in different experiment locations.

The present investigation was conducted with the following objectives: (1) to determine stable high-yielding and ascochyta-resistant bean genotypes that could be adapted for wider and/or

specific environments and make recommendations for their possible release; and (2) to evaluate and select bean genotypes for high-yield and other agronomic traits, focusing mainly on ascochyta disease resistance through the participation of farmers.

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6.2 Material and methods

6.2.1 Germplasm, site and experimental design

Twenty advanced F_6 bean lines were evaluated against four standard checks (Table 6.1), to test for ascochyta resistance, the stability of seed yield and other important traits, at five different locations, during the short rainy season of 2016. The controls were newly released genotypes with a high yield, but which were susceptible to ascochyta blight. The advanced bean genotypes used in this study were developed by crossing popular (large-seeded), but ascochyta-susceptible, genotypes with ascochyta-resistant genotypes from Rwandan germplasm and CIAT. A typical bulk selection was applied in the breeding process up to F_6. The selection criteria to improve bean genotypes were ascochyta resistance, earliness and seed yield, together with other important agronomic traits.

The trials were carried out in the following locations: Rwerere, Musanze, Nyamagabe, Muhanga and Rubilizi. With the exception of Rubilizi and Muhanga, the others are situated in the highland regions of Rwanda, as shown in Figure 6.1.

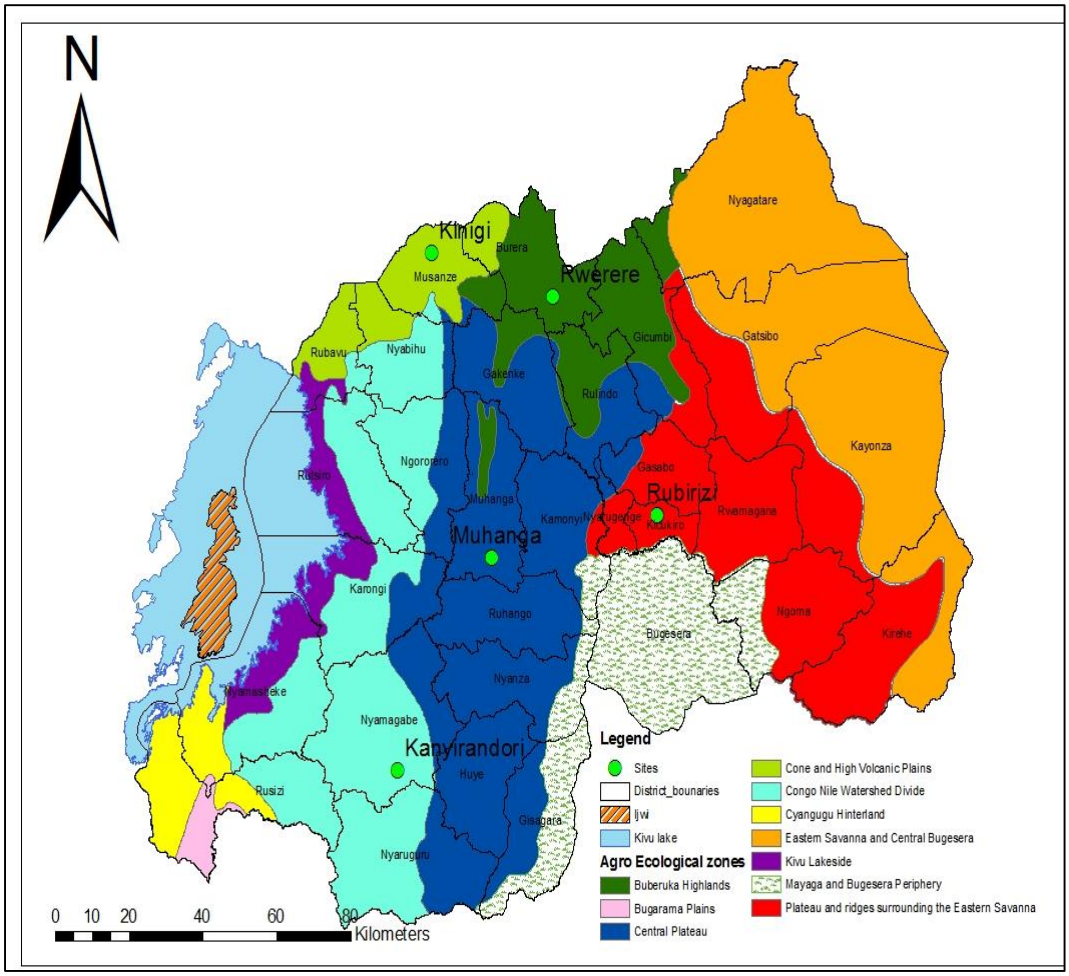

Figure 6.1: Experimental locations

Table 6.1 List of advanced lines used in the study

Genotype	Pedigree	Growth habit	Seed colour	Seed size
Bush				
Line 1B	RWR 2245 X ICTA Hunapu	Type I	Dark red	Medium
Line 2B	RWR 2245 X ASC 87	Type II	Red	Large
Line 3B	RWK 10 X ASC 87	Type III	Red mottled	Medium
Line 4B	RWK 10 X MIB 755	Type I	Red mottled	Large
Line 5B	RWR 1668 X MIB 755	Type III	Dark red	Large
Line 6B	RWR 1668 X ICTA Hunapu	Type I	Red mottled	Medium
Line 7B	RWR 1668 X ASC 87	Type I	Red	Medium
Line 8B	RWR 275 X ASC 87	Type II	Red	Small
Line 9B	RWR 275 X MIB 755	Type III	Black	Small
Line 10B	RWR 275 X CMS 17	Type I	Kaki	Small
RAB 487 (Checks)		Type II	Red mottled	Large
RWR 2154 (Check)		Type II	sugar	Large
Climber				
Line 1C	MAC 44 x G10747	IV	Red mottled	Medium
Line 2C	MAC 49 x G35084	IV	Red mottled	Medium
Line 3C	MAC 44 x G35034	IV	Black	Small
Line 4C	MAC 49 x G10747	IV	Red mottled	Large
Line 5C	Rwibarura x G10747	IV	Black	Small
Line 6C	MAC 49 x G35034	IV	Dark red	Large
Line 7C	MAC 44 x G35084	IV	Black	Small
Line 8C	MAC 49 x RWV 1348	IV	Red	Large
Line 9C	Rwibarura x RWV 1348	IV	Dark red	Small
Line 10C	MAC 44 x RWV 1348	IV	Red	Medium
RWV 1129 (Check)		IV	Light Pink	Large
GASILIDA (Check)		IV	Purple	Large

Large: weight of 100 seeds > 40g; Medium: weight of 100 seeds 25-40g; small: weight of 100 <25g.

