

MAKERERE



UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

SCHOOL OF AGRICULTURAL SCIENCES

**SCREENING OF SCHELEROTIUM ROT ROOT RESISTANT BEAN LINES FOR
FUSARIUM SPP RESISTANCE AT NACRRI IN WAKISO DISTRICT**

SSENTONGO ROY

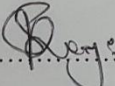
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**A SPECIAL PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF
AGRICULTURAL PRODUCTION, SCHOOL OF AGRICULTURAL SCIENCES IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF A
BACHELORS OF SCIENCE IN AGRICULTURE**

AUGUST, 2018

DECLARATION

To the best of my knowledge and awareness, I SSENTONGO ROY declare that the work submitted in this Special Project report is my own and original. The report has never been submitted in any other institution for any academic award.

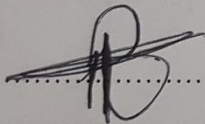
Signature.......... Date.....1st/09/2018.....

SSENTONGO ROY

BSc Agriculture IV

APPROVAL

This special project report has been submitted to College of Agricultural and Environmental Sciences, School of Agricultural Sciences, Department of Agricultural Production with my approval;

Signature.......... Date.....1/09/18.....

PROFESSOR PHINEHAS TUKAMUHABWA

ACADEMIC SUPERVISOR

DEDICATION

I dedicate this report book to my parents Mr. & Mrs. Ssentongo, my siblings Lukwago Raymond, Kawuki Raphael, Namugerwa Ritah and Nakanjjako Rosette, Mr Allan, and my dear friends Owachigui Job and Openytha James for the financial, emotional and physical support they have offered me. May the Almighty God bless and reward your generosity accordingly.

ACKNOWLEDGEMENT

My greatest appreciation and thanks go to the Almighty God the provider of life and wisdom. I also greatly appreciate my parents who have managed to sponsor my academics.

I pass my sincere gratitude to Professor Phinehas Tukamuhabwa, for giving me the theoretical and practical background and guidance I needed to successfully carry out my experiment and write this report. I also thank Dr. Narisi Mubangizi, Dr. Richard Edema, Dr. T L Odong and all my lecturers at the School of Agricultural Sciences for the efforts and contributions to my agricultural knowledge and skills.

I extend my sincere thanks to National Crops Resources Research Institute (NaCRRI), Legumes Program which accepted, welcomed and facilitated my research topic and allowed me to carry out the study using institute and program facilities.

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ABSTRACT

Fusarium root rot (*Fusarium solani f.sp. phaseoli*) is one of the most important disease affecting common beans (*Phaseolus vulgaris L.*). This research was conducted at National Crops Resources Research Institute (NaCRRI) to determine the bean lines resistant to *Fusarium spp* and the virulence of the *Fusarium spp* isolates. A complete randomized experimental design was used with two replications of each treatment. There were two factors under investigation which are *Fusarium spp* isolates F890 and F386.

Thirty (30) treatments comprising 30 selected bean varieties were used in the study. Each experimental unit comprised of 10 sampling units. Thirty accessions for the study were randomly selected from those that were found to have moderate to high tolerance to *Schlerotium rolfsii f. sp phaseoli* the causal agent for *Schlerotium* root rot disease. Disease evaluation was assessed basing on the symptoms of the hypocotyl and roots then scored using the CIAT scale of 1-9. Data analysis was done using GenStat and Micro-Soft Excel computer programmes to obtain differences in the mean disease severity. Analysis of Variance (ANOVA) and t-Test for paired means were used to address the study objectives.

The findings of this study revealed that *Fusarium spp isolate F890* had a significant effect on genotypes ADP 98, ADP 112, ADP 43, ADP 20, ADP 1, Lira U00030, Lira U00205 and NABE 17 obtained from NaCRRI in Wakiso District at 5 percent level of significance. On the other hand, ANOVA results also showed that *Fusarium spp isolate F386* had a significant effect on genotypes ADP 98, ADP 110, ADP 20, ADP 12, ALB 153, NABE 17 and KWP 9. Furthermore, t-test results from the experiment showed that there is no significant difference in the virulence of the two *Fusarium spp* isolates F890 and F386 at 5 percent level of significance.

CHAPTER ONE:

INTRODUCTION

1.1 Background

Common bean (*Phaseolus vulgaris*. L, 2n=22) is classified as a pulse and is the most important pulse in the human diet (Jones, 1990) as cited by (Larochelle *et al.*, 2016). They were introduced into East Africa by Portuguese traders in the sixteenth century (Greenway, 1945 as cited by Wortmann *et al.*, 1998).

The common bean is in class **Magnoliopsida**, order **Fabales**, family **Fabaceae**, genus **Phaseolus** and species ***Phaseolus vulgaris* L.** Gepts (1998), found out that there are two primary gene pools that actually represent sub-species of *Phaseolus vulgaris* L. and these are the Mesoamerican and the Andean gene pools. These gene pools are the basis for genetic improvement in common beans globally.

Common beans are very important in ensuring nutritional security in the developing countries (Garden-Robinson, 2013) since they are a source of Vitamin B complex, iron, calcium, zinc, and proteins (Ilse de Jager, 2013). In Uganda, 20% of the percapita total protein intake is from pulses which dominantly include beans. (FAO, 2005 & 2007). Common beans once consumed by an individual lower susceptibility to cardio-vascular diseases, diabetes and cancer (Heller, 2011; Munoz, 2002 as cited by Katungi *et al.*, 2009). They are also appetite suppressants thus used in weight loss programs. (Katungi *et al.*, 2009)

Common beans are one of the cheap protein sources available on the world food market with a percapita availability of approximately 3kg/ person/ year (FAO, 2011) making them the most consumed legume by man (Broughton *et al.*, 2003 as cited by Pamela *et al.*, 2014). Globally, the common bean yield increased at a rate of 0.4% between 1994 and 2008 (FAO, 2011).

In Africa, common beans are mainly grown in the sub-Saharan regions of East and Southern Africa (Katungi *et al.*, 2009) where it's consumed as leaves, pods, fresh grains and dry beans literally providing edible products during most of its growth period thus ensuring that the people have a certain degree of food security throughout the growth season.

Over 200 million people depend on common beans in sub-Saharan Africa (CGIAR, nd) rendering it a crop of high significance in terms of food and protein security. This large market is mainly satisfied by Uganda because it is among the top producers and net sellers of common beans on the East African regional market (FAO statistics, 2013).

