ADAPTABILITY OF SELECTED SNAP BEAN GENOTYPES AND RESISTANCE TO BEAN RUST (*Uromyces appendiculatus* (Pers.:Pers.) Unger var. *appendiculatus)* IN SOUTHERN HIGHLANDS ZONE OF TANZANIA

OWEKISHA, HERMAS KWIGIZILE

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ABSTRACT

Snap bean (*Phaseolus vulgaris* L.), also widely known as French bean or Green bean, is of growing importance in the socio-economic systems of East and Central Africa. It is a crop with great potential for addressing food insecurity, improving incomes and alleviating poverty in East and Central Africa. Most of the commercial varieties grown locally are highly susceptible to bean leaf rust caused by *Uromyce appendiculatus* fungus. Development of disease resistant varieties with acceptable pod quality is a feasible preference to alleviate the constraint. The present work centered on evaluating the adaptability and performance of snap bean genotypes and resistance to bean rust in the Southern highlands zone of Tanzania. Ten advanced bushy snap bean genotypes (HAB 404, HAB 425, HAB 449, HAB 423, HAB 240, HAB 427, HAB 442, HAB 403, HAB 414, and HAB 419), and the check variety Teresa were evaluated for agronomical characteristics, reaction to bean leaf rust, yield potential and pod quality using the Randomized Complete Block Design with three replications, at each location. The plot size was 1.5 m x 1.0 m, with two rows of plants spaced at 50 cm x 10 cm. Data was collected on growth habit, yield and yield components, and bean leaf rust score beginning 3, 5 and 7 weeks. Pod characteristics (color, shape, length (cm), width (mm) and snap-ability) were also assessed. The entries were scored for the bean rust at vegetative growth, flowering and at pod filling stages. Data was subjected to ANOVA using GenStart 14 Edition (2012) and means were separated using the Fisher's Protected LSD at P = 0.05. Genotype HAB 427, HAB 425, and HAB 423, and HAB 240 were high yielder and showed resistance to bean leaf rust. Significant and positive correlations were also

observed among genotypes on the yield and yield components. There were highly significant differences at ($P \leq 0.001$) across sites on number of pods per plant, total pod yield and pod weight (g), 50% days of flowering and maturity. Interaction between genotypes and location showed also significant differences at ($P \leq 0.01$) for the number of pods per plant and total pod yield (kg) per 10 plants harvested. Therefore; genotypes HAB 427, HAB 425, and HAB 423, and HAB 240 promised can be used as donor parental materials.

DECLARATION

I, Owekisha Hermas Kwigizile, do hereby declare to the senate of Sokoine University of Agriculture that this dissertation is my original work and has not been submitted for a degree award in any other University.

20th October, 2014.

Owekisha Hermas Kwigizile **Date**

MSc. (Candidate)

The above declaration is confirmed:

20 - 10 - 2014

Prof: Paul Mbogo Kusolwa **Date**

(Supervisor)

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DEDICATION

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LIST OF ABBREVIATIONS AND SYMBOYS

%	Percent
*	Significant at 5%
**	Significant at 1%
***	Significant at 0.1%
ANOVA	Analysis of variance
SUA	Sokoine University of Agriculture
CV	Coefficient of variation
Ns	Not signifiant
CIAT	Centro International de Agricultural Tropical
Cm	Centimeter
g	Grams
Kg/ha	Kilogram per hectare
Kg	Kilogram
LSD	Least Significant Difference
TSP	Triple Sulphate Phosphate
CAN	Calcium Ammonium Nitrate
ALS	Angular Leaf Spot
SARI	Selian Agricultural Research Institute
EU	European Union
SSG	Small Scale Growers
^0C	Degree-Celsius
R	Coefficient of determination
P	Probability

K	Potassium
O_2	Oxygen
CO_2	Carbon dioxide
RH	Relative humidity
Mn_2	Manganese
Cl	Chlorine
Mg	Magnesium
F_2	Faint
SE	Standard error
≤	Less or equal to
>	Greater than
m.a.s.l	Meters above sea level
SED	Standard error of difference
S	South
E	East
RCBD	Randomized Complete Block Design
Mg/kg	Milligram per kilogram
Fig	Figure
UK	United Kingdom

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Snap bean (*Phaseolus vulgaris* L.), commonly known as French beans or Green bean, is of growing importance in the socio-economic systems in East and Central Africa. It is a crop with great potential for addressing food insecurity, improving incomes and reducing poverty especially among smallholder farmers in urban family (Ugen *et al.* 2005).

In East, Central and North Africa, production is dominated by bushy type's varieties. However; climbing varieties are generally more productive and have a longer harvest period compared to bushy varieties. Snap beans could be of particular interest to small scale producers desiring to strengthen earnings on both family labours and or hired. Commercial snap beans varieties currently grown among smallholder farmers, have a short harvest duration (3-4 weeks) with yields ranging between 6 and 8 tons per hectare and most are susceptible to major biotic constraints (Ndegwa, 1999).

According to Tanzania's horticultural export production data; Small Scale Growers (SSGs) are supplying vegetables to Europe for wholesale markets. Appendix 1 shows that exports of the target products to the target markets to date from Tanzania are very small, but strongest in green beans. Green beans represented the major export product by far in 2005. Other produce includes peas, chilli pepper, sweet corn, and gourds (DAI, 2007). Appendix 2 shows that although supply market still small,

volumes of fresh vegetable exports from Tanzania to the target EU markets have increased significantly since 2000, and that the main markets for success have been the UK and the Netherlands.

Production in Tanzania is mainly dominated by small to medium scale farmers and the enterprise creates on-farm employment opportunities for the rural communities, household consumption, family source of income, and urban family for smallholders. However, access to the European Union markets for small scale producers in East Africa is becoming increasingly difficult as a result of strict safety and quality standards (Monda *et al.* 2003). According to FAOSTAT (2012), the area harvested was 460 hectares, and the total production was 2300 tonnes which provides the total of 5 tonnes per hectare compared to the year of 2011 where by the area harvested were 450 hectares, and the total production was 2200 tonnes.

Bean production faces several constraints, which include pests, diseases and stringent quality requirements (Monda *et al.* 2003). One of the most widespread and important an economical disease of the green beans is bean leaf rust caused by the fungus *Uromyces appendiculatus*. However; snap beans varieties grown by smallholder farmers, have been reported to be susceptible to bean leaf rust disease. Bean rust is the most important and widely distributed disease of both Common beans and green beans in Eastern Africa, the disease is second to bean anthracnose, caused by (*Colletrichum lindemuthianum*) in the Southern highlands zone of Tanzania (Karel,*et al.* 1981*)*. Bean rust caused by *Uromyces appendiculatus* is one of the biotic factors reported by Muigai and Ndegwa, (1991) in bean fields. The disease is endemic and

severe in Eastern and Southern Africa and causes yield and quality reductions ranging from 18 to 100% in humid and tropical areas (Kimani, 2002; Monda *et al.* 2003).

Yield losses caused by bean rust depends on the degree of susceptibility of dry or green beans variety grown, the climatic conditions favouring rust infection, disease development, and earliness of the infection. Early infections occurring during the pre-flowering and flowering stages of bean crop development usually result in higher yield losses (Stavely and Pastor-Corrales, 1989).

1.2 General Characteristics of the Crop

Snap bean (*Phaseolus vulgaris* L), also referred to as green bean, is an annual leguminous plant that belongs to the genus, *Phaseolus,* with pinnately compound trifoliate large leaves. It is largely a self-pollinated plant though cross-pollination is possible if the stigma contacts with pollen coated bee when extended. Seeds are non-endospermic and vary greatly in size and colour from the small black wild type to the large white, brown, red, black or mottled seeds of cultivars, which are 7-16 mm long (Cobley and Steele, 1976). Snap bean shows variation in growth habits from determinate bush to indeterminate, and extreme climbing types. The bush type bean is 20-60 cm tall with most of the pods held above the ground while climbers may grow 2-3 m tall if they have support. The bush type green bean is the most predominant type grown in Africa (Buruchara, 2007).

1.3 Agronomy

Snap bean is a warm season crop that does not accept frost or long periods of exposure to near-freezing temperatures at any stage of growth. Typically high temperatures do not affect it if sufficient soil water is present, although high night temperatures will slow down pollination. The crop requires moderate amounts of rainfall (300 – 600 mm) but adequate amounts are essential during and immediately after the flowering stage. Generally, green bean is considered a short-season crop with most varieties maturing in a range of 50 to 70 days from emergence to physiological pod maturity. In Tanzania, crop cultivation is concentrated at altitude above 1000 masl, with adequate amounts of precipitation (> 400 mm of rain) during crop growing season and soil pH above 5.5; However, crop area in low elevation area (<1000masl) has also been increasing following population pressure (Buruchara, 2007).

1.4 Economic Importance of the Crop

The economic importance of the crop in Tanzania cannot be underestimated, both as a source of protein and as a high-fiber, low-fat, and low-sodium content ingredient of modern diets for the prevention and treatment of degenerative diseases, such as diabetes mellitus, heart disease, low blood sugar, and obesity (Hughes, 1991; Vorster and Venter, 1994; Holden and Haytowitz, 1998). Pachico (1993) reported that beans as the second most important source of dietary fiber for humans and the third most important source of calories among all agricultural products in Eastern and Southern Africa. It is an excellent source of vitamin A. Like most of vegetables; it provides important minerals such as Calcium (Ca), Magnesium (Mg), Phosphorus (P), and Potassium (K) and is an important source of income to smallholder farmers in

developing countries (Silbernagel *et al.* 1993). For the resource poor farmers, green bean plays a strategic role in alleviating malnutrition but other health related functions exist. Regular consumption of green bean and other pulses is now promoted by health organizations because it reduces the risk of diseases (Leterme and Munoz, 2002). This is because Snap bean is low in fat and is cholesterol free. It is also an appetite suppressant because it digests slowly and causes a low sustained increase in blood sugar. It is consumed as boiled green leaves, and green immature pods. The fresh form of pods is the most preferred because of its fresh flavour, good taste, and requires considerably little time to cook (approximately 40 min).

1.5 Problem Statement and Justification

The most commonly grown varieties of snap bean in Tanzania are Samantha, Teresa, Monel and Amy for either fresh market or processing. Teresa and Monel varieties are reported to have high yielding and resistance to anthracnose disease, but they are susceptible to bean rust. In the baseline study report conducted in 2006, by SARI Bean Project, discovered that about 46% of farmers grows Army variety, 35% Monel, 17% Samantha, and 2% grows Teresa variety, probably few are growing Teresa due to its disease susceptibility.

Most snap bean cultivars grown in East Africa are very susceptible to rust (Hillocks *et al.* 2006; CIAT, 2008). Monda *et al.* (2003) reported that, severe infection can be estimated to cause yield losses of 37% to 65% in various production areas. Smallholder snap bean farmers mainly rely on fungicides and insecticides to reduce production and post-harvest losses associated with diseases (Wasonga *et al.* 2010).

However, the use of chemicals makes the produce less marketable due to limitation on chemical residue level requirements set by the European markets (Kimani, 2002). Continued use of chemicals also leads to emergence of disease resistant pathotypes, increased production costs and negative effect on the environment and human health (Burkett *et al.* 2008). Chemical control using expensive fungicides has been the standard disease management strategy among farmers (Monda *et al.* 2005). Cultural practices such as crop rotation, intercropping, elimination of plant debris, adjustment of planting dates, use of compost, and merger heterogeneous cultivars can reduce diseases severity (Deeksha *et al.* 2009). However, the use of host plant resistance is by far the most economic and environmentally sustainable method for controlling bean diseases.

Development of cultivars with high resistance to disease has been the goal in bean breeding (Milkas *et al.* 2002; Sharma *et al.* 2007). Resistance and tolerant cultivars to bean leaf rust is recommended as the most cost effective management method (Sanders and Schwarts, 1980). This will also assist farmers to meet stringent export requirements for residue levels and to attain food safety and quality. Therefore this work aimed at screening snap bean advanced lines for resistance to bean leaf rust, and eventually identify breeding lines for resistance to the disease to be utilized in the genetic improvement of cultivars presently grown in Tanzania.

1.6 Objectives

1.6.1 Overall objective

To screen the snap bean genotypes resistant to bean rust as a source of breeding materials in Tanzania

1.6.2 Specific objectives

i. To evaluate snap bean genotype with high yielding, good horticultural characteristics for performance and adaptation under natural prevalence of bean leaf rust

ii. To screen snap bean genotypes in the screen house for resistance to bean rust under artificial disease inoculation

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Bean leaf rust is a major fungal disease affecting most dry and snap bean in many tropical and subtropical region of the world. The pathogen is known to have high virulence diversity, and this has been reported from many different regions of the world like Brazil, Australia, United States, Mexico and Colombia (Araya *et al.* 2004). It is characterized by its high degree of pathogenic variability more than a hundred races have been identified worldwide, and several races can occur in a single field crop. Virulence diversity of the pathogen first was reported by Harter *et al.* 1935. Several other virulence phenotypes over 300 races were identified from 1952 to 1996 (Liebenberg *et al.* 2003). The rust pathogen normally produces pustules on the upper and lower leaf surface and sometimes on the pods and stems.