At each location, field experiments were arranged in a randomized block design with three replications. The plot was 4 m^2 (2 × 2 m) in size, comprising four rows of 2 m each.

Planting was done by farmers during the short rainy season. Farmers participated from the planting stage to management, from the physical observation of the growth of bean genotypes to the final evaluation of foliar incidences of pests and diseases, agronomic stresses and yields. The farmers' role was to learn the bean plant growth habits, to observe any changes occurring to beans plants in terms of disease expression on the leaves, to record the days taken to flowering and the attainment of physiological maturity, pod bearing and filling, and yield. During the trial management, no chemicals were applied to manage diseases or pests. The assessment of bean cultivar performance started 21 days after planting. Fertilizers were applied before planting, at a rate of 50 kg ha^{-1} N and 100 kg ha^{-1} P_2O_5. Experiments were carried out between January and June 2016. Throughout the experiment, weeds were controlled by hand.

6.2.2 Data collection

Data collected included plot yield, agronomic data on seed (seed color, shape, brilliance), as well as the evaluation of fungal, bacterial and viral pathogens and pests.

The agronomic data collected included a participatory assessment of tolerance to drought, the number of days to flowering, pod development and the filling and physiological maturity period. Seed traits, such as seed colour, shape, size, brilliance and desirability were recorded. After ascertaining the physiological maturity of the plants, harvesting was done with full farmer participation.

The foliar diseases that were assessed included ascochyta blight [*Phoma exigua var. diversispora* (Bubak) Boerema], angular leaf spot (*Phaeoisariopsis griseola (*Sacc*)* Ferrais) and anthracnose (*Colletotricum lindemuthianum (*Sacc*)* Magnus). The disease severity levels were assessed, using a scale from 1 to 9 for disease severity, as characterized by CIAT (1987).

The most predominant pests that were observed to damage the plants included the legume pod borer (*Acanthomyyia* spp) and the bean fly (*Ophiomyia* spp). All collected data were subjected to analysis of variance (ANOVA), using the statistical programme Genstat, 17th Edition.

6.2.3 Data analysis

The analysis of variance (ANOVA) and other statistical analyses were performed with the statistical package Genstat 17th Edition.

6.3.3.1 Additive main effect and multiplicative interaction model

The additive main effect and multiplicative interaction (AMMI) model equation is as follows:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n Y_{gn}\, \delta_{en} + \varepsilon_{ger} + \rho_{ge}$$

Equation ……1

Where: Y_{ger} is the observed yield of genotype (g) in environment (e) for replication (r); additive parameters: μ is the grand mean; α_g is the deviation of genotype g from the grand mean, β_e is the deviation of the environment e; multiplicative parameters: λ_n is the singular value for interaction principal component analysis (IPCA), γ_{gn} is the genotype eigenvector for axis n, δ_{en} is the environment eigenvector; ε_{ger} is error term and ρ_{ge} is PCA residual.

Accordingly, genotypes with a low magnitude, regardless of the sign of IPCA scores, have general or wider adaptability, while genotypes with a high magnitude of IPCA scores, have specific adaptability.

6.3.3.2 AMMI stability value

The AMMI stability value (ASV) is the distance from the coordinate point to the origin in a two-dimensional plot of IPCA1 scores against IPCA2 scores in the AMMI model (Purchase, 1997). Because the IPCA1 score contributes more to the GXE interaction sum of squares, a weighted value is needed. This weighted value was calculated for each genotype and each environment, according to the relative contribution of IPCA1 to IPCA2 to the interaction sum of squares as follows:

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

Equation ……2

Where, SSIPCA1 / SS IPCA2 is the weight given to the IPCA1-value, by dividing the IPCA1 sum of squares by the IPCA2 sum of squares. The larger the ASV value, either negative or positive, the more specifically adapted a genotype is to certain environment. Smaller ASV values indicate more stable genotypes across all environments (Purchase, 1997).

6.3.3.3 Genotype selection index

Stability is not the only parameter for selection, as most stable genotypes would not necessarily give the best yield performance. Therefore, based on the rank of the mean grain yield of genotypes (RYi) across environments and rank of AMMI stability value (RASVi), the genotype selection index (GSI) was calculated for each genotype as:

$$GSIi = RASVi + RYi$$

Equation……..(3)

A genotype with the lowest GSI is considered as the most stable (Farshadfar and Sutka, 2008).

6.3.3.4 Eberhart and Russell regression model

The stability of yield performance for each genotype was calculated by regressing the mean grain yield of individual genotypes on the environmental index and calculating the deviation from regression, which is suggested by Eberhart and Russell (1966) to be:

$$Y_{ij} = \mu_i + b_i I_j + S^2 d_{ij}$$

Equation ……(4)

Where Y_{ij} is the mean performance of i^{th} genotype in j^{th} environment, μ_i is the mean of i^{th} genotype over all environments; b_i is the regression coefficient which measures the response of i^{th} genotype to varying environment; I_j is the environmental index of i^{th} environment; and s^2d_{ij} is deviation from regression of i^{th} genotype in the j^{th} environment.

The regression coefficient (bi) was considered as an indication of the response of the genotype to varying environments. If the regression coefficient is not significantly different from unity (b = 1.0), the genotype is adapted to all environments, whereby genotypes with b > 1.0 are more responsive, or adapted to high-yielding environments, whereas any genotype with b significantly

lower than 1.0, is adapted to low yielding environments (Eberhart and Russell, 1966). Both AMMI and Eberhart and Russel models were computed, using Genstat software 17 version.

6.3 Results

6.3.1 Additive main effects and multiple interaction model

A combined analysis of variance revealed significant (P≤0.01) variations among environments for bush and IPCA1 (bush and climber), while GXE interaction was significant at 5% (Table 6.2). The variation among genotypes was not significant for both bush and climbers. The mean grain yield across geographic locations ranges from 1.8 to 2.7 t ha^{-1} for bush and 4.4 to 5.8 t ha^{-1} for climbers. The environmental variations were significant for bush (P≤0.01) and climbers (P≤0.05), which implies that locations greatly influence the grain yield performance.

