BACTERIAL BLIGHT (*XANTHOMONAS AXONOPODIS* PV. *PHASEOLI*) TOLERANCE IN COMMON BEAN VARIETIES (*PHASEOLUS VULGARIS* L.) IN ZIMBABWE

A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR DEGREE OF BACHELOR OF SCIENCE HONORS IN AGRICULTURE AND NATURAL RESOURCES

BY

KUDZAI SIGAUKE

FACULTY OF AGRICULTURE AND NATURAL RESOURCES

AFRICA UNIVERSITY

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ABSTRACT

Bacterial blight disease of common bean has devastating effects on the production of bean varieties among smallholder farmers in Zimbabwe. Bacterial blight disease caused by the bacterium *Xanthomonas axonopodis* pv. *phaseoli* causes substantial yield losses in the smallholder sector hence the need to conduct the study to determine bacterial blight tolerance levels in bean varieties. The different bean varieties belong to different classes namely Ex Rico Navy (small white canning variety), Bonus (Sugar bean variety), Natal sugar (brown speckled bean variety), White kidney (large white kidney variety) and Carioca (Carioca class). The objectives of the study were to determine the different levels of tolerance in the five bean varieties and to prove the existence of bacterial blight tolerance traits in the white bean varieties. The study was carried out at Africa University Research Block located at Africa University Farm in Mutare. The study commenced on 28 February 2012 and was completed on 10 May 2012. Data on disease severity was collected from the field trials. The data was recorded as scores based on the CIAT scale. The average scores for each of the three replications of the varieties was subjected to ANOVA analysis using MINITAB version 13 software. Results of the study showed that Ex Rico Navy was the most tolerant and was significantly different from the other four varieties with an AUDPC of 14.35cm^2. Bonus variety was the second most tolerant with an AUDPC of 73.85cm^2 followed by Natal Sugar with an AUDPC of 102.78cm^2. Carioca with an AUDPC of 128.66cm^2 and white kidney variety with an AUDPC of 130cm^2, were not very significant from each other and had the least tolerance to bacterial blight. Farmers should make use of the bacterial blight tolerant varieties such as Ex-rico. Research institutes and plant breeders should manipulate the bacterial blight tolerance genes in the white bean varieties and insert them in other bean varieties of other classes for varietal diversity.

DECLARATION

I,……………………………………………, hereby declare that this dissertation is the result of my authentic effort and investigations and such work has not been previously presented elsewhere for any B.Sc. Honors Degree level. All additional sources of information have been acknowledged by means of references.

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Student's signature

Date…………………………………………………

Approved for submission

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Name of supervisor

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Supervisor's signature

Date……………………………………………

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The research project was a success, all attributes given to my project supervisor Dr Manyangarirwa. W; the Sigauke Family and; the Africa University Faculty of Agriculture and Natural Resources.

DEDICATION

This project is dedicated to the Sigauke Family

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CHAPTER 1

1.0 INTRODUCTION

This particular research project focussed on the effect of *Xanthomonas axonopodis pv. Phaseoli* on bean varieties from different categories namely, small white canning variety, large white kidney variety, red kidney variety, speckled sugar variety and carioca variety. The common dry bean (*Phaseolus vulgaris L.*), is the most important food legume for direct consumption in the world. Phaseolus vulgaris is produced in a range of crop systems and environments in regions as diverse as Latin America, Africa, the Middle East, China, Europe, the United States, and Canada. The leading bean producer and consumer is Latin America, where beans are a traditional, significant food, especially in Brazil, Mexico, the Andean Zone, Central America, and the Caribbean. In Africa, beans are mainly grown for subsistence, where the Great Lakes region has the highest per capita consumption in the world (Jones, 1999). In Africa, beans are grown in Kenya, Tanzania, Malawi, Uganda, Zambia and Zimbabwe.

In Zimbabwe, bean production in the smallholder sector is characterized by low yields averaging about 500 to 700 kg/ha under dryland conditions in Zimbabwe. The bean plant (*Phaseolus vulgaris*) is vulnerable to diseases and common bacterial blight is one of the most common diseases that affect many bean varieties. Common bacterial blight (*Xanthomonas axonopodis pv phaseoli*) affects bean foliage, pods, and seedlings. Early foliar symptoms are small, angular, light green, water-soaked or translucent spots. During warm and wet conditions, these lesions rapidly enlarge and merge. Gradually, the centers of the lesions become dry and brown, and are surrounded by a distinct, narrow zone of yellow tissue. In highly susceptible varieties, the lesions continue to expand until the leaves appear scorched, ragged, and torn by wind and rain. Pod symptoms consist of lesions that are generally circular, slightly sunken, and dark red-brown. In severe cases, entire pods may shrivel and die. Seeds in less severely affected pods may show no symptoms of disease or may be slightly wrinkled

1.1 Statement of the Problem

Bean production in the smallholder sector is characterized by low yields averaging about 500 to 700 kg/ha under dryland conditions in Zimbabwe. The bean plant (*Phaseolus vulgaris*) is vulnerable to diseases and bacterial blight is one of the most common diseases that affect many bean varieties. Common Bacterial Blight is caused by *Xanthomonas phaseoli* and is one of the major constraints in dry bean production in the world. Bacterial blight has devastating effect on the final yield's quality and quantity and the plant's growth performance. The seeds infected by bacterial blight have a very poor germination rate; and the infected seedlings have injured growing tips with the primary leaves having watersoaked spots. Infected leaves have watersoaked spots with lemon-yellow or bright-yellow colored margins. As the disease progresses, the spots turn brown and the leaf may fall prematurely. Infected pods have watersoaked spots with reddish-brown edges. When the infected tissue dries out, a bacterial crust is formed on the surface of the older pods lesions as a result of the drying of the bacterial discharges (Hall, 1991).

1.2 Justification of Study

The project helped assess and evaluate the level of bacterial blight tolerance in bean cultivars and hence ensured which cultivars can be grown with minimum risk of bacterial blight damage . Some of the cultivated bean varieties that were used in this research are said to possess a certain degree of tolerance to bacterial blight. With reference to the Pan-Africa Bean Research Alliance (2010); Teebus - a small white canning variety - is resistant to bacterial blight and Liebenberg *et al*., (2007), have complemented this. The assessment and evaluation of bacterial blight tolerance in the selected bean varieties (The Control – Ex Rico Navy - small white canning variety, Natal Sugar bean variety, White kidney variety, Bonus variety and Carioca variety) to test the different levels of tolerance has given an assurance on what varieties to grow in a bid to curb the bacterial blight problems in the farming sector and has provided a base for the recommendations given to farmers in par with the research.

Different methods to curb the problem of bacterial blight have been suggested but, these have proved to be either costly or insignificantly effective especially with small holder farmers.

These include; Crop rotation with crops that are not susceptible to the bacteria; Use of diseased-free seeds, and use of resistant cultivars; Hot water seed treatment; Proper fertilization and water management; Proper land preparation for better drainage; Proper plant spacing for proper air circulation and sunlight penetration within plants; Insect pest control as they may serve as the carrier of the bacteria; Weed control; No farm activities when plants are wet; Clean farm tools; Field sanitation; Removal and proper disposal of infected plant parts; Deep ploughing to bury plant debris and followed by fallowing the area; Lemongrass extract; Garlic extract and the use of chemical sprays (Brooklyn Botanic Garden, 2000). These might not provide a long term, low cost, efficient solution.

A bacterial blight tolerant bean variety is a cheaper and long term solution towards bacterial blight control for small holder farmers and commercial farmers. Evaluating bacterial blight tolerance in the bean varieties helped assure a long term alternative and more natural solution towards the problems posed by bacterial blight. The problems include yield losses in tonnage and reduced yield quality. It in turn helps reduce chemical use and eliminate problems of chemical residues on bean products which pose problems on human health and the environment.