Between 1994 and 2008, the percapita consumption of common beans in the sub-Saharan region increased at a rate of 1.67% although the yield had a slow increase of 0.3% (Akibode & Maredia, 2012 as cited by Larochelle *et al.*, 2016) suggesting that the rate of increase in demand for common bean products is higher than the rate of increase of supply of the common beans. This in turn has increased the threat of protein insecurity of the over 200 million people dependent on common beans.

In Uganda, the highest producing areas are: Ntungamo with 137,899 tons, Mubende with 78,027 tons, Amuru with 74,671 tons and Mbale with 23,637 tons (Anon, nd). Akibode (2011) reported that between 2006 and 2008, 0.87 million hectares of common beans were cultivated in Uganda yielding 0.43 million tons translating to a 0.5 tons ha⁻¹. Comparison between the production periods 1994-1996 and 2006-2008 shows a 1.04% decline in common bean production from the earlier period to the later period because of the various biotic, abiotic and socio-economic constraints.

Fusarium wilt is caused by *Fusarium oxysporum f.sp phaseoli* which is a fungus classified under the Hyphomycetes (Groenewald, 2005) basing on structures that possess the conidiogenous hyphae. Hyphomycetes lack closed fruit bodies. It is a filamentous soil borne fungus (Booth, 1971 as cited by Groenewald, 2005) just like other Fusaria. It was first discussed and reported by Harter in 1929 in California (Kraft, Burke & Haglund, 1981; Schwartz & Pastor-Corrales, 1989; Woo *et al.*, 1996) and later was reported in other common bean growing areas like Brazil, Colombia, Peru, United States and Costa Rica.

Fusarium root rot is caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder and is designated as a formae specialis of *Fusarium solani* (Snyder & Hansen, 1941). It was first reported by Burkholder in 1916 in New York State (Zanmevcr & Ihormas, 1957 as cited by Schwartz & Pastor-Corrales, 1989; Kraft *et al.*, 1981). O'Donnell (2000) found out that *Fusarium solani* had 9 formae speciales and all these were phylo-genetically distinct. He also

suggests the centres of origin for the various formae speciales to be India-Sri Lanka, Africa, New Zealand and South America.

Common bean is basically known as a women's crop because its majorly grown by women (Xavery *et al*, 2005) which is responsible for many of the socio-economic constraints in common bean production.

1.2 Problem statement

With the area allocated to bean production in average household land increasing by 151% and the yield declining by 64% with in the period from 1999 to 2006 in Uganda (Kraybill, Bashaasha & Betz, 2012), the common bean productivity per hectare has generally declined over the years threatening protein security of both urban and rural living Ugandans. Akibode & Maredia (2012) also found out that the average yield of common beans in Uganda is 500kg ha⁻¹ which is far below the 1500-3000 kg ha⁻¹ potential yield of common beans under the required bean production conditions (Hillocks *et al.*, 2006). Common bean production in Uganda is also constrained by various factors as listed under chapter two of this report. One of these constraints are the *Fusarium spp* diseases namely; Fusarium wilt and Fusarium root rot diseases.

The interaction between the pathogenic *Fusarium spp* and the genetic potential of the various common bean lines in Uganda results into up to 84% yield loss (Miller & Burke, 1986; Abawi & Pastor Corrales, 1990; Park & Tu, 1994). The yield loss due to *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* can go even up to 86% in presence of other stress factors like excess soil moisture, drought, herbicide injury, use of the ammonium form of nitrogen, toxic metabolites of crop residue decomposition, unfavorable temperatures for bean germination, soil compaction (Abawi & Corrales, 1990; Harveson, Smith & Stroup, 2005), and *Pythium ultimum var ultimum* (Pieezarka & Abawi, 1978a as cited by Schwartz & Pastor-Corrales, 1989; Abawi & Corrales, 1990)

1.3 Justification

Fusarium solani is responsible for about 50% of the *Fusarium spp* infection cases followed by *Fusarium oxysporum* which is responsible for about 20% of infection cases (Kosmidis & Denning, 2017) making these two Fusaria very important plant pathogens worth further in depth studies.

This study will thus be able to identify those common bean races that are tolerant to *Fusarium spp* thus build on knowledge of how to reduce the yield losses due to *Fusarium spp* diseases through breeding for resistance thus increase the yield per annum to a level that satisfies the percapita consumption per annum of common beans hence food and protein security in Uganda and Africa at large.

1.5 The objectives of this study

- i. Screen selected bean lines at National Crops resources research institute (NaCRRI) for tolerance and/or resistance to F890 and F386 *Fusarium spp* isolates.
- ii. Determine the virulence of the *Fusarium spp* isolates on the selected bean lines at NaCRRI.

1.6 The hypotheses of this study

- i. There is no significant effect of the *Fusarium spp* isolates on the selected bean lines at NaCRRI.
- ii. There is no significant difference in the virulence of the two *Fusarium spp* isolates F890 and F386 to the selected bean lines at NaCRRI.

CHAPTER TWO: LITERATURE REVIEW

2.1 Constraints to common bean production in Uganda

Drought stress (Beebe *et al.*, 2011; 2013) which affects the nutrient up-take by plants and is caused by the climatic change (as cited by Namugwanya *et al.*, 2014). Furthermore, low quantity of available phosphorus is another key constraint (Beebe *et al.*, 2011; 2013; Wortman *et al.*, 2004). Lunze *et al.*, (2007) suggests that the average yield of local varieties in P-deficient soil is 750kg ha⁻¹ which is below the common bean plant's genetic potential (Hillocks *et al.*, 2006). Athanase *et al.*, (2013) suggest that water logging especially in Kisoro is a key constraint in that region causing about 28.9% yield loss.

Pests and diseases have been observed to have field interaction with low available soil phosphorus levels and soil moisture (Namugwanya *et al.* 2014). Buruchara, Mukaruziga & Ampofo (2010) reported the key common bean insect pests as; cutworms, bean fly, bean foliage beetle, flower and pollen beetles, pod borers, pod sucking bugs, aphids, white flies and bean bruchids. The diseases include; common bacterial blight, halo blight, angular leaf spot, bean anthracnose, ascochyta leaf spot, web blight, powdery mildew, floury leaf spot, white mould, leaf rust, bean common mosaic virus disease, common bean mosaic necrotic virus disease, Pythium root rot, Fusarium root rot, Rhizoctonia root rot, sclerotium root rot, charcoal rot and Fusarium wilt.

2.2 *FUSARIUM SPP*

Fusarium spp utilize food and nutrients of the plant that they invade (Pegg & Manners, 2014). Some species also form an endophytic association in the xylem. *Fusarium spp* is basically known for causing vascular wilt and root rot diseases in host specialized forms in plants.