Table 6.2: Analysis of variance for grain yield of bean lines tested, using the AMMI model

Source	df	Mean squarre		% GXE interaction	
		Bush	Climber	Bush	Climber
Environments	4	1462196**	3054202*		
Genotype	11	NS	NS		
GxE interaction	44	117162*	547418*		
IPCA I	14	200239**	705676**	43.8	64.6
IPCA II	12	98468	238962	33.1	18.7
IPCA III	10	77081	224815	8.5	6.8
Residual	110	49920	36404		
Total	179	138631	374731		

*,** = significant different at 5% and 1%, respectively

Amongst the bush beans, a considerable percentage of GXE interaction (43.8%) is explained by IPCA1, followed by 33.1% and 8.5% for IPCA2 and IPCA3, respectively. Except for the first IPCA1, the remaining five IPCAs axes were not significant and contributed 56.2% to the GXE interaction (Table 6.2). A significant percentage of GXE interaction (64.6%) is explained by IPCA1 for the climbers, followed by 18.7% and 6.8% for IPCA2 and IPCA3, respectively. Apart

from the first IPCA1, the remaining five IPCAs axes were not significant and contributed 35.3% of the GXE interaction.

In the present study, the first two $IPCA_S$ were used to portray GxE interaction and placement on the biplots. Accordingly, bush genotype Lines1B, 8B and 9B attained values (of both IPCAs) relatively close to zero, and hence are better and more widely adaptable genotypes across all locations (Figure 6.2). Genotypes with low magnitude IPCA scores have general adaptability, while those with high magnitude IPCA scores have specific adaptability (Gauch and Zobel, 1996). However, Lines 7B, 3B, 5B and the control RWR 2154, attained IPCA values closer to one, either for both, or for IPCAI alone (Figure 6.2).

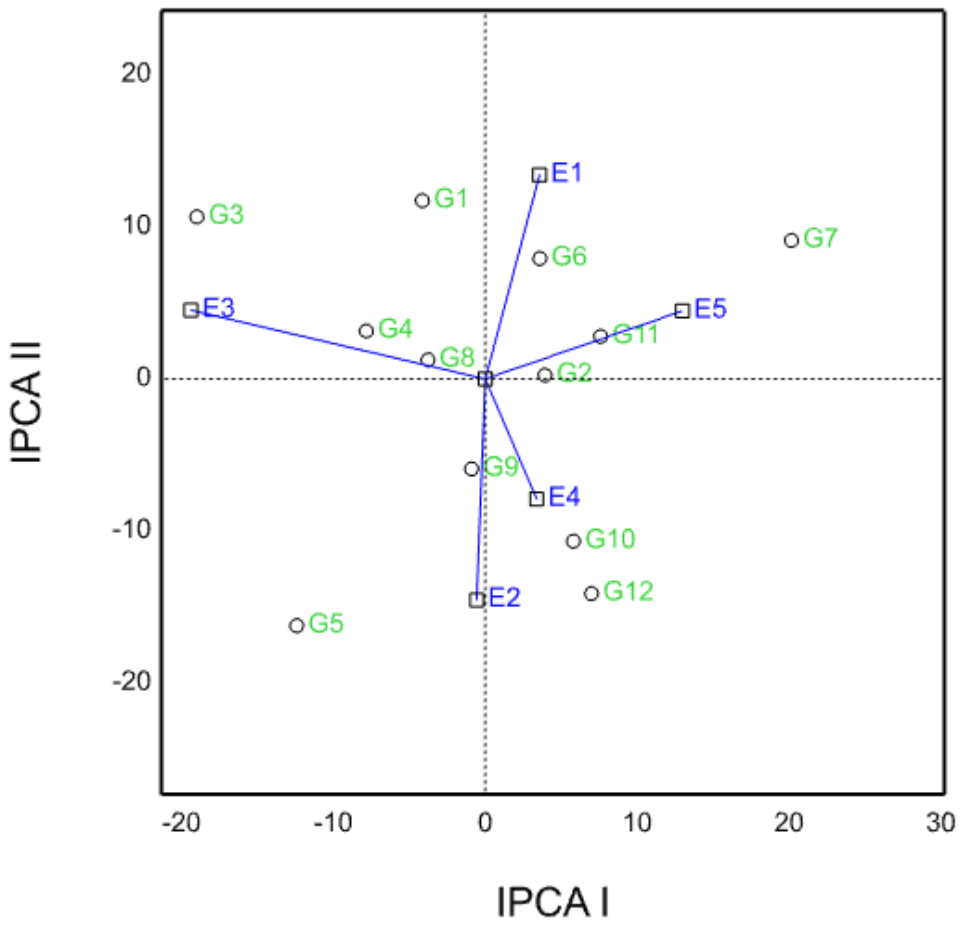

Figure 6.2: Biplot showing bush genotypes grain yield stability and preferential adaptation environment using the first two IPCAs. (Key: E1=Rwerere site, E2=Musanze site, E3=Nyamagabe, E4=Muhanga site, and E5 Rubilizi site).G1=Line 1B, G2=Line 2B, G3=Line 3B, G4=Line 4B, G5=Line 5B, G6=Line 6B, G7=Line 7B, G8=Line 8B, G9=Line 9B, G10=Line 10B, G11= RAB 487, G12=RWR 2154.

Amongst the climbers, Line 6C and 2C attained IPCA values (of both IPCAs) relatively close to zero, and hence, they are stable and widely adaptable genotypes across all locations (Figure 6.3), whereas Lines 4C, 7C and 1C attained IPCA values closer to one (Figure 6.3).