1.3 Objectives

- To determine different levels of Bacterial blight tolerance among the selected bean varieties of four different bean classes namely, Ex Rico Navy(Small white canning variety), Natal Sugar(Brown speckled Sugar bean Variety), Bonus(Sugar bean variety), Carioca (Carioca class) and White kidney(Large white kidney variety)
- To demonstrate the existence of the bacterial blight tolerance trait in the white bean varieties and to assess their level of tolerance to the disease
- To determine the significance of using a tolerant bean variety in bean production compared to the expenses of using other non-tolerant varieties in a non- chemical application situation.

1.4 Hypothesis

The selected bean varieties have different levels of tolerance to Bacterial Blight infection.

CHAPTER 2

2.0 LITERATURE REVIEW

The common dry bean (*Phaseolus vulgaris L.*), is the most important food legume for direct consumption in the world. Phaseolus vulgaris is produced in a range of crop systems and environments in regions as diverse as Latin America, Africa, the Middle East, China, Europe, the United States, and Canada. Among major food crops, it has one of the highest levels of variation in growth habit, seed characteristics (size, shape, colour), maturity, and adaptation. It also has a tremendous variability (> 40,000 varieties). Germplasm collection in bans compares well with other important commodities on a worldwide basis (Jones, 1999).

2.1 Origin of *Phaseolus Vulgaris* L

Phaseolus vulgaris L., known as Common bean belongs to the plant family Fabaceae or Leguminosae and is also known as French bean, haricot bean, dry bean, navy bean, sugar bean or string bean. The origin of sugar bean cultivation was proven archaeologically by findings of sugar bean in Central America and Mexico dating back to 7000 B.C. During the time of the discovery of the Americas and the European expansion into the new world, *Phaseolus vulgaris* was grown throughout the tropical and sub-tropical areas of South America. The early Spanish and Portuguese explorers found Indians cultivating sugar beans in several West Indian islands, Mexico, Brazil, Argentina, Paraguay, Bolivia and Peru. From these regions sugar bean was disseminated to Europe, Africa, Asia and the Pacific Islands (Michaels, 1991)

2.2 Uses of common bean

Phaseolus vulgaris is a pulse crop hence a major source of protein. In temperate regions, the green immature pods are cooked and eaten as a vegetable. Immature pods are marketed fresh, frozen or canned, whole, cut or french-cut. Mature ripe beans, variously called navy beans, white beans, northern beans, or pea beans, are widely consumed. In lower latitudes, dry beans furnish a large portion of the protein needs of low and middle class families. In some parts of the tropics leaves are used as a pot-herb, and to a lesser extent the green-shelled beans are eaten. In Java, young leaves are eaten as a salad. After beans are harvested, straw is used for fodder (Duke, 1983)

Common bean is an annual legume having 22% protein; with considerable variation in growth habit, vegetative characters, flower colour, flower size, shape and colour of pods and seed. Plant types vary from the climbing to erect/bush cultivars (Hall, 1991). Beans are said to be used for acne, bladder, burns, cardiac, carminative, depurative, diabetes, diarrhea, diuretic, dropsy, dysentery, eczema, emolient, hiccups, itch, kidney, resolvent, rheumatism, sciatica, and tenesmus.

2.3 Nutritive value of common bean

Beans are a high nutritive, relatively low-cost protein food. Green snap beans contain 6.2% protein, 0.2% fat, and 63% carbohydrate. Analysis of a sample of dried beans marketed under the name 'Rajmah' gave the following values: moisture, 12.0%; protein, 22.9%; fat, 1.3%; carbohydrates, 60.6%; and minerals, 3.2%; Ca, 260 mg; P, 410 mg; and iron, 5.8 mg.; 346 calories/100 g. The vitamin contents of the dried beans are: thiamine, 0.6; riboflavin, 0.2; nicotinic acid, 2.5; and ascorbic acid, 2.0 mg/100. Analysis of dried beans from another source yielded: Na, 43.2; K, 1160; Ca, 180; Mg, 183; Fe, 6.6; Cu, 0.61; P, 309; S, 166; and Cl, 1.8 mg/100 g. Beans also contain I (1.4 μg/100 g), Mn (1.8 mg/100 g), and arsenic (0.03 mg/100 g). Raw immature pods of green, and yellow or wax snap beans are reported to contain per 100 g, 32 and 27 calories, 90.1 and 91.4 g moisture, 1.9 and 1.7 g protein, 0.2 g fat, 7.1 and 6.0 g total carbohydrate, 1.0 g fiber, and 0.7 g ash, respectively. Raw pods of kidney beans contain (per 100 g edible portion): 150 calories, 60.4% moisture, 9.8 g protein, 0.3 g fat, 27.8 g total carbohydrate, 2.3 g fiber, 1.7 g ash, 59 mg Ca, 213 mg P, 3.6 mg Fe, 10 μg vitamin A, 0.38 mg thiamine, 0.12 mg riboflavin, 1.5 mg niacin, 7 mg ascorbic acid. Raw dried mature seeds of white, red, and pinto beans are reported to contain per 100 g: 340, 343, and 349 calories, 10.9, 10.4, and 8.3% moisture, 22.3, 22.5, and 22.9 g protein, 1.6, 1.5, and 1.2 g fat, 61.3, 61.9, and 63.7 g total carbohydrate, 4.3, 4.2, and 4.3 g fiber, 3.9, 3.7, and 3.9 g ash, respectively. Whole seeds of kidney beans contain (per 100 g): 86 mg Ca, 247 mg P, 716 mg Fe, 5 μg vitamin A, 0.54 mg thiamine, 0.19 mg riboflavin, 2.1 mg niacin, 3 mg ascorbic acid. Whole seeds cooked contain: 141 calories, 68.0% moisture, 5.9 g protein, 5.7 g fat, 17.9

g total carbohydrate, 1.1 g fiber, 2.5 g ash, 46 mg Ca, 120 mg P, and 1.9 mg Fe. Raw leaves contain (per 100 g): 36 calories, 86.8% moisture, 3.6 g protein, 0.4 g fat, 6.6 g total carbohydrate, 2.8 g fiber, 2.6 g ash, 2 74 mg Ca, 75 mg P, 9.2 mg Fe, 3,230 μg β-carotene equivalent, 0.18 mg thiamine, 0.06 mg riboflavin, 1.3 mg niacin, 110 mg ascorbic acid. After harvest, plants can be fed to cattle, sheep, and horses. It is satisfactory as a part of the roughage when fed with good hay and is comparable to corn and sorghum fodder in nutritive value. Analysis of a sample gave the following values: moisture, 10.9; protein, 6.1; fat, 1.4; N-free extract, 34.1; fiber, 40.1; ash, 7.4; Ca, 1.7; P, 0.1; K, 1.0; digestible protein, 3.0; and total digestible nutrients, 45.2%; nutritive ratio, 14.1. After pod removal, silage may be prepared from green vines. Dehydrated bean vine meal prepared from green plants after pod removal is comparable to alfalfa meal as a vitamin supplement for chicks. It contains protein, 18.3; digestible protein, 12.3; and total digestible nutrients, 46.3%; nutritive ratio, 2.8. Meal made from vines with mature leaves is inferior in quality. Leaves contain carotene (178.8 mg/100 g), thiamine, riboflavin, nicotinic acid, folic acid, and pantothenic acid. They contain also a quercetin glycoside. The hull is said to yield 0.13% rubber. The leaves, are said to contain allantoin. (Duke 1983)