Rust epidemics in snap bean have been reported in many different regions of the world, and severe losses occur in tropical and subtropical climates (Mmbaga *et al.* 1996). According to Mmbaga and Stavely, (1988), out of 17 single uredinium isolates evaluated, nine distinct races were identified in Tanzania. These races have been progressively numbered, from narrowest to broadest in virulence, as Tanzanian (T) race 1 through 9.

2.1.1 Distribution

Rust is one of the most widespread diseases of bean crop and has been reported from all bean production areas of the world. (Steadman. 1995). Africa by (Allen. 1995). the first report of the fungus in South Africa was in 1909 in the Transvaal province and it was noted over the years to be widespread and destructive by (Doidge *et al.* 1953). By 1945, it had been reported in all provinces as well as in Zimbabwe and Mozambique on *P. vulgaris* and on *P. acutifolius* Gray var. *latifolius Freem.* (tepary bean) in the Eastern Transvaal, and was cited as common and widespread on *Phaseolus coccineus* Linn. Doidge, (1950)

It occurs widely in Africa in at least 19 of the 20 Eastern and Southern African countries (Allen, 1995) but regularly reaches epidemic proportions in the cooler, more humid highland areas, on the Eastern escarpment, and in the islands of Madagascar and Mauritius (Steadman *et al.* 2002a). According to Wortmann *et al.* (1998), bean leaf rust is responsible for causing an estimated 191,400 tonnes per annum yield loss in sub-Saharan Africa.

2.2 Pathogen Nomenclature, Morphology, and Life Cycle

2.2.1 Nomenclature

The pathogen is basidiomycete *Uromyces appendiculatus* (Pers.:Pers.) Unger var. *appendiculatus* (Boerema *et al.* 1993) was first described in Germany in 1795 by Persoon as *Uredo appendiculata phaseoli*. Frequent name changes of the pathogen usually have been confusing. The names according to Boerema *et al.* (1993) are *Hypodermium appendiculatum* (Pers.:Pers.) Link (1915), *Puccinia phaseoli* (Pers.)

Rebentisch (1804), *Uromyces phaseoli* (Pers.) winter, (1980), and *Uromyces phaseolorum* Tulasne (1854).

In Africa common name used are ''roes'' (Afrikaans: South Africa), ''ferrugem (Portuguese: Angola, and Mozambique), ''kutu'' (Swahili: Tanzania, Kenya, and the Democratic Republic of the Congo, ''la rouille (du haricot)'' (French-speaking countries), and ''chiwau'' (Chichewa: Malawi), which denotes the general burning or scorching effect, the name ''chiwau'' is also used for other bean leaf diseases, such as Angular leaf spot (ALS). In Spanish-speaking parts of Latin America, bean leaf rust pathogen is known as ''la roya.

2.2.3 Morphology and life cycle

2.2.3.1 Asexual stage

Bean rust pathogen is an autoecious and macrocyclic obligate parasite. Life cycle is restricted to a single host, which means completing its entire life cycle on the common bean (*P. vulgaris*) (Harter and Zaumeyer, 1941). It has all five spore states include the urediniospores, teliospores, basidiospores, pycnyospores and aeciospores. The pathogen cannot live independently of its common bean host. For this reason, it cannot be cultured on artificial media, but the possible urediniospores can be preserved for long period in the laboratory in the liquid nitrogen, where spores are not able to germinate. Urediniospores normally; are contained within the reddish brown uredinia (known as pustules) which are observed on infected leaves and sometimes on pod and stem of bean plants during most of the bean crop growing season.

Urediniospores germinate on the surface of the leaf or other aerial part, and the germ tube, tightly appressed to the leaf surface, grows over the epidermis until it reaches stomata (Wynn, (1976); Allen *et al.* 1991). Oxygen (O_2) or Carbon dioxide (CO_2) concentration, or pH gradients are reported to involve in the urediniospores germination (Von Alten, 1983).

An infection hypha grows into the sub stomatal cavity, after which the intercellular spaces are colonized, and nutrients are extracted from host mesophyll cells by means of haustoria Wynn, (1976); Mendgen, (1978); and Von Alten, (1983). The surrounding cells are stimulated and preserved at the expense of the undivided tissue Wingard, (1935).

Resistant accessions often react to haustorial formation with a hypersensitive reaction, which involves dissolution of the contents of the infected cell, collapse of the infected cell leading to necrosis, or collapse of the haustorium itself without visible cell damage. This usually results in the death of the fungus before spore production Wingard, (1935); Mendgen, (1978). After colonization, the mycelium aggregates to form sores. This enlarges, and gives rise to thin walled, single cell echinulate urediniospores, Harter and Zaumeyer, (1941). It has reported that 7 to 10 days after infection, the epidermis burst open, and this is a result of the pressure of volatile metabolites exposing the developing spores to the atmosphere (Last and Schein, 1973).

According to Yarwood, 1961; darken from golden to cinnamon brown, forming the characteristic uredinia, or pustules, on both the adaxial and abaxial leaf surfaces, but

more frequently on the abaxial. Uredinia characteristically range from 0.2 to 0.9 mm in diameter, but can reach 2 mm and even 4.8 mm. For susceptible genotypes, secondary and sometimes tertiary sori can develop in concentric circles around the primary pustule (Harter and Zaumeyer, 1941), but their formation found not to be a very consistent characteristic.

The mycelial area within the leaf can reach less than 5mm within 40 days, its area being greater than that of the sporulating area (Yarwood, 1961). According to Harter *et al.* 1935, the Urediniospores are released on a continuous basis and are relatively short lived depending on the relative humidity (RH) during sporulation; a potential urediniospore production of less than 20 000 per pustule per day has been calculated (Aust *et al.* 1984). A susceptibility temperature density interaction plays an important role. Urediniospores, which are primarily wind dispersed, often in clusters can germinate as soon as they mature, completing the asexual cycle approximately every 10 to 15 days by re infecting the host (Harter *et al.* 1935; Zaumeyer and Thomas, 1957).

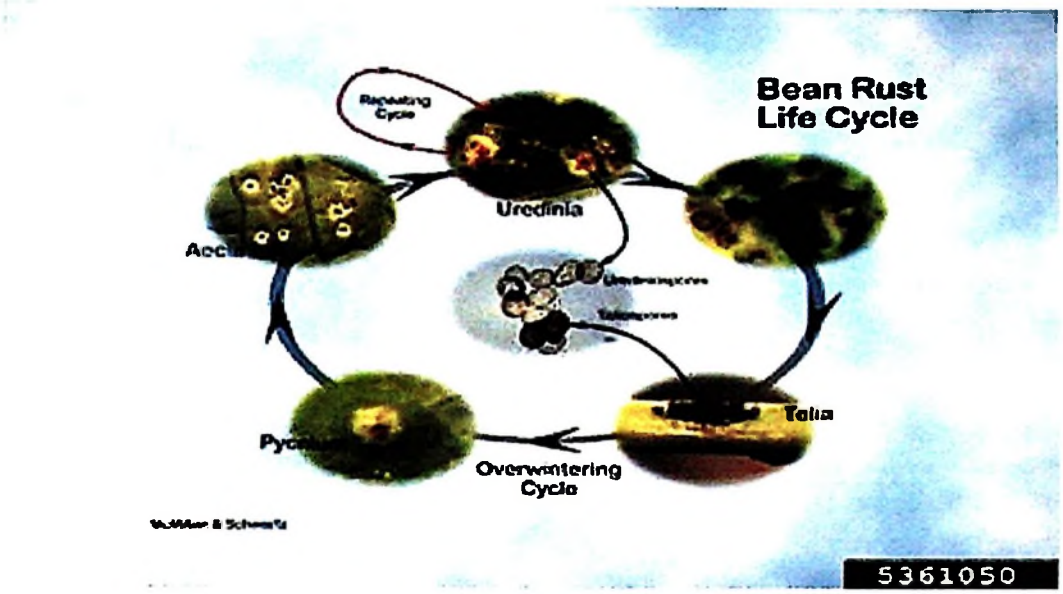

Figure 1: Life cycle of bean rust pathogen

Source: Howard F. Schwartz and Mark S. McMillan, Bugwood.org

2.2.3.2 Sexual stage

According to Waters, (1928), environmental factors such as light intensity, temperature, and moisture, either singly or in combination, indirectly influence the life cycle of bean leaf rust fungi, including *U. appendiculatus*. These factors affect the metabolism of the host (particularly when the host is weakened) and in this way induce the change over from uredinial to telial production. Factors such as plant maturity, leaf age, and host response may also play a role (Stavely and Pastor-Corrales, 1989). In the field, the replacement (within the same pustule) of urediniospores by dark brown teliospores occurs toward the end of the growing season on older leaves and is common in more temperate climates. (Mc Millan *et al.* 2003). Most isolates collected from temperate areas can be induced to form teliospores in the greenhouse by manipulating host metabolism (Harter *et al.* 1935), but teliospores will form spontaneously on leaves kept for longer periods in the

greenhouse (Linde *et al.* 1990). Teliospores are single celled, smooth, sparsely, striate and thick walled, with a fragile, hyaline pedicel (Laundon and Waterston, 1965).

McMillan *et al.* (2003) concluded that conditions conducive to the emergence of volunteer bean plants are also favorable for basidiospore germination and subsequent bean plant infection. Basidiospores germinate on any aerial surface of the bean plant, forming a single germ-tube that grows primarily along or toward the epidermal cell junctions.

The fungus adheres to the plant surface by means of an appressorium. The appressorium gives rise to a penetration peg that, in contrast to the uredinial germ tube, ruptures the epidermal cell wall, eventually forming an intra and intercellular hyphal network in the epidermis and underlying tissue; the entire process takes up to 72 hrs (Gold and Mendgen, 1984a). From this network, spermogonia (pycnia), which first become visible as small chlorotic spots, 0.5 to 1.0 mm in diameter, are formed 4 to 5 days after infection (Gold and Mendgen, 1984c). After 6 to 7 days, the epidermis is ruptured and spermogonia reach 3 to 5mm in diameter. These cause localized light yellow chlorosis and malformation of the leaf, giving it a blistered appearance (Schwartz *et al.* 1990; Mc Millan *et al.* 1990).

On leaves, spermogonia generally form on the adaxial leaf surface, and aecia on the abaxial side. On stems, petioles, and veins, the two structures are adjacent, elliptical, and less than 10mm long (McMillan *et al.* 1990, 2003). The sexual stage has been

reported on the primary leaves, petioles, stems, and the hypocotyl near or just under the soil surface (McMillan *et al.* 2003). However, pods can also be infected (McMillan and Schwartz, 1994). Venette *et al.* (1978) found both spermogonia and aecia within 60cm of the soil surface.

The sexual stage has also been observed outdoors under simulated natural conditions (Rijkenberg, 1994). The complete life cycle often has been induced in the greenhouse (McMillan *et al.* 2003). It is therefore that the sexual stage is common in temperate climates. However, some races for example, those occurring in areas with warmer climates appear to have lost the ability to form teliospores (Linde *et al.* 1990; Liebenberg, 2003). Gross and Venette, 2001 reported survival of urediniospores on bean leaves left outdoors overwintered, therefore indicating that, even in temperate climates, these could also be a source of initial inoculums in the following crop.

2.3 Disease Symptoms

The disease can be found on all aerial parts but is mostly common on leaves. Initial symptoms are white to cream colored circular specks under the epidermis. Rust colored pustules develop as urediniospores break through the epidermis on both the adaxial and abaxial leaf surfaces, but are more common on the abaxial.

Larger pustules often are surrounded by a chlorotic halo. A ring of secondary, and even tertiary, pustules may develop on susceptible genotypes. Circular or irregular brown or gray to black necrotic lesions, ranging from less than 0.3mm to 3mm or more in diameter, are also found but often are difficult to detect in the field. In some

cases, necrotic lesions may contain sporulating pustules (Harter and Zaumeyer, 1941). These pustules are generally small (Stavely *et al.* 1989b) but can reach 0.8mm or more Liebenberg, (2003). Green pastules without sporulating have also been observed in the field and greenhouse.

Premature chlorosis, senescence, and defoliation can also take place due to high respiration rates. Pustule type is a function of the race host interaction (Harter and Zaumeyer, 1941); consequently, where more than one race is present, more than one type can occur simultaneously on the same leaf. Pustule size, normally also a function of the race host interaction, can be negatively affected by overcrowding resulting from large numbers of infection points and by extreme temperatures and overshadowing.