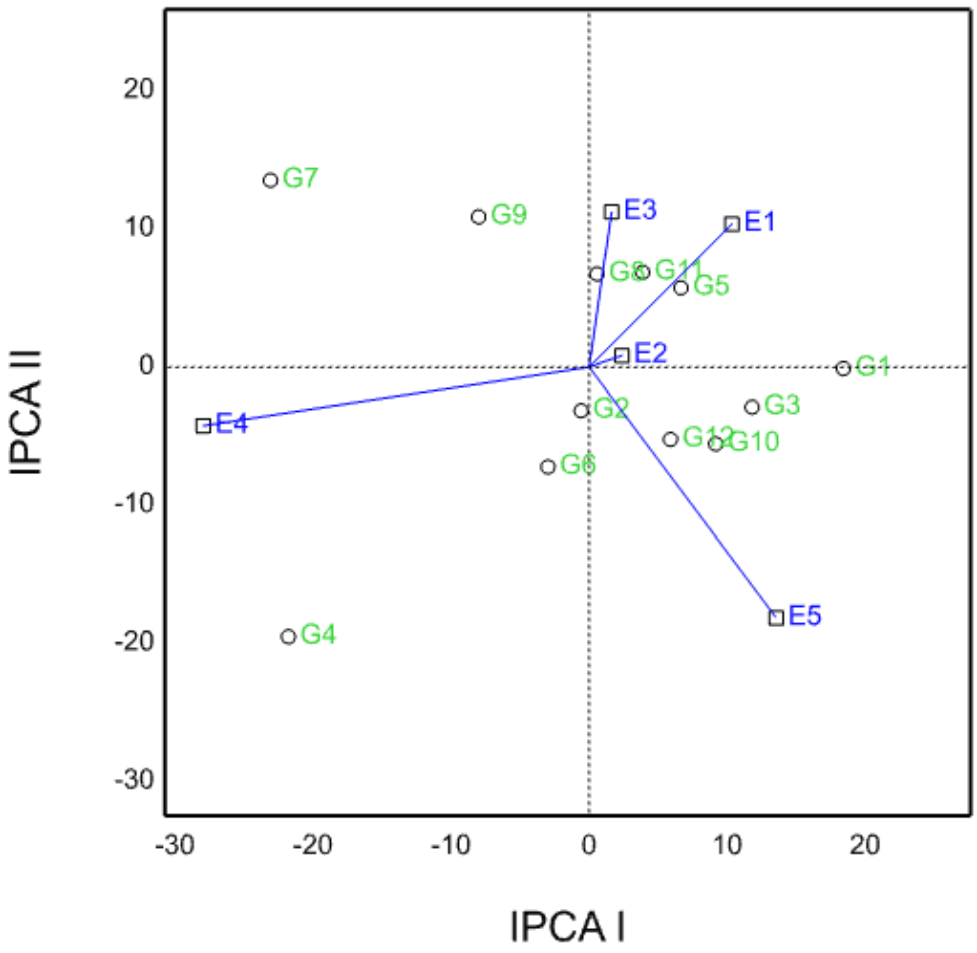

Figure 6.3: Biplot showing climbing genotypes grain yield stability and preferential adaptation environment, using the first two IPCAs. (Key: E1=Rwerere site, E2=Musanze site, E3=Nyamagabe, E4=Muhanga site, and E5 Rubilizi site) G1=Line1C, G2=Line 2C, G3=Line 3C, G4=Line 4C, G5=Line 5C, G6=Line 6C, G7=Line 7C, G8=Line 8C, G9=Line 9C, G10=Line10C, G11= RWV1129, G12=Gasilida.

The above biplot shows the unique grain yield performance of a genotype at a specific site. For instance, bush Line 3B gave a higher grain yield at Nyamagabe than it did across all locations, and hence it is placed nearest the test environment (E3) on the PC axis (Figure 6.2) Similarly, the climbing Line 4C gave a higher grain yield at the Muhanga site than it did at other locations (Figure 6.3).

6.3.2 AMMI stability value and genotype selection index

The analysis, using the AMMI stability value, indicated that RWR 2154 (0.09), Line 2B (0.11), Line 8B (0.12), Line 5B (0.33) and Line 6B (0.48) were among the bush genotypes with the lowest ASV values, in order of importance. This revealed that these genotypes are relatively more stable than others. However, RAB 487 (2.39), followed by Line 10B (2.36), were classified as the least stable bush genotypes (Table 6.3).

For climbers; the AMMI stability value indicated that Line 6C (0.28), Line 1C (0.30), Line 7C (0.34), RWV 1129 (0.35) and Line 2C (0.36) were among the genotypes with the lowest ASV values, in order of importance. GASILIDA (2.81) and Line 3C (2.80) were classified as the least stable climbing genotypes (Table 6.3).

The genotype selection index (GSI) revealed that Line 3B and Line 1B are the best and top-ranking bush genotypes, integrating both stability and grain yield performance parameters, followed by Line 2B, Line 8B and RWV 2154 (Table 6.3). Climbing Lines 6C and 5C are ranked as the best genotypes, integrating both stability and grain yield performance parameters, followed by Line 3C and 2C (Table 6.3).

Table 6.3: AMMI stability value (ASV) and genotype selection index (GSI) for test genotypes and locations

Bush				Climber			
Genotype	**Mean yield (t ha^{-1})**	**ASV**	**GSI**	**Genotype**	**Mean yield (t ha^{-1})**	**ASV**	**GSI**
Line 1B	2.85	1.99	13.00	Line 1C	4.88	0.30	18.00
Line 2B	2.36	1.11	14.00	Line 2C	5.11	0.36	19.00
Line 3B	2.31	0.51	12.00	Line 3C	5.17	2.80	17.00
Line 4B	2.03	1.73	24.00	Line 4C	5.24	2.14	24.00
Line 5B	1.60	0.33	20.00	Line 5C	5.27	2.49	16.00
Line 6B	2.10	0.48	15.00	Line 6C	5.35	0.28	12.00
Line 7B	2.17	0.62	16.00	Line 7C	4.86	0.34	18.00
Line 8B	2.46	0.12	14.00	Line 8C	4.81	2.79	24.00
Line 9B	2.05	0.57	19.00	Line 9C	4.53	2.12	23.00
Line 10B	2.29	2.36	19.00	Line 10C	4.10	2.47	19.00
RAB 487 (Checks)	2.05	2.39	26.00	RWV 1129 (Check)	4.60	0.35	24.00

	Bush				Climber		
Genotype	**Mean yield (t ha^{-1})**	**ASV**	**GSI**	**Genotype**	**Mean yield (t ha^{-1})**	**ASV**	**GSI**
RWR 2154 (Check)	2.41	0.09	14.00	GASILIDA (Check)	4.93	2.81	17.00
Mean	**2.22**	**1.02**	**17.17**		**4.90**	**1.60**	**19.25**
Environment							
Rwerere	2.70	0.44	7		5.80	0.83	5
Nyamagabe	2.60	0.93	8		4.56	0.23	8
Musanze	2.42	2.06	7		5.32	0.56	7
Gitarama	1.60	0.90	8		4.50	0.67	4
Rubilizi	1.80	0.58	7		4.40	0.88	5

6.3.3 Analysis based on Eberhart and Russell regression model

Based on the Eberhart and Russell (1966) analysis model, bush genotypes Line 1B, 2B and 8B were the most acceptable candidates, with a better grain yield (2.85, 2.36 and 2.46 t ha^{-1}), regression coefficients approaching one (1.17, 1.26 and 0.15) and an acceptable deviation from regression (-0.46, 0.33 and -0.30), implying that they are stable and more widely adaptable than the other genotypes (Tables 6.3 and 6.4).