2.4 Ecology of common bean

Beans tolerate most environmental conditions in tropical and temperate zones, but do poorly in very wet tropics where rain causes disease and flower drop. Rain is undesirable when dry seeds are harvested. Frost kills plant. There are both short-day and day-neutral cvs. Excessive water will injure plants in a few hours, but some black-seeded cvs will grow well in standing water. Beans grow best in well-drained, sandy loam, silt loam or clay loam soils, rich in organic content, but are sensitive to concentrations of Al, B, Mn, and Na. Below pH 5.2 Mn toxicity may be a problem. In calcareous soils, zinc deficiencies can be serious in sandy acid soils, Mg and Mo deficiencies may arise. At EC (conductivity) 1500 (EC of saturation extract) garden bean yields are decreased by 10%, by 25% at EC of 2000, and by 50% at EC of 3500. French or snap beans seem more sensitive to Na than many other cvs. Temperatures of -5° to -6°C are harmful at germination, -2° to -3°C at flowering and -3° to -4°C at fruiting. Some cvs withstand short frosts as low as -3°C. The optimum monthly temperature for growth is 15.6°C–21.1°C, the maximum ca 27°.C, the minimum ca 10°C. Blossum-drop is serious above 30°C, and can completely prevent seed set above 35°C. Beans are traditionally a subtropical or temperate crop. In the tropics they are normally found in montane valleys (800–2,000 m). Very few beans are grown in hot humid tropics where cowpeas fare better. Five different writers give five different pH ranges. Our computer program reported 4.2–8.7; average of 144 cases was 6.4. Other values were 5.5–6.8, 5.5–7.5, 6.0–7.0, and 6.0–7.5. Ranging from Boreal Moist to Wet through Tropical Very Dry to Wet Forest Life Zones, common bean is reported to tolerate annual precipitation of 0.9–42.9 dm (mean of 217 cases = 12.8), annual mean temperature of 5.7°–28.5°C (mean of 216 cases = 19.3), and pH of 4.2–8.7 (mean of 144 cases = 6–4) (Duke 1983).

2.5 Cultivation of common bean

Common bean is most widely cultivated of all beans in temperate regions, and widely cultivated in semitropical regions. Germination of *Phaseolus vulgaris* is rapid at soil temperatures above 18°C. In pure stands, bush cvs give good yields at 30 by 30 cm spacings, but wider spacing facilitates weeding. Pole beans are usually planted 4–6 seeds in hills spaced about 1 m apart at a seeding rate of nearly 80 kg/ha. Seed rates are 20–115 kg/ha depending on the cv, seed size, and width of row; 'Red Kidney', 'Marrow', and 'Yellow Eye' at 75–100 kg/ha; 'Pea Beans', 'Black Turtle Soup', at 30–40 kg/ha; row widths 70–75 or 80 cm. Some pole beans are sown at rates as low as 25 kg/ha. Seed of good quality is essential for production of dry beans. Susceptibility to diseases, mechanical injury, frost damage, and wet weather damage at harvest time, and cracked seedcoats should be considered. With a corn, bean or beat drill with removable plates, beans are usually planted 5–8 cm deep, deep enough to give good coverage and sufficient moisture to promote fast germination and growth. Plants should be cultivated to control weeds; care should be taken late in the season to avoid injuring roots extending out between the rows just beneath soil surface. Inoculation of seed with nitrogen-fixing bacteria is unnecessary for dry beans. Beans should be rotated with other crops to maintain high yields and quality and to reduce the hazard of diseases which may survive in the soil or on plant refuse in the soil. In the tropics beans are often interplanted with such crops as coffee, corn, cotton, sweet potatoes, and little or no fertilization is employed, although the plant does respond to nitrogen.(Duke, 1983.)

Bean production in the smallholder sector is characterized by low yields averaging about 500 to 700 kg/Ha under dryland conditions in Zimbabwe and the farmers predominantly grow the erect-bush types. In normal situations, bean varieties produce an average yield of 1tonne per hectare and 3tonnes per hectare on a high yield basis. Factors limiting bean productivity include among others; Lack of appropriate skills and knowledge on agronomic practices that optimize yields; lack of well adapted cultivars with tolerance to diseases and insect pests; Lack of irrigation facilities and poor water management practices and poor produce marketing channels that act as a disincentive for farmers to grow beans (Schwartz, et.al, 1989).

Beans mature very quickly and green beans may be harvested 4–6 weeks after sowing. In early snap bean cvs, harvest begins in 7–8 weeks, 1 or 2 weeks after flowering. Beans should be picked every 3–4 days. Bush beans mature over a short time; pole beans continue to bear for a long time. Dry beans should be harvested when most pods are fully mature and have turned colour. In mechanized harvesting, to minimize shatter, harvesters should not shake the vines. The cutter consists of 2 broad blades set to cut 2 adjacent rows about 5 cm below the ground. Then prongs pull plants from both rows into one windrow in wet weather; plants are forked into field stacks ca 1.3 m in diameter and 2–3 m high that are supported by a center stake. This is done mostly in developed countries. In small holder farming, beans are usually hand harvested, or manually gathered and windrowed. Plants are pulled, dried, and threshed; sometimes beans are handshelled. (Duke, 1983)

2.6 Major Diseases of Common Bean

Diseases tend to pose major problem in the production of beans in smallholder farmers and even commercial farmers. Major diseases affecting bean varieties are mainly viral diseases, fungal diseases nematodes and bacterial diseases. Bacterial diseases include Bacterial brown spot (*Pseudomonas syringae*), Common bacterial blight (*Xanthomonas axonopodis pv. phaseoli)*, Bacterial wilt (*Curtobacterium flaccumfasciens pv. fluccumfasciens*), Halo blight (*Pseudomonas syringae phaseolicola*), Wildfire (*Pseudomonas syringae pv. tabaci*). Fungal diseases include *Sclerotinia sclerotiorum*, *Fusarium solani*, *Rhizoctonia solani, Macrophomina phaseolina*, and *Phaseoisariopsis griseola*. Nematodes include Leison

(*Paratylenchus brachyurus*) and Root knot (*Meloidogyne incognita, Meloidogyne javanica*). Viral diseases include Bean Common Mosaic Virus (BCMV) and Bean Golden Mosaic Virus (BGMV) (Hall, 1991).

2.7 Focus of the Research

This particular research project focussed on *Xanthomonas axonopodis pv. phaseoli*. The pathogen incites common bacterial blight, one of the five most important biotic constraints to bean production in sub-Saharan Africa (Gridley, 1994). According to Allen (1995), 19 of the 20 bean producing countries in Eastern and Southern Africa have reported the presence of the disease. Giga (1989) reported the disease as one of the major constraints to bean production in Zimbabwe. This bacterium is a major pathogen of common bean not only in Zimbabwe, but also all over the world (Mabagala and Saettler, 1992; Opio et al., 1996). Common bacterial blight of bean (*Phaseolus vulgaris* L.) is caused by the seed-borne bacteria *Xanthomonas axonopodis pv. phaseoli* (Smith) (Vauterin et al., 1995) and *X. axonopodis pv. phaseoli var. fuscans* (Burkholder) Starr and Burkholder, as the brown-pigmented variant (Leben, 1981; Schaad, 1982). The disease causes yield losses ranging between 10 and 40%, depending on bean cultivar susceptibility and environmental conditions (Saettler, 1989). Common blight has been reported in most bean-producing areas as one of the major limiting factors in bean production for instance in Serbia(Todorovic, 2006; Popovic et al., 2007; Popovic, 2008). The management of common bacterial blight is difficult and is based mainly on pathogen-free seed and resistant cultivars (Zaumeyer and Thomas, 1957). Bacterial blight occurs in both tropical and temperate environments, particularly in irrigated and rained lowlands. It is commonly observed when strong winds and continuous heavy rains occur (Ou 1985, Mew et al 1993). Bacterial blight is a gram negative aerobic rod known by the scientific name, *Xanthomonas axonopodis pv. phaseoli* (Smith) and its synonyms are; *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye; *Xanthomonas axonopodis pv. phaseoli* var. *fuscans* (Burkholder) Starr & Burkholder. The bacteria's principal host is *Phaseolus vulgaris*, but other legume species are also naturally infected (Bradbury, 1986). The bacteria can survive for six to eighteen months in plant residue on the soil surface. Infected or contaminated seed is a source of inoculum. The bacteria can spread from plant to plant and field to field in many ways, including wind-driven and splashing rains, overhead irrigation, surface-drainage water, and farm machinery. Bacteria enter plants through natural openings or injuries caused by insects, or even accompany other diseases such as rust. Once inside the plant, the bacteria may move systemically to the leaves, stems, and pods and into the seed. Under ideal conditions, even a few infected seeds per hectare may be sufficient for a severe outbreak of common blight. Conditions that favour development of *X. phaseoli* include warm temperature of 27.8˚C to 32.2˚C; frequent rain and high humidity; over-crowded plants with poor air flow and low sunlight penetration among plants; improper soil nutrition and irrigation management; poor soil drainage; and diseased seeds and planting materials.