Pustules on the pods, stems, and petioles, when they occur, are elongated. In some cases, pustules become black as urediniospores are replaced by the darker and more robust teliospores on older leaves near the end of the growing season see figure 5 (Zaumeyer and Thomas, 1957). Although the uredinial stage can occur on the primary leaves of seedlings, it is generally most prolific during and after flowering (Stavely and Pastor-Corrales, 1989).

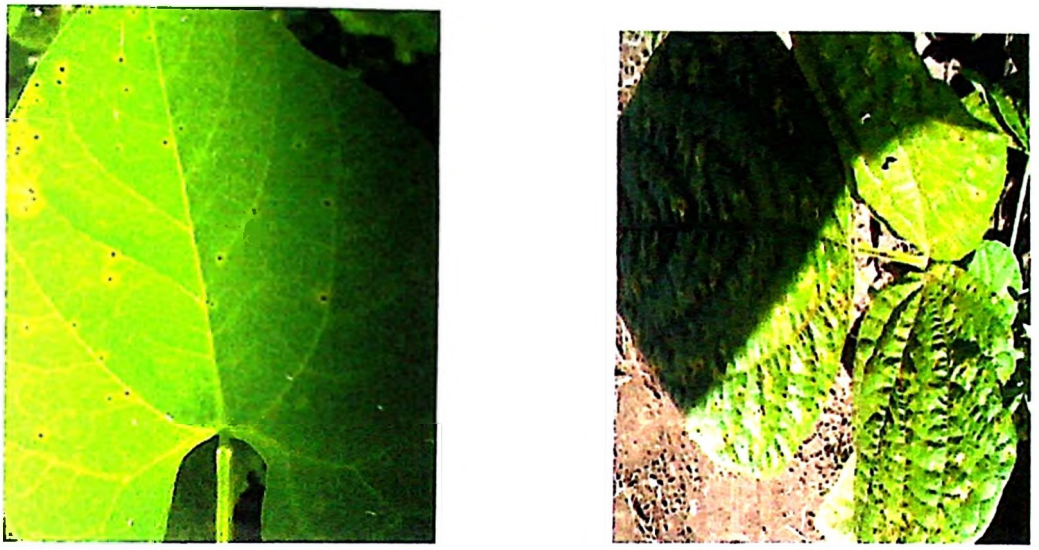

Plate 1: **Early uredinial pustules *Uromyces appendiculatus* consisting of small raised white spots and advanced uredinial pustules *Uromyces appendiculatus* surrounded with yellow borders containing reddish-drown repeating spores**

Plate 2: Bean plants severely infected with *Uromyces appendiculatus*

Source: ARI Uyole, Mbeya 2012

2.4 Epidemiology of Bean Rust

2.4.1 Dissemination

The chief dissemination method of bean rust is wind, and in particular gustiness, which enables the escape of spores from the canopy Aylor, (1990). Irregular periods of high humidity and windiness at fairly cool temperatures are therefore, important. Although wind accounts for dissemination over long distances, other agents, such as migratory birds, animals, insects, clothing, water, vehicles, and implements, can also play a role (Nagarajan and Singh, 1990). The disease is not, however, seed borne (Zaumeyer and Thomas, 1957). Heavy rain or overhead irrigation can retard dissemination (Mc Millan, 1994).

Under normal circumstances, urediniospores are short lived, but dehydration, followed by exposure to low temperatures, is known to considerably extend their survival period. These conditions would be experienced at high altitudes during dissemination and may account for the ability of the fungus to infect isolated bean fields and the occurrence of severe epidemics in the absence of local teliospore production. Teliospores, and to a lesser degree also urediniospores, can survive for considerable periods in bean debris (Gross and Venette, 2001). Under these circumstances, urediniospores probably are responsible for the propagation of the fungus.

2.4.2 Environmental influences

Ideal conditions for bean leaf rust development are temperatures ranging from 17 to 25^0C, occurring simultaneously with high Relative humidity (>95%) for at least 7 to

8hrs (dew formation is critical for infection), interspersed with dryer periods that favor dispersal (Stavely, 2005). Although both temperature and humidity in particular, appear critical, it is the interaction between these two factors that is important. Due to local adaptation, genotypes originating from different climatic regions may exhibit different environmental optimal.

2.4.2.1 Temperature

Temperature is an important factor in the development of rust. Changes of only 5 to 6^0C for 4 to 8hrs can significantly influence the epidemiology of the disease. The presence of bean leaf rust under natural condition is strongly influenced by temperature. Bean rust spores will germinate at 10°C, optimum germination occurs at 18°C - 20°C (Gold and Mendgen, 1985). The optimal temperature for disease development is 15°C to 20°C for the pre penetration stage and 24 to 26°C for post penetration stage (Code *et al.* 1985). As temperatures increases above 20°C optimum, germ tube exhibit disorientation and failure to penetration the host (Alten, 1983), making susceptible host plants appears partially resistant. Davison and Vaughan, (1963b) obtained minimal germination of urediniospores stored at 3 to 4^0C for 446 days and speculated that urediniospores could overwinter on bean debris and staking poles considering that, in the spring, early rust pustules are found on bean plants closest to stakes.

2.4.2.2 Humidity and leaf surface moisture

Infection caused by *Uromyces appendiculatus* is favoured by prolonged periods (10-18 hrs) of moisture condition greater than 95% Relative humidity (RH), but rarely

occurs at humidity below 95%. High relative humidity is necessary for infection by basidiospores (Groth and Mogen, 1978), which can occur at a very young stage, probably at or just after seedling emergence.

Leaf surface moisture plays a key role in both germination and infection, and the disease causes yield loss only where sufficient humidity to promote leaf surface moisture occurs. Hydration of dry urediniospores is found to increase germination and shorten germination time (Curtis, 1966). Harter *et al.* (1935) reported that within appropriate temperature ranges, high levels of infection obtained when plants exposed to a RH of 96% or higher, provided free moisture present on the leaves.

Air movement, which caused evaporation of moisture from the leaves preceding penetration, also inhibits infection. Yarwood, (1961) and Imhoff *et al.* 1982) observed that a greater percentage of pustules erupted under humid conditions, and more spores are produced by pustules exposed to humid conditions than those exposed to low RH. Rehydration followed by a period of high RH is also found to be a prerequisite for teliospore germination.

2.4.2.3 Light

Von Alten, (1983) reported that appressorium formation is maximal under intermittent light-dark conditions but is reduced by exposure to continual light, and Harter *et al.* (1935) found that exposure to less than 48hrs of subdued light during the infection period led to reduced infection and delayed pustule development. Any factor adversely affecting the condition of the plant, including low light levels, is also

detrimental to the development of the rust fungus. Wei, (1937) reported that the infection type on highly resistant and highly susceptible plants is the least affected by light intensity. Low light intensities tended to lengthen the incubation period and increase necrosis on susceptible hosts. Low light intensity or decreased day length can also stimulate the change over from urediniospores to teliospores (Waters, 1928). Alternating periods of darkness and light (15 000 to 26 000 lux with an optimum intensity of 17 000 lux) are reported to be a prerequisite for teliospore germination. No germination of teliospores occurrs under constant light or constant darkness (French *et al.* 1993). In southern highlands light factor changes much, which may influence the germination of teliospore. Light intensity is not constant, varies from period during growing season.

2.4.2.4 pH and ion concentration

Both pH and ion concentration of the medium in which the urediniospores germinate affect germination levels. A pH of 6 to 7 is optimal for germination; very low germination levels occurrs below pH 5 and above pH 8 (Bell and Daly, 1962). Baker *et al.* (1987) determined that calcium ions (Ca_2) (at 0.1 to 3mM) stimulate the germination of urediniospores. Magnesium ions (Mg_2) (at 1m M) have a slight stimulatory effect. The effect of the monovalent sodium (Na) and potassium (K) ions was negligible and that of manganese (Mn_2) is negative at a concentration of above 200 M.

Soil pH has been reported to influence pustule diameter, with larger pustules forming on plants in low pH (5.8) potting medium compared to those in soils of pH 6.5 or pH

7.9. This appears to result from the effect of pH on the Cl-, Mn^{2+}, and K^{+} concentrations in leaves. Pustule diameter was positively correlated with Cl- (2.0 to 17.9 g kg^{-1}) and Mn^{2+} (51 to 332mg kg^{-1}) in leaves. There was also a host genotype soil ion interaction (Zaiter *et al.* 1991).

2.4.2.5 Leaf age influences (Host factor)

Tissue age strongly influence receptivity (defined as the number of visible pustules forming per unit of applied inoculums). Smaller or undeveloped pustules are observed for very young (unfolding) leaves. Primary leaves is most receptive while rapidly expanding, with maximum receptivity is reached when leaves is approximately 20 to 40% expanded, depending on the genotype. Receptivity declined sharply after approximately 40% expansion, and older leaves generally developed smaller and fewer pustules. The age of leaves, leaf type, physiological condition of the host, and cultivar type, influences rust spore production. The fastest infection occurs in leaves that are a few days old due to a high number of stomata per unit area. In old leaves, only a few germ tubes manage to penetrate stomata to reach the mesophyll. However, once the germ tube reaches the leaf interior, it has the same changes of pustule formation as on young leaves. Susceptibility of bean leaves to infection at the time of unfolding will increase with age until they are 20-40% expanded (Alten, 1983).

2.5 Disease Inoculation Preparation and Inoculation Methods

Although Waterhouse, (1954) inoculated fully expanded primary leaves, in concurrence with research findings reported on leaf age that, the standard procedure

to inoculate leaves is between one and two thirds expanded leaves (Liebenberg and Pretorius, 2004a).

A urediniospore concentration of 2 x 10^4 ml has proven adequate for race identification (Faleiro *et al.* 2004), although 2.5 x 10^4 ml^{-1} can be used for routine screening inoculations. This ensures sufficient infection points and enables maximal development of uredinia. Concentrations of 4 x 10^4 ml^{-1} and higher lead to overcrowding, resulting in smaller pustules and misleading the results (Davison and Vaughan, 1964).

Screening or race identification can also be done using a fixed mass of spores, either suspended (10mg in 10 ml 0.01% Tween 20 wetting agent) (Venette *et al.* 1998), or applied dry (2.5 mg per leaf) by means of a settling tower (Mmbaga *et al.* 1994). Various inoculum application methods have been used. Spraying with an apparatus creating a fine mist has been found to be very effective, the most popular being low pressure ''spray guns'' designed for artist's or spray paint application (Stavely, 1983), an atomizer by (Waters, 1928; Faleiro *et al.* 2004), or a nasal pump sprayer by (Gross and Venette, 2002). Harter and Zaumeyer, 1941) used an atomizer or a camel's hair brush, but reported that dusting the leaves with dry spores was equally, or even more, effective. Waterhouse, (1954) rubbed spores gently on leaves atomized with water also reported the results to be the same. These methods are particularly useful when testing segregating breeding material or screening germplasm.

2.5.1 Incubation periods

Incubation periods used vary from 16 hrs (Stavely, 1983), 24 to 48 hrs (Faleiro *et al.* 2004) to 40 to 48 hrs (Ogle and Johnson, 1974). Incubation periods of less than 24 hrs are found to be insufficient, leading to low uredinium density. Incubation periods of greater than 24 hrs are not significantly increase uredinium density. Low light intensity (2×10^5 mμ Einstein cm^2 sec^1) during incubation (18 hrs) favors infection, whereas high light intensity (6 hrs darkness followed by 12 hrs high intensity, or 18 hrs high intensity) is detrimental (Augustin *et al.* 1972).

2.5.2 Disease reaction and rating

Ratings are generally done 14 to 15 days after inoculation (Harter and Zaumeyer, 1941; Stavely, 1983). A number of variables, such as soil pH and leaf age, have been reported to influence uredinium pustule formation and size, this in turn, can influence ratings. Various rating scales, summarized in appendix 3 and 4, have been used for greenhouse and field evaluation, the 1 to 6 pustule size scale (Stavely *et al.* 1993), the 1 to 9 CIAT intensity scale (Van Schoonhoven and Pastor-Corrales 1987).

Mmbaga *et al.* (1996) developed a quantitative disease score for use with statistical analysis, based on the 1 to 6 pustule size scale. Mmbaga *et al.* (2003) reported that progressions of subjective classes for rating scales were exponential and for this reason based his 1 to 9 rust scales on this model. McMillan and Schwartz, (1993) investigated the feasibility of assessing rust by means of image analysis and reported promising results for simulated bean rust (the standard grading scale), covering all aspects of disease evaluation. Shaik and Steadman, (1989a) stated that the spread of

disease is largely determined by the number of spores produced, Although this parameter is very difficult and tedious to measure, a good estimation of spore number (for susceptible accessions only) can be obtained by measuring colony size, which includes secondary uredinia, opposed to primary uredinium size.

