STUDY OF ANTHRACNOSE (*Colletotrichum lindemuthianum*) RESISTANCE AND ITS INHERITANCE IN UGANDAN DRY BEAN GERMPLASM

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DEDICATION

To my dear wife Rachael and son Tendo, for all those long periods of time I was away from home. Thank you for being patient and understanding.

GENERAL ABSTRACT

The common bean (*Phaseolus vulgaris* L.) is an important crop grown widely in Uganda. It is also an important source of income for smallholder farmers particularly women. Despite its importance, production in the cool highland regions is constrained by anthracnose disease which causes losses in both the quantity and the quality of beans produced. The principal aim of this research was to elucidate on the status of dry bean anthracnose and the genetics governing its resistance.

A participatory rural appraisal study was conducted to explore farmers' knowledge, experience, problems and cultivar preferences in association with managing dry bean anthracnose disease. This study revealed that anthracnose is an important constraint to production which is not controlled in any way. Although farmers have varying cultivar preferences, they use mostly home saved seed and only 1% could access improved seed. The study suggested the need for practical approaches in the provision of quality anthracnose resistant seed in consideration of farmers' preferences and the dynamics of their rural livelihoods.

A study was conducted to determine the variability of the anthracnose (*Colletotrichum lindemuthianum*) pathogen in some of the major bean growing regions of Uganda. Use was made of a set of 12 internationally accepted anthracnose differential cultivars to identify the physiological races present. The results obtained indicated the presence of eight races with one race (767) being dominant and most aggressive. Differential cultivars AB 136 and G2333 were resistant to all the eight races, and can be utilised as potential sources of resistant genes.

A germplasm collection of mostly Ugandan accessions was screened for anthracnose resistance. Using the area under disease progression curve as the tool for assessing disease severity, eleven accessions were identified that posses good levels of anthracnose resistance.

The yield loss attributed to the anthracnose disease was determined on three susceptible Ugandan market-class dry bean cultivars and two resistant cultivars. The results showed that the yield of susceptible cultivars was reduced by about 40% and an almost equivalent yield was lost due to poor quality seed. In comparison, the yield lost by the resistant cultivars was not significant. The study suggested the use of resistant cultivars as the best solution in combating anthracnose resistance.

Three susceptible Ugandan market class dry bean cultivars and six resistant cultivars were used for the study of the inheritance of resistance to the anthracnose pathotype 767 in a complete 9x9 diallel design. The results clearly indicated that the resistance was predominately conditioned by additive gene action. It was also established that epistatic gene action was important. More than one pair of genes displaying partial dominance were responsible for determining resistance and the maternal effect did not have an influence on resistance. Additionally, the result showed that some of susceptible cultivars combined very well with the resistant cultivars and that anthracnose resistance heritability estimates in both the narrow and broad sense were high. These results suggested that the use of simple pedigree breeding procedures such as backcross selection could be useful in improving anthracnose resistance levels in the Ugandan market class varieties.

DECLARATION

The work described in this dissertation was carried out at Namulonge Agriculture and Animal Research Institute (NAARI), and Kachwekano Agricultural Research and Development Centre in Uganda from December 2003 to August 2006 under the supervision of Dr. Fina Opio (Mrs.). The write up was done at the Faculty of Agriculture, University of KwaZulu-Natal Pietermaritzburg, South Africa under the Supervision of Prof. Rob Melis; and Prof. Mark, D Laing. The studies represent original work by the author and have not otherwise been submitted in any form for any other degree or diploma at any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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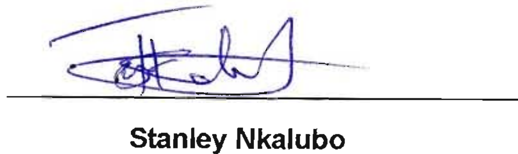
Student: 

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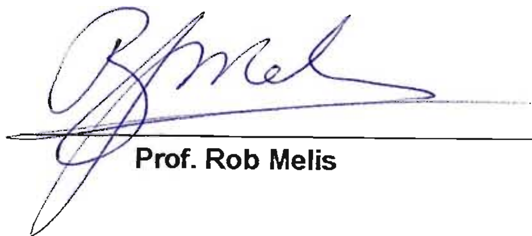
Supervisors: 

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Introduction

Agriculture is the backbone of Uganda's economy, accounting for about 40% of gross domestic product (GDP), over 90% of exports and employs about 84% of the household population (World Bank, 2002; MAAIF, 2003). According to the World Bank (2003) agricultural output comes almost exclusively from about 2.5 million smallholder farmers, 80% of whom have less than 2 ha. Only tea and sugar are grown on large estates. Food crop production accounts for 71%, and export crops 5% of the agricultural GDP. Uganda's population growth rate is between 2.8 - 3.0%. This growth is expected to continue and it is predicted that the population will reach 55 million by 2025 (World Resources Institute, 1990). Consequently, there will be continued pressure on the limited natural resources to produce ever larger amounts of food for the expanding population. Therefore, any attempts to reduce food losses through disease and pest control are essential in ensuring increased food production in the country.

The common dry bean *Phaseolus vulgaris* L., is one of the major food crops grown in Uganda, on about 600,000 ha of land (FAO, 2003). The dry bean is produced by nearly all farmers and is a basic component of most traditional diets (David *et al.*, 2000; Opio *et al.*, 2001), where it is consumed in the form of immature pods, green-shelled seeds and dry seed. Also green tender leaves are sometimes harvested before pod set and eaten as vegetables in some regions (Grisley and Mwesigwa, 1990). Beans occupy an important niche in Uganda's agricultural sector and farm household economy. The crop is an important source of calories and the most important source of protein for all rural and the majority of urban people who cannot afford beef, poultry or fish (Opio *et al.*, 2001). Beans are utilised as either food, sold in the markets for cash or exported to earn foreign exchange. According to Grisley and Mwesigwa (1990), it is increasingly becoming competitive with maize in terms of land allocation. Uganda has undertaken a strategy of promoting non-traditional agricultural export crops to further develop and expand its agricultural export base. Within the context of the export crops diversification initiative, beans have gained a major dominance in terms of tonnage and monetary value of exports compared to other non-traditional export crops (Anon., 2000).

The cultivated varities of *P. vulgaris* originated in Latin America (Singh, 1992) and according to Purseglove (1969), were introduced in Eastern Africa by the Spanish and Portuguese traders probably in the sixteenth century. It is further suggested that these traders called at

Zanzibar and Mombasa and their goods were transported to the interior by Arab slave traders and Swahili merchants. The beans are believed to have been established as a food crop well before the colonial era. Literature does not provide for precise dates as to when beans were first introduced in Uganda. However, considering the fact that the Spanish and Portuguese influence on the East African coast lasted between 1498 and 1740 (Matson, 1962), it would not be wrong to believe that dry beans arrived in Uganda between the 17-19th Century. In addition, Ugandan history has no documentation on bean production before the 19th Century, the possible reason for this being that beans had a very low status as a food crop before the beginning of the last century. The Department of Agriculture, which is now referred to as the Ministry of Agriculture Animal Industry and Fisheries (MAAIF), did not refer to bean cultivation in Uganda until 1913 when J.D. Snowden reported various edible beans grown as green manure in a Kampala plantation (Rubaihayo *et al.*, 1981). Serious consideration of beans as a food crop in Uganda started in the 1920s, when most of the cultivar introductions were made (Mukasa, 1970). But Leakey (1970) believes that the wealth of local names given to distinctive cultivars in, for example western Uganda, gives evidence of its long establishment as a crop.