2.8 Impact of Common Blight on Yield

Common bacterial blight affects bean foliage, pods, and seedlings. Early foliar symptoms are small, angular, light green, water-soaked or translucent spots. During warm and wet conditions, these lesions rapidly enlarge and merge. Gradually, the centers of the lesions become dry and brown, and are surrounded by a distinct, narrow zone of yellow tissue. In highly susceptible varieties, the lesions continue to expand until the leaves appear scorched, ragged, and torn by wind and rain. Pod symptoms consist of lesions that are generally circular, slightly sunken, and dark red-brown. In severe cases, entire pods may shrivel and die. Seeds in less severely affected pods may show no symptoms of disease or may be slightly wrinkled.

2.9 Integrated Disease Management

Considering Integrated Disease Management, chemical use should be the last resort towards controlling bacterial blight. Crop rotation and clean tillage help reduce the risk of disease by reducing the amount of inoculum in the immediate area. However, ploughing may increase soil loss through erosion. The risk of the disease can also be reduced by planting certified, pathogen-free seed from reputable suppliers and seed produced in semiarid regions. Risk can also be reduced by avoiding entering fields to cultivate or handle plants wet with dew or rain. Equipments should be sanitized by spraying with a disinfectant before moving from an infected to a blight-free field. Sprays provide moderate control of common blight. Small holder farmers growing beans will most probably fail to protect their crops from *X.phaseoli* because of their limited ability to possess the capital they need for chemicals; which are also costly. Although Commercial farmers have a higher chance of affording the chemicals, utilize them and still come up with a profit, farmers would prefer lower costs of production hence they would opt not to use chemicals if there was another natural and cheaper way to help them realize high profits, low costs of production per unit and a long term solution to bacterial blight disease problems – a tolerant cultivar. Consumers would prefer bean products that are free of chemical residues for their own health's security and for the sake of the environment. (Draper 1995)

CHAPTER 3

3.0 MATERIALS AND METHODS

The materials and methods used in this project are given in detail in this chapter. These include the geography of the site of experiment, the field setup, experimental units and the detection of bacteria.

3.1Descripton of the experimental site

The research project on Bacterial Blight (*Xanthomonas axonopodis pv. phaseoli*) tolerance in bean varieties was carried out at Africa University Farm Research Block. The soil type at the site is sand clay loam (SCL) meaning that it is well drained and suited for bean production. The soil has an average pH of 5.1. (Africa University Agricultural Advisory Services 2011). The Africa university farm area is in Natural region II having annual rainfall of 818 mm. Rain falls mostly in the months December to February although heavy showers are possible before and after this period (*Retrieved from: Google earth*). The average annual temperature is 19°C. The coldest month is July with minimum temperatures of 8.5°C and maximum 20.5°C. The hottest month is January with minimum temperatures 17°C and maximum 26°C.

3.2 Experimental units and Management

The project was executed in the open field. The selected bean varieties for this project were five, each variety distinct from the other on the basis of their classification by use and species. These selected varieties were:

1) The Control – Ex Rico Navy - small white canning variety
2) Natal Sugar – A brown speckled sugar bean variety
3) White kidney – A large kidney variety
4) Bonus – A sugar bean variety
5) Carioca – A carioca class variety

There were three replications for the above treatments.

To measure disease tolerance, weekly readings (seven-day intervals) were taken on disease incidence severity for each treatment. Over time, due to the increase in the rate of incidence, the readings were taken on a three-day interval. The data was used to construct disease progress curves for each variety and the Area Under Disease Progress Curves (AUDPC) were subjected to ANOVA to see if there were significant differences in terms of disease tolerance between the varieties. The variety with the smallest AUDPC was the most tolerant.
Disease severity of *X. axonopodis* on each variety was evaluated basing on a scoring scale developed by CIAT. It is attached in annexure 1.

Benomyl was used to suppress fungi to avoid its interference on assessing Bacterial blight in the field trial.

3.3 Field Setup

For field beans, the plots were set at dimensions, 2m (width) × 3m (length) having 5 rows in each plot. The inter-row spacing was 45cm and in-row spacing depended on the bean variety as shown in Table 1.

Table 3. 1 Plant spacing for beans sown on 45 cm rows

Class of beans	in row spacing	plant population (plants/ha)
Small white	7	300 000
Speckled sugar	10	220 000
Large white kidney	10	220 000
Sugar bean	10	220 000
Carioca	10	220 000

The bean varieties were planted on 28 January 2012 to evade the December heavy rains that might have caused an uncontrollable incidence and severity of the disease. There were 3 replications for each treatment with each replication presented in a single plot out of the five plots in each of the three blocks; thus there were 15 plots all together. The area that was used for the field experiment was 299m^2 or 0.03 Hectares considering the 1 meter paths that separate the plots and the 1.5m guard rows (Plate 3.1).

In the field, the bean plants were naturally infected by the bacteria. Scores of disease severity were recorded from the first week of disease occurrence after emergence.

Land preparation

The area was tilled using a tractor pulled rom-harrow two weeks before planting. To control weeds that were growing, the field was sprayed with Gramaxone (Contact herbicide) on 26 January 2012 and on 28 January 2012.

The three blocks were marked using string for straightness; a field tape measure for measurement accuracy and pegs to mark the boundaries and corners of the blocks. Plots were also marked in the process.

Using a string for straightness, inter-row markings were set using hoes for each plot in all three blocks to come up with five 45cm spaced inter-row markings or lines for each plot.

Planting of experimental units

Planting was done on 28 January 2012. Using the Shuffling and picking traditional randomization method, the bean varieties were randomly assigned to distinct plots in each block. Each bean variety was hand planted at estimated depths of 5 - 8cm; and according to their relative inter-row spacing shown in table 1 using marked strings tied to pegs at each end. Hand hoes were used to cover the seed.

Irrigation, emergence and gap filling

Initial irrigation for germination was initiated on 30 January 2012 for 6 continuous hours a day for the first 3 days. Irrigation became irrelevant after full emergence because the rainfall was enough for the growth of the bean plants after emergence.

The first emergences were observed on the 3 February 2012 and full emergence was noted on 5 January 2012. Some been seeds, mainly the Ex rico Navy, did not emerge due to the inefficient low-pressure irrigation, hence gap filling was done on 18 February 2012.

The experimental design that was used for the field experiment was the Randomized Complete Block Design (RCBD).