Climbers Lines 6C, 3C and 2C were the most acceptable candidates with a good grain yield (5.35, 5.17 and 5.11 t ha^{-1}), regression coefficients (1.12, 0.78 and 0.87) and acceptable deviation from regression (-0.30, 0.16 and -0.18), implying that they are stable and more widely adaptable than the other genotypes (Tables 6.3 and 6.4). An ideal genotype has the highest average grain yield, a regression coefficient (bi) value of approximately one and a mean square deviation from regression (s^2di) value close to zero (Becker and Leon, 1988; Eberhart and Russell, 1966).

Table 6.4: Regression coefficient (bi) and squared deviation from linearity of regression (s^2di) of the tested genotypes, using Eberhart and Russell model

	Bush				Climber		
Genotype	**Regression coefficient (bi)**	**Squared deviation from regression (s^2di)**	**Pr.>F**	**Genotype**	**Regression coefficient (bi)**	**Squared deviation from regression (s^2di)**	**Pr.>F**
Line 1B	1.17	-0.46	0.7	Line 1C	0.95	-0.02	0.46
Line 2B	1.26	0.33	0.65	Line 2C	0.87	-0.18	0.82
Line 3B	0.88	-0.17	0.81	Line 3C	0.78	0.16	0.19
Line 4B	1.29*	0.49	0.03	Line 4C	0.78*	0.40	0.04
Line 5B	0.82	-0.23	0.93	Line 5C	0.64	-0.14	0.73
Line 6B	1.42*	0.06	0.02	Line 6C	1.12	-0.30	1.00
Line 7B	0.99	0.13	0.23	Line 7C	0.91	0.13	0.23
Line 8B	0.15	-0.30	1.00	Line 8C	1.42*	0.06	0.02
Line 9B	0.64	-0.14	0.73	Line 9C	0.82	-0.23	0.93
Line 10B	1.15*	0.45	0.04	Line 10C	1.25*	0.49	0.03
RAB 487 (Checks)	0.98	-0.23	0.91	RWV 1129 (Check)	0.88	-0.18	0.81
RWR 2154 (Check)	1.01	-0.15	0.75	GASILIDA (Check)	1.14*	0.46	0.04

Standard error of beta = 0.176

The regression coefficients were significantly (P≤0.05) different from unity for Lines 4B, 6B and 10B for bush, and 4C, 8C, 10C, and the standard check Gasilida, for climbers (Table 6.4). This indicates that the above genotypes are less stable and characterized by specific adaptability.

6.3.4 Farmer's' perception on bean seed appearance characteristics

Harvested seeds were subjected to appearance analysis for acceptance, based on the farmers' perceptions of the desired qualities. Selected attributes that were considered as important, included seed colour, shape, size, brilliance and seed weight (Tables 6.5 and 6.6). Similar characteristics were evaluated by Garcia et al. (1997).

Table 6.5: Seed yield, seed characteristics and farmers' acceptance of seed characteristics

Genotype	Yield (t ha^{-1})	Seed color	Seed size	Acceptance	
Bush				**High altitude sites**	**Low altitude sites**
Line 1B	2.85	Dark red	Large	Good	Excellent
Line 2B	2.36	Red	Large	Good	Very Good
Line 3B	2.31	Red mottled	Medium	Good	Very Good
Line 4B	2.03	Red mottled	Large	Good	Excellent
Line 5B	1.60	Dark red	Large	Fair	Fair
Line 6B	2.10	Red mottled	Medium	Fair	Good
Line 7B	2.17	Red	Medium	Fair	Good
Line 8B	2.46	Red	small	Good	Very Good
Line 9B	2.05	Black	small	Fair	Fair
Line 10B	2.29	Kaki	small	Fair	Fair
RAB 487 (Checks)	2.05	Red mottled	Large	Good	Excellent
RWR 2154 (Check)	2.41	sugar	Large	Good	Very Good
Climber					
Line 1C	4.88	Red mottled	Medium	Good	Fair
Line 2C	5.11	Red mottled	Medium	Very Good	Fair
Line 3C	5.17	Black	small	Good	Very Good
Line 4C	5.24	Red mottled	Large	Excellent	Good
Line 5C	5.27	Black	small	Fair	Very Good
Line 6C	5.35	Dark red	Large	Excellent	Good
Line 7C	4.86	Black	small	Fair	Good
Line 8C	4.81	Red	Large	Good	Fair
Line 9C	4.53	Dark red	small	Good	Good
Line 10C	4.10	Red	Medium	Good	Fair
RWV 1129 (Check)	4.60	Light Pink	Large	Very Good	Fair
GASILIDA (Check)	4.93	Purple	Large	Very Good	Fair

Table 6.6: Attributes considered by farmers in selection of genotype

Attribute	Bush selected lines	Climber selected lines
Drought Resistance	Line 1B, Line 3B, Line 5B	Line 10C; Line 9C
Earliness	Line 5B, Line 6B; Line 4B; Line 9B	Line 10C; Line 9C; Line1C
Shattering	Line 1B; Line 8B	Rwv 1129 (Check); Line 2C; Line 3C; Gasilida (Check); Line 4C
Seed color	Line 1B; Line 2B; RWR 2154 (Check)	RWV 1129 (Check); Gasilida (Check); Line 4C; Line 6C; Line 5C
Seed brilliance	RAB 487 (Check); Line 7B	Line 4C; Line 6C; RWV 1129 (Check)
Seed size	Line 1B; Line 10B; RAB 487 (Check); RWR 2154 (Check); Line 6B	RWV 1129 (Check); Gasilida (Check); Line 4C; Line 2C; Line 5C; Line 6C
Disease resistance	Line 1B; RAB 487 (Check); Line 8B; Line 2B; RWR 2154 (Check)	Gasilida (Check); RWV 1129 (Check); Line 6C; Line 5C; Line 1C; Line 4C
Insect resistance	RAB 487 (Check); Line 7B; Line 1B; Line 4B; Line 9B	Line 1C; Line 6C; Line 5C; Line 10C; Line 2C; RWV 1129 (Check)
Yield	Line 1B; Line 8B; RWR 2154 (Check); Line 2B	Line 6C; Line 5C; Line 4C; Line 3C; Line 2C; Gasilida (Check)