2.6 Management of the Bean Rust

Although several strategies exist for the management of bean leaf rust, rust management under field conditions in most bean producing areas is accomplished using disease resistant cultivars and fungicides. Bean rust can be controlled by cultural practice which include crop rotation, removal of volunteer plants, deep plowing to remove bean debris from the soil surface, encourage decomposing and avoidance by choosing planting time, also use of fungicides (chemical applications) has been one of the disease control method in most of African farmers. Use of resistant cultivars as a best disease control method is recommended as a most effective and cost reduction means (Liebenberg and Pretorius, 2010).

2.7 Cultural Practices

2.7.1 Intercropping and multiple cropping

Intercropping of beans with non-host crops, such as maize, is popular in many parts of Africa (Fininsa and Yuen, 2001). In some circumstances, maize in particular may decrease rust levels, possibly due to the provision of a physical barrier influencing factors such as spore dispersal and temperature (Fininsa, 1996).

2.7.2 Sanitation

Reduction of the number of volunteer bean plants, including those within rotation crops or weed canopies, thorough harvesting of bean seed, weeding, and the use of herbicides is recommended to remove potential hosts for the establishment of the sexual stages of the pathogen in highlands areas climates and to prevent early buildup of inoculums. Tillage practices are also important to avoid potential hosts for the sexual stage, and include deep plowing to incorporate bean debris into the soil. Bean plants that germinated through bean debris were reported to have a higher incidence of aecial infection than those that germinated through a clean surface, indicating that minimum tillage practices encourage the development of the sexual stage in bean rust. Effective weed control and wider spacing can also minimize the formation of a dense canopy, which creates a humid microclimate conducive to bean leaf rust (McDonald and Linde, 2002; and McMillan *et al.* 2003).

2.7.3 Crop rotation

Rotation of two or more growing season between bean crops is widely recommended, especially in areas where the sexual stage is known to occur. To be effective, this crop rotation must be combined with good sanitation practices, which have the additional advantage of also reducing bacterial and other fungal diseases. (McMillan *et al.* 2003).

2.7.4 Planting time

Conducive conditions during the most vulnerable stages of the host can be avoided by judicious selection of planting date (Steadman, 1995). This method is widely practiced by small-scale farmers in Africa (Mohamed and Teri, 1989).

2.7.5 Overhead irrigation

McMillan, (1994) obtained a significant decrease in rust severity accompanied by a fourfold yield increase by applying overhead irrigation four times per week. This created the effect of intermittent heavy rain, which most likely washed the spores off the leaves and into the soil, thus preventing spore dissemination by wind (Chupp and Sherf, 1960).

2.7.6 Host resistance

The incorporation of multiple resistance genes into locally adapted cultivars remains the most cost effective control measure for bean leaf rust (Liebenberg *et al.* 2005). However, many cultivars are susceptible, and the disease remains a problem where the environment is favorable. In some cases, cultivars/genotypes lose their resistance when new races appear. Resistance to bean leaf rust is controlled by a series of several genes that to date all are single and dominant (Kelly *et al.* 2003; Miklas *et al.* 2006). Mienie *et al.* 2005 reported that resistance to this disease is conditioned by a considerable number of genes including Ur- 3, Ur- 4, Ur-5 and Ur- 9 which control disease resistance.

In general many commercial cultivars acquire resistance to one or more races, but no cultivar or variety source have been found that is immune or resistance to all reported races or population structure of bean rust (Kelly and Miklas, 1998). This need to have multiple races resistance incorperated into local grown cultivars.

2.7.7 Use of fungicides (chemicals)

The effectiveness of fungicides to reduce yield losses due to rust on Common and Snap bean has been well studied and documented. However, changes in fungicide registrations in many countries occur every time. Gent *et al.* 2003) reported that use of organo silicone based adjuvant improved fungicide leaf coverage by 26% to 38% in bean leaf rust control. The addition of certain commercial adjuvant to Maneb lowered rust incidence up to 62%. The use of food additives as an alternative control measure for *U. appendiculatus* was investigated by Arslan *et al.* (2006).

Disadvantages of fungicides and other chemicals; include the increase in production costs, practically widespread lack of application technology, expertise, and correct timing of application, as well as lack of availability, particularly in Africa (Arslan *et al.* 2006). These problems turn into fungicides generally unpractical for use by subsistence farmers. Potential phytotoxicity due to using incorrect rates, time of applying, and possible detrimental effects of the chemicals on the environments are further negative factors that make indispensable use unadvisable.

Prohibition of fungicide application within a certain period before harvesting to meet residue limits, the presence of chemical residues particularly applicable to green beans, is of paramount importance farmers to take into account in snap bean market.

The development of fungicide resistance may limit also the long term usefulness of these fungicides, and over use, especially prevalent in some Snap bean production areas, is a threat to their stability. (Stavely and Steadman, 1992).

2.8 Economic Importance of Bean Leaf Rust in Relation to snap Bean Production

Losses due to bean leaf rust can be dramatic. Damage to snap bean not only causes a decrease in the number of seeds but also leads to a loss of pod quality and decreased seed size resulting from poor pod fill due to loss of photosynthate (Steadman *et al.* 1986).

Pod lesions can also blemish pods in the case of green/snap beans (Foster, 1947). Steadman *et al.* 1986a) reported a highly significant correlation between bean leaf rust severity and yield, total number of pods, total number of seeds and seeds per plant, and mentioned that early rust intensity could be used to predict yield loss. The most accurate yield loss prediction is, however, obtained when rust severity is recorded approximately 2 weeks before maturity (Lindgren and Steadman, 1992). These authors also found indications that yield losses became significant when bean leaf rust infection levels reached 5% to 20%. Lindgren *et al.* (1995) reported that the relationship between bean leaf rust severity and yield loss due to bean leaf rust is linear and that an estimate of rust induced yield loss (in kg /ha) for any given growing season could be obtained by multiplying by 19 the percentage leaf area with rust symptoms at 72 days.

Lopes and Berger, (2001) compared the effects of anthracnose caused by *Colletotrichum lindemuthianum* and rust on photosynthesis in bean. At all disease levels, anthracnose had a significant positive effect on the photosynthesis of the leaf area not covered by lesions. Rust, however, followed a different pattern. When rust

severity is low (<30%), reduction in photosynthesis is proportional to the leaf area covered by pustules. However, when rust severity is >30%, chlorophyll content decrease markedly, and photosynthetic rate of leaves with rust severity of between 70% and 90% is near zero. When comparing the effects of Angular Leaf Spot (ALS) and bean leaf rust on yield loss, De Jesus *et al.* (2001) reported that, although in their experiments rust did not cause significant defoliation (in contrast to ALS), did significantly decrease yield. These authors suggested that this is due to the drain on carbohydrates brought about by rust. Both of the above groups of authors observed no interaction between the two diseases studied.

The strongly detrimental effect of bean leaf rust appears to be due to the creation of a "pathological sink" operating at the expense of new tissue formation. Transpiration rate and average stomata aperture of *P. vulgaris* are adversely affected by bean leaf rust. During the pre-sporulation stage, daylight transpiration rate of diseased leaves is significantly less than that of the control, and a linear decrease in average stomata aperture to a minimum of 1 mm at 65 pustules in the light was observed as infection density increased, the latter reaching a plateau at 75 pustules. At maximum effect, average stomata aperture of diseased plants is approximately 30% that of the control (Wittmann and Schonbeck, 1995).

Therefore, due to the points above, it is clear that more research is needed in this area. However, the most important aspect is the detrimental effect of bean leaf rust on yield and pod quality of snap bean, which appears to be generally more serious than for most other fungal diseases in the snap bean production. This may also be

partly due to the fact that, in Africa, bean leaf rust generally appears at an earlier stage than angular leaf spot and Anthracnose, which is already competing with the host for nutrients from the early pod, filling stage.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Introduction

This study was mainly based on two experiments, field studies and screen house studies. Field experiment was for the purpose of evaluating ten snap bean genotypes, under natural condition for adaptability and performance to bean leaf rust disease. Second experiment was conducted in the screen house, for screening ten snap bean genotypes for resistance to bean leaf rust under an artificial inoculation for the purpose of identifying future breeding lines to be used as a donor parent. Therefore this chapter explains the methodology used to achieve both two objectives of the study.

3.2 Field Experiment

3.2.1 Experimental sites

The experiment was conducted during the rainy growing season January to May of 2011/2012 in Southern highlands of Tanzania at three locations. Seatondale in Iringa urban 07.79059 ^{0}S, 035.698770 E at 1539 m above sea level, and the other sites were Uyole in Mbeya urban, 8^{0}55`00.76``S, 33030`59.08``E at 1795 m above sea level, and Mbimba in Mbozi 8057 S, 033^{0}13 E at 1241 m above sea level.

3.2.2 Experimental design

The experiment was in randomized complete block design (RCBD) with three replications at each location. Ten bushy snap bean genotypes were the experimental

treatments and the local variety "Teresa" as a check for the bean leaf rust disease and replicates being the blocks. The ten snap bean genotypes used in the experiments; were HAB 404, HAB 425, HAB 449, HAB 423, HAB 240, HAB 427, HAB 442, HAB 403, HAB 414, and HAB 419, and the check variety Teresa, all study materials were provided by the University of Nairobi, Kenya.

3.2.3 Crop husbandry

Fields were disc ploughed and harrowed in early January, 2012. Hand hoes were used for field levelling. At Uyole, the experiment was planted on 28th, February 2012, then at Mbimba on 22nd, February 2012 and Seatondale on 10th, January 2012. These planting dates were observed when the weather (rain) is well set for the disease to develop.

Each experimental plot at each site per entry per replication was 1.5 m by 2.0 m giving 3.0 m^2. In each trial plot two rows were planted and each plant row contained fifteen bean plants which make thirty plants per plot. The spacing of seeds within a row was 0.1 m and the distance between rows in a plot was 0.5 m.

Ten plants in the middle of two rows were used for data collection while the other plants were used as guard rows plants. A recommended agronomic package such as hand weeding was done two weeks after emergence and the second one just before flowering. Insect pests were controlled by alternate application of Dimethoate® and Karate® (Lambda cyhalothrin) at the rate of 1.5 ml L^{-1} every week. Fungicides were not applied for the purpose of evaluating the varieties for resistance to major

diseases, (notably bean rust). Fertilizers Triple Sulphate phosphate (TSP) and Calcium Ammonium Nitrates (CAN) were applied in the rate of 19.02 g of TSP and 17.20 g of CAN per plot at planting. At green pod stage, pods were harvested twice a week at three day intervals for 2 weeks.

Plate 3: Vegetative stage of snap bean genotypes at Uyole site, Mbeya

3.3 Screen House Experiment

3.3.1 Screening snap bean genotypes for resistance to bean leaf rust

Screening process was conducted in the screen house; forty (44) pots of about 5 litres volume were used and filled with a mixture of forest soil and animal manure about three quarter. The pots were arranged in the greenhouse in randomized complete block design (RCBD) with five (4) replications. Two seeds were sown in each pot, which made a total of twenty (22) seeds per replicate or block. Regular irrigation

(daily) was imposed to smooth the progress of seed germination and emergence. Three replicates contained selected snap bean genotypes, and the check variety Teresa, and the other replicate contained with no inoculums selected snap bean, as a control.

3.3.2 Inocula preparations and inoculation

Urediniospores of *Uromyces appendiculatus* were collected from rust affected bean leaves in a nearby bean field at Uyole for inoculation. Two out of three primary leaves from each plant in a pot was inoculated in both sides of the leaves (upper and lower side). According to Waterhouse, (1954), inoculation was done at 8 - 10 days, when the plants were fully expanded two primary leaves. According to (Ariyarathne and Pradeep, 2001), direct spore rubbing method was used for inoculation. A magnifying glass and a fine millimetre ruler were used to measure the pustules diameter. Inoculum concentration was prepared by mixing 2 x 104 ml per litre with 10 ml of 0.01% tween 20, a wetting agent as proposed by (Faleiro *et al.* 2004). According to Faleiro *et al.* 2004 the inoculated plants were covered by white transparent polythene bags to create and maintain relative humidity condition of (95%) for disease development and progress, then pressed in the cold room for incubation periods of 48 hrs, at temperature of 17^0C to 21^0C, while relative humidity were maintained at 95% according to Harter *et al.* (1935). After incubation period, the plants were removed from the cold room, allowed to dry, and then shifted into greenhouse for disease score.

3.3.3 Disease reaction and score

According to Stavely *et al.* 1983; and Mmbaga *et al.* 1996), the disease reaction started at 10 to 14 days after inoculation. Disease score was done 15 days after inoculation, three times per week for three weeks. Rust symptoms on leaves were scored 15 days after inoculation, using bean rust grading scale for 1-6 (Stayvel and pastor- corrales, 1989) and (Stavely, (1993) for the evaluation of rust in the greenhouse, based on both pustule size and type See appendix 3. Pustules diameters were converted into quantitative disease scores for statistical analysis Mmbaga *et al.* 1996). See appendix 3. Analysis of variance (ANOVA) was calculated to determine the main effect of genotypes. F- Test was used to determine if this effect was significant.