Plate 3. 1Layout of plots at the experimental site

3.4 Confirmation of bacterial isolates in the Laboratory

The Bacteria was isolated in the laboratory from leaf samples (Plate 3.2). The leaf samples were washed thoroughly in running tap water in order to remove soil and debris from sample. The leaves were surface disinfected with 2% NaOCl for six minutes and were rinsed with water to remove excess disinfectant. 3 sub-samples leaves were tested by separately cutting the tissue showing the symptoms of bacterial blight. The cut tissue was placed onto a glass – slide in a drop of water to chec for bacterial ooze. After observing ooze, the sample was cut into pieces in a drop of sterile saline (0.85% NaCl) and left for 15minutes. The sample ws then streaked using a glass rod, on 2 plates of Nutrient Agar(NA). The plates were incubated at 25˚C for 7 days. The colonies, which were yellow were recorded. The suspected colonies were transferred to fresh NA medium plates by streaking with bacteriological loop. The plates were then incubated at 28˚C for 72hrs.

Plate 3. 2 Leaf with signs of infection with bacterial blight

3.5. Data collection

To measure disease tolerance, weekly readings (seven-day intervals) were taken on disease severity for each treatment. Over time, due to the increase in the rate of incidence, the readings were taken on a three-day interval. Data collection started in the week when disease occurrence was observed on 4 April 2012. Direct observations were made and scores were taken basing on the CIAT scoring scale (annexure 1) for scoring bacterial blight severity.

Five readings were taken hence the last day for data collection was 20 April 2012 The data was recorded on recording sheets and entered on Microsoft excel as row data (annexure 2) to be used in data analysis. The data was used to construct disease progress curves for each variety.

3.6 Data Analysis

Area Under Disease Progress Curves (AUDPC) were determined using the average scores derived from data collected. Graphs showing Area Under Disease Progress for each replication. The graphs were plotted on standard graph papers with grids of $4cm^2$. The AUDPCs were obtained by physically counting the grids and calculating the area using a scientific calculator (annexure 4) (Table 4.2). AUDPCs were subjected to ANOVA to determine significant differences in terms of bacterial blight disease tolerance between the varieties. The variety with the smallest AUDPC was the most tolerant. The programme used to derive the ANOVAs analysis was MINITAB version 13.

CHAPTER 4

4.0 RESULTS

The scores for each variety were recorded on recording sheets as row data (Annexure 2). For each plot; data recorded was taken from five random plants and averaged to give a single average score for each day of data collection. The average scores were used to plot graphs showing Area Under Disease Progress for each replication. The graphs were plotted on standard graph papers with grids of $4cm^2$. The AUDPCs were obtained by physically counting the grids and calculating the area using a scientific calculator (Annexure 4) mean AUDPCs were derived using MINITAB (Table 4.2).

4.1 Mean AUDPCs for the bean varieties

The Ex Rico had a mean AUDPC of 14.35 and this was significantly different from the other varieties ($P<0.05$) hence the variety is significantly tolerant to bacterial blight compared to the other four varieties. The Bonus variety had a mean AUDPC of 73.85 which meant the variety had better tolerance levels compared to the preceding three varieties but had a significantly lower level of tolerance compared to Ex Rico. The Natal sugar variety had a mean AUDPC of 102.78 and ranked third after the Bonus variety in terms of disease tolerance. The White kidney variety had a mean AUDPC of 130 and The Carioca variety had a mean AUDPC of 128.66 which were not significantly different from each other at $P<0.05$. The AUDPCs were recorded in cm^2.

The standard error (Tukey's) in the ANOVAs analysis was 0.05 and the individual error rate(Tukey's individual error rate) after subjecting the data to ANOVA was 0.00818 hence error significantly less than 0.05 shows that there is a significant difference between the varieties.

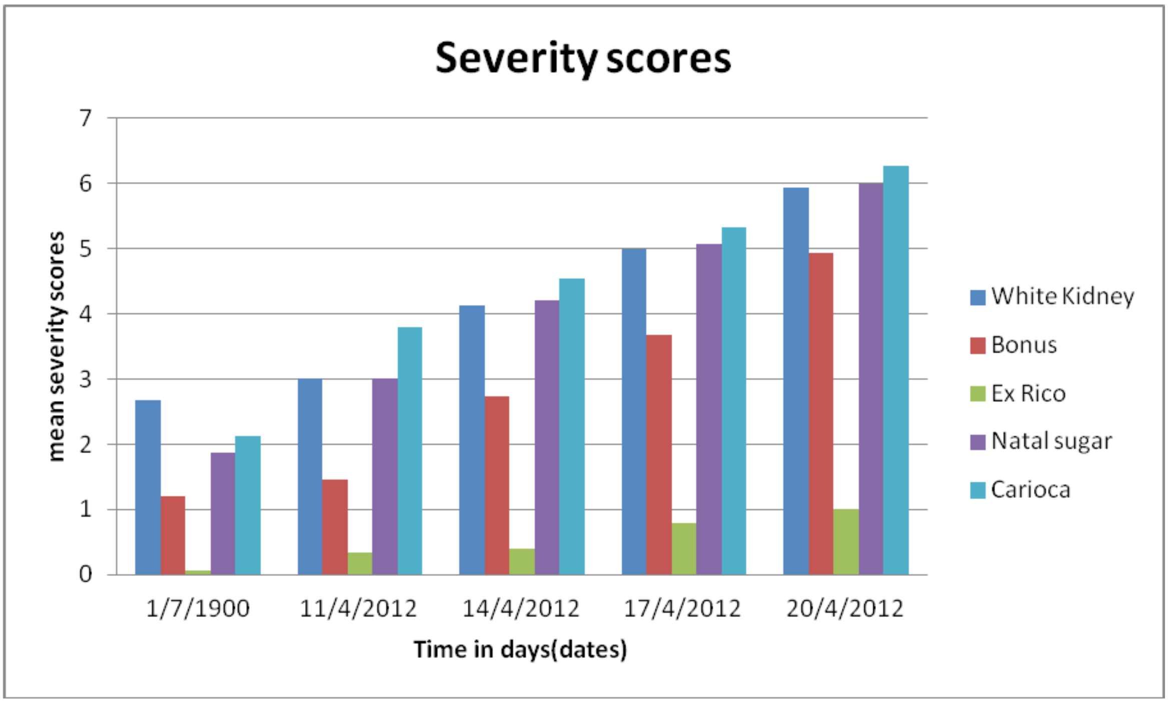

Figure 4. 1 Mean severity scores for bean varieties

Table 4. 1 Mean AUDPCs for the bean varieties

Variety	Mean AUDPC (cm^2) ± st dev		
White Kidney	130	± 1.68	d
Bonus	73.85	± 7.85	b
Ex Rico	14.35	± 3.8	a
Natal sugar	102.78	± 1.18	c
Carioca	128.66	± 8.26	d

Means with the same letter in the same column are not significantly different at $P<0.05$ using Tukey's comparison.

4.2 Rainfall data

Rainfall patterns during the time of study correspond to the data collected on disease severity on the bean varieties. Rainfall data in mm/month for the period of February to April were recorded during the period of the experiment from the time the beans were planted. Full data on rainfall in mm/day for the period are presented in Annexure 3. There was a general increase in the rate of disease severity between day 7 and day 15 after date of first observation of disease occurrence. This was noticeable in all the five varieties in all replications. Rainfall patterns show that there low rainfalls (mm/month) in the month of February and they increased to 82mm/month in the month of March. Rainfalls gradually decreased in the period March – April and dropped to 24mm/month in April.