6.3.5 Participatory evaluation of bean genotypes for most occurring diseases

The reaction of genotypes was significantly different to naturally-occurring pathogens (Table 6.7). The manifestation of diseases on some advanced lines was generally low, indicating disease tolerance. The diseases that were evaluated included ascochyta blight. The severity of ascochyta on plants was relatively high at highland sites (Table 6.7). High severity rates were observed on bush Line 5B, RWR 2154, RAB 476 and Line 10, at a 6.8, 6.6, 5.2 and 5.0 score of severity, respectively. The least affected genotypes included Line 1B and 2B. Climber entries Lines 3C, 6C, 8C and 7C were the least affected by ascochyta. Lines 2C and 5C, as well as controls GASILIDA and RWV 1169, showed high ascochyta infection.

The severity of angular leaf spot, one of the most serious and widely distributed diseases of beans, was low on most climbing genotypes, with scores ranging from 1.0 – 2.3 (Table 6.7). High susceptibility was observed in lowland areas and bush genotypes showed the highest level

of infection. Bush Lines 10B, 7B and 5B were highly infected by angular leaf spot among bush genotypes, while Lines 1C, 4C, 9C and 10C were highly infected amongst climbers (Table 6.7).

Another foliar disease known to affect bean productivity is bean anthracnose. However, its evaluation in highland sites showed a low severity score (Table 6.7). Some bush lines exhibited the same characteristic foliar symptoms, while the climbers appeared to be tolerant.

Table 6.7: Genotypes reaction (severity) to naturally occurring diseases

Genotype	Aschochya blight	Angular leaf spot	Anthracnose	Farmer preference	
Bush				**High altitude sites**	**Low altitude sites**
Line 1B	2.60	2.50	1.20	Good	Excellent
Line 2B	3.20	1.70	2.50	Good	Very Good
Line 3B	3.80	4.30	4.10	Good	Very Good
Line 4B	4.00	1.40	2.20	Fair	Very Good
Line 5B	6.30	4.70	6.10	Fair	Fair
Line 6B	3.80	4.40	4.20	Fair	Good
Line 7B	4.10	4.60	4.40	Fair	Good
Line 8B	3.90	4.30	4.20	Good	Very Good
Line 9B	4.00	4.50	4.10	Fair	Fair
Line 10B	5.00	5.50	5.30	Fair	Fair
RAB 487 (Checks)	5.20	2.60	1.50	Good	Excellent
RWR 2154 (Check)	6.60	4.10	3.90	Good	Very Good
lsd	0.38	0.28	0.23		
CV	26.4	25.3	27.0		
Climber					
Line 1C	1.50	2.30	3.10	Very Good	Fair
Line 2C	2.70	1.50	1.20	Fair	Fair
Line 3C	1.30	1.10	1.00	Good	Very

					Good
Line 4C	2.50	2.30	2.00	Good	Good
Line 5C	3.10	1.00	1.00	Very Good	Good
Line 6C	1.40	1.00	1.90	Excellent	Good
Line 7C	1.60	1.40	1.10	Fair	Good
Line 8C	1.40	1.20	3.10	Good	Fair
Line 9C	2.50	2.30	1.00	Fair	Good
Line 10C	2.50	2.30	2.00	Good	Fair
RWV 1129 (Check)	3.70	1.50	1.20	Good	Fair
GASILIDA (Check)	2.10	1.90	1.60	Good	Fair
lsd	0.30	0.20	0.15		
CV	25.1	24.0	25.7		

Lsd = least significant difference; CV = coefficient of variation; Scores 1-9 where 1 = Healthy, 9 = highly affected) (CIAT, 1987)

6.4 Discussion

The main objectives of this study were to measure the breeding progress by identifying new stable, high-yielding, ascochyta-resistant advanced bean genotypes, which could be recommended for further testing before their possible release in a specific or wider environment. The new advanced lines were also evaluated, together with the participation of the farmers.

The twenty advanced F_6 bean lines were evaluated against the four standard checks, to test the stability of seed yield and other important traits at five different locations during the short rainy growing season of 2016.

The analysis of variance for yield revealed highly significant (P≤0.01) variations among environments and the principal component (IPCA1), while the GXE interaction was significant at 5%. The variation for yield among genotypes was non-significant for both bush and climbers. The mean grain yield across geographic locations ranged from 1.8 to 2.7 t ha^{-1} for bush and 4.4 to 5.8 t ha^{-1} for climbers. The results indicated that locations greatly influenced the grain yield performance. In line with the present findings, statistically significant differences between environments, genotypes and GXE interactions were reported for the grain yield of bean genotypes evaluated across six environments by Carbonell et al. (2004).

A considerable percentage of GXE interaction (43.7%) is explained by IPCA1, followed by IPCA2 (33.0%) and IPCA3 (8.4%) for bush beans. The remaining five IPC axes were not significant and contributed 54.2% of the GXE interaction (Table 6.2). For the climbers, a significant percentage of GXE interaction (64.6%) was explained by IPCA1, followed by 18.7% and 6.8% for IPCA2 and IPCA3, respectively. Apart from the first IPCA1, the remaining five IPC axes were not significant and contributed 33.3% of the GXE interaction. Several authors working on various crops reported that a significant and greater percentage of GXE interaction was explained by the first IPCA score; for example, for maize (Wende and Labuschangne, 2005), wheat (Farshadfar and Sutka, 2008), the common bean (Abeya et al., 2008) and the field pea (Mengistu et al., 2011).

In the present study, the first two IPCAs were used to portray genotypes by their interaction with the environment and their placement on the biplots. Accordingly, bush genotypes Lines 1B, 8B and 9B attained IPCA values relatively close to zero and were therefore stable and widely adaptable genotypes across all locations (Figure 6.2). Genotypes with low magnitude IPCA

scores have a good general adaptability, while those with a high magnitude of IPCA scores have specific adaptability (Gauch and Zobel, 1996). Bush Lines 7B, 3B, 5B and the released genotype RWR 2154, attained IPCA values closer to one (Figure 6.2). Amongst the climbers, Line 6C and 2C attained IPCA values relatively close to zero, and they are hence stable and widely adaptable genotypes across all locations (Figure 6.3), whereas Lines 4C, 7C and 1C, attained IPCA values closer to one. The biplot also shows the specific yield performance of a genotype at a particular site. For instance, bush Line 3 gave a higher grain yield at Nyamagabe than it did across locations, and hence it is placed nearest the test environment (E3) on the PC axis (Figure 6.2). Similarly, climbing Line 4C gave a higher yield at the Muhanga site than it did at other locations (Figure 6.3). Pan et al. (2001) and Harer et al. (2000) reported results that are in agreement with the present study.