3.4 Data Collection

3.4.1 Field experiment

Data in the field were collected from ten middle bean plants. Plant growth habit and yield were recorded; include plant stand counts at 2 weeks after emergence, plant stand counts at harvest stage, days to 50% flowering, crop vigor score on a scale of 1-9 (CIAT, 1987), days to 50% maturity of green pods, pod length, pod width, and bean leaf rust score on a scale of 1-9 CIAT, (1987) see appendix 4 & 5.

3.4.2 Plant stand after emergence and at harvest

Number of plants emerged were recorded ten days after sowing (germination data), and number of plants stand at the stage of harvest, by using standard system for the evaluation of beans from CIAT, (1987).

3.4.3 Plant vigour

Plant vigour were scored on a 1 to 9 scale, where 1=excellent and 9= very poor. Vigor was scored within the first three weeks after emergence.

3.4.4 Days to 50% flowering and days to 50% maturity

Days to 50% flowering were measured as actual number of days from planting to initiation of developmental stage R6, when approximately 50% plants in a plot have at least one opened flower. For days to 50% maturity were measured as actual number of days from planting to the initiation of developmental stage R9, when approximately 50% of plants in a plot have at least one mature green pod (Hall, 1991). These data were used in analysis.

3.4.5 Plant height (cm)

Plant height was taken by measuring distance from the plant base (soil surface) to the tip of the main shoot. The mean value of 10 plants in each plot was recorded, and used in analysis.

3.4.6 Number of pods per plant and pod weight (g)

Number of pods per plant was recorded by counting total number of pods present from 10 plants and average of the plot computed for the ten plants. Total weight in grams of pods from 40 plants which obtained after four times of harvesting, and each harvest only 10 plants as a sample were taken, giving a total of 40 plants. Harvesting mature pods from 10 plants in two rows, equivalent to 3 m^2, all harvesting pods were weighed without grading. The fresh weight of each genotype was determined at each

harvest and the cumulative yield for the three weeks calculated at the end of the harvest period.

Plate 4: Snap bean pods in the field before harvesting (65 days) at Seatondale. Iringa site

3.4.7 Pod length (cm) and width (mm) measurements

At each harvesting time, 10 pods were randomly selected and their length in centimetres was measured, and average computed. Pod width was taken after harvesting of the pod as extra fine (6 mm), fine (6-8 mm) and bobby (>8 mm) and length of the pods above 10 cm (HCDA, 2009), by measuring 10 pods from randomly selected sample of each plot using a Vanier calliper, and average computed.

Plate 5: Harvested pods from different snap bean genotypes for data collection (Source: ARI uyole, 2012)

3.4.8 Bean pod fibres

Fiber content of harvested Snap bean pods was determined or assessed by random selection of 10 pods from each genotypes, then broke the pod, and observe visually and rating on a scale of 1-5 where (score 1 = snapped clean, score 5 = did not snap, had excessive string and seed development) then recorded (CIAT, 1987).

3.4.9 Disease severity score in the field

Disease scores were recorded every two weeks until maturity (Visual estimates), using 0 to 9 scales, developed by Van Schoonhoven and Pastor-Corrales, (1987). Mean disease scores were calculated for each genotype and used to determine the level of reaction to the pathogen. See appendix 4 and 5.

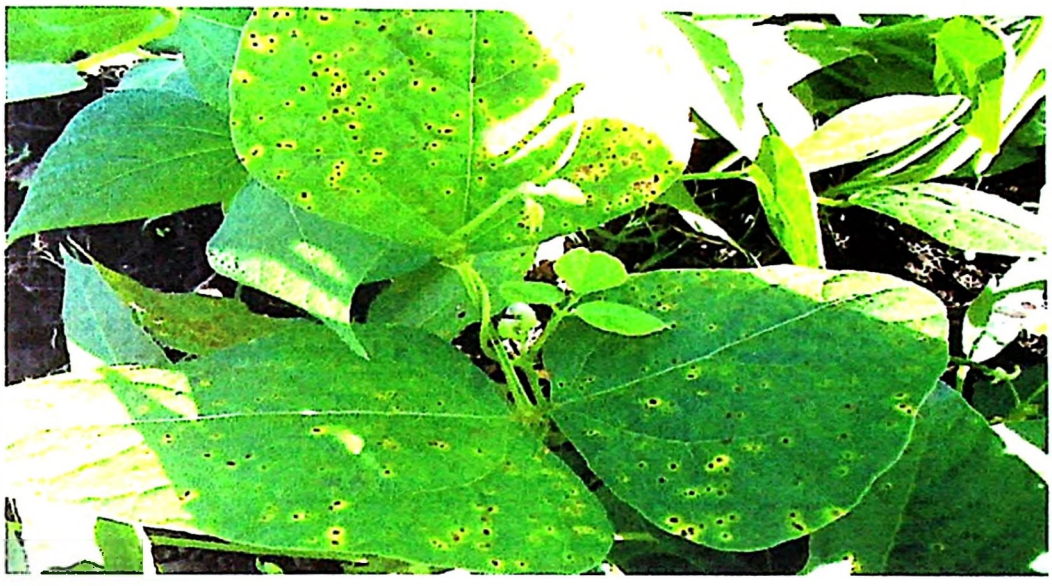

Plate 6: Infected snap bean (Teresa variety) in the field at Mbimba, Mbozi site

3.5 Screen House Experiment

Disease severity data was collected by using descriptive grading scale for 1-6 in appendix 3 (Stavely and pastor- corrales, 1989).

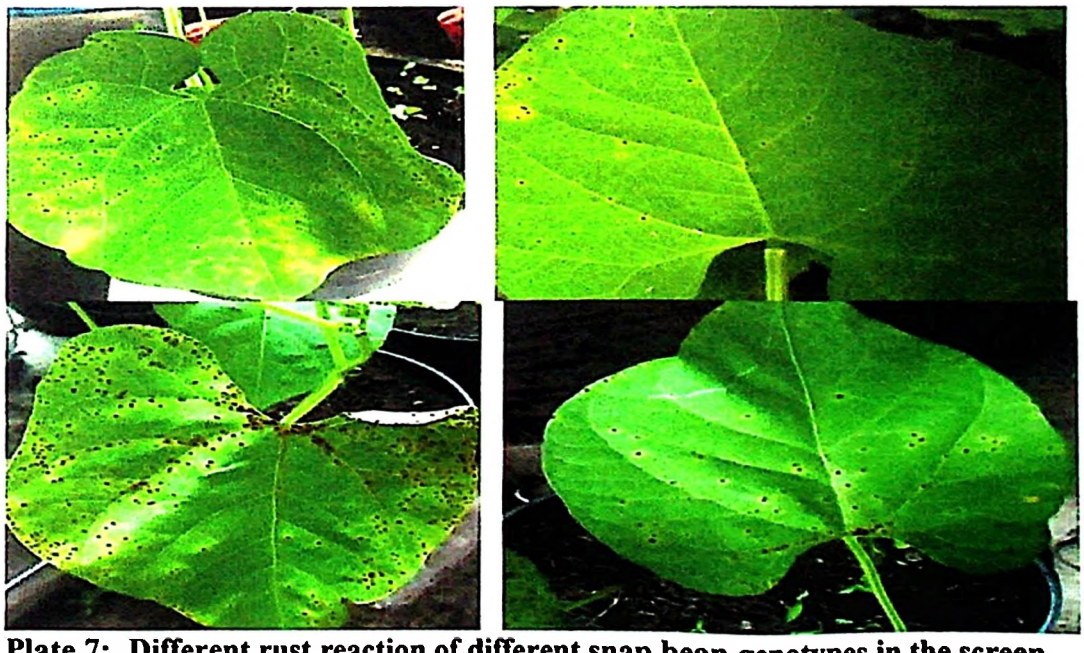

Plate 7: Different rust reaction of different snap bean genotypes in the screen house after inoculation at Uyole 20 days after inoculation

3.6 Data Analysis

Quantitative data collected from the experiment were subjected to combined analysis of variance (ANOVA) using the ANOVA procedure of GenStat 14 Edition, 2012. Genotypes treatment was treated as fixed effects. Location was considered to be a random effect. Differences among the genotypic means were compared using the Fisher's protected LSD test at 5% probability level.

3.6.1 Analysis of variance

3.6.2 Single site analysis

Data was analyzed using ANOVA in GenStart 14.2 Edition software and means were separated using the Fisher's Protected LSD Test. Data were firstly subjected to analysis of variance for each location using the procedure given by Gomez and Gomez (1984) for RCBD.

3.6.3 Combined analysis

Combined analysis of variance was performed. The statistical model used was $Y_{ijkl\ m}$

$= \mu + L_{(i)} + R_{j(i)} + T_{(k)} + LT_{(ik)} + E$

$Y_{ijkl\ m}$ = Yield effect

μ= mean effect

$L_{(i)}$ = i^{th} location effect

$R_{j(i)}$ = j^{th} replication (Block) within i^{th} location

$T_{(k)}$ = k^{th} Treatment effect (Genotypes)

$LT_{(ik)}$ = interaction effect of i^{th} location and ik genotypes

$E_{(a)}$ = Error (random error)

3.6.4 Correlation analysis

Simple correlations among yield components and disease severity variable were computed using GenStart 14.2 Edition software package for each location and for combined location

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Genotypes and Location on Growth Parameters of Selected Snap Bean Genotypes for 50% Flowering, 50% Maturity, and Plant Height and Bean Rust Score (mean square)

Summary of combined analysis of variance of the studied variables is shown in Table 1. Locations showed highly significant variations ($P \leq 0.001$) for all the variables. Snap bean genotypes displayed significant difference in all variables, except plant height. Significant effects were also observed on location x genotype (interaction between genotypes, and location), for all variables except plant height (Table 1).

Table 1: Summary of analysis of variance (ANOVA) for effect of genotypes and location on 50% flowering, 50% maturity, and plant height (cm) mean square (Field studies)

Source of variation	df	Days to 50% flowering	Days to 50% maturity	Plant height (cm)	Bean rust score%
Location $_{(L)}$	2	526.37***	362.31***	7261.68***	18.4793***
Replication	2	3.465ns	0.01ns	54.74ns	2.3459ns
Genotype $_{(G)}$	10	6.22***	1.98***	72.17ns	9.5021***
Error $_{(a)}$	4	1.42	0.01	19.01	0.6612
L x G	20	2.118*	1.98***	25.2ns	1.6239***
Error $_{(b)}$	60	1.08	0.01	23.41	0.5949

*, **, and *** indicate level of significance at 5%, 1% and 0.1%, respectively

Locations differed significantly ($P \leq 0.001$) from each other on days to 50% flowering where Mbimba was the latest at 43.6 days, followed by Uyole (42.3 days) and finally Seatondale was the earliest (36.1 days). For days to 50% maturity, location showed again highly significant difference ($P \leq 0.001$) in which Mbimba was the latest at 59

days followed by Uyole at 55 days while Seatondale was the earliest in maturity at (52.4 days). For plant height, highly significant different ($P \leq 0.001$) was observed, whereby Seatondale had the tallest plants (54.6 cm); Uyole had (29.1 cm) plants while Mbimba produced the shortest plants (28.7 cm). Mbimba differed significantly from other sites (Table 2). For bean leaf rust disease, location showed significant different ($P \leq 0.001$), whereby Uyole had less infestation (1.56) compared to Seatondale (2.02) and Mbimba (2.42) sites (Table 4).

Table 2: Effect of genotypes on days to 50% flowering, days to 50% maturity, plant height (cm), and rust scores for locations (Field studies)

Locations	Days to 50% flowering	Days to 50% maturity	Plant height (cm)	Bean rust severity score%
Uyole	42.3	55.0	29.1	1.56
Mbimba	43.6	59.0	28.7	2.42
Seatondale	36.1	52.4	54.6	2.02
Mean	**40.7**	**55.5**	**37.4**	**1.99**
SE±	**1.04**	**0.10**	**4.84**	**0.154**
SED	**0.86**	**0.08**	**3.92**	**0.45**
CV%	**2.6**	**0.2**	**12.9**	**22.3**
$LSD_{0.05}$	**1.72**	**0.16**	**7.82**	**0.84**

Genotypes differed significantly from each other across locations and the interaction. At seatondale; HAB 240 and HAB 403 tend to flower earlier than the check variety. At Uyole; HAB 414 and HAB 240 was among genotypes flowered earlier than others. At Mbimba; HAB 240, HAB 404, and HAB 449 were earlier in flowering than others (Table 3). Days to 50% maturity; there were significant different observed on genotypes across location and the interaction, whereby all genotypes matured at 55.33 days followed by the check variety (Teresa), which matured at 56.89 days.