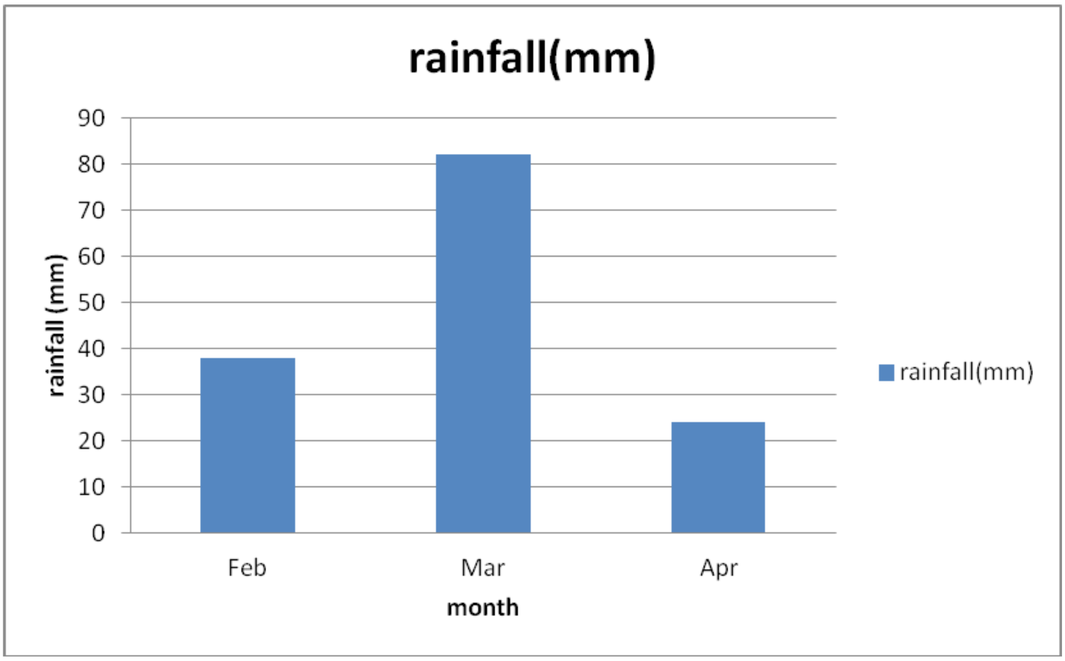

Figure 4. 2 Rainfall in mm/month for months of February, March and April 2012.

CHAPTER 5

5.0 DISCUSSION

Results were obtained and the data collected during the research period was analyzed. The results from the One way ANOVA analysis showed significant differences in the five bean varieties therefore, the results were interpreted to derive the meaning of the results in comparison with the alternative hypothesis and literature.

Symptoms of the disease were visible from week nine after emergence. The results showed that there were significant differences in the levels of tolerance to bacterial blight in the five bean varieties. The disease progress was slow the first seven days of observation and increased rapidly after. This means that the bean varieties will not be at much risk from bacterial blight infection if conditions for bacterial blight germination are not induced hence weather conditions and climate are an important factor to consider for management of the disease in beans. In terms of AUDPCs, the variety with the least AUDPC was Ex Rico Navy variety. Although the conditions for the bacteria to infect the plants were conducive, the bacteria did not show any effect on the Ex Rico variety in all three replications hence the variety is not susceptible to the disease. Following, was the Bonus variety; followed by Natal sugar; and then White kidney and Carioca varieties which did not have much significant AUDPC differences. The other four varieties are susceptible to bacterial blight although the susceptibility levels differ. The Bonus variety appears to be more tolerant compared to carioca, white kidney and Natal sugar. The bonus variety has great chance of being improved to be more tolerant up to the levels of Ex Rico, henceforth, with its large seed size, the Variety can be improved to have high levels of tolerance and high yield potentials.

The AUDPCs were subjected to ANOVA analysis using MINITAB software. They showed that the Ex-Rico variety was significantly different from the other varieties having the least AUDPC hence the highest tolerance level. Bonus variety and Natal sugar had the second least AUDPCs compared to Carioca and White kidney varieties. The ANOVA analysis showed that there was no significant difference between the White kidney variety and the Carioca variety.

The variety with the least AUDPC is the variety with the highest tolerance to Bacterial blight. The variety with the most AUDPC has the least tolerance. This therefore means that Ex Rico Navy had a high level of tolerance to Bacterial blight infection such that it is significantly different from the other varieties. The highest possible score of disease severity in the field trials for Ex Rico variety was recorded as 1.2 according to the CIAT scale showing that the effects of the disease were insignificant on the cultivar. The Ex Rico Navy therefore has tolerance genes that are able to block the active site of the Bacteria such that it was not able to cause any severe disease symptoms on the crop plants. The Bonus variety had the second lowest AUDPCs with the a highest recorded score of 4.8 based on the CIAT scale. Natal Sugar had the third lowest AUDPC with a highest recorded score of 5.8 on the CIAT scale. The former and the latter did not have much difference in terms of AUDPC with Carioca and White kidney as compared to Ex Rico Navy. The highest recorded score for White kidney was 6.8 and 7.4 for Carioca. The results show that the four varieties, Bonus, Natal sugar, White kidney and Carioca are significantly susceptible to Bacterial Blight infection, albeit levels of tolerance to the disease differ among the four varieties. Disease occurrence was initially observed at the same time when conditions for germination of the bacteria had been induced. From week 1 up to week 8 after germination, the beans grew disease free and only showed signs of insect damage. Conditions induced by irrigation in the initial stages of the plants' growth were not ideal for the bacteria to germinate hence in the first nine weeks there was no sign of disease. Blight occurs if the number of rainy days and the amount of rainfall lower (Mohiuddin et al 1977). Mean minimum and maximum temperature of 24.3 and 34.0˚C respectively favour disease spread (Premalatha Dath et al., 1978). In the month of March, the rainfall in mm/month were as high as 82mm. Bacterial blight develops more at high temperatures of 28-34˚C (Hsieh and Chang 1977). The temperatures were ideal, ranging from 24 - 34˚C and sometimes higher. These conditions were necessary to create the right atmosphere for germination of *X.axonopodis pv phaseoli.* The conditions initiated germination and ingression of the bacteria on the bean plants. Signs of infection were visible on the bean varieties at the beginning of the month of April when rainfall was gradually declining. The optimum temperatures for infection are 23.9˚C – 26.1˚C (Yang 1997).Visible brown lesions with yellow edges were observed on the leaves. The disease infection was first noticeable on the upper part of the plants because of the younger leaves being more susceptible than the older leaves. The new lesions were small yellow to brown spots on leaves. The lesions then dried out to turn brown and were surrounded by a yellowish halo. The rate of spread of these lesions was higher on Carioca and White kidney bean varieties. Larger proportions of the leaf tissue were covered with blight in short periods such that for the Carioca variety, the second readings had jumped to 5.2 on the CIAT scale in Rep 2 compared to the first reading of 2.8 on the CIAT scale.

An increase in disease severity was witnessed during the month of April. This was so because the rainfalls and temperature created adequate moisture conditions for the germination, ingression and infection of the bacterial spores on the bean plants. The increasing amount of dead tissue led to senescence of the plants such that plots with the most susceptible varieties, Carioca and White Kidney were the first to show signs of senescence. This might have been caused by the act of ingression and invasion of the bacterial germinated spores on the plant tissue. The time to physiological maturity was therefore to some extent influenced by the disease. Harvest maturity was consequently visible but the pods were not affected much by the disease. Consequently, Natal sugar and Bonus Variety were affected by bacterial blight although the rate was less than that of Carioca and White Kidney. The rate of infection also increased with inducement of adequate conditions but the rates were slower than those of Carioca and White kidney thus showing difference in levels of bacterial blight tolerance. Bacterial blight infection rate was insignificant on the Ex Rico Navy variety such that a few plants were affected and a small proportion of the leaves were. The highest score recorded for the Ex Rico was 2 and the highest recorded average score was 1.2 on the CIAT scale.