The analysis using AMMI stability value indicated that RWR 2154 (0.09), Line 2B (0.11), Line 8B (0.12), Line 5B (0.33) and Line 6B (0.48) were among the bush genotypes with lower ASV values, in order of importance. These genotypes are, therefore, relatively more stable than others. RAB 487 (2.39), followed by Line 10B (2.36), were classified as the least stable bush genotypes (Table 6.3). Amongst the climbers, the AMMI stability value indicated that Lines 6C (0.28), 1C (0.30), 7C (0.34), RWV 1129 (0.35) and 2C (0.36) were among the genotypes with low ASV values, in order of importance. GASILIDA (2.81), followed by Line 3C (2.80), were classified as least stable climbing genotypes (Table 6.3).

Stability is not the only parameter for selection of high yielding genotypes, as the most stable genotypes would not necessarily give the best yield performance. The genotype selection index revealed that Lines 3B and 1B are the best and top-ranked bush genotype, integrating both stability and grain yield performance, followed by Lines 2B, 8B and RWR 2154 (Table 6.3). Climbing Lines 6C and 5C are ranked as the best genotypes, integrating both stability and grain yield performance, followed by Lines 3C and 2C.

Based on the Eberhart and Russell (1996) analysis model, bush genotypes Lines 1B, 2B and 8B were the most acceptable candidates, with high grain yield (2.85, 2.36 and 2.46 t ha^{-1}), regression coefficients approaching one (1.17, 1.26 and 0.15) and an acceptable deviation from regression (-0.46, 0.33 and -0.30), implying that they are more stable and widely adaptable than the other genotypes (Tables 6.3 and 6.4). Amongst the climbers, Lines 6C, 3C and 2C were the most acceptable candidates with best grain yield (5.35, 5.17 and 5.11 t ha^{-1}), regression coefficients (1.12, 0.78 and 0.87) and acceptable deviation from regression (-0.30, 0.16 and -

0.18), implying that they are more stable and widely adaptable than the other genotypes (Tables 6.3 and 6.4). An ideal genotype has a high average grain yield, a regression coefficient (bi) value of approximately one and a mean square deviation from regression (s^2di) value close to zero (Becker and Leon, 1988; Eberhart and Russell, 1966).These results are consistent with those reported by Pereira et al. (2016) and Pan et al. (2001). The regression coefficients were significantly (P≤0.05) different from unity for Lines 4B, 6B and 10B for bush, and Lines 4C, 8C, 10C and the standard check, GASILIDA, for climbers (Table 6.4). This indicates that the above genotypes are less stable and characterized by specific adaptability.

The result obtained, using the Eberhart and Russell (1996) model, is corroborated by the AMMI model. Bush genotype Line 1B (2.85 t ha^{-1}) and Line 8B (2.11 t ha^{-1}) gave the higher grain yield, but the regression coefficient (bi) was significantly (P≤0.05) higher than unity (Table 6.4,) and the deviation from regression was positive (Table 6.4). This is also observed for climber Lines 2C (5.11 t ha^{-1}) and 6C (5.35 t ha^{-1}). This implies that these genotypes are highly responsive to changes in the environment and hence are recommended for specific environmental conditions, with appropriate agronomic practices. Likewise, bush genotypes Lines 7B and 5B and climber Lines 2C and 7C gave grain yields below the average, regression coefficients (bi) significantly (P≤0.05) different from one and squared deviations from regression (s^2di) higher than zero, and they are hence poorly adapted to all environments (Table 6.4).

Among the important attributes that farmers considered, when adopting a new genotype of interest, is yield. However, the farmers' perceptions differed, depending on the location of trials. In the highlands, bush beans have been abandoned, whereas in the lowland areas, climbers are not often adopted because of drought and the lack of staking materials. Other traits of importance that were mentioned by farmers included the maturity period which is reflected in the days to flowering and physiological maturity. Such traits are important for farmers, in view of planning the time for planting, executing management practices and effective harvesting. The results from the PVS showed that bush Lines 5B, 6B, 4B, 9B and climber Lines 10C, 9C and 1C, if planted early, can have good yields (Table 6.6), leading to abundant food supply for consumption.

Seed shape, weight and whether the seed is shiny or dull are characteristics that determine the commercial value of the beans. Bush Lines 1B, RAB 487, Line 8B and climber Lines 6C, 5C and 4C possess the most desired attributes (Table 6.5), which are considered to be important. The same preferred genotypes are the maturity period, tolerance to drought stress, shiny seed and

good yield (Tables 6.5 and 6.6). Similar traits were also found important in bean evaluation by Teshale et al. (2005) in Ethiopia.

Genotypes reacted significantly to naturally-occurring pathogens (Table 6.7). The manifestation of diseases on plant parts was generally low on some genotypes, indicating the possibility of tolerance to the pathogens. A high severity of ascochyta blight was observed on bush Lines 5B and 10B. The least affected genotypes included Lines 1B and 2B and the standard check RWR 2154. Climbers Lines 3C, 6C, 8C and 7C showed good tolerance to ascochyta blight.

In summary, both the AMMI and Eberhart and Russell models showed that bush Lines 1B and 8B, and climbing genotypes Lines 2C and 6C, were ascochyta tolerant, stable and high yielding. Based on both the AMMI, Eberhart and Russell models and the ascochyta severity evaluation, Lines 1B and 8B for bush and Line 6C for climber are recommended as being stable, ascochyta resistant and having wider environmental- adaptability. The average ascochyta severity score for Lines 1B, 8B and 6C was 2.6%, 3.9% and 1.4% respectively, whereas for the control bush genotypes RAB 487, RWR 2154 and climber RWV 1129 and GASILIDA, it was 5.2%, 6.6%, 3.7% and 2.1% respectively. This shows that, the ascochyta severity score for developed breeding lines was low, compared to the controls, indicating the genetic gain obtained from the breeding strategy used in this study. However, further studies in different regions are needed, before the release of these lines.