Table 3: Variation in flowering of selected snap bean genotypes at three locations (Field study)

Genotypes	Mbimba	Seatondale	Uyole
HAB 240	42.0a	36.0a	41.0ab
HAB 403	45.3cd	36.0a	42.3abc
HAB 404	42.3ab	35.3a	41.0abc
HAB 414	44.0bc	36.3ab	41.0a
HAB 419	44.0bc	35.0ab	43.0cd
HAB 423	43.3ab	36.3ab	42.7bcd
HAB 425	43.7abc	35.7abc	41.7abc
HAB 427	42.7ab	37.0abc	42.3abc
HAB 442	43.0ab	36.3abc	42.3abc
HAB 449	42.3ab	35.3bc	43.3cd
Teresa (check)	46.3d	37.7c	44.3d
Grand mean	**43.6**	**36.1**	**42.3**

Means within sites/column followed by same letter(s) are not significantly different from each other according to Fisher's protected LSD at 5% level.
$LSD_{0.05}$ for site = 0.81, $LSD_{0.05}$ for among Snap bean genotypes = 0.98
$LSD_{0.05}$ for site by genotype interaction = 1.72

Table 4: Variation in bean leaf rust severity scores of selected snap bean genotypes at three locations (Field study)

Genotypes	Mbimba	Seatondale	Uyole
HAB 240	2.14ab	1.19a	1.19a
HAB 403	2.33bc	2.53d	1.51a
HAB 404	3.11de	1.97bc	1.44a
HAB 414	2.97cde	2.14bcd	1.41a
HAB 419	1.50a	1.78b	1.01a
HAB 423	2.87bcde	2.28cd	1.22a
HAB 425	2.79bcd	1.22a	1.32a
HAB 427	1.50a	1.25a	1.44a
HAB 442	1.56a	2.06bc	1.60a
HAB 449	2.47bcd	2.25cd	1.42a
Teresa (check)	3.47e	3.58e	3.56b
Grand mean	**4.42**	**2.02**	**1.56**

Means within sites/column followed by same letter(s) are not significantly different from each other according to Fisher's protected LSD at 5% level.

4.2 Effect of Genotypes and Location on Pods per Plant, Pod length (cm), Pod Yield, and Pod Weight (g) Growth Parameters (mean square)

There was highly significant difference at ($P \leq 0.001$) across sites on number of pods per plant, pod yield, and pod weight (g), except pod length (cm). Selected snap bean genotypes showed highly significant difference at ($P \leq 0.001$) on the number of pods per plant and pod yield, across locations, while pod weight (g) and pod length (cm) had no significant at ($P \leq 0.05$). Interaction between genotypes and location were significantly different at ($P \leq 0.01$) for the number of pods per plant and pod yield (g) per 10plants (Table 5).

Table 5: Summary of analysis of variance (ANOVA) for effect of genotypes and location on pods per plant, pod length (cm), pod yield, and pod weight (g)

Source of variation	df	Pods per Plant	Pod yield	Pod weight (gm)	Pod length (cm)
Location $_{(L)}$	2	631.78***	252711***	5021832***	41.463ns
Replication	2	6.07ns	2428ns	217358ns	7.498ns
Genotype $_{(G)}$	10	17.23***	6893***	264226ns	9.383ns
Error $_{(a)}$	4	3.64	1458	98507	14.973
L x G	20	10.75**	4302**	143874ns	4.182ns
Error $_{(b)}$	60	4.54	1816	83907	4.369

*, **, and *** indicate level of significance at 5%, 1% and 0.1%, respectively

Effects of location on the variables studied are indicated in (Table 6). Locations displayed highly significant difference from each other at ($P \leq 0.001$). Uyole had the highest number of pods per plant (7.71), Seatondsale had (6.52), while Mbimba produced the lowest (3.47) number of pods per plant, the difference was significant from the other sites (Table 6).

Table 6: Effect of genotypes on pods per plant, pod yield, and pod weight (kg), pod length (cm) and bean rust disease score for locations (Field study)

Variety/ location	Number of pods per plant	Pod yield	Pod weight (Kg)	Pod length (cm)	Bean rust score%
Uyole	7.71	308.5	1326	11.72	1.56
Mbimba	3.47	138.9	661	10.89	2.42
Seatondale	6.52	260.9	1348	11.95	2.02
Mean	**5.90**	**236.1**	**1112**	**11.52**	**1.99**
SE±	**0.288**	**11.51**	**94.6**	**0.58**	**0.15**
SED	**0.235**	**9.40**	**77.3**	**0.48**	**0.45**
CV%	**4.9**	**4.9**	**8.5**	**5.10**	**22.30**
$LSD_{0.05}$	**0.652**	**26.10**	**214.5**	**1.32**	**0.84**

Highly significant variations at ($P \leq 0.001$) was also observed on pod yield whereby; Uyole yielded the highest (308.5 pods) followed by Seatondale (260.9 pods) and lastly was Mbimba site (138.9 pods) from ten middle plants. Highly significant difference at ($P \leq 0.001$) was also observed on pod weight (g), where Seatondale had the heaviest (1348 g) followed by Uyole (1326 g) which did not differ significantly from Seatondale, and the least was Mbimba (661g).

There was highly significant difference at ($P \leq 0.001$) among selected snap bean genotypes across locations on number of pods per plant, and pod yield. At Uyole; HAB 427 was the genotype with the highest number of pods per plant (9.19), followed by HAB 442 (8.36), compared to the check variety Teresa (6.55). At Seatondale; HAB 425 had the highest number of pods per plant (10.47), followed by HAB 404 (7.56), compared to Teresa (6.20). At Mbimba; HAB 425 had produced the highest number of pods per plant (4.03), and all genotypes produced low number of pods per plant compared to the check variety Teresa (5.08). Generally; genotype

HAB 425 produced highest number of pods (7.52), followed by HAB 414 (5.88) and HAB (442), and while HAB 419 was the lowest produce at all sites (Table 7).

At Uyole; HAB 427 was the genotype with the highest total pod yield per plant for entire growing season (367.7), while the lowest was HAB 419 (274.7), At Seatondale; HAB 425 (418.7) had the highest followed by HAB 404 (302.3). The lowest total pod yield was obtained with HAB 419 (209), produced lower than the check variety (248.0). At Mbimba; total pod yield was low compared to other sites, HAB 425, and HAB 427 were the only genotypes produced high, though did not exceed the check.

Generally; HAB 425 (300.9), HAB 427 (264.0), and HAB 404 (246.2) had the highest total pod yield in all sites. The lowest total number of pods was obtained in genotypes HAB 419, produced lower than the check variety in all sites except at Uyole (Table 7).

Table 7: Variations among snap bean genotypes and locations on pod yield

Genotypes	Mbimba	Seatondale	Uyole
HAB 240	34.4ab	56.7a	71.8ab
HAB 403	39.2ab	53.7a	80.8abc
HAB 404	33.1ab	75.6ab	76.0abc
HAB 414	30.9ab	63.3ab	82.1abc
HAB 419	25.8a	52.3a	68.7ab
HAB 423	34.3ab	58.3ab	73.3ab
HAB 425	40.3ab	104.0b	80.7abc
HAB 427	37.1ab	69.0ab	91.9c
HAB 442	26.6a	61.2ab	83.6bc
HAB 449	29.3a	60.9ab	74.2ab
Teresa (check)	50.8b	62.0ab	65.5a
Grand mean	**34.7**	**65.2**	**77.1**

Means within column followed by same letter(s) are not significantly different from each other according to Fisher's protected LSD at 5% level.

At Seatondale; HAB 425 obtained the highest pod weight (g) (508.2), followed by HAB 404 weighed at (460.2), while the lowest genotype was HAB 240 (220.3), and weighed lower than the check variety (267.8). At Uyole; HAB 442 (412.1) had the highest pod weight followed by HAB 403 (369.1). The lowest pod weight was obtained with HAB 240 (243.2), produced lower than the check variety (255.5). At Mbimba; total pod weight was low compared to other sites. HAB 404 and HAB 404 had the same pod weight (179.3) and (179.3) respectively; genotypes HAB 419 (141.7) displayed the lowest pod weight, while HAB 404 and HAB 425 genotypes had the highest pod weight, though did not differ significantly from each other (Table 8).

Table 8: Variations among snap bean genotypes and locations on pod weight (g)

Genotypes	Mbimba	Seatondale	Uyole
HAB 240	173.4bc	220.3a	243.2a
HAB 403	178.3bc	254.2a	369.1ab
HAB 404	179.3bc	460.2bc	397.5b
HAB 414	160.0ab	336.6abc	368.0ab
HAB 419	141.7a	368.0abc	258.6a
HAB 423	173.3bc	281.2ab	305.2ab
HAB 425	160.0ab	508.2c	355.9ab
HAB 427	169.2ab	285.9ab	329.7ab
HAB 442	160.8ab	331.2abc	412.1b
HAB 449	168.3ab	393.5abc	350.7ab
Teresa (check)	155.6a	267.8a	255.5a
Grand mean	**165.4**	**337**	**331**

Means within sites/column followed by same letter(s) are not significantly different from each other according to Fisher's protected LSD at 5% level.

4.3 Effect of Genotypes and Location on Disease Severity (mean square)

Some disease incidences, Rust (*Uromyces appendiculatus* (pers.), Angular leaf spot (*Phaeoisariopsis griseola*) and Ascochyta (*Ascochyta boltshauseri*) were observed

especially from late vegetative stage to maturity on all selected Snap beans genotypes and the check variety Teresa. Bean leaf rust disease showed highly significant difference at ($P \leq 0.001$) on the genotypes and the interactions, while angular leaf spot (ALS) and ascochyta diseases were no significant differences on location, genotypes and interaction (Table 9).

Table 9: Summary of analysis of variance (ANOVA) for effect of genotypes and location on rust severity (Field study)

Source of variation	df	Bean rust (1-9)	Angular leaf spot (1-9)	Ascochyta (1-9)
Location $_{(L)}$	2	18.4793ns	13.52502ns	2.5567ns
Replication	2	2.3459ns	0.08948ns	26.1339ns
Genotype $_{(G)}$	10	9.5021***	3.17590ns	2.1704ns
Error $_{(a)}$	4	2.5251	0.53691	0.5369
L x G	20	1.6239***	2.92581ns	3.3050ns
Error $_{(b)}$	60	0.5949	1.70448	3.0206

*, **, and *** indicate level of significance at 5%, 1% and 0.1%, respectively

There was highly significant different observed among genotypes and the interaction at ($P<0.001$), but all Snap beans genotypes showed least attack by all diseases. At Uyole, mean bean rust disease scored at (1.56), Angular leaf spot at (2.30), and Ascochyta disease at (3.44). At Mbimba site, mean bean rust scored (2.42), Angular leaf spot (2.82), Ascochyta (3.21). At Seatondale, rust scored (2.02), Angular leaf spot, (2.56), and Ascochyta disease at (3.33) (Table 10).

Table 10: Means of disease scores from three diseases on snap bean genotypes at each of the three locations (Field study)

Location	Rust severity (1-9)	Angular leaf spot (1-9)	Ascochyta (1-9)
Uyole	1.56	2.30	3.44
Mbimba	2.42	2.82	3.21
Seatondale	2.02	2.56	3.33
Mean	**1.99**	**2.56**	**3.33**
SE±	**0.28**	**0.13**	**0.13**
SED	**0.23**	**0.10**	**0.10**
CV%	**13.8**	**5.00**	**3.80**
$LSD_{0.05}$	**0.63**	**0.45**	**0.45**

Bean leaf rust disease scores were highly significantly different among genotypes ($P<0.001$). HAB 240 genotypes was the least attacked by rust at Seatondale and Uyole followed by HAB 427, but at Mbimba site, the genotypes was highly affected by the disease, while the check variety Teresa was highly affected by rust at all the sites. HAB 419 and HAB 442 were relatively least infected by bean leaf rust at all locations. All genotypes showed least severity of bean rust compared to the check variety (See fig 2). Generally; HAB 240, HAB 425, and HAB 427 were the least attacked by rust. HAB 419 showed relatively least infected by the disease. HAB 403, HAB 449, and HAB 423 had a higher severity of the disease at all sites (Fig. 2).