There is a highly significant difference in levels of tolerance to bacterial blight infection between the Ex Rico Navy variety and the other four varieties. The small white bean varieties have genotypic traits that allow them to be tolerant to *X.axonopodis*. the trait of major economic importance that has successfully been transferred from teparies to common bean varieties is tolerance to common bacterial blight. Tolerance to *X.axonopodis* was noted in white bean cultivars from Nebraska as have been derived from *P.acutifolius* crosses (Hucl and Scoles 1985) hence the high level of tolerance to bacterial blight in the Ex Rico Navy White canning Variety.

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Bean varieties are generally susceptible to *X. axonopodis*. The disease becomes significantly severe when the pathogen exposed to optimum moisture conditions and ideal temperatures. The classes of bean: Sugar bean, Large White Kidney and the Carioca class, are all susceptible to Bacterial blight. White bean class has high tolerance to bacterial blight due to the transferred tolerance trait from P. acutifolius crosses. Smallholder farmers in Zimbabwe face problems induced by Bacterial blight infections. The most common class of beans grown by smallholder farmers in Zimbabwe is the sugar bean class because the varieties are readily available on the market and within the smallholder farming communities. There is the need for the farmers to engage into production of more tolerant cultivars namely the white canning varieties. Although they are expensive to grow, they also have higher returns due to reduced use of chemicals. Diversity is an important factor thus breeders should work to manipulate the tolerance genes and insert them in all the bean classes to preserve these classes. The experiment showed that the Ex Rico Navy is significantly tolerant to Bacterial blight and it is different from the other varieties.

6.2 Recommendations

Smallholder farmers and Commercial farmers

- Smallholder farmers widely grow sugar bean varieties that are susceptible to bacterial blight. Farmers should engage into production of small white varieties that are tolerant to Bacterial blight of common bean like Ex rico. This will help them reduce the costs induced by bacterial blight effects. The white bean varieties are not readily available to these smallholders in Zimbabwe. For such farmers, they can make use of varieties which are fairly tolerant to the disease like BONUS bean variety, which has a better level of tolerance compared to White Kidney, Carioca and Natal Sugar.

- Commercial farmers have the capital and resources to engage into production of the white bean varieties. Engagement into white bean variety production is recommended as this will ensure a reduction in the use of chemicals. The reduction in the use of chemicals means a reduction in air, earth and water pollution thus enhancing harmony between farming practices and the environment. In turn, the farmers will benefit from the reduction in the use of chemicals meaning a reduction in the production costs hence a favourable increase in income for the farmers. This also benefits consumers in that consumer health will be secure. Reduced use of chemicals means reduced toxicity in the final product.

Breeders and Research institutes

- It is recommended that breeders should manipulate *X.axonopodis* tolerance genes and incorporate them in other bean varieties so that farmers can grow a wide range of been varieties that are tolerant to the disease with reduced use of chemicals, without being limited to one class of beans. The bonus variety has great chance of being improved to be more tolerant up to the levels of Ex Rico, henceforth, with its large seed size, the variety can be improved to have high levels of tolerance and high yield potentials.

- Seed companies should encourage farmers to grow bacterial blight tolerant white bean varieties through increased production of the tolerant varieties and marketing of the varieties to both smallholder and commercial farmers.

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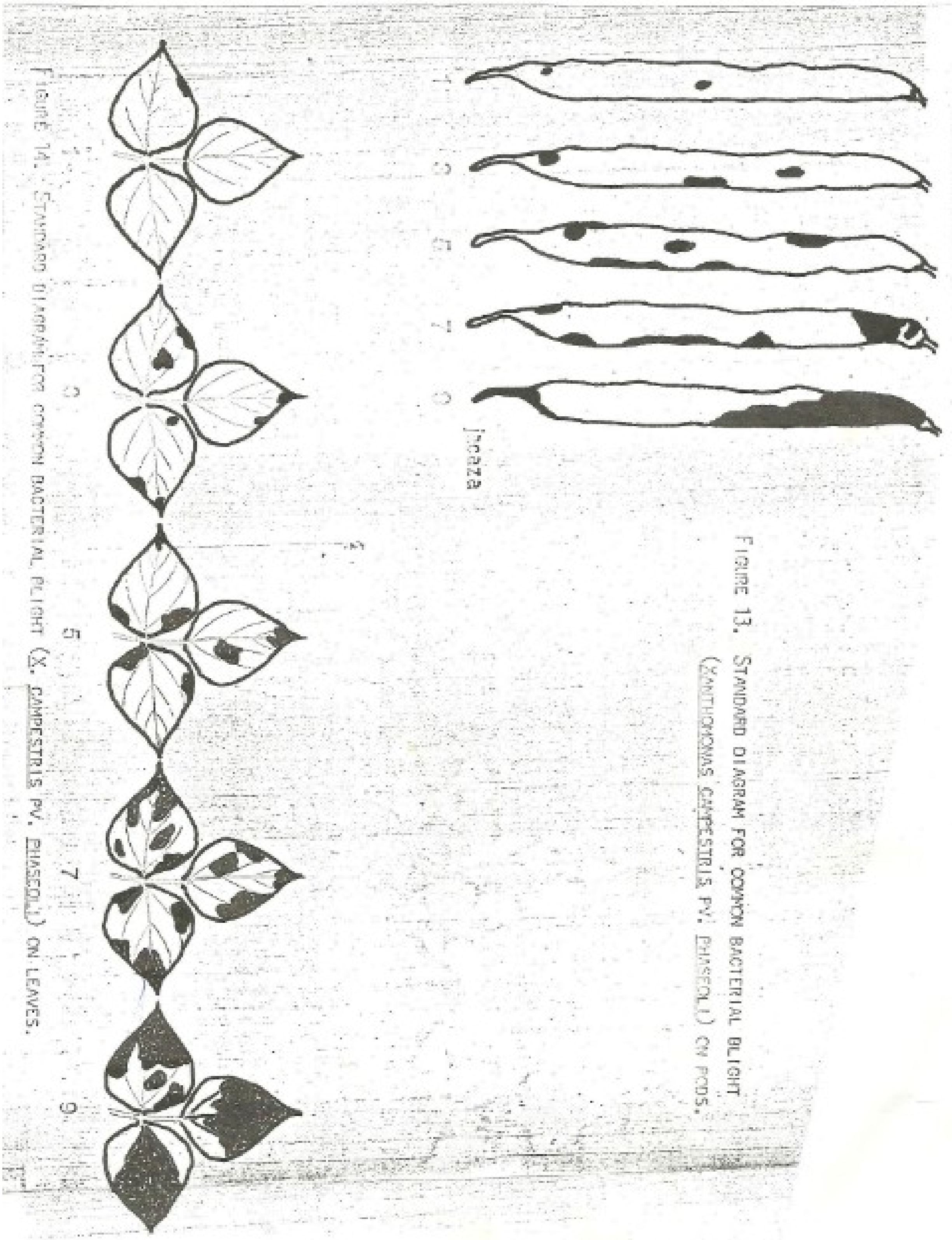
APPENDICES