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Chapter Seven: Overview of the study

The common bean (*Phaseolus vulgaris* L.) is the most important grain legume crop grown worldwide. The crop is also an important source of income for smallholder farmers in the rural areas, particularly the women. However, production in Rwanda is affected by several constraints. Bean ascochyta blight is one of the major biotic constraints to bean production and was the main focus of this study. The study aimed at contributing to food security in Rwanda, by improving resistance to ascochyta blight in the major market class bean genotypes that are preferred by local farmers. The study included the following: a participatory rural appraisal (PRA), to identify farmers' perceptions of common bean production and ascochyta blight; a study of the yield loss caused by the ascochyta blight; an evaluation of bean germplasm for its resistance to ascochyta blight; a genetic analysis of resistance to ascochyta in the identified sources; and finally, a yield performance evaluation of advanced breeding lines.

A participatory rural appraisal was conducted at four sites covering four major districts (Burera, Musanze, Kamonyi and Rwamagana), where the common bean is widely grown. The study was carried out with the aim of obtaining information on farmers' perceptions of bean varieties, the reasons for their variety choices, the farmers' knowledge of bean diseases and pests and other major production constraints.

Farmers were aware of the damage caused by ascochyta blight to their bean crop, but were not knowledgeable about the disease itself. Farmers associated the disease with poor soils, excessive rainfall and poor crop management practices. Most farmers did not attempt to control the diseases. However, a few farmers practiced the roguing of infected plants. The use of resistant genotypes to control the disease was not recognized by most of the farmers. Generally, farmers confirmed their preference for large-seeded genotypes over small-seeded types. High yield, marketability, resistance to diseases, tolerance to excessive rainfall and drought, and taste were the most important criteria considered before the adoption of a new bean genotype by farmers. Bush beans are the preferred type in lowland regions, because they are early maturing and require less labour and staking materials for their production than the climbing beans. Climbing beans are grown extensively by farmers in northern Rwanda for their high yields and tolerance to diseases.

The yield loss assessment quantified the yield loss as a result of bean ascochyta blight of 64 common bean genotypes, including the bush and climbing types. Using a split plot design, trials were conducted at three locations, where the ascochyta disease is prevalent. The

different genotypes that were used had variable levels of susceptibility and were compared with resistant genotypes ICTA Hunapu and ASC 87 for the bush types, and G 35034 G 35306 for the climbing types. The results showed that the local market class genotypes recorded higher disease severity and higher yield losses than the resistant controls. There was a strong positive correlation between the relative area under the disease progress curve (RAUDPC) values and the yield losses. It was established that the use of susceptible cultivars, in the presence of the ascochyta pathogen, may result in a yield loss of up to 75%. This loss is much lower, if a resistant genotype is used or if a fungicide is used to protect the crop against the effects of the pathogen. The bush genotypes showed a larger reduction in yield, due to ascochyta blight, than the climbing genotypes. Unfortunately, the available resistant genotypes are not as marketable as the susceptible genotypes.

The germplasm evaluation was conducted to quantify the impact of the ascochyta blight on phenotypic and agronomic traits, under natural conditions. Field screening trials of 39 bush (Types I, II and III) and 36 climbing (Type IV) genotypes were conducted at three sites, namely, the Rwerere, Nyamagabe and Musanze Research Stations, for two seasons. The findings from these studies showed that there were some local and recently-introduced common bean genotypes that were resistant to the ascochyta blight. The study indicated that out of the 75 germplasm genotypes, 13 gave a consistent resistant reaction to the ascochyta pathogen, 29 gave an intermediate resistance reaction and 23 were susceptible. Some of the identified resistant genotypes can be used to introgress ascochyta resistance into susceptible Rwandan market-class common bean genotypes.

The study of the inheritance of the resistance to ascochyta established that the resistance is primarily governed by additive gene action, with a degree of dominance in a few crosses. Resistance was shown to be governed by 2-8 additive genes, with some genotypes probably having dominant genes with recessive minor genes, while the other sources of resistance have mainly recessive resistance genes. Resistance genes are located on more than one locus. Heritability estimates of ascochyta resistance indicated the quantitative nature of this trait. The influence of maternal effects on the traits was also highlighted and care must be taken of the parents for improving resistance to ascochyta, in order to avoid delays in achieving progress due to complications posed by maternal and non-maternal effects.

The study of the yield performance in advanced common bean genotypes revealed that the analysis of variance of GXE interaction was highly significant. The variations among the test locations were also significant. Both the AMMI and the Eberhart and Russell models, revealed that advanced bush genotypes Lines 1B and 8B, and advanced climbing genotype 6C were resistant to ascochyta blight; widely adapted, stable and high yielding. The

genotypes selected by farmers were those that exhibited a high tolerance to both abiotic and biotic stresses. These promising genotypes are therefore recommended for possible release.

The above findings have several implications for future breeding strategies for resistance to ascochyta blight.

- Breeding for resistance to ascochyta should involve farmers and take into account the farmer's preferred traits. There is also a need to develop new varieties that are adapted to the local farming system. This approach will ensure the adoption of the new varieties by farmers.
- The study identified several genotypes that have a good level of resistance to ascochyta blight and that can be used as parents in future ascochyta breeding programmes.
- The significant differences between the yield loss of susceptible and resistant cultivars suggested that the use of resistant cultivars would be the most economic option for controlling and managing the ascochyta disease. The main challenge will be to develop farmers' preferred varieties that have a durable resistance to ascochyta blight.
- The choice of the female parents in crosses in an ascochyta resistant breeding programme is important, as maternal effects play a significant role in the resistance to ascochyta in beans.
- The heritability estimates that were obtained for ascochyta resistance were low in this study, indicating the quantitative nature of the ascochyta resistance. The presence of many minor additive genes that govern resistance to ascochyta, creates an opportunity to develop ascochyta varieties with durable, horizontal resistance to ascochyta blight, using a breeding strategy with recurrent selection.

In conclusion, the findings clearly show the potential to develop locally adapted varieties, with farmer-preferred traits for durable resistance to ascochyta blight. Rapid breeding progress could be realized by the careful control of the test environment, and taking care of which parent should be used as female or male, when designing crosses during the breeding. This study has generated important information on breeding for ascochyta blight resistance. Several advanced lines have been developed in this study that could be of immediate benefit to bean farmers in Rwanda. These advanced lines need further testing in the regional trials, before they can be recommended for release.