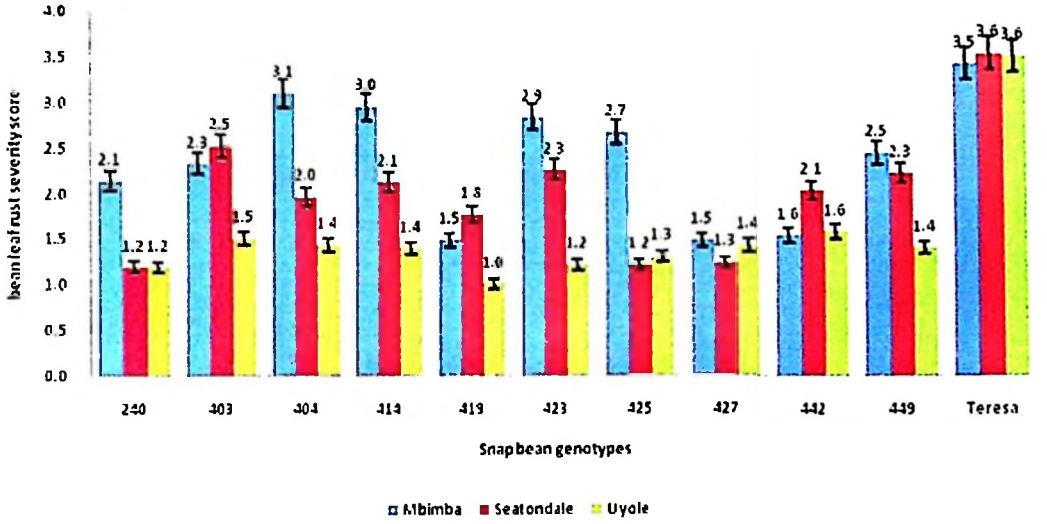

Figure 2: Bean leaf rust disease reaction over the entire growing periods of the three locations P≤ 5% value (Field study)

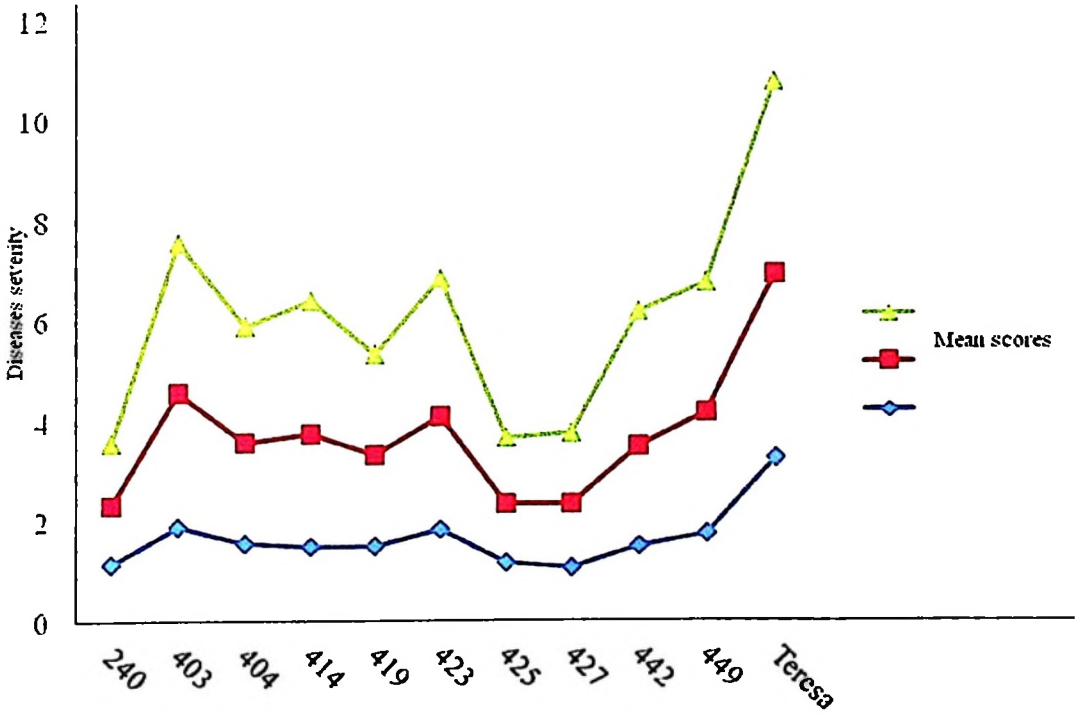

Figure 3: Epidemiology of bean leaf rust on snap bean genotypes at different disease score date (Field study)

4.4 Relationship Between Days to 50% Flowering, Days to 50% Maturity, Plant Height (cm), Total Pod Yield, Total Pod Weight (g), Number of Pods Per Plant, Pod Length (cm), and Bean Leaf Rust Severity

4.4.1 Correlation analysis

Results of combined correlation analysis among studied variables are shown in Table 13. Yields in terms of total number of pods were found positively and significant correlations with number of pods per plant ($r = 0.831^{**}$), total pod weight (g) ($r = 0.831^{**}$), and bean leaf rust ($r = 0.660^{**}$). There was also positive and significant correlations observed between days to 50% flowering with total pod weight ($r = 0.501^{**}$), days to 50% maturity ($r = 0.805^{**}$), bean leaf rust ($r = 0.701$), total pod yield ($r = 0.317^{**}$), and number of pods per plant ($r = 0.501^{**}$). There were also positive and significant correlations displayed between plant height (cm) with pod length ($r = 0.418^{**}$), total pod yield ($r = 0.218^{*}$), total pod weight ($r = 0.402^{**}$), and number of pods per plant ($r = 0.402^{**}$).

Positive significant correlation was also observed between days to 50% maturity with bean leaf rust ($r = 0.519^{**}$), total pod yield ($r = 0.609^{**}$), and total pod weight ($r = 0.641^{**}$). Total pod weight showed positive significant correlations with number of pods per plant ($r = 0.519$), and pod length ($r = 0.206^{*}$). Bean leaf rust severity from all sites displayed significantly and negatively correlated, except on total pod yield, days to 50% flowering, and days to 50% maturity (Table 11).

Table 11: Simple correlation among studied variables in combined analysis for various bean genotypes

		1	2	3	4	5	6	7
1.	Days to 50% flowering							
2.	Days to 50% maturity	0.805**						
3.	Plant height (cm)	-0.879**	-0.755**					
4.	Total pod yield	0.317**	0.609**	0.218*				
5.	Total pod weight (g)	0.501**	0.641**	0.402**	0.831**			
6.	No. of pods per plant	0.501**	-0.641**	0.402**	0.831**	1.000**		
7.	Pod length (cm)	-0.371**	-0.300**	0.418**	-0.107	0.206*	0.206*	
8.	Bean leaf rust	0.701	0.519**	-0.079	0.660**	-0.323**	-0.323**	-0.017

*, **, correlation is significant at the P≤ 5% and P≤ 1% respectively

4.5 Effect of Bean Leaf Rust on snap Bean Genotypes after Artificial Inoculation in the Screen House

Table 12: Analysis of variance for bean leaf rust quantitative disease score on bean leaves 14 days after inoculation in screen house studies

Source of variation	df	Mean squares
Replication	2	13.7098ns
Genotype $_{(G)}$	10	5.3546***
Error $_{(a)}$	4	0.9830

*, **, and *** indicate level of significance at 5%, 1% and 0.1%, respectively

Statistical analysis shows that there was a highly significant difference at ($P \leq 0.001$) in disease reactions between snap bean genotypes Table 12. The disease symptoms showed in leaves were significantly different among genotypes used. Leaves of control (Not inoculated) did not show any disease symptoms. All snap bean genotypes showed disease symptoms in different rates. HAB 427, HAB 425, and HAB 240, showed high levels of resistance to bean leaf rust, compared to other selected snap bean genotypes, Genotypes HAB 423, HAB 419, and 442. Variety Teresa and HAB 414 were the most susceptible with a score of up to 5.0 Table 13.

Table 13: Average means of bean leaf rust disease score of half expanded primary leaves 17 days afterinoculation with *Uromyces appendiculatus* in screen house

Genotypes	Cumulative average scores for three weeks
HAB 240	2.8abc
HAB 403	4.8de
HAB 404	3.96 fed
HAB 414	5.46 fe
HAB 419	3.5abc
HAB 423	3.4abc
HAB 425	2.6ab
HAB 427	2.3a
HAB 442	3.5bc
HAB 449	4.0cd
Teresa	5.0de
$LSD_{0.05}$	**1.31**
CV%	**20.6**

CHAPTER FIVE

5.0 DISCUSSION

Results of this study showed significant differences among snap bean genotypes for all the studied variables across locations, and within locations except for pod length (cm) and disease severity. The performance of genotypes was different from site to site (different environments) due to genetic variations and environmental. When genotypes are compared in different environments, their performance relative to each other may not be the same. One genotype may have a higher yield in some environments, while other genotype may do extremely well in others. The environment usually; relates to the set of climatic, soil, abiotic stress, biotic stress (insect pests and diseases) and management conditions in an individual trial that are carried out at a given location Gebeyehu and Assefa, (2003).

Falconer and Mackay, (1996) stated that changes in the relative performance of genotypes across environments (genotype x environment interactions) automatically imply that the behaviour of the genotypes depends upon the particular environment in which they are evaluated. Information on genotype x environment interactions is important to plant breeders for the development, selection and recommendation of cultivars that are suitable for growth in different environments. In this study, the significant genotype x location interaction revealed that there is existence of genetic differences among the 10 snap bean genotypes assessed, thus, superiority of a genotype is clearly conditioned by the environment and to the genotype x environment interaction. Genotype HAB

5.1 Number of Days to 50% Flowering and 50% Maturity

Highly significant differences displayed in the number of days required to reach 50% flowering and 50% maturity at Uyole, Seatondale, and Mbimba sites, as well as when the data were combined from both locations ($p \leq 0.001$). These results suggest that in addition to the presence of genetic variations, the flowering time among snap bean genotypes was also influenced by the environment, possibly due to variability in the temperature and rainfall of the three locations. Genotypes at Seatondale flowered and matured earlier because of a bit higher temperature, compared to other sites. In March during flowering time Uyole and Mbimba had mean temperature of 23.4°C and 21.3°C respectively while Seatondale had 24.5°C. Flowering is influenced by increase in temperature. High temperature facilitates node development hence earlier flowering and maturity. Low temperatures retard growth and development of some common bean genotypes. Ohashi *et al.* (2000) reported that there was decrease in growth, development and flowering duration in beans grown at low temperature. Variation in maturity may also be caused by altitude and temperature. Lower altitude induces earlier flowering, while a higher altitude causes later flowering. Seatondale was at lower altitude compared to Uyole location.

5.2 Yield and Yield Components Characteristics

The study showed significant differences between genotypes for the various characters across locations and within location. For plant height the study revealed that, Mbimba site had shortest plants compared to Seatondale and Uyole sites. This was possibly due to poor performance of snap bean genotypes and less adaptability, this resulted to a significant reduction of growth quality of the genotypes which is reflected by condensed plant height, number of pods per plant, total number of pods

and total pod weight. Type of the soil might be contributed to poor adaptability of genotypes. According to ARI- Uyole, Mbimba constitute a clay soil of compact characteristics with insufficient soil moisture. Vergara, (1976) and Ohashi, *et al.* (2000) reported insufficient soil moisture on the soil causes reduction of plant height due to decreased photosynthetic production and translocation to plant parts. Also Mbimba was the only site showed highly susceptibility in bean leaf rust severity. Possibly this also contributed to lower yield and other parameters. Steadman *et al.* (1986) stated the physiology and effect of bean leaf severity on snap bean especially on yield components that losses due to bean leaf rust can be dramatic, and damage to Snap bean is not only that causes a decrease in the number of seeds but also leads to a loss of pod quality and decreased seed size resulting from poor pod fill due to loss of photosynthate, but in other hand; Foster, (1947) observed that pod lesions caused by bean leaf rust, can also blemish pods in the case of green/snap beans which in turn result into poor yield.

The most accurate yield loss prediction is, however, obtained when rust severity is recorded approximately 2 weeks before maturity Lindgren and Steadman, (1992). Uyole site yielded higher almost all genotypes in terms of number of pods per plant, compared to other locations; this might be because of good adaptability of genotypes for the various qualities. This is of the same mind that the environment at Uyole appears to consent to easy favouritism of the best genotypes for expression of the potential and thus; prove to be a good breeding and production location. Despite of the fact that all genotypes at Uyole yielded high, these snap bean genotypes, HAB 425, HAB 427, and HAB 240 was the best yielder in number of pod per harvesting date, early in flowering, and maturity, while the same genotypes showed also good

resistance to bean leaf rust disease severity at all locations; therefore this seem to be best genotypes to grow in these areas due to their yield potential and resistance to diseases, could as well be grown in all localities where rust is a problem. On the pod quality, the genotypes observed to have thick and large pods which in terms of markets could be problem. Other genotypes were susceptible to diseases and thus management practices like use of fungicides on appropriate planting times that fall within periods of low infection rates, should be employed if at all are selected for production. Based on total pod weight, HAB 425, HAB 404, HAB 423 and HAB 442 displayed high pod weight compared to other snap bean genotypes in all locations. This also give chance to recommend these genotypes, when yield is measured in terms of pod weight.