Snyder *et al.* (1941); O'Donnell (2000) stated out that the different Fusaria can be identified and classified on basis of their morphological characteristics like colony morphology and structure morphology for example the macroconidia of *Fusarium solani f. sp. phaseoli* are less curved, have one blunt end, and are slightly larger than those of *Fusarium oxysporum f. sp. phaseoli*. (Abawi & Corrales, 1990). Classification can also be done using anamorph of the Fusaria (O'Donnell, 2000) thus being a differentiating factor.

Formae speciales are classified basing only on their pathogenicity (Snyder *et al.*, 1941). Messiaen & Cassini (1981) suggested that *Fusarium solani* is less specialized in its hosts compared to *Fusarium oxysporum* and thus its formae speciales is determined by the symptoms it incites while *Fusarium oxysporum* obtains its formae speciales through host specialization as sited by (Roy, 1997). The reddish discolouration/lesions are on above ground tissue in infections of *Fusarium oxysporum f. sp. phaseoli* while in *Fusarium solani f. sp. phaseoli* infections, the reddish-brown discolourations/lesion are on below ground tissue. (Abawi & Corrales, 1990).

2.2.1 Symptomology of *Fusarium spp* in common beans

2.2.1.1 *Fusarium oxysporum f. sp phaseoli*

The time for appearance of above ground symptoms is influenced by factors like the inoculum density and environmental conditions like temperature (Ribeiro & Hagedorn, 1979b as cited by Schwartz & Pastor-Corrales, 1989; Abawi & Corrales, 1990). Chlorosis is the initial symptom followed by wilting of the plant starting with the lower leaves and later progressing to the upper younger leaves (Buruchara *et al.*, 2010). The margin of the infected leaves may become necrotic and stunting may also be seen (Schwartz & Pastor-Corrales, 1989). The leaves may show epinasty and also water soaked lesions develop on leaves (Abawi & Corrales, 1990) and pods (Goth, 1966 as cited by Schwartz & Pastor-Corrales, 1989). The symptoms continue to develop rapidly resulting into premature defoliation and later death of plant.

Dead stem and petiole tissue are pinkish orange (Schwartz & Pastor-Corrales, 1989; Abawi & Corrales, 1990) because of the pathogen's spore masses. Plants also have a brown vascular discoloration in the stem and root tissue after the initial appearance of foliar symptoms. The reddish-brown discolouration in the stem, root and petiole tissue varies with disease severity, plant cultivar and environmental conditions.

Abawi & Corrales (1990) point out that the symptoms usually develop in a uni-lateral manner because of the location and arrangement of the infected xylem vessels.

2.2.1.2 *Fusarium solani* f. sp *phaseoli*

Symptoms appear on the hypocotyl and tap root 1-2 weeks after infection as longitudinal narrow reddish lesions (Schwartz & Pastor-Corrales, 1989; Abawi & Corrales, 1990; Buruchara *et al.*, 2010) which later enlarge, coalesce and the entire root system is covered by the reddish lesions. This discoloration can extend up to the soil surface however rarely does it go beyond. The lesions lack definite margins, remain superficial, and may show longitudinal fissures. The lateral roots are continuously killed by the fungus however remain attached as dried and/or decomposed remains. Under severe infection, the tap root and lateral roots die, the lower stem becomes hollow or pithy and lateral adventitious roots develop above the infected area to support the plant although they too can get infected. The above ground symptoms include stuntedness, chlorosis, and premature defoliation.

2.2.2 Control measures

The major control measures for the *Fusarium spp* include;

Use of resistant varieties (Schwartz & Pastor-Corrales, 1989; Silbernagel & Mills, 1990; Abawi & Corrales, 1990; Fravel, Olivain, & Alabouvette, 2003; Ongom *et al.*, 2012; Cross *et al.*, 2000 and Pereira *et al.*, 2009 as cited by Batista *et al.*, 2016) and soil chemical fumigation is also recommended however these chemicals are dangerous to the environment, humans and are costly (Abawi & Corrales, 1990; Groenewald, 2005). Furthermore, soil chemical fumigation has been found not to be effective (Mukankusi *et al.*, 2012). Other control measures include; use of recommended cultural practices like adjusting planting depth and planting time (Schwartz & Pastor-Corrales, 1989; Abawi & Corrales, 1990), proper irrigation by sprinklers (Naseri, Shobeiri, & Tabande, 2016), organic manure application (Abawi & Corrales, 1990; Naseri *et al.*, 2016), prevention of introduction of the pathogen into new clean fields (Groenewald, 2005), using clean planting materials since the pathogen can be transferred from one geographical location to another through contaminated planting materials (Woo *et al.*, 1996) and biological control of *Fusarium solani* f sp *phaseoli* with *Bacillus subtilis* CA32 and *Trichoderma harzianum* RUO1 (Abeysinghe, 2012)

Kraft *et al.*, (1981) also suggested that providing soil conditions that promote optimum plant growth reduces the severity of *Fusarium solani* f. sp *phaseoli* in the field.

Harveson, Smith & Stroup (2005) also pointed out that reducing soil compaction through zero tillage reduced root rot disease primarily of *Fusarium* root rot by minimizing the plant's stress. Proper crop rotation with non-host plants is also effective in controlling *Fusarium spp* (Abawi & Corrales, 1990).

Schwartz & Pastor-Corrales (1989) stipulated that monoculture of common beans and improper crop rotation were also some of the factors that caused the prevalence and high severity of root rot diseases. Robert & Lana, (1992) further clarified this by showing that a crop rotation system that introduced common beans into the *Fusarium solani f.sp phaseoli* infected soils every after 3 or less years was not effective because *Fusarium spp* has chlamydospores which allows it to survive long in crop debris and soil thus is not completely eliminated from the soil (Schwartz *et al.*, 2005). Silbernagel & Mills (1990) suggest that deep soiling and narrow row spacing increase crop yield under infected fields with *Fusarium solani f.sp phaseoli*.

Abawi & Corrales (1990), Buruchara *et al.*, (2010) and Batista *et al.*, (2016) suggested Integrated control as another effective control measure of *Fusarium* wilt and *Fusarium* root rot diseases.

2.2.3 Life cycle

Pegg & Manners (2014) suggested that *Fusarium spp* produce up-to five propagules from which new individuals can develop. The propagules include; mycelium which is a mass of tubular filaments, *Nectria heamatococca* (Agrios, 1997 as cited by Mukankusi, 2008) which is a perithecial stage produced by *Fusarium solani f.sp phaseoli* under certain conditions, macroconidia and microconidia that are the asexual spores, ascospores which are the sexual spores, Chlamydospores which are structure that can survive for a long time under un-favorable conditions. Nelson, Toussoun, and Marasas (1983) & Abawi and Corrales (1990) however classified chlamydospores as asexual spores.

The fungus over-winters in host or non-host plant debris saprophytically, in infected plant tissue as spores or mycelium, in seeds and soil as chlamydospores (Mukankusi, 2008; Batista *et al.*, 2016). Propagules germinate when favorable conditions that reverse fungistasis prevail like presence of nutrient exudates from germinating bean seeds and root tips

2.2.5 Epidemiology

2.2.5.1 *Fusarium oxysporum f. sp. phaseoli*

The *Fusarium spp* pathogen invades underground roots and stems directly through the epidermis, stomates and/or wounds (Toussoun, 1970; Schwartz & Pastor-Corrales, 1989; Abawi & Corrales, 1990) usually near the root tip, just behind the root cap. This root infection is more rapid under conditions of high rainfall due to increased root growth (Robert & Lana, 1992).

Root invasion is followed by the development of systemic vascular invasion (Pegg & Manners, 2014) and involves passive movement of the micro-conidia through the xylem. The host defense responses cause blockage of water conducting vessels resulting into wilt. In advanced stages of disease, the fungus grows out of the vascular tissue and forms a multitude of conidia and chlamydospores. The chlamydospores are returned to the soil when the dead plant decays and they can remain dormant for several years. The cycle is repeated when chlamydospores grow saprophytically or by invading a host.

Dissemination of the pathogen occurs through the movement of colonized debris, infested soil, infected plant tissue by wind, irrigation/rain water, animals, etc. (Abawi & Corrales, 1990)

2.2.5.2 *Fusarium solani f. sp. phaseoli*

The pathogen's inoculum is maintained in the soil over long time periods through the chlamydospores (Schwartz & Pastor-Corrales, 1989) and the pathogen's ability to colonize roots of non-host crops without causing disease (Abawi & Corrales, 1990). The chlamydospores germinate when stimulated by exudates, presence of bean seed or when close to fresh organic matter. The pathogen is disseminated through movement of infected host tissue or infected soil by water, implements, wind, humans and animals or through planting contaminated seed. It penetrates the host plant directly through tissues, wounds, and natural openings like stomata. Kraft *et al* (1981) suggests that upon penetration, the pathogen grows intercellularly through the cortex tissue until when its stopped by the endodermis layer. During moist environmental conditions, the spores can be seen growing on the stem above ground. Chlamydospores are produced throughout the roots and hypocotyl tissue.

2.3 Mechanisms of resistance to *Fusarium* spp.

Mukankusi (2008) describes the mechanism of resistance to *Fusarium solani f. sp. phaseoli* as “A hypersensitive reaction to invasion by *Fusarium solani f. sp. phaseoli* as reported by Pierre & Wilkinson (1970). They observed browning of cortical cells in the advent of invasion by the hyphae of *Fusarium solani f. sp. phaseoli*, which limited the growth of hyphae in resistant varieties. Browning of the peridium of the roots was also observed, but this was reported not to limit hyphal growth. A vigorous root system has often been suggested to increase tolerance to root rot (Snapp et al., 2003; Román-Avilès et al., 2004).

The division of carbohydrates between shoots and roots is influenced by both genetic and environmental factors. This may imply that the genes governing root system vigour also influence resistance to root rot such that varieties with genetically vigorous root systems are more resistant to Bean root rots compared to those with weak root systems. The colour of seed and hypocotyls has also been related to the level of resistance to *Fusarium solani f. sp. phaseoli*.

Statler (1970) observed higher resistance to *Fusarium solani f. sp. phaseoli* in black seeded varieties and varieties with purple-coloured hypocotyls, and related it to the greater production of phenolic compounds inhibitory to fungal growth in the early stages of seedling growth. Phytoalexins such as phaseolin have been identified and reported to be produced in response to infection by *Rhizoctonia solani* (Pierre & Bateman, 1967) and *Fusarium solani f. sp. phaseoli* (Kendra & Hadwiger, 1989), with production of these phytoalexins being shown to be greater and more rapid in resistant varieties. Similarly, Beebe et al. (1981) recorded more resistance to *Fusarium solani f. sp. phaseoli* in the small and black seeded varieties compared to large red mottled ones. Selection, either direct or indirect, aimed at enhancing these traits should allow for rapid improvement of resistance to Fusarium Root Rot in Andean bean genotypes.”

2.3.1 Cultivar tolerance to *Fusarium spp*

Batista *et al.*, (2016) showed in their breeding experiment that different common bean cultivars have varying *Fusarium spp* tolerance potentials which signified that it is possible to improve *Fusarium spp* tolerance in a desired susceptible cultivar through conventional breeding. This also further justifies that resistance of common beans towards *Fusarium spp* is race specific in nature due to the fact that the resistance genes are many and are located on different loci in a plant's genome (Mukankusi *et al.*, 2011). Mukankusi (2008) cites that resistance/tolerance to *Fusarium spp* is achieved through vigorous plant root development (Snapp *et al.*, 2003; Román-Avilès *et al.*, 2004) and production of fungal growth inhibitory phenolic compounds (Statler, 1970) which are in line with VanEtten & Smith (1975), Abawi & Corrales (1990) and Dakora & Phillips (1996).

Woo *et al.* (1996) grouped the pathogenic isolates of *Fusarium spp* into 5 races using common bean cultivars' response to inoculation with *Fusarium spp* as the differentiating factor thus further dividing the two races suggested by (Ribeiro & Hagedorn, 1979).

Ribeiro & Hagedorn (1979) and Woo *et al.* (1996) both suggested that common bean resistance and/or tolerance to *Fusarium spp* was race specific. This means that breeding for resistance is an effective method of control of Fusarium root rots however not a lasting solution since resistance can break when the resistant cultivar is exposed to a new race of *Fusarium spp*.

Woo *et al.* (1996) used five races each from a different country in three continents i.e. North America, South America and Europe and these races had genetic variation with in themselves. The variation caused a difference in the pathogenicity of these races on the various common bean cultivars originating from South America. They also went ahead to find out that of the 20 selected pathogenic isolates used, 45% of them were non-pathogenic to any of the common bean cultivars used in the study. This further verifies the findings of Ribeiro & Hagedorn (1979).

CHAPTER THREE: METHODOLOGY

3.1 Scope of the study

3.1.1 Geographical and Environmental scope

The research project was conducted at National Crops Resources Research Institute (NaCRRI) which is located in the bimodal rainfall region at latitude 0032° north and 3237° east. NaCRRI is North of Kyaddondo constituency, Kyaddondo County, Wakiso District and 27km North of Kampala city, central Uganda. It has a tropical wet and dry mild climate with slightly humid conditions of 65% on average and has a savannah vegetation with tall trees and the most dominant type of grass is *panicum maximum*

The experiment was carried out in a greenhouse to minimize environmental interference with the *Fusarium spp* genetic resistance (Schneider & Kelly, 2000)

3.1.2 Time scope

The research project ran for six months starting from January 2018 to July 2018. Five months were used for the experiment and data collection and one month for data analysis and discussion of the results.

3.1.3 Content Scope

The research focused on characterizing the common bean lines in Uganda according to their level of resistance against *Fusarium spp*.

3.2 Experimental Layout

A complete randomized experimental design was used with two replications of each treatment (Nakedde *et al.*, 2016). The two factors under investigation were the germplasm and the *Fusarium spp* isolates F890 and F386.

Thirty (30) treatments were used which were the 30 selected varieties to be used in the study. Each experimental unit comprised of 10 sampling units. The sampling unit was composed of a row of a particular germplasm shown in the table below:

Table 3.1: Sampling unit of a row of a particular germplasm

No	Line	Nursery	Category
1	ALB 171	Interspecific Hybrid	Resistant
2	ADP 98	Andean Diversity Panel	Resistant
3	KWP 12	Pythium Root Rot	Resistant
4	ADP 115	Andean Diversity Panel	Resistant
5	ALB 184	Interspecific Hybrid	Resistant
6	KWP 17	Pythium Root Rot	Resistant
7	ALB 191	Interspecific Hybrid	Resistant
8	ADP 110	Andean Diversity Panel	Resistant
9	KWP 9	Pythium Root Rot	Resistant
10	ALB 155	Interspecific Hybrid	Resistant
11	ALB 153	Interspecific Hybrid	Resistant
12	ADP 122	Andean Diversity Panel	Resistant
13	ALB 151	Interspecific Hybrid	Resistant
14	ALB 200	Interspecific Hybrid	Resistant
15	ALB 2	Interspecific Hybrid	Resistant
16	KRO 15	Pythium Root Rot	Moderate
17	ADP 112	Andean Diversity Panel	Moderate
18	Kamuli U00244	Local Germplasm	Moderate
19	ADP 43	Andean Diversity Panel	Moderate
20	ADP 20	Andean Diversity Panel	Moderate
21	ADP 114	Andean Diversity Panel	Moderate
22	ALB 115	Interspecific Hybrid	Moderate
23	Lira U00030	Local Germplasm	Moderate
24	ADP 1	Andean Diversity Panel	Moderate
25	Lira U00205	Local Germplasm	Moderate
26	Kamuli U00245	Local Germplasm	Moderate
27	NABE 2	Released Market-Class	Moderate
28	ADP 12	Andean Diversity Panel	Moderate
29	ADP 32	Andean Diversity Panel	Moderate
30	Kamuli U00309	Local Germplasm	Moderate

3.3 Materials.

Materials used included; Plastic Petri-plates, auto-clavable bottle, measuring cylinders, surgical blades, forceps, surgical gloves, tape, Bunsen burner, tissue paper, weighing machine, plastic bags, incubator and loam-sand soil mixture at 3:1 ratio. Other materials used included; JIK (3–8% sodium hypochlorite), 80% ethanol and Potato dextrose agar.

3.3.1 Germplasm selection

Thirty accessions for the study were randomly selected from those that were found to have moderate to high tolerance to *Schlerotium rolfsii f.sp phaseoli* the causal agent for schlerotium root rot disease (Paparau *et al.*, 2016). Upon random selection, the germplasm was grouped basing on the Nursey as shown below;

Table 3.2: Germplasm for Evaluation

NURSERY	ORIGIN	NUMBER OF LINES
Andean Diversity Panel	Accessions and released lines from East and South Africa, CIAT, North America, Ecuador, Angola and Caribbean	11
ALB	Interspecific Hybrid (SER 16 x G35346)	9
Local Germplasm	Land races and released lines from Uganda	6
Pythium Root rot.	Kenyan Root Rot Nursery for development for Pythium resistance	4
TOTAL		30

Source: 1 National Crops Resources Research Institute, Namulonge

3.3.2 Fungal Isolate

The *Fusarium* spp isolates used were F890 and F386 obtained from infected plant hypocotyl tissue at an early stage of disease development (Abawi & Pastor-Corrales, 1990). The isolates were cultured on sterilized Potato dextrose agar (Khilare & Rafi, 2012) which is most suitable for *Fusarium* spp growth.

3.3.3 Preparation of Growth medium

Weighed 9.75g of potato dextrose agar and made a mixture by adding 250ml of distilled water, followed by heating the mixture while frequently agitating it to dissolve the potato dextrose agar. Boiled for 1 minute until complete dissolution and placed mixture in an autoclave bottle, autoclaved at 121⁰C for 15 minutes and then cooled the mixture to 48⁰C. Dispensed the liquid mixture into plates and immediately cover with lids. The potato dextrose agar solidified within 15 minutes.

3.3.4 Operation of the Autoclaving machine

ILRI (2014) identified the various safety prerequisite precaution procedures of operating an autoclave machine which was followed throughout the usage of the autoclaving machine.

The lid of the autoclaving machine was opened by opening locks diagonally starting with the major diagonal. The materials to be sterilized were placed into the autoclaving machine and closed the lid starting with the major diagonal. The autoclaving machine was switched on, closed the valve and let the pressure build up to 1-1.2 bars then opened the valve. The process was timed for 15 minutes followed by switching off the autoclaving machine at end of 15 minutes and allowed it to cool.

3.3.5 Isolation and culturing of *Fusarium spp* from host plant tissue

Abawi & Pastor-Corrales (1990) suggested the aseptic and prerequisite procedures of isolation and culture transfer of *Fusarium spp* which was followed in this experiment.

JIK was poured into a clean plate and distilled water into two other plates followed by placing two clean pieces of tissue paper A & B on the working table. Using forceps to hold the infected bean plant in a fixed position on the filter paper next to the tissue paper, small pieces of the shoot area just above the point the root system commenced were cut using the surgical blade, soaked into JIK for two minutes to destroy bacteria then transferred through the first and second plates of water to wash off excess JIK and placed on tissue papers A & B to drain off water. The dry sample pieces were placed on the prepared potato dextrose agar in plates and sealed with tape. Incubation was done at 25-35°C for four days. (Khilare & Rafi, 2012). The pure cultures of each isolate were transferred to 250g of double-sterilized millet (CIAT, nd) in plastic bags and incubated for 7 days at 25-35°C.

3.3.6 Screening Protocol for *Fusarium* spp in the Screenhouse (Papar, unpublished, nd)

20 kilograms of sterile loam and sand in a 3:1 ratio were mixed thoroughly and placed in trays. 100grams of the inoculum prepared on millet were added to each of the trays and mixed in the soil. The inoculum-soil mixture was made by placing the inoculum in a polythene with a small portion of the soil, thoroughly mixing these then adding the mixture to the larger portion of soil in the tray. The soil was covered for a week to enable the pathogen's full saturation in the soil. Cal 96 was then planted in all trays to increase the pathogen's concentration in the soil. After three weeks, the Cal 96 was uprooted, the soil taken out of the crates, mixed together evenly, redistributed among all the crates (Ongom *et al.*, 2012) and the test germplasm planted. Watering was done on a daily basis (Scheider & Kelly, 2000; Mukankusi *et al.*, 2011). The experiment was harvested after 28 days for data collection.

3.4 Severity Evaluation

Disease evaluation was assessed basing on the symptoms of the hypocotyl and roots then scored using the CIAT scale of 1-9 (Abawi & Pastor-Corrales, 1990); 1= No visible symptoms, 3= light discoloration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissues covered with lesions, 5= approximately 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm with deterioration of the root system, 7= approximately 50% of the hypocotyl and root tissue covered with lesions combined with considerable softening, rotting and reduction of root system, 9= approximately 75% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with severe reduction in the root system.

Plants with disease severity of 1-3 were considered resistant, 3.1-5.9 were moderately resistant and those below 6 were susceptible (Mukankusi *et al.*, 2012; Nakedde *et al.*, 2016).

3.5 Data Analysis

Data analysis was done using SPSS to obtain differences in the mean disease severity (Payne *et al.*, 2007) as cited by (Mukankusi *et al.*, 2011). Analysis of Variance (ANOVA) and t-Test for paired means were used to address the study objectives.

3.6 Assumptions

- i. All experimental units are under the same environmental conditions.
- ii. The concentration of microconidia, macroconidia and chlamydozoospores in every *Fusarium spp* inoculum weighing 100 – 100.04 grams is equal.

3.5 Limitations of the Study

- i. The identification and characterization of the *Fusaria* causing the common bean root rot and wilt in Uganda has not been done and thus I have to generalize the pathogen as *Fusarium spp*. This was later solved by laboratory diagnosis and analysis of the selected isolates for this study and identified the isolates as *Fusarium solani f. sp phaseoli*.
- ii. Excessive rainfall which caused water logged conditions in the experiment and thus making the experiments progress slow. This was solved by inserting a polythene sheet over the experiment to protect it from rain water.

CHAPTER FOUR:

RESULTS

4.1 General Observations

The isolates F890 and F386 were observed to be of *Fusarium solani f. sp phaseoli* and not *Fusarium oxysporum f. sp phaseoli* basing on the morphological characteristics like colony morphology and anamorphs thus this made my experiment more direct when it came to scale selection for data collection and discussion of the obtained results. According to the nature of the symptoms on and in the hypocotyl of the infected common bean plants, there was confirmation that the pathogen was indeed *Fusarium solani f. sp phaseoli* basing on the symptoms identified by (Abawi and Corrales, 1990). *Fusarium solani f. sp phaseoli* and *Fusarium oxysporum f. sp phaseoli* are highly similar in their behavior and this is evident in (Schwartz and Pastor-Corrales, 1989; Abawi and Corrales, 1990) where the methodologies of extraction, evaluation, culturing, isolation are similar.

4.2 Effect of the *Fusarium spp* isolates on the selected bean lines at NaCRRI

Figure 4.1 below shows the respective severities of the 30 bean lines that were considered in this experiment. Of the 30 beans lines, Lira U00205 and ALB 153 bean lines had the most severe effects by *Fusarium spp isolates F890 and F386* with means of 7.1 and 6.5 respectively. The results from the experiment further showed that *Fusarium spp isolates F890 and F386* had the least effects on KWP 12 and ALB 171 bean lines with both having means of 2.3. Additionally, *Fusarium spp isolates F890* also had more severe effect on the Lira U00030, ADP 112, and ADP 1 bean lines with means of 7.0, 6.9 and 6.9 respectively.

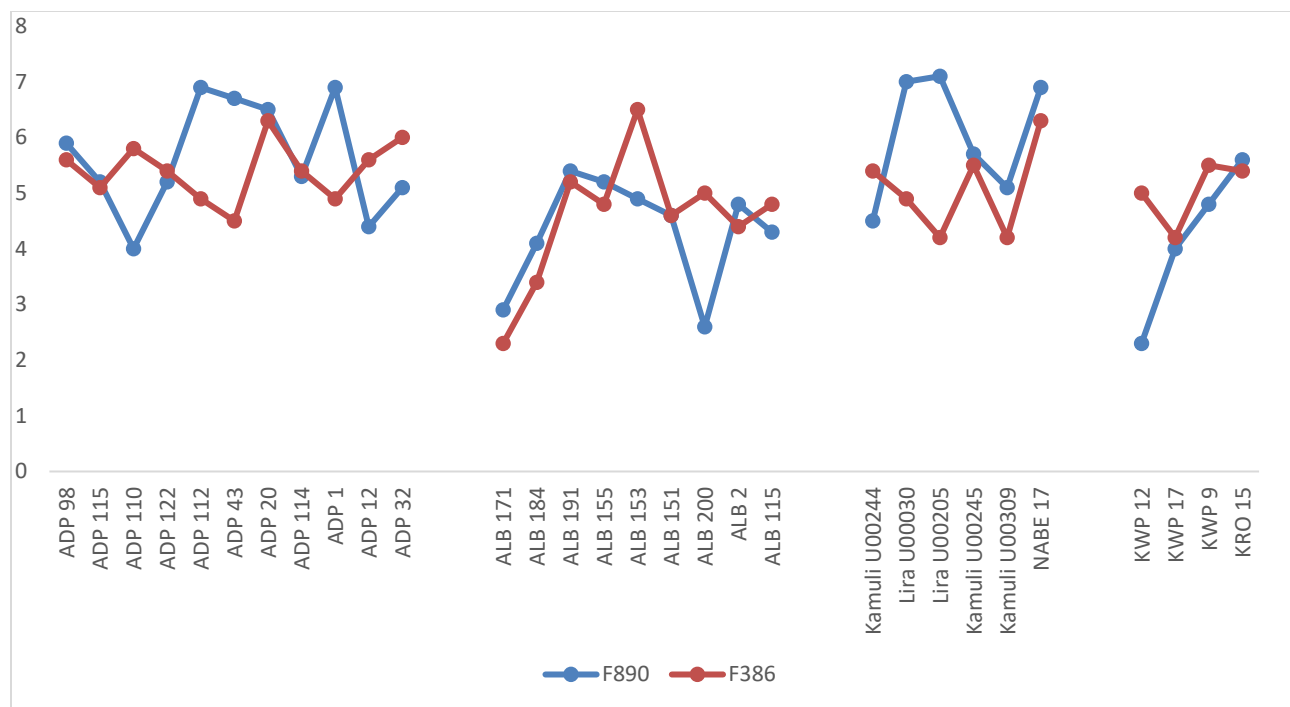


Figure 4.1: Disease severities of the *Fusarium spp* isolates F890 and F386 on the selected bean lines

As seen from table 4.1 below, ANOVA results from the experiment showed that *Fusarium spp* isolate F890 had significant effect on the ADP 98, ADP 112, ADP 43, ADP 20, ADP 1, Lira U00030, Lira U00205 and NABE 17 bean lines obtained from NaCRRI in Wakiso District at 5 percent level of significance since all their p-values were less than 0.05. Furthermore, ANOVA results also showed that *Fusarium spp* isolate F386 had significant effect on the ADP 98, ADP 110, ADP 20, ADP 12, ALB 153, NABE 17 and KWP 9 bean lines obtained from NaCRRI in Wakiso District at 5 percent level of significance since all their p-values were less than 0.05. In summary, all the accessions that had a mean severity score greater than 5.4 were significantly affected by the *Fusarium spp* isolate F890 and F386. 45.45% of the Andean Diversity Panel germplasm and 50% of the local germplasm were susceptible to the *Fusarium spp* isolate F890. 25% of the Pythium Root Rot Nursery germplasm, 16.67% of the local germplasm and 45.45% of the Andean Diversity Panel germplasm were all susceptible to the *Fusarium spp* isolate F386.

Table 4.1: ANOVA results of the effect of the *Fusarium spp* isolates F890 and F386 on the selected bean lines

Bean Line	F890		F386	
	Mean	P-value	Mean	P-value
ADP 98	5.9	.029**	5.6	.049**
ADP 115	5.2	.333	5.1	.654
ADP 110	4	.260	5.8	.036**
ADP 122	5.2	.489	5.4	.749
ADP 112	6.9	.024**	4.9	.145
ADP 43	6.7	.017**	4.5	.847
ADP 20	6.5	.011**	6.3	.021**
ADP 114	5.3	.123	5.4	.843
ADP 1	6.9	.001**	4.9	.141
ADP 12	4.4	.214	5.6	.004**
ADP 32	5.1	.123	6	.303
ALB 171	2.9	.999	2.3	.260
ALB 184	4.1	.523	3.4	.449
ALB 191	5.4	.327	5.2	.333
ALB 155	5.2	.061	4.8	.214
ALB 153	4.9	.078	6.5	.000**
ALB 151	4.6	.123	4.6	.199
ALB 200	2.6	.945	5	.111
ALB 2	4.8	.661	4.4	.074
ALB 115	4.3	.128	4.8	.840
Kamuli U00244	4.5	.612	5.4	.074
Lira U00030	7	.000**	4.9	.800
Lira U00205	7.1	.001**	4.2	.561
Kamuli U00245	5.7	.741	5.5	.741
Kamuli U00309	5.1	.121	4.2	.121
NABE 17	6.9	.024**	6.3	.005**
KWP 12	2.3	.845	5	.555
KWP 17	4	.336	4.2	.661
KWP 9	4.8	.845	5.5	.018**
KRO 15	5.6	.777	5.4	.612

4.3 Difference in the virulence of the two *Fusarium spp* isolates F890 and F386.

Fusarium solani f. sp. *phaseoli* is the most common of the *Fusarium* disease causing species (Bilgi *et al.*, 2008; Abeysinghe, 2012). The severity is highly dependent on the virulence of the pathogenic race (Burlakoti *et al.*, 2012). The difference in the virulence of the two *Fusarium spp* isolates F890 and F386 was investigated using a T-test for paired sample for means as seen in table 4.2 below. Due to no significance, a T-test of transformed means was used to test for significance. (Table 4.3)

Table 4.2: T-test results for Paired Two Sample for Means of F890 and F386

	F890	F386
Mean	5.13	5.04
Variance	1.64	0.75
Observations	30	30
Pearson Correlation	0.29	
Hypothesized Mean Difference	0	
Df	29	
t Stat	0.39	
P(T<=t) one-tail	0.35	
t Critical one-tail	1.70	
P(T<=t) two-tail	0.70	
t Critical two-tail	2.05	

Table 4.3: T-test results for Paired Two Sample for Transformed Means of F890 and F386

	F890	F386
Mean	0.22	0.29
Variance	0.37	0.30
Observations	30	30
Pooled Variance	0.34	
Hypothesized Mean Difference	0	
Df	58	
t Stat	-0.47	
P(T<=t) one-tail	0.32	
t Critical one-tail	1.67	
P(T<=t) two-tail	0.64	
t Critical two-tail	2.00	

CHAPTER FIVE

DISCUSSION

5.1 Reaction of the germplasm to the *Fusarium spp* isolates F890 and F386

Considering the classification of resistance by severity of Mukankusi *et al.*, (2012) and Nakedde *et al.*, (2016), there were three resistant, twenty moderate and seven susceptible to F890 isolate and one resistant, twenty-five moderate and four susceptible to isolate F386 (Figure 4.1). However, these results are not sufficient enough to show whether this effect is statistically significant because *Fusarium spp* severity scores and resistance cannot be classified into discrete categories (Schneider, Grafton & Kelly, 2001) since it is a quantitative trait (Baggett *et al.*, 1965 as cited by Schneider *et al.*, 2001; Schneider & Kelly, 2000; Obala *et al.*, 2012; Nakedde *et al.*, 2016)

The obtained results were continuous in nature and not discrete (Table 4.1) signifying a typical quantitative trait (Falconer and Mackay, 1996) thus in line with the findings of Baggett *et al.*, (1965) as cited by Schneider, Grafton & Kelly (2001), Schneider & Kelly (2000), Obala *et al.* (2012) and Nakedde *et al.* (2016).

From the ANOVA results (Table 4.1), the classification of resistance by severity (Mukankusi *et al.*, 2012; Nakedde *et al.*, 2016) was modified such that germplasm that were not significantly affected by *Fusarium solani f. sp phaseoli* were grouped into the resistant with mean severity scores from 1-3 and moderate resistance with mean severity scores of 3.1-5.4. Those with mean severity scores greater than or equal to 5.5 were considered susceptible. Therefore, there were three resistant, seventeen moderately resistant and ten susceptible to *Fusarium spp* isolate F890 and one resistant, twenty moderately resistant and nine susceptible to *Fusarium spp* isolate F386.

The ADP accession and Andean bean variety NABE 17 had from moderate resistance to susceptible to both the *Fusarium spp* isolates F890 and F386 because they are from the Andean gene pool which is a shallow gene pool with low genetic diversity with only a limited population of them showing resistance to *Fusarium solani f. sp phaseoli* (Cichy *et al.*, 2015). These accessions used in this study didn't completely lack the resistance genes because they on average performed better than the susceptibility check (Mukankusi *et al.*, 2012).

The Interspecific Hybrids had from high resistance to moderate resistance to both the *Fusarium spp* isolates F890 and F386 (Figure 4.1). The Interspecific Hybrids have parents (Table 3.2) SER 16 with drought resistance through its good shoot and root traits (Beebe, Rao, Blair, & Acosta-Gallegos, 2013) and G35346 which is a *P. coccineus* (CIAT, 2018) with resistance to *Fusarium solani f. sp phaseoli* (Porch *et al.*, 2013; Redden *et al.*, 2015 as cited by Mukankusi *et al.*, 2018) that significantly transferred these resistance genes to the resultant hybrid populations. However, the absence of a severity score of 1 shows that these hybrids don't have all the sources of resistance (Mukankusi, 2008; Mukankusi *et al.*, 2010; Mukankusi *et al.*, 2012) which is in line with the ideology of Mukankusi *et al.*, (2012). ALB 153 reacted differently to *Fusarium spp* isolates F386 and was susceptible with a mean score of 6.5 because of changes in environmental conditions (Mukankusi, 2018).

The Mesoamerican accessions in this study which included the land races (5) and those from the Kenyan Root Rot Nursery for development for Pythium resistance (4) had varying resistance levels to both the *Fusarium spp* isolates. The landraces from Kamuli had moderate resistance to both isolates and this is in line with the fact that Mesoamerican germplasm have a moderate resistance to *Fusarium solani f. sp phaseoli* (Mukankusi *et al.*, 2011; Mukankusi *et al.*, 2012, Table 1). The Lira landraces were moderately resistant to F386 but susceptible to F890. This was probably due to environmental condition changes given the fact that resistance to *Fusarium solani f. sp phaseoli* is highly influenced by environmental factors (Schneider & Kelly, 2000) since there was no significant difference in virulence between the isolates (Table 4.2; Table 4.3). The mentioned reasons also explain the un-coordinated variations in the severities of the germplasms from the Kenyan root rot nursery for development for Pythium resistance.

In summary, the accessions from the Andean genepool tended to be more susceptible to *Fusarium solani f. sp phaseoli* than the accessions from the Mesoamerican genepool (Abawi & Pastor-Corrales, 1990).

5.2 *Fusarium spp* virulence in common beans

As observed in Table 4.2 and Table 4.3, results from the experiment show that p-value is 0.70 and 0.64 respectively were greater than our alpha, 0.05. We therefore accept the null hypothesis that there was no significant difference in the virulence of the two *Fusarium spp* isolates F890 and F386 at 5 percent level of significance. This is probably because the two isolates were collected from the same ecological region of South-Western Uganda although at different geographical places thus have very little variation. Furthermore, Groenewald (2005) suggests that virulence in *Fusarium spp* is controlled by several genes thus the quantity of these genes present in a given race's genome determines its level of virulence. Therefore, both *Fusarium spp* isolates F890 and F386 probably have relatively equal quantities of the genes for virulence thus making their virulence not significantly different (Table 4.2; Table 4.3). The difference in the variances and a P- value that is less than 1 (Table 4.2; Table 4.3) are evidence that there is a small variation between the two isolates that can be due environmental conditions (Groenewald, 2005), genetic factors or experimental error.

CHAPTER SIX:

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The major objective of this study was to Screen selected bean lines at National Crops resources research institute (NaCRRI) for tolerance and/or resistance to F890 and F386 *Fusarium spp* isolates. The findings of this study revealed that *Fusarium spp isolate F890* had a significant effect on genotypes ADP 98, ADP 112, ADP 43, ADP 20, ADP 1, Lira U00030, Lira U00205 and NABE 17 bean lines obtained from NaCRRI in Wakiso District at 5 percent level of significance. ANOVA results also showed that *Fusarium spp isolate F386* had a significant effect on genotypes ADP 98, ADP 110, ADP 20, ADP 12, ALB 153, NABE 17 and KWP 9 bean lines. The experiment shows that accessions that had a mean severity score between 1 and 5.5 had some level of resistance to *Fusarium solani f. sp phaseoli* isolates F890 and F386 thus considered resistant. Furthermore, t-test results from the experiment showed that there is no difference in the virulence of the two *Fusarium spp* isolates F890 and F386 and thus the effects of each isolate on the common bean are relatively the same.

6.2 Recommendation

Following the study, further research is recommended to focus on the following;

- Creating common bean ideotype with *Fusarium* root rot, schlerotium root rot and *Pythium* root rot resistances.
- Parameters for growth retardation (shoot fresh weight and shoot height), root weight changes and yield should be integrated into the screening protocol for *Fusarium solani f. sp phaseoli* in order to increase accuracy in identifying resistant germplasm on a basis of multiple indicator factors i.e. yield, growth rate reduction and root characteristics like roots fresh weight. The use of the hypocotyl scoring scale (Abawi & Pastor-Corrales, 1990) is not sufficient considering the fact that hypocotyl characteristics are not a conclusive indicator of root injury in common beans (Schneider & Kelly, 2000)
- Resistance classification scale (Mukankusi *et al.*, 2012; Nakedde *et al.*, 2016) should be used with caution and justifiable reasons in future common bean screening experiments for *Fusarium solani f.sp. phaseoli* resistance.

- Increasing the number of replications of this experiment to investigate whether there is a significant difference between those germplasms reported as not being significantly affected by the pathogen in this experiment to establish a statistically valid scale for classification of resistance on basis of severity means and thus accept or reject the suggestion of Schneider, Grafton & Kelly (2001)
- Gene mapping for the virulence genes in the Ugandan races of *Fusarium solani f. sp phaseoli*.

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APPENDIX;

Figure 1: Mycelial growth on soil surface before planting of test germplasm



Figure 2: *Planting of test germplasm.*



Figure 3: Test germplasm at 28 days after planting.



Figure 4: Uprooting test plants during disease scoring.



Figure 5: Injury on common bean roots due to *Fusarium solani* f.sp phaseoli