Uganda lies within 900-1500 metres above sea level (masl), except for Kabale district, which largely lies within 1500-2100 masl. Bean cultivation is practiced within 900 and 2100 masl (Lindblade *et al.*, 1998). The climate of Uganda, with the exception of the mountains, is typically tropical and it is favourable for bean cultivation. The mean monthly maximum temperature ranges between 18.2-23.9° C in Kabale, the coldest zone, and 28.8-35.2° C in Kitgum, the hottest zone (NEMA, 2001). Most of the country experiences two rainy seasons extending from March to May and August to December and sowing is normally done between the dates of March-April and August-September for the first and second season crops, respectively. However, in the Lake Victoria crescent zone, rain falls almost throughout the year and here farmers tend to plant beans whenever they feel there will be sufficient moisture for the crop.

In Uganda beans are grown country-wide and are distributed and produced according to the agro-ecological zones. According to Wortman and Allen (1994), the production areas are defined based on the amount of rainfall per bean growing season, mode of rainfall and soil pH. These three factors are responsible for the bean yield variation among the different districts. The greatest production is in the areas with adequate, well distributed rainfall and

productive soils; these include the districts of Kabale, Kapchorwa, Mbale, Apac and Lira, each of which produces over 20,000 ton per year (Opio *et al.*, 2001). Production figures by 2002 were 496,000 ton of grain on about 618,000 ha of land (FAO, 2003). Considering that beans constitute a major part of the diet of over 80% of all Ugandan households (FAO, 2003), this is still low production. This low production could be attributed to the dependence on nature for production, poor cropping systems, diseases and insect pests.

Dry beans are grown in environments that are eminent for constraints like diseases, insects, drought, low soil fertility (Kyamanywa 1997; Opio *et al.*, 2001) and most farmers lack improved cultivars. In addition, farmers cannot afford and lack knowledge in the use of agrochemicals. According to Singh (1992), breeding for resistance to production-limiting factors helps recover yield potential of commercial cultivars, minimises production losses, reduces production costs, and stabilises yields. It also permits resource poor farmers to take advantage of improved cultivars and minimise the spread of seed borne diseases.

The cultivated species of *Phaseolus* in Uganda fall in four main groups; first is *P. vulgaris L.*, which includes the common bean, haricot, navy, and french or snap bean. Although the latter are newly introduced, they are becoming increasingly popular among smallholder farmers because of their high yield potential and market value (Anon., 2000). Second is *P. lunatus L.*, which includes the lima, sieva and butter or madagascar beans. The third is *P. coccineus L.*, and in this group, only the scarlet runner bean is grown in Uganda. The last group is *P. acutifolius A* but beans in this group are not common in Uganda, the only varieties available are found at research institutes and are only used for experimental purposes (Opio *et al.*, 2001).

Dry bean cultivars grown in Uganda vary considerably in their plant growth habits, agronomic and yield characters (Leakey, 1970; Mukasa, 1970). It is not surprising to find all the different growth types being grown in one farmer's field at the same time. The choice of a certain growth habit of beans is mainly determined by the type of crop associated with it. For example, in Kigezi (western Uganda), climbing beans are grown in association with sorghum whereas, in central Uganda, bush beans are preferred for interplanting with *Solanum* potatoes, maize and bananas (Rubaihayo *et al.*, 1981; David *et al.*, 2000). The determinate bush beans are more popular than the climbers because of being early maturing and their suitability in the intercropping systems used by most farmers (Rubaihayo *et al.,* 1981).

The cultivars which have been selected at research stations have made little impact due to seed multiplication and distribution problems. In the early 1960s Banja, Mutike 4, Canadian Wonder, Bukalasa and Abundance were the cultivars improved and recommended for better seed type and yield (Leakey, 1970). However, these cultivars were commonly found at experimental and other government stations and were rare on farmers' fields. This trend has been reversed in recent years as researchers are working hand in hand with farmers to develop cultivars suitable for their requirements. New and better yielding cultivars, suitable for market have been released since 1994 and these include K132 (CAL 96), K131 (MCM 5001), NABE 1, 2, 3, 4, 5 6, 7C, 8C, 9C, 10C, 11 and NABE 12C (David and Sperling, 1999; David *et al.*, 2000).

The most widely adopted cultivars are the bush types, Kanyebwa, K20 and K132, and they are highly marketable. The oldest of these is Kanyebwa, a large seeded pink mottled landrace, which is grown mainly in the central region of Uganda. K20 was released in 1968 and was the first product of bean research activities and is currently widely grow in Uganda, Kenya and Tanzania (Grisley, 1994; David and Sperling, 1999) because of its marketability and yield stability. Cultivar K132, released in 1994, is characterized by dark red mottled, large seed and is the most widely adopted and marketable of the three cultivars. The main hinderance to the use of these three cultivars is their susceptability to diseases.

Bean production in Uganda is constrained by pests, diseases, lack of improved cultivars, poor quality seed and poor cultural and agronomical practices (Leakey, 1970; Rubaihayo *et al.*, 1980; David *et al.*, 2000, Opio *et al.*, 2001). Furthermore, constraints are experienced from the adverse weather conditions, since all bean farmers in Uganda depend heavily on nature to produce beans and as such, any changes in the weather directly affect production.

Considering all the above constraints collectively, diseases are reported as the number one constraint to bean production (Kyamanywa, 1997; David *et al.*, 2000, Opio *et al.*, 2001). Most of the available cultivars are susceptible to diseases caused by fungi, bacteria and viruses. In many cases, farmers' fields may have more than one disease at the same time giving rise to disease complexes. About 20 bean diseases have been identified and the most important include angular leaf spot (*Phaeoisariopsis griseola* Sacc. Ferraris), anthracnose (*Colletotrichum lindemuthianum* Sacc. et. Magn.), rust (*Uromyces appendiculatus* Pers.

Unger), bacterial blights (*Xanthomonas phaseoil; X. phaseoli* var. *fuscans; and Pseudomonas phaseolicola*), bean common mosaic virus and root rot (Buruchara and Opio, 1996). The damage caused by diseases alone or in combination with other constraints varies with cultivar and environmental conditions. At the worst the crop may be completely destroyed.

Diseases have continually persisted in Uganda's dry bean industry mainly because of lack of improved resistant cultivars and a clean seed production and distribution system. Most of the seed producers in Uganda lack the technology for certifying seed against diseases and in many cases the seed quality and viability is questionable. As noted by DeVries and Toenniessen (2001), the root cause of all seed problems is the lack of proper monitoring of seed stock to keep them free of undesirable traits. Many seed companies also use the same breeder's seed stock for longer than is recommended. Uganda has very few plant breeders, especially bean breeders, and work on bean breeding is still in its infant stages.

Although work on dry beans started as early as 1920, breeding did not begin until after 1960 (Leakey, 1970; Rubaihayo *et al.*, 1981) and was halted in the early 1970s because of the civil strife that engulfed the country for nearly fifteen years. During this time, there was a complete destruction of the country's economy and the whole agricultural research system. Before the civil strife, several varieties had been bred and released but most varieties had not yet reached farmers and most of them were still at the research stations (Opio *et al.*, 2001). The reasons for the delayed distribution were the absence of an efficient extension system and seed market outlets by that time (Dr. Fina Opio, *pers. com.*).

Bean breeding was restarted in 1985/86 with the aim of increasing productivity of the crop by developing acceptable varieties (MAAIF, 2003). But unlike the other parts of the world where breeding objectives are broad based, in Uganda, dry bean breeding is still mainly focused on the production of cultivars resistant to pests and diseases which are seen as the major limiting factors to high yield production. At present, breeding for resistance to various diseases is underway at the National Agriculture Research Stations of Namulonge and Kawanda. Some of the diseases so far tackled include angular leaf spot and root rot (Opio *et al.*, 2001). It is therefore hoped that with the training of more breeders, more constraints will be tackled and more improved cultivars will be made available to cater for the diverse requirements of the Ugandan farmers and also help improve the country's economy.

Anthracnose as the main point of focus of this thesis, is a fungal disease caused by the fungus *C. lindemuthianum*. The disease is widely spread in all bean growing regions and often causes severe damage which affects yield, seed quality, and marketability of beans. It is one of the most important bean diseases worldwide and yield losses can reach 100%, especially when infected seeds are use (Allen *et al.*, 1996). In Uganda, anthracnose disease is most important in the high altitude low temperature areas (Opio, *et al.*, 2006). In most of these areas, susceptible cultivars still predominate and the disease has been observed to cause severe yield losses.

Anthracnose is known to be transmitted through seed, and considering that a large percentage of farmers in Uganda depend on farm-saved seed (David *et a*l., 2000; Opio *et a.l*, 2001), disease severity on farmer fields is normally high. In addition, there is a lot of sharing of the farm-saved seed between farmers, and this increases chances of disease transmission between farmer fields and regions.

The use of clean seed can greatly decrease disease severity but this strategy may be impractical, as very few seed companies are available. The use of fungicidal seed dressing and preventive spraying with protective or systemic fungicides (e.g., Benomyl, Zineb and Captafol) may be partially effective, but this is too costly for the resource poor farmers and the fungicides have adverse effects to the environment. In this case, the best strategy would be the use of resistant cultivars.

With the above consideration in mind, this research was set out with the principal aim of elucidating on the status of dry bean anthracnose in Uganda and the genetics governing anthracnose resistance and inheritance in relation to a sample of the Ugandan dry bean germplasm. The thesis is set out in different chapters with each new chapter tackling a different dry bean anthracnose related topic. The chapters are designed in such a way that each is independent and can easily be 'pulled out' and modified into a journal article. For that reason, one is bound to find a certain level of overlap and repetition between chapters.

The thesis is comprised of seven chapters. The first chapter is allocated to a literature review on the dry bean anthracnose disease. Chapter Two is allocated to the findings of the participatory rural appraisal conducted in one of Uganda's major bean growing areas. The results on the status of anthracnose physiological races in Uganda, dry bean germplasm

evaluation, yield loss associated with anthracnose and the anthracnose resistance genetics study, are given in Chapters Three, Four, Five and Six, respectively. The last chapter is dedicated to an overview of the research findings and their implication on the management of dry bean anthracnose in Uganda.

References

Allen, D.J., Ampofo, J.K.O., and Wortman, C.S. 1996. Pest, disease, and nutritional disorders of the common bean in Africa: A Field Guide. *Centro Internacional de Agricultura Tropical,* Cali Colombia. 132p.

Anonymous 2000. Marketability and new bean varieties in Uganda. *ASARECA AGRICFORUM*. Vol. 2.

Buruchara, R.A. and Opio, F. 1996. Pathogen diversity and screening for resistance to *Phaseolus griseola* in Uganda. In: *Uganda Bean Program Annual Report*.

David, S., and Sperling L., 1999. Improving technology delivery mechanisms: Lessons from bean seed systems research in eastern and central Africa. *Agriculture and Human values* 16:381-388.

David, S., Kirkby R., and Kasozi S. 2000. Assessing the impact of bush bean varieties on poverty reduction in Sub-Saharan Africa: Evidence frown Uganda. Network on Bean Research in Africa, occasional Publication Series, No. 31, *Centro Internacional de Agricultura Tropical,* Kampala, Uganda.

David, S., Mukandala L., and Mafuru J. 2002. Seed availability, an ignored factor in crop varietal adoption studies: a case study of beans in Tanzania. *Centro Internacional de Agricultural Tropica,* Kampala Uganda.

DeVries, J. and Toenniessen, G. 2001. Securing the Harvest. Biotechnology, Breeding and Seed Systems for African Crops. CAB Publishing, NewYork, USA. 208p.

FAO. 2003. Food and Agriculture Organisation quarterly bulletin of statistics. *FQBS* 12:49.

Grisley, W. 1994. The bean revolution. Ceres 149 (5): 27-30.

Grisely, W. and Mwesigwa F. 1990. Consumer preference for fresh and dry beans in Uganda: Implications for bean breeders (unpublished).

Kyamanywa, S. 1997. Current Status of Integrated Pest Management in Uganda. In: Gebrekidan, B., Amirault, J.P., and Abate, T. (eds.) *Proceedings of the IPM Networking in Sub-Saharan Africa Workshop*, October 1996, Addis Ababa/Nazreth, Ethiopia.

Leakey, C.L.A 1970. The improvement of beans (*Phaseolus vulgaris*) in East Africa. In: Leakey, C.L.A (ed.). *Crop improvement in East Africa*. London, The Commonwealth Agricultural Bureaux. pp. 98-128.

Lindblade, K.A, Carswell G., Tumuhairwe J.K., 1998. Mitigating the relationship between population growth and land degradation: Land-use change and farm management in Southwestern Uganda . *Ambio* 27: 565-571.

Matson, A.J. 1962. The history of East Africa. In: Russels, E.W. (ed.). *The Natural Resources of East Africa*. D.A. Hawkins Ltd. E.A. Lit. Bureau, Nairobi. pp. 10-16.

Ministry of Agriculture, Animal Industry and Fisheries (MAAIF). 2003. Operationalization of the Medium Term Plan for the Modernisation of Agriculture in Uganda, 1997-98, 2001-02, a Government Task Force Report, Entebbe, Uganda.

Mukasa, S.K. 1970. Beans (*Phaseolus, vulgaris* L.). In: Jameson,J.D. (ed.). *Agriculture in Uganda*. OUP. pp. 240-242.

NEMA. 2001. Republic of Uganda, 2001. State of the Environment Report: Kabale 2000/2001. National Environment Management Authority.

Opio, F., Ugen, M.A., Kyamanywa, S., David, S., and Mutetikka, M. 2001. Beans. In: Mukiibi, J.K, Esete, J.P., Musoli, P.C., Oryokot, J.O.E., Twinamasiko, E., Odongkan, O.K., Esegu, J.F.O., Otim-Nape, G.W., Ssali, H., and Odogola, W. (eds.). *Agriculture in Uganda. Volume II Crops*. NARO. Kampala, Uganda.

Opio, F., Mugagga., Mawejje, D., and Nkalubo, S. (2006). Progress report on bean anthracnose research in Uganda. *MUARIK bulletin.* (I*n press*)

Purseglove, J.W. 1969. Tropical Crops: Dicotyledons. Longman. pp. 304-310.

Rubaihayo, P.R., Milidwa, D, Songoba, T., and Kamugira, F. 1981. Bean production in Uganda. *In: Regional workshop on Potential for Field Beans in Eastern Africa, Lilongwe, Malawi, 1980 proceedings.* Centro Internacional de Agricultural Tropical. Cali, Colombia, 226p.

Singh, S.P. 1992. Common bean improvement in the tropics. In: Janick, J. (ed.). *Plant Breeding Reviews.* 10:199-270. John Wiley and Sons Inc.

World Bank. 2002. Uganda: Growing Out of Poverty, World Bank, Washington, DC.

World Bank. 2003. Uganda Agriculture: A World Bank Country Study. The World Bank Washington, D.C.

World Resources Institute. 1990. World Resources 1990-91. Oxford University Press, UK.

Wortman, C.S. and Allen, D.J. 1994. African bean production environments: their definition, characteristics and constraints. *Network on Bean Research in Africa.* Occasional paper series No. 11, Dar Salaam, Tanzania.

CHAPTER ONE
LITERATURE REVIEW

1.1 The bean crop

Dry bean is a leguminous grain food high in protein, phosphorus, iron, vitamin B_1, fiber, with no cholesterol and is an important staple food in many areas of the world, especially Central and South America, and Africa (Berglund and Brucher, 1976). Dry bean is a crop that requires special cultural management and attention by the producer. For maximum profitability, proper management in terms of field selection, planting, harvesting through to marketing, are essential.

There are two basic dry bean growth habit types; the determinate (bush) and the indeterminate (vining or trailing) and cultivars may be classified according to these two types (Singh, 1992; Walters *et al.*, 1997). For example, navy beans may be either of the bush or vining type. In the determinate type, stem elongation ceases when the terminal flower racemes of the main stem or lateral branches have developed. On indeterminate types, flowering and pod filling will continue simultaneously or alternately as long as temperature and moisture permits growth to occur. In addition to the distinction between determinate and indeterminate plant types, four plant growth habits have been identified. These are: Type I – determinate bush; Type II – upright short vine, narrow plant profile, three to four stems; Type III – indeterminate, prostrate vine; Type IV – indeterminate with strong climbing tendencies (Singh, 1992). These growth habits have become useful in identification and classification of new upright bean cultivars (Palmer, 1967; Rutger and Beckham, 1970; Singh, 1992). All these growth habit types are available in the different regions of Uganda and grown widely. A farmer can grow more than one growth type on the same field at the same time.

1.2 Dry bean yields

Dry bean yields vary with cultivar type but are mainly affected by agronomic practices and management. Improved varieties may give yields ranging between 1500-4500 kg ha^{-1} (Singh 1992). In Uganda, where home saved seed is mainly used, yields may be as low as 600 kg ha^{-1} (UEPB, 2005) but this can be lower depending on the number of constraints faced by the farmer.

Worldwide dry bean production has been constrained by a number of mostly fungal diseases which has led to significant reductions in seed quality, viability and eventually poor yields. One of the leading fungal diseases is bean anthracnose (*Colletotrichum lindemuthianum* (Sacc. et. Magn.) Lams. Scrib.) which has been reported to cause total yield losses in suitable environments.

1.3 Bean anthracnose (*Colletotrichum lindemuthianum*): Its biology and physiology

Bean anthracnose caused by the fungus *C. lindemuthianum* is a serious seed borne disease, capable of inducing complete yield losses on susceptible genotypes (Pastor-Corrales *et al.*, 1995). According to Tu (1983), the seedborne nature of bean anthracnose was demonstrated by Barrus in 1921 and the parasite, *C. lindemuthianum*, is a filamentous *Deuteromycetes* fungus with no known sexual stage. It causes anthracnose on the common bean (*Phaseolus vulgaris* L.), and under favourable conditions epidemics are severe, and yield losses high (Allen *et al.*, 1996). Anthracnose affects yield, seed quality, and marketability of beans. It is more prevalent in the temperate zones than in the tropics. There are multiple races of the fungus which are characterized by the cultivars they attack and as such, bean cultivars have been bred for resistance to one or more of these races. The anthracnose pathogen is spread through seed and can also be disseminated by rain splash, wind, and physical contact between plants. Infection and spread are favoured by cool temperatures (17-24° C) and high relative humidity.

Symptoms caused by the fungus initially appear on the lower surface of the leaf, which lead to indefinite enlarging reddish or dark brown lesions along the veins. Lesions are also commonly developed on cotyledons, petioles, branches, stems and pods (Allen *et al.*, 1996). With time these lesions are covered with sunken black fungal fruiting bodies that resemble tiny pin cushions containing black spines that are easily seen with a hand lens (Holiday, 1980). The seeds within diseased pods also become infected and seedlings developing from these infected seeds normally show severe symptoms (Allen *et al.*, 1996). Infected seed may be shriveled and mouldy, or near normal in appearance. It is within these infected seeds that the fungus survives as mycelium, it can also over-winter within the crop debris (Dillard, 1990).

Controlling anthracnose may be achieved through the use of clean seed and by doing so, one can greatly decrease disease severity but this strategy may be impracticable in much of

Africa, since farmers save seed. Alternatively, for suspected incidences, seed coat infestation can be controlled chemically, or for an already planted crop, preventive spraying can be done with protectant or systemic fungicides (e.g., Benomyl, Zineb and Captafol). All the former methods provide partial protection as the crop can be attacked by the fungus once it is susceptible to any of the prevailing races of the fungus. Although fungicidal seed dressing and other plant chemical protections can be effective, the use of a resistant cultivar is considered the best strategy.

1.3.1 Biology

The anthracnose fungus is a *Deuteromycetes* which is ensured by asexual reproduction and uses conidia for further infection and spread (Bailey *et al.*, 1992). Splashing raindrops carry conidia to the surface of the host. It is from here that the conidia germinate and the fungus enters the host by means of appressoria formation (Király *et al.*, 1970). The infective hyphae then penetrate the host through the appressorial and towards the end of the disease cycle, acervuli are formed on the necrotic sunken spots induced by the fungus in the host (Király *et al.*, 1970; Bailey *et al.*, 1992).

Although the continuity of *C. lindemuthianum* is ensured in nature by asexual reproduction, sexual forms of the pathogen have been observed in laboratory experiments, but these are not reproducible due to low viability of the ascospores (Bryson *et al.*, 1992). It is thus thought that sexual or parasexual reproduction occurs in natural populations, leading to recombination between different loci (Sicard *et al.*, 1997).

In nature the fungus has been found to withstand unfavourable conditions by overwintering in plant residues (Dillard, 1990), but the most common survival mechanism for the fungus is through infected seed, which are passed on in the next season (Tu, 1981; Bailey *et al.*, 1992; Young and Kelly, 1997). The development of the disease requires high humidity (87-93%), a temperature range of 15-28°C and rainy conditions. Furthermore, windy weather is necessary for epidemic spread (Tu, 1981; Bryson *et al.*, 1992).

1.3.2 Physiology

In *C. lindemuthianum*, the conidia are embedded in a matrix of moist hydrophilic mucilaginous material (Griffiths and Campbell, 1972; Nicholson and Moraes, 1980), comprised mostly of polysaccharides and high molecular weight glycoproteins (Ramadoss *et al.*, 1985; Louis *et*

al., 1988). These matrices are readily soluble in water, with spores being released and dispersed by the action of free water and as such, rainfall availability mainly determines the survival and severity of field infections. The matrix maintains the viability of conidia under adverse conditions such as extreme temperature, ultraviolet light, and low humidity (Nicholson and Moraes, 1980; Nicholson *et al.*, 1986; Louis *et al.*, 1988), and protects spores from toxic material produced in the host tissues during lesion development (Nicholson and Moraes, 1980; Nicholson *et al.*, 1986). There is also evidence that it can inhibit conidial germination, thus ensuring that conidia do not germinate until they are dispersed from the acervulus (Louis and Cooke, 1985; Seebach *et al.*, 1989; Leite and Nicholson, 1992).

On germination of conidia, the spore, germ-tubes and appressoria formed are surrounded by mucilage, which may take the form of water-soluble fluids (Nicholson and Moraes, 1980) or more rigid, polymerized materials (Kwon and Epstein, 1997; Sugui *et al.*, 1999). These mucilages mediate the adhesion of conidia germ-tubes and appressoria to the host surface (Mercure *et al.*, 1994; Hughes *et al.*, 1999). For the penetration of the host tissue, the appressorium wall contains melanin at an extraordinarily high osmotic pressure which is converted into a penetration force, concentrated at the tip of a thin penetration hyphae/peg (Bechinger *et al.*, 1999). *Colletotrichum lindemuthianum* being a classical example of a hemibiotrophic fungus (O'Connell *et al.*, 2000), after penetration of the host cuticle and the epidermal cell wall, it initially grows as a biotroph with primary intracellular hyphae for the first few days (Pain *et al.*, 1996; O'Connell *et al.*, 2000). This ensures reliable infection, while causing minimal damage to host cells. Subsequently, secondary, narrower hyphae are formed that kill the host cells and proliferate by necrotrophic growth (O'Connell *et al.*, 2000) to other plant tissues. As new host cells are colonized by primary hyphae, the sequence of a transient biotrophic phase followed by cell killing is repeated. This relationship ends as soon as narrow secondary hyphae develop, which are not surrounded by the host membrane and lack an interfacial matrix (Bailey *et al.*, 1992; Bechinger *et al.*, 1999). Host walls break down because of the secretion of large amounts of cell-wall-degrading enzymes by the secondary hyphae. In very severe cases the entire plant may be killed.

1.4 Genetic variability of *Colletotrichum lindemuthianum*

Colletotrichum lindemuthianum has been reported to posses a very high degree of genetic variability in different parts of the world (Menezes and Dianese, 1988; Drijfhout and Davis, 1989; Tu, 1992; Sharman, *et al.*, 1999). This variability has been investigated by a number of researchers in Mesoamerica, and the South and North Andes, which are the three centres of diversity of common bean. It has been suggested that co-evolution between this pathogen and the common bean may have led to parallel gene pools (Gepts *et al.*, 1986; Gepts, 1988, Sicard, *et al.*, 1997). During a study on *Phaeoisariopsis griseola*, another important bean pathogen, Guzmán *et al.* (1995) established that its isolates are divided into two molecular groups one appears to consist of isolates that attack Mesoamerican beans and the other consists of isolates that attack southern Andean cultivars. This same hypothesis had been suggested for *C. lindemuthinuml,* although the findings of Sicards *et al.* (1997) suggest that there is an adaptation of pathogenic strains to attack cultivars of the same geographic origin. Sicards *et al.* (1997) further subdivided the *C. lindemuthianum* isolates, based on molecular and virulence markers, into three groups corresponding to the host gene pools. In other studies to determine the variability of resistance to *C. lindemuthianum* in the three centres of diversity of common bean, it was established that there was polymorphism for resistance both between and within the three centres of diversity (González *et al.*, 1998).

A better resolution of the structure of the variability in *C. lindemuthianum* is obtained by combining virulence and molecular analyses. Unfortunately, virulence analysis based on local codes and different cultivar differential series has limited the understanding of the broad variability within this pathogen worldwide (Beebe and Pastor-Corrales, 1991; Sicards *et al.*, 1997). The use of differential cultivars in regions where their optimal growth may not be favoured by the environmental conditions may jeopardize their resistance or susceptibility to different physiological races of the disease. Also the imbalance among cultivars from the two *P. vulgaris* gene pools in the new differential series (Pastor-Corrales, 1992) might favour identification of races belonging to the Middle American reaction group (Balardin and Kelly, 1998). In addition, the multigenic resistance in some differentials such as G 2333 might select races with multiple avirulence genes (Pastor-Corrales *et al.*, 1994). Therefore, information related to virulence may still be biased. Susceptibility of plants to local strains indicates local adaptation of the pathogen towards hosts from the same region. Resistance of plants to allopatric strains reveals the existence of different resistance genes within a given region and this suggests that the region can be differentiated for resistance genes. It is this differentiation

in resistance to different bean cultivars that classifies the different physiological races of the disease.

1.5 Identification of physiological races of *Colletotrichum lindemuthianum*

Identification of physiological races is based on their pathogenicity on certain host cultivars. Considering that cultivars are approximately homogeneous from a genetic point of view, they are parasitalised differently by different physiological races of the same pathogen (Király *et al.*, 1974). Therefore, pathogenic races of plant pathogens are determined on the basis of their pathogenicity on certain cultivars of the host plant. Black *et al.* (1953) devised an international system of classifying races and genes for resistance. According to this system, a particular race reflects the number of the resistance gene possessed by the plant that it is able to infect. For example, the "common field race", race 0 can infect only cultivars of potato with no R genes. Race 1 can infect plants with gene R1 in addition to the cultivars with no R genes. Race 2 can infect plants with gene R2 plus the plants with no R genes, and so on. Races which infect plants with dominant genes R1 and R2 as well as those with no R genes are designated as race 1.2 and so forth.

The procedures for identifying pathogenic races were suggested by Stakman *et al.* (1962). They involve the collection of samples of the pathogen from different locations, which may then be increased in the greenhouse or laboratory conditions. Isolates are made from these samples which are then tested for pathogenicity on a differential set of crop cultivars whose susceptibility and resistance to the different races of the pathogen differ (Király *et al.*, 1974; Pastor-Corrales *et al.*, 1985). Each member of the differential set shows a reaction after infection, which may be designated as resistant, susceptible or mesothetic (Drijfhout and Davis, 1989; Pastor-Corrales *et al.*, 1995; Tu, 1992). The reactions of the differentials are compared after infection with the different pathogen isolates to determine the pathogenicity of each isolate. It is on the basis of these reactions that different physiological races are distinguished. Reaction of differentials may be changed depending on the environmental conditions and in this case, determination of races is conducted under controlled light and temperature, as these two conditions are very influential in the host-pathogen reactions.

1.6 Races of *Colletotrichum lindemuthianum* and their differentials

Colletotrichum lindemuthianum exhibits many physiological races which are identified by their differential virulence on a range of bean cultivars as defined by Pastor-Corrales *et al.* (1985). It has been proposed that the race-cultivar specificity observed between the two organisms results from the interaction of the products of single avirulence genes, with those of the corresponding resistance genes (O'Connell and Bailey, 1986; Bailey, 1991). This system exemplifies the gene-for-gene concept defined by Flor (1971). In the absence of sexual reproduction in this fungus, the isolates cannot be characterised for the purposes of genetic control of their virulence. Seven avirulence genes have been postulated corresponding to seven resistance genes known in *P. vulgaris* (Farbre *et al.*, 1995).

The anthracnose pathogen shows extensive physiological differentiations which gives rise to various races. Several physiological races of the *C. lindemuthianum* fungus have been identified (Drijfhout and Davis, 1989; Tu, 1992). According to Sharman *et al.*, (1999) more than 15 races have been characterized so far. These include; alpha, beta (Barrus, 1911); gamma (Burkholder, 1923); delta (Andrus and Wade, 1942); Mex I, II and III (Yerkes and Ortiz, 1956); epsilon (Blondet, 1963); Brazil I, II (Oliari *et al.*, 1973); lambda and kappa (Hubbeling, 1976); alpha Brazil (Fouillox, 1976); jota (Hubbeling, 1977); and C236 (Schwartz *et al.*, 1982), their composition varying among countries and different regions. The *C. lindemuthianum* races are identified using different bean differential cultivars.

The differential cultivars used for the identification of the races are meant to react differently to different physiological races and while AB 136 cultivar is used as a general resistant, Michelite acts as general susceptible cultivar (Drijfhout and Davis, 1989), although it has been known to be resistant to some isolates of beta and gamma races (Yerkes and Ortiz, 1956; Hubbeling, 1957; Bannerot, 1965; Goth and Zaumeyer, 1965). The various differential cultivars show clear vertical resistance to anthracnose but vary greatly in the breadth of pathotypes to which they confer resistance. For instance, Cornell 49-242, which has the *ARE* anthracnose resistance gene (Mastenbroek, 1960), is resistant to races alpha, beta, delta, epsilon, gamma and lambda (Muhalet *et al.*, 1981) and susceptible to a great number of isolates in Latin America, while Mexico 222 (Mexique I resistance gene), TO (Mexique II resistance gene), TU (Mexique III resistance gene), Ecuador 299, and P.I 207262 are susceptible to far fewer races (Beebe and Pastor-Corrales, 1991). On the other hand, AB 136, though reported to be resistant in field tests in many locations in Latin America and

Costa Rica, has also been seen to be susceptible to a few Costa Rican isolates in greenhouse seedling tests (Schwartz *et al.*, 1982). This exemplifies the high variability and pathogenic capacity of the *C. lindemuthianum* fungus. According to Beebe and Pastor-Corrales (1991), some newly identified resistance sources such as G2333, G811, and G2641, are resistant to all known races or isolates under all conditions. This conclusion was made after having evaluated these new resistance sources using field tests in several countries and in greenhouse seedling tests in Colombia. It is therefore believed that their resistance may well be controlled by major genes, but as yet, cannot be classified as vertical (Beebe and Pastor-Corrales, 1991).

It is evident from some experiments conducted by certain researchers that not all cultivars universally used to differentiate races of *C. lindemuthianum* are the most suitable ones for differentiation of the known races of this fungus. For example, in an experiment conducted by Drijfhout and Davis (1989), the reaction of Perry Marrow to beta, delta, kappa and C236 races were unclear and as such, this cultivar would be less suitable as a differential because of genetic complexities. Normally plant selections out of a cultivar used as a differential may differ in reaction to a race of the pathogen, due to genetic impurity within the differential cultivar (Tu, 1985; Pastor-Corrales *et al.*, 1985). Also any cultivar used as a differential may give intermediate reaction with some races, while other cultivars always give clear susceptible or resistant reactions (Drijfhout and Davis, 1989). Only the latter should be used as differentials.

A standardized binary nomenclature system and set of twelve differential cultivars has been developed (Pastor-Corrales, 1991) and according to Balardin and Kelly (1997), the major objectives of the standardized system were to generate an unbiased description of *C. lindemuthianum* populations, and to facilitate the international exchange of information.

Using this methodology, 15 races previously characterised on traditional differential sets and named with Greek letters or local codes (traditional nomenclature), were re-characterised (Table 1). A binary value was assigned to each race (Balardin and Kelly, 1997). For example, Race Beta (Race 130) was previously characterised as Race 3, whereas Race Epsilon Kenya (Race 19) and Race Epsilon (Race 65) both were previously assigned Race 1. Also, Race Kappa (Race 31) had been shown to overcome Mexico 222 cultivar, although only the first five differentials should be susceptible. It is believed that one possible cause of error in

classifying races might be the lack of genetic purity within the differential cultivars (Drijfhout and Davis, 1989; Balardin and Kelly, 1997). This misclassification of races has resulted in a false assessment of the variability present in *C. lindemuthianum* and has generated bias in the research results reported in certain countries (Tu, 1994; Balardin and Kelly, 1997; Balardin *et al.*, 1997).

Table 1: Races of *Colletotrichum lindemuthianum* characterised, based on the international set of differentials, and named, based on the binary nomenclature system (Balardin and Kelly, 1997)

Race designation		**Differential cultivars***											
Binary	**Traditional**	1	2	3	4	5	6	7	8	9	10	11	12
17	Alpha	S	R	R	R	S	R	R	R	R	R	R	R
19	Epsilon Kenya	S	S	R	R	S	R	R	R	R	R	R	R
23	Delta	S	S	S	R	S	R	R	R	R	R	R	R
31	Kappa	S	S	S	S	S	R	R	R	R	R	R	R
55	Lambda	S	S	S	R	S	S	R	R	R	R	R	R
65	Epsilon	S	R	R	R	R	R	S	R	R	R	R	R
81	Eta	S	R	R	R	S	R	S	R	R	R	R	R
87	Mu	S	S	S	R	S	R	S	R	R	R	R	R
89	Alpha Brazil	S	R	R	S	S	R	S	R	R	R	R	R
99	Teta	S	S	R	R	R	S	S	R	R	R	R	R
102	Gamma	R	S	S	R	R	S	S	R	R	R	R	R
130	Beta	R	S	R	R	R	R	R	S	R	R	R	R
141	C236	S	R	S	S	R	R	R	S	R	R	R	R
337	Mex II	S	R	R	R	S	R	S	R	S	R	R	R
453	Zeta	S	R	S	R	R	R	S	S	S	R	R	R

*1. Michelite (1), 2. Michigan Dark Red Kidney (2), 3. Perry Marrow (4), 4. Cornell 49-242 (8), 5. Windusa (16), 6. Kaboon (32), 7. Mexique 222 (64), 8. PI 207262 (128), 9. TO (256), 10. TU (512), 11. AB136 (1024), 12. G2333 (2048). The numbers between parentheses represents the binary value for each differential. The binary value for each race is obtained by summing the value of cultivars with susceptible (S) reaction.

1.7 Host-pathogen interaction

The interaction between the bean plant and *C. lindemuthianum* pathogen, although not proven, seems to conform to Flor's gene-gene model (Beebe and Pastor-Carrales, 1991; Neema *et al.*, 1994). In this case, for each gene that confers avirulence to the pathogen, there is a corresponding gene that confers resistance to the host, and vice versa. Within a plant lies the basis for dynamic interaction between host and parasite. Plants have innate chemical and structural defenses, and their cells and tissues have the capacity, in most instances, to recognize a foreign presence within minutes to hours after initial contact and to respond with a succession of defense processes by which they thwart most attempts to penetrate and colonize them (Beckman, 1987). Thus, if we are to understand, and indeed to

appreciate, the mechanisms by which the few successful parasites are able to penetrate and colonize a host plant, it must be in terms of their capacity to circumvent or overcome this complex and well-integrated set of defences. It will also be understood that the failure of the pathogen to gain entry or cause infection to the plant would be as a result of the pathogen's inability to overcome the plants defence mechanism or the ability of the plant to suppress the effects of the pathogen. This is what the host-pathogen relation is all about and it can be enhanced or depressed by the prevailing environment in which the plant is growing.

The effects of environment to the host-pathogen interaction include among other things, relative humidity, temperature, nutrition levels and the amount and quality of the initial pathogen inoculum. In this respect, and in the case of laboratory experiments, low aggressiveness of the pathogen may be a result of continuous propagation on artificial culture media. The pathogen is said to lose its aggressiveness when grown in the artificial environment for prolonged periods of time (Drijfhout and Davis, 1989). Therefore, re-isolation of the fungus from the host plant at regular intervals is needed to maintain the pathogen aggressiveness to host attack. Low initial spore concentration causes a weak host reaction and many researchers have considered a concentration of not less than 1×10^6 cells ml^{-1} to be adequate for host reaction (Tu, 1985; Bigirimana and Höfte, 2001). Any spore cell concentration below that may have no effect on the host. Also temperatures lower than 15°C and higher than 28°C and/or growth conditions which are too dry may result in a weak or an early termination of the fungus (Bailey *et al.*, 1992). A relative humidity greater than 80% is required for successful disease development (Drijfhout and Davis, 1989).

1.8 Dispersion and mode of infection of anthracnose

The initial infection comes from fungus propagules that are carried in the seed or, to a lesser extent, in bean straw. The fungus remains alive in the seed as long as the seed is viable. In the field, recent research shows that the fungus can survive over the winter only in dry straw, not in wet or buried straw (Dillard, 1990). After this initial infection, the disease is spread by spores carried in splashing raindrops, by machines, or by people. Splashing raindrops containing spores are blown by gusting winds and this causes the disease spread to follow the direction of the prevailing winds.

Anthracnose disease has been observed to appear on initially healthy plants 3-7 days after major rains and where temperatures are conducive for its development (Tu, 1981; 1983). It is

believed that rain aids the release of anthracnose spores from their gelatinous substance on the acervuli. The spread of bean anthracnose fungus from an infection focus appears to be limited to the travel distance of splashing raindrops (Nicholson and Moraes, 1980), the spread between regions is thought to originate from infected seeds in stock that are distributed to various growers.

The first essential feature of successful pathogenesis is the attachment of dispersed fungal propagules to the host plant surface (Hamer *et al.*, 1988; Nicholson and Epstein, 1991). Studies have shown that *Colletotrichum* conidia will adhere rapidly to a wide range of plant and artificial surfaces, including cellophane, polystyrene, polycarbonate and glass (Young and Kauss, 1984; Sela-Buurlage *et al.,* 1991; Mercure *et al.*, 1994; 1995), suggesting that adhesion is non-specific. Furthermore, studies by Mercure *et al.* (1995) showed that the respiration inhibitor sodium azide and the transcription inhibitor, actinomycin D, had no effect on adhesion, suggesting that adhesion of ungerminated conidia is largely a passive process.

The ultrastructure of the conidia of several species of *Colletotrichum* (*C. lindemuthianum*, *C. truncatum*, and *C. graminicola*), has been examined after preparation by cryofixation and freeze-substitution (Van Dyke and Mims, 1991; Mims *et al.*, 1995; O'Connell *et al.*, 1996). These studies showed that the walls of ungerminated and germinated conidia are coated with a layer of fibrillar material, the 'spore coat', which was more electron dense than the underlying cell wall. It has been suggested that the spore coat in *Colletotrichum* is responsible for the hydrophobicity of the cell surface and controls the adhesion of this pathogen to the host cell (Young and Kauss, 1984; Sela-Buurlage *et al.*, 1991; Mercure *et al.*, 1994). The spore coat appears to be a preformed structure, present on ungerminated, unimbibed conidia (Van Dyke and Mims, 1991), which is consistent with a role in the initial attachment of conidia to the plant surface.

Although several methods could be used by *Colletotrichum* species to penetrate its hosts, according to Bailey *et al.*, (1992), the direct penetration method is the most common. He further states that the infection through wounds is not common, and is not usually a prerequisite for infection, but it is well known that for some other diseases infection through wounds is very essential (Krantz *et al.*, 1978; Agrios, 1988). In the majority of *Colletotrichum* species, appressoria are usually, but not always, a prerequisite for penetration of host cuticles (Bailey *et al.*, 1992). There is evidence that the penetration peg of *C. lindemuthianum* can exert sufficient mechanical force for the penetration peg to penetrate host cuticles

(Wolkow *et al.*, 1983; Rasmussen and Hanau, 1989; O'Connell *et al.*, 1992; Pascholati *et al.*, 1993). Appressoria are often sessile but may form at the end of distinct germ-tubes and are sometimes produced at the tips of mycelial branches (Parbery, 1981). Adhesion of appressoria to the plant surface is essential for successful penetration of the cuticle and underlying cell wall by the penetration peg (Bailey *et al.*, 1992). Adhesion ensures that the pathogen remains in contact with the host for sufficient time for penetration to occur, as well as placing the infection hypha at a site where penetration, whether mechanical or enzymatic, can be achieved. Firm anchorage is also essential for the penetration peg to exert the mechanical force required for penetration (Bailey *et al.*, 1992). Appressorium formation is often accompanied by the secretion of a mucilaginous matrix, which surrounds the appressorium (O'Connell *et al.*, 1996; Pain *et al.*, 1996). This matrix extends outwards over the host surface, and appears to attach the appressorium to it (O'Connell *et al.*, 1985; O'Connell and Ride, 1990; Bailey *et al.*, 1992).

After penetration of the cuticle, the fungus is restricted within the epidermal layer until it colonises and causes death to the underlying tissues (Dron and Bailey, 1999). During this period, called the latent or dormant period, the host undergoes physiological changes of fruiting and ripening, leading to further pathogen development (Brown, 1977; Prusky and Plumbley, 1992). According to Dron and Bailey (1999), the infection process involves an initial biotrophic phase, in which spherical infection vesicles are produced within the initially infected epidermal cells. This is followed by a destructive necrotic lesion forming phase. The latent period of *C. lindemuthianum* within the host varies greatly depending on environmental conditions, especially moisture content. The fungus was found to survive for as long as five years in infected pods and seeds of *P. vulgaris* that had been air-dried and kept in storage at 4° C or in dry infected plant materials left in the field in sealed polyethylene envelopes that had no contact with water (Tu, 1983; Dillard, 1990). In summary, the anthracnose fungus is well suited to infect its host and has the ability to overwinter during the harsh conditions and re-infect its host at the earliest opportunity.

1.9.1 Leaf infection and symptoms

Under moderate temperature and high humidity, the fungus penetrates the leaf tissue and, at first, undergoes biotrophic growth without inducing symptoms (Meyer *et al.*, 2001). Several days later, infected cells die, the colonized dead tissue turns brown, visual symptoms appear

and the fungal growth becomes necrotrophic (Bailey *et al.*, 1992). Lesions are most common on leaf petioles and on the lower surfaces of leaves and leaf veins (Hall, 1991).

Although infection may occur on both sides of the leaf and on the petiole, early signs of infection usually appear on the lower leaf surface along the veins, which show brick red to purplish red discoloration. Later, such discoloration also appears on the upper leaf surface. At the same time, brown lesions of various sizes, with black, brown, or purplish red margins, develop around small veins (Allen *et al*, 1996). During disease progression, vein necrosis appears first, then wilting and bleaching often occurs at the tip of the leaflet before spreading over the margin and finally over the centre of the blade (Godoy *et al.*, 1997)

During this stage hyphae proliferate throughout host tissues, inside cells, in walls and through walls and in intercellular spaces. Colletotrichum *lindemuthianum* produces cell wall degrading enzymes and low molecular weight phytotoxins that may, by killing cells in advance of the invading hyphae, contribute to the necrotrophic growth of this pathogen (O'Connell *et al.*, 1985; Bailey *et al.*, 1992). Eventually conidiophores rupture through the host cuticle and form acervuli on the plant surface (Bailey *et al.*, 1992).

Because of the necrotic lesions in the leaf tissue, there is a decrease in the leaf photosynthetic activity (Meyer *et al.*, 2001), but the studies conducted by Bassanezi *et al.* (1997) revealed that the reduction of the photosynthesizing green area caused by the fungus does not account for the large inhibition of net CO_2 assimilation. It can thus be said that the necrotic patches created by the pathogenic activities of the fungus cause a decrease in the photosynthetic activity in the remaining green part of the infected leaves and this has far reaching consequences regarding crop yield.

1.9.2 Pod infection and symptoms

Before attacking the pods, the anthracnose fungus will infect the stems first. Stem infection is manifested by dark brown eyespots which develop longitudinally along the stems (Tu and Aylesworth, 1980; Tu, 1981; 1983). In the young seedling, if the eyespots enlarge, the stem may break off, but for older stems, the eye-shaped lesion is limited to an approximate length of 5-7 mm, and the lesion often has a sunken cankerous centre (Allen *et al.*, 1996). After infecting the stems, the infection will then be passed on to the pods.

On pods, the most striking disease symptoms are small brown specks on rusty brown spots. As these spots enlarge, their centres turn brown and many tiny black specks appear randomly on the brown area, replacing the brown specks (Bailey *et al.*, 1992). Each of the tiny black specks contains a mass of pinkish spores, often visible as a viscous droplet in humid conditions. The lesions on the pod usually reach a diameter of 5-8 mm, are slightly sunken at the centre and have a dark brown or purplish brown margin (Tu and Aylesworth, 1980).

1.9.3 Seed infection and symptoms

Seed infection is the major source of anthracnose transmission to the next crop generation and provides conditions which enable the fungus to survive unfavourable weather conditions. The fungus will remain alive as long as the seed remains viable, although not all infested and infected seed is capable of transmitting the disease (Bailey *et al.*, 1992). The variation in seed transmission relates to the degree of infestation as well as the severity and site of infection in the seed (Tu, 1983). Infection of the seed is as a result of infections passed on from the pods. The higher the number of pods infected, the higher is the number of seeds infected.

On the seed, anthracnose is displayed as brown to light chocolate-coloured spots on the seed coats and in highly infected seed, the lesions may extend into the cotyledons (Bailey *et al.*, 1992; Allen *et al.*, 1996). Infected seeds, when planted will serve as the primary source of infection for successive crops and in environments where anthracnose infrequently overwinters or where the disease is non existant.

1.10 Mechanism of resistance

Plants have a wide array of physical and chemical strategies to defend themselves from invasion by pathogens. Host resistance can be based upon physical factors such as surface features (e.g., topography, leaf hairs, and epicuticular waxes), that impede the formation of infection structures; structural barriers such as papillae that impede penetration; and secondary metabolites that are toxic or otherwise inhibitory to fungal growth (Heath, 1981). Physical barriers such as the mechanical strength of the cuticle and epidermal wall and the resistance of their structural polymers to enzymatic degradation, represent the first line of defense to fungal pathogens. These may contribute to the greater resistance in several species of mature leaves and stems compared to young plant organs (Mercer *et al.*, 1974). However, according to Beckman (1987), the active biochemical defence strategies adopted

by plants to ward off fungal attack are generally considered to be more important as these often determine the success of an infection.

A common feature of a host resistance reaction expressed in response to infection is the rapid localised death and browning of host cells, known as a hypersensitive reaction (HR) (Bailey, 1991). O'Connell and Bailey (1986) distinguished two kinds of resistance in the bean anthracnose pathosystem. These were a form of resistance involving the early death of epidermal cells, and a form associated with delayed death of infected cells. In many resistant cultivars, single epidermal cells died as soon as they were in contact with the pathogen. This type of resistance, which seems to depend on the localised accumulation of phytoalexins, has been referred to as an example of HR based on host cell incompatibility (Bailey *et al.*, 1992). Where resistance was associated with the delayed death of infected cells, initial events resembled those of susceptible interactions. However, after a biotrophic phase of varying duration, the infected cells died rapidly, turned brown and further fungal growth was inhibited (O'Connell *et al.*, 1985). The extent of symptom and pathogen development appears to depend on the time at which the infected cells died, turned brown and accumulated phytoalexins (O'Connell and Bailey, 1986). However, a critical investigation of the HR by Bailey *et al.* (1992) indicated that early death of infected cells occurred before phytoalexin formation.

Hydroxyproline-rich glycoproteins (HRGPs) are another group of metabolites often associated with host resistance. It has since been reported that HRGPs accumulate in bean seedlings infected with the *Colletotrichum* fungus, and that greater amounts are produced in resistant tissues than in susceptible ones (Mazau and Esquerré-Tugayé, 1986). HRGPs have also been shown to accumulate especially in the papillae of *P. vulgaris* and are produced in response to infection by *C. lindemuthianum* (O'Connell *et al.*, 1992). The precise role of HRGPs in plant defense is unknown, but it has been suggested that they act by increasing the structural resistance of cell walls to pathogen penetration. In addition to HRGPs, other plant defense proteins are thought to be secreted at the host-pathogen interface (Esquerré-Tugayé *et al.*, 1992). These include protein inhibitors of fungal hydrolases, and plant hydrolases which exert their hydrolytic activity towards bacterial and fungal cell walls, causing lysis and/or release of elicitor-active fragments (Esquerré-Tugayé *et al.*, 1992).

Formation of wall appositions (papillae) is often observed in the living cells of both resistant and susceptible hosts. These papillae are largely composed of callose, together with HRGPs

and phenolics, and are deposited between the plasma membrane and outer wall of epidermal cells at sites of attempted fungal penetration (Bushnell and Gay, 1978; O'Connell *et al.*, 1985). It has been suggested that the production of papillae may be stimulated by events which take place before the cuticle has been fully penetrated, such as the diffusion of fungal metabolites or by-products of cuticle degradation (O'Connell and Bailey, 1986; Mould *et al.*, 1991). Although a proportion of intracellular hyphae can become completely encased by wall appositions and fail to develop further, papillae do not prevent all infections. It is probable that papilla deposition occurs in all penetrations, but in successful infections, the response is 'switched off' and the fungus grows through the papilla. This would account for the collar-like structure formed around the neck of haustoria and infection vesicles. Unlike grasses and cereals, where the proportion of the hyphae encased by papillae is greater than 70%, in dry beans the proportion is only between 5-15% (Sherwood and Vance, 1980). This is possibly why beans are infected and easily destroyed by the anthracnose fungus.

1.11 Sources of resistance

An understanding of the mode of infection is a prerequisite for developing effective control strategies, particularly those based on host plant resistance. Knowledge of the factors influencing infection processes also provides breeders with information which can be developed into potential sources of resistance. Painter (1951) described a plant's resistance as the relative amount of its heritable qualities that influence the ultimate degree of damage done by any pest or pathogen. In the same manner, the common bean would be expected to express a similar phenomon against all pathogens. Therefore, the use of genetic resistance would be the most practical and economical way to manage and control anthracnose in this crop.

There are two kinds of host resistance to crop pathogens, i.e. vertical and horizontal resistance. While the inheritance of vertical resistance is conditioned by single genes that are part of a gene-for-gene relationship (Flor, 1971), horizontal resistance is conditioned by multiple genes (Vanderplank, 1968; 1975). According to Robinson (1997), horizontal resistance functions equally against most strains of the pathogen and that it cannot fail to the extent that vertical resistance fails. Horizontal resistance is assumed to be durable and that it is the resistance that invariably occurs in the absence of vertical resistance, or after a vertical resistance has been matched (Vanderplank, 1968). Unfortunately, it tends to be lost when

crops are bred for vertical resistance, or when they are bred under the protection of chemicals.

The genetic control of resistance to different races of anthracnose has been studied by several investigators. Burkholder (1918), McRostie (1919) and Andrus and Wade (1940) were the first to provide evidence that resistance to different races of *C. lindemuthianum* were controlled by either single, double or triple factors (Muhalet *et al.*, 1981) depending on the race under investigation. However, plant breeders have historically relied upon race-specific resistance to control specific races of *C. lindemuthianum*. For this reason the reactions of many bean cultivars and accessions have been tested to find diverse sources of resistance (Beebe and Pastor-Corrales, 1991; Pastor-Corrales *et al.*, 1992).

The main drawback to this type of resistance source is the possible loss of resistance caused by the adaptation of the pathogen to host resistance (Fry, 1982; McDermott, 1993). *Colletotrichum lindemuthianum* is a highly variable pathogen (Kelly *et al.*, 1994; Sicard *et al.*, 1997; Balardin and Kelly, 1998; Sharman *et al.*, 1999), and there are no single resistance genes that are effective against all known races of this pathogen. For example, cultivars that carry the *Are* or A resistance genes have failed in North America (Krüger *et al.*, 1977; Kelly *et al.*, 1994; Pastor-Corrales *et al.*, 1994; Tu, 1994; Pastor-Corrales *et al.*, 1995). The protection offered by single genes is potentially short lived. Therefore, diverse sources of genetic resistance should be sought by bean breeders. Alternatively, durable resistance should be acquired through the exploitation of horizontal resistance breeding.

Genetic resistance to some pathotypes of *C. lindemuthianum* is conferred by different single, duplicate, or complementary dominant genes (Schwartz *et al.*, 1982; Young and Kelly, 1996), and is available in numerous germplasm accessions (Schwartz *et al.*, 1982; Sharman *et al.*, 1994; Pastor-Corrales *et al.*, 1995; Fernández *et al.*, 2000). The available resistance is not effective against all known races from the same or different regions. For example, some of the well-known resistance genes that are effective in Europe (Co-2, Co-3, Co-4, and Co-5) (Kelly *et al.*, 1994) are not effective in Colombia, Brazil, Costa Rica, and Mexico (Kelly *et al.*, 1994; Krüger *et al.*, 1977; Menezes and Dianese, 1988; Tu, 1994). Also the breakdown of resistance in Cultivar G2333, which has three resistance genes (Co-4^2, Co-5 and Co-7) and once thought to be effective against all races (CIAT, 2000), only reflects the need for diversification of resistance genes.

According to Mahuku *et al.* (2002) the secondary gene pool of *Phaseolus* species is highly resistant to a range of *C. lindemuthianum* pathotypes, whose resistance has been transmitted successfully to interspecific progenies. This is evident in a number of interspecific lines with the plant type of *P. vulgaris* that are highly resistant to various