5.3 Bean Rust Reaction

Appearance of highly significant differences for the bean leaf rust disease severity among the genotypes shows that variability for resistances existed among the genotypes for bean leaf rust. Araya *et al.* (2004) reported that over 300 races or pathotypes of bean leaf rust are recognised, indicating the broad inconsistency of the rust fungus under favourable environment found at the study sites, this also was observed that, the pathogen is capable of rapidly overcoming newly deployed resistance races. The significant difference observed among genotypes, indicates a possibility of obtaining snap bean genotypes with genes conferring resistance to *Uromyces appendiculatus* (Ur) for resistance from their parents, mean scores for locations, showed to be low, this may be either most of the genotypes consists genes for resistance or the level of disease was very low, as reported by Markell *et al.* (2009) and Pastor-Corrales *et al.* (2010). The severity of bean leaf rust on all

genotypes was less at Uyole and, Seatondale while at Mbimba the disease severity was higher, this confirm that different location might have different pathotypes. The high pathogenic variability of the bean leaf rust fungus has been reported to make most of snap bean cultivars grown in Eastern and Southern Africa to be susceptible by Hillocks *et al.* (2006) and Monda *et al.* (2003). Therefore, improvement of snap bean varieties with several genes of resistance to bean leaf rust is consequently essential in order to have power over the disease.

Based on the mean disease severity scores, in the screen house evaluation, with bean leaf rust resistance, HAB 425, HAB 427, and HAB 240, were observed among introduced snap bean genotypes to be resistant to the disease. Such genotypes that showed resistance to bean leaf rust, and possess trait of resistance might be used as a breeding materials in bean improvement strategies; However, the parental variety used as check in the current study did not exhibit resistance to the bean leaf rust, although according to Pastor-Corrales, (2006) reported that Teresa has intermediate resistance to bean leaf rust and had highest total pod yield.

5.4 Simple Linear Correlation

Results from this study indicated positive correlations between yield (total pod yield) and number of pods per plant, Pod weight, and bean leaf rust. These results have the same opinion with those of Niehuis and Singh, (1988); Adams, (1973) who found that yield was positively correlated with number of pods per plant. Effect of bean leaf rust on yield was also reported by Steadman *et al.* (1986a) that there was a highly significant correlation between bean leaf rust severity and yield, total number of

pods, total pod weight, total number of seeds and seeds per plant, and mentioned that early rust intensity could be used to predict yield loss.

Lindgren *et al.* (1995) reported the same result; that the relationship between bean leaf rust severity and yield loss due to bean leaf rust is linear and that an estimate of rust induced yield loss (in kg /ha) for any given growing season could be obtained by multiplying by 19 the percentage leaf area with rust symptoms at 72 days. These positive correlation traits are indicators for yield component, hence could be selected simultaneously in breeding programs.

Highly positive and significant correlations were also observed between number of days to 50% flowering and number of days to 50% maturity. This early flowering among genotypes is an indicator of early maturity, therefore; genotypes tend to flower early than others in all sites could be also considered in bean improvement. This result also consent with those of Mduruma and Nchimbi, (1994); Cerna and Beaver, (1990) who observed positive correlations between days to flowering and days to maturity, indicating a possibility of simultaneous selection for both traits. Significant correlation between days to 50% flowering and total pod yield and bean leaf rust, indicate that, early flowering in genotypes has influence on the yield, and this situation may lead for genotypes escape from attacked disease. This was also agreed by Stavely and Pastor-Corrales, (1989), who stated that yield losses caused by bean rust depends on the degree of susceptibility of Snap beans variety grown, the climatic conditions favouring rust infection, disease development, and earliness of the infection. He also concluded that, early infections occurring during the pre-

flowering and flowering stages of bean crop development usually result in higher yield losses. Therefore those genotypes flowered early and yielded high, it is the evidence that the severe disease infection occurred after flowering.

Significant correlation was between plant height and total pod yield and total pod weight, this is perhaps due to the fact that the higher the plant height, the higher the number of pods, thus; increased in pod yield and weight. Thus these pairs of variables are not antagonistic in their influence on yield. Also significant and positive correlations were observed between days to 50% maturity with total pod weight, total pod yield, and bean leaf rust. These results imply that early maturity among snap bean genotypes, lead into lowering disease infection and increased yield in terms of number and weight of pods, hence pod quality. Generally; bean leaf rust from all site expressed negative correlations, except in the total pod yield, days to 50% flowering and days to 50% maturity. This could be influenced by either low level of disease severity or late emergence of disease. Steadman *et al.* 1986a) reported a highly positive and significant correlation between bean leaf rust severity and yield, total number of pods, total number of seeds and seeds per plant, and mentioned that early rust intensity could be used to predict yield loss.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The study conducted during the season 2011/2012, to assess the adaptability of ten snap bean genotypes and resistance to bean leaf rust. The adaptability were evaluated on days to 50% flowering, 50% maturity, plant height, number of pods per plant, total pod yield, total pod weight and bean leaf rust incidences and severities. Pod yield data were analyzed to get descriptions of genotype adaptability across environments and unfairness among genotypes for each of the environments. Bean leaf rust incidences and severities differed among genotypes and across sites.

High yielding genotypes were identified as HAB 425, HAB 427, and HAB 423, while the same genotypes and HAB 240 inclusive were shown promising in bean leaf rust resistance. These genotypes could be recommended to be grown in high yielding areas and where bean rust is the serious biotic factor. Results show HAB 427, HAB 240, HAB 419, HAB 404, and HAB 423 as being stable for the flowering variable and they are earliest hence could be recommended to be grown in marginal areas where rain period is short, hence can escape both disease (notably bean rust) and water stress which affect flowering.

It was also revealed that plant height, number of pods per plant, and days to 50% maturity have high and positive correlations with yield. The genetic improvement of these characteristics is necessary in order to confer better yield. The psychological

effect of diseased beans on farmers without a doubt has an influence on their choice of planting material, but the final determining factors must be yield and quality of pods, and field resistance is perhaps best measured in these terms without relying on greenhouse results (Artificial condition). This would benefit from an understanding of the mechanisms responsible for stable yield in cultivars in spite of significant amounts of rust. Perhaps researchers should study "durable yield" instead of "durable resistance" and Selection of superior genotypes based on yield as such, is not effective. For a rational approach towards the improvement of yield, selection has to be made for the components of yield such as days to flowering, days to maturity, plant height, and pods per plant, pod size, pod shape, and pod colour. These results donate significant information about the range of the genetic material from Southern Highlands of Tanzania and information from the evaluated genotypes will be relevant to breeders fascinated in widening genetic base of current Snap bean cultivars.

It was observed, the perfect moisture condition provided with humidifiers and mist or dew chambers can be provided simply by covering the plant with polythene bags in the incubation period. This simplified method is an easy and economic procedure for rust resistance screening of beans or determining *uromyces appendiculatus* races.

6.2 Recommendations

These results and observations have been based on only one growing season data. It is recommended that, data for more seasons be included in evaluations due to some of the snap bean genotypes were still segregating, in which a certain genotype may show promising traits while later it will lose it. This will give a better estimate of the

snap bean genotypes adaptability and resistance to bean leaf rust, thus bring more viable recommendations.

There is, however, a need for effective sources of true pathotypes and nonspecific forms of resistance to be undertaken. Although the experience of field resistance has received attention, the need for the understanding and application of this resistance type in breeding is challenging.

In Tanzania, where population growth often exceeds food production, and soils are rapidly becoming depleted of nutrients, reliable yield under natural conditions, without the intervention of fungicides, is of paramount importance. Therefore the techniques used to study the mechanisms of durable resistance of bean leaf rust pathogen are often difficult to use, and new methods that enable quick and easy screening are needed.

For example the use of molecular markers (Mass Assisted selection) breeding for disease resistance not only for bean leaf rust but also for other diseases and other crops; although the real measure of success is yield and quality under field conditions. Markers and greenhouse screening do not provide an easy way out, and they must remain a tool and not the aim of any breeding program.

The development of a set of near isogenic lines as differentials, each carrying a single resistance gene, is needed. This will facilitate race characterization and mapping as well as effective resistance breeding. This will go hand to hand with an

increasing demand for organically grown food that might be an added incentive for resistance breeding, integrated with cultural practices that lower inoculum and disease levels. Finally; breeders in bean improvement programs, should now focus on pyramiding breeding where by multiple disease resistance genotypes will be obtained and made available.

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APPENDINCES

Appendix 1: Fresh vegetable exports from Tanzania to target countries, 2000-2005 (tonnes)

	Snap beans	Peas	Sweet corn	Leeks	Totals
UK	2,077	609	227	31	2,994
Netherlands	516	296	2	0	814
France	124	31	0.5	0	155.5
Germany	5	5	0	0	10
Total	2,722	941	229.5	31	3 923.5

Source: Euro stat.

Appendix 2: Tanzanian fresh vegetable export growth, 2001 – 2005 (tonnes)

	2000	2001	2002	2003	2004	2005
UK	47	296	390	221	855	1,134
Netherlands	0	13	13	2	379	408
France	0	0	0	44	105	6
Germany	0	3	0	0	5	2
Total	47	312	403	267	1344	1550

Source: Euro stat.

Appendix 3: Description of reaction types (grades) of *Uromyces appendiculatus* virulence and their conversion to quantitative disease score for statistical analysis

Reaction type	Description	Quantitative disease score
1	Immune, no visible symptoms	1.1
2	Necrotic spots, without sporulation	2.1
3	Uredinia less than 0.3 mm in diameter	3.1
4	Uredinia 0.3 – 0.5 mm in diameter	4.1
5	Uredinia 0.5 – 0.8 mm in diameter	5.1
6	Uredinia larger than 0.8 mm in diameter	6.1

Appendix 4: Standard ratings scale (1 – 6) for the evaluation of rust in the Greenhouse and Field as recognized at the First International Bean Rust Workshop, based on both pustule size and type

Reaction type and value	Descriptions and size
1	Immune: absence of pustules or flecks
2	Necrotic spots without sporulation (HR)
2	<0.3mm
2+	0.3–1mm
2++	1–3mm
2+++	>3mm
3	Sporulating pustules <0.3mm
4	Sporulating pustules 0.3–0.5mm
5	Sporulating pustules 0.5–0.8mm
6	Sporulating pustules >0.8mm

Source: Stavely *et al.* 1993

C (or c) = Small faint chlorotic halo

C+ = Large, intensely yellow chlorotic halo

N= (with appropriate number of '+' signs) for necrosis plus sporulating pustule size (3–6)

f2 = Faint "2"; used to indicate very small, pale necrotic spots

Appendix 5: Standard ratings scale (1 – 9) for the evaluation of rust intensity in the Greenhouse and Field as recognized at the First International Bean Rust Workshop, based on both pustule size and type

Reaction type	Descriptions
0 or 1	No visible sign of rust
2	Very little, isolated pustules. Not easy to sight, often only underside of leaves
3	Pustules few but easy to sight, often only on underside
4	±7.5% of the leaf covered with pustules, very easily seen, may be limited to underside of leaves
5	±10% of the leaf covered with pustules, very easily seen, may be limited to underside of leaves
6	Many pustules – very little or no defoliation
7	Many pustules, premature defoliation has started (1/3 of leaves)
8	Leaves full of pustules, premature defoliation (1/3 – 2/3 of leaves)
9	Leaves very full of pustules, premature defoliation is general (>2/3 of the leaves)

Source: Van Schoonhoven and Pastor-Corrales 1987

2 = Necrosis without pustules

N = Necrosis with sporulating pustule, e.g N++4

Appendix 6: Experimental sites

Location	District	Latitude	Longitude	Altitude (m)
Uyole	Mbeya urban	S 08^0 56'	E 033^0 06'	1795
Mbimba	Mbozi	S 8^0 57'	E 033^0 13'	1241
Seatondale	Iringa urban	S 57^0 79'	E 035^0 77'	1539

Appendix 7: Data for soil analysis from three sites (Uyole, Mbimba and Seatondale)

	UYOLE		MBIMBA		SEATONDALE	
Parameter	Value	Comments	Value	Comments	Value	Comments
Sand	40		33		62	
Clay	34	Clay loam	55	Clay	12	Sandy loam
Silt	26		12		14	
Soil pH (H_20)	6.83	Slightly acidic	5.91	Slightly neutral	6.80	Slightly acidic
%TN mg/kg	0.140	Low	0.154	Low	0.19	Low
%OC mg/kg	2.30	medium	2.11	medium	2.20	medium
Brl Ext.P Mg/kg	5.69	Low	3.23	low	19.60	high
CEC $cmol_ckg$	20.6	high	22.8	high	13.6	medium

Appendix 8: Weather data for Uyole and Mbimba sites

Month	Uyole		Mbimba	
	Rainfall (mm)	Temperature (max.)	Temperature (min)	Rainfall (mm)
September 2011	0.0	24.60	11.2	0.0
October	26.00	26.70	13.2	22.4
November	52.00	25.50	14.7	237.80
December	243.80	22.70	14.6	387.10
January 2012	209.00	23.30	14.9	376.20
February	123.00	22.90	14.9	406.80
March	201.80	23.40	13.2	176.00
April	107.90	23.10	12.2	91.80
May	27.60	22.30	11.1	4.0
June	7.90	21.40	7.8	6.5