Annexure 1 CIAT Scoring scale



Annexure 2 Disease severity scores taken in the field

		disease progress		
		Date	ralative scores/plant	Avg plot score
BLOCK 1	**PLOT 1 (Ex rico)**	4/4/2012	0;0;0;0;1	0.2
		11/4/2012	0;0;1;0;1	0.4
		14/4/2012	1;0;0;1;0	0.4
		17/4/2012	1;1;0;1;0	0.6
		20/4/2012	0;1;2;0;1	0.8
	PLOT 2 (white kidney)	4/4/2012	4;0;1;3;2	2
		11/4/2012	4;2;1;3;1	2.2
		14/4/2012	7;3;0;2;3	3
		17/4/2012	4;3;7;5;2	4.2
		20/4/2012	6;4;7;5;3	5
	PLOT 3 (Carioca)	4/4/2012	2;1;2;3;1	1.8
		11/4/2012	2;0;2;3;4	2.2
		14/4/2012	5;0;3;3;0	2.2
		17/4/2012	2;6;4;2;2	3.2
		20/4/2012	4;6;5;3;4	4.4
	PLOT 4 (Natal Sugar)	4/4/2012	0;2;3;2;2	1.8
		11/4/2012	2;2;1;3;2	2
		14/4/2012	7;4;3;4;4	4.4
		17/4/2012	6;5;4;7;3	5
		20/4/2012	7;4;5;5;6	5.4
	PLOT 5 (Bonus)	4/4/2012	2;0;1;1;0	0.8
		11/4/2012	3;0;1;2;0	1.2
		14/4/2012	6;3;0;5;0	2.8
		17/4/2012	7;3;4;2;2	3.6
		20/4/2012	6;4;7;4;8	5.8

		disease progress		
		Date	ralative score/plant	Avg plot score
BLOCK 2	**PLOT 1 (Bonus)**	4/4/2012	1;1;1;2;2	1.4
		11/4/2012	2;2;1;2;2	1.8
		14/4/2012	3;4;3;1;1	2.4
		17/4/2012	2;5;3;2;2	2.8
		20/4/2012	3;5;3;4;6	4.2
	PLOT 2 (Ex rico)	4/4/2012	0;0;0;0;0	0
		11/4/2012	0;0;1;0;0	0.2
		14/4/2012	0;1;1;0;1	0.6
		17/4/2012	1;0;0;2;1	0.8
		20/4/2012	1;1;0;2;1	1
	PLOT 3 (Natal sugar)	4/4/2012	3;1;2;2;1	1.8
		11/4/2012	7;6;4;3;3	4.6
		14/4/2012	6;8;6;3;3	5.2
		17/4/2012	5;7;8;3;4	5.4
		20/4/2012	6;5;8;8;7	6.8
	PLOT 4 (White kidney)	4/4/2012	4;2;3;3;4	3.2
		11/4/2012	5;6;2;2;3	3.6
		14/4/2012	3;5;7;5;4	4.8
		17/4/2012	4;5;7;6;3	5
		20/4/2012	8;5;7;4;6	6
	PLOT 5 (Carioca)	4/4/2012	5;3;2;2;2	2.8
		11/4/2012	3;9;5;6;3	5.2
		14/4/2012	6;4;3;7;8	5.6
		17/4/2012	6;5;8;5;5	5.8
		20/4/2012	7;7;8;6;7	7

disease progress

		Date	ralative score/plant	Avg plot score
BLOCK 3	**PLOT 1 (Natal sugar)**	4/4/2012	3;1;3;2;1	2
		11/4/2012	2;4;0;3;3	2.4
		14/4/2012	7;6;0;1;1	3
		17/4/2012	6;3;4;6;5	4.8
		20/4/2012	7;5;7;6;4	5.8
	PLOT 2 (Bonus)	4/4/2012	1;2;2;1;1	1.4
		11/4/2012	3;2;0;2;0	1.4
		14/4/2012	6;2;3;0;4	3
		17/4/2012	3;5;6;5;4	4.6
		20/4/2012	4;7;6;3;4	4.8
	PLOT 3 (Ex rico)	44/2012	0;1;0;0;0	0.2
		11/4/2012	1;1;0;0;0	0.4
		14/4/2012	0;1;0;0;2	0.6
		17/4/2012	0;1;2;0;2	1
		20/4/2012	1;2;1;0;2	1.2
	PLOT 4 (Carioca)	2/4/2012	2;1;2;3;1	1.8
		11/4/2012	2;3;4;6;5	4
		14/4/2012	7;8;6;6;2	5.8
		17/4/2012	9;5;7;6;8	7
		20/4/2012	7;9;7;8;6	7.4
	PLOT 5 (White kidney)	4/4/2012	3;4;2;2;3	2.8
		11/4/2012	0;6;0;7;3	3.2
		14/4/2012	2;7;8;6;0	4.6
		17/4/2012	3;8;5;9;4	5.8
		20/4/2012	5;8;7;9;5	6.8

Annexure 3 Rainfall data

	RAINFALL 2011-12							Location Kies house at Africa University				

	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1			24									
2				6		18		8	22			
3			11	20					2			
4			8		13		11					
5						5						
6												
7				4				2				
8						8		42				
9						1		2				
10												
11												
12					4							
13					40							
14					16	2	9					
15					6	8	2					
16							14					
17					34		2					
18						10						
19						14						
20						10						
21			13			15						
22				6		2						
23												
24												
25				45	6							
26												
27												
28												
29					26							
30												
31					26			28				
Total	0	0	56	81	171	93	38	82	24	0	0	0
Total to date	0	0	56	137	308	401	439	521	545	545	545	545

Annexure 4Average scores and AUDPCs

	White Kidney			Bonus			Ex Rico			Natal sugar			Carioca		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
4/4/2012	2	3.2	2.8	0.8	1.4	1.4	0.2	0	0	1.8	1.8	2	1.8	2.8	1.8

11/4/2012	2.2	3.6	3.2	1.2	1.8	1.4	0.4	0.2	0.4	2	4.6	2.4	2.2	5.2	4
14/4/2012	3	4.8	4.6	2.8	2.4	3	0.4	0.2	0.6	4.4	5.2	3	2.2	5.6	5.8
17/4/2012	4.2	5	5.8	3.6	2.8	4.6	0.6	0.8	1	5	5.4	4.8	3.2	5.8	7
20/4/2012	5	6	6.8	5.8	4.2	4.8	0.8	1	1.2	5.4	6.8	5.8	4.4	7	7.4
AUDPC	**130**	**128**	**131.7**	**69.4**	**69.2**	**82.9**	**13.4**	**11.1**	**18.5**	**104**	**103**	**102**	**129**	**137**	**120**

Annexure 5 ANOVA Analysis results on MINITAB

One-way ANOVA: C2 versus C1

```
Analysis of Variance for C2
Source     DF        SS        MS        F        P
C1          4   27726.1    6931.5   233.30    0.000
Error      10     297.1      29.7
Total      14   28023.2
```

```
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ---------+---------+---------+-------
1           3    130.00      1.68                              (-*)
2           3     73.85      7.85                (*-)
3           3     14.35      3.80   (-*)
4           3    102.78      1.18                       (-*)
5           3    128.66      8.26                             (-*-)
                                   ---------+---------+---------+-------
Pooled StDev =     5.45                   40        80       120

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.00818

Critical value = 4.65

Intervals for (column level mean) - (row level mean)

                 1           2           3           4

       2       41.51
               70.78

       3      101.02       44.87
              130.29       74.14

       4       12.59      -43.56     -103.07
               41.85      -14.29      -73.80

       5      -13.29      -69.44     -128.95      -40.51
               15.97      -40.17      -99.68      -11.25
```

Annexure 6 Dates for activities during the study

DATES

DESCRIPTION	DATE	COMMENT
land prep	27'01/2012	
planting	28/01/2012	

emergence	3/2/2012	
full emergence	5/3/2012	
gap filling	19/02/2012	
herbicice spraying	26/01/2012	Gramaxon
	28/06/2012	Gramaxon
weeding	16/02/2012	
	7/3/2012	
fungicide spraying	8/3/2012	Benomyl

Annexure 7 Photographs of Bacterial blight infected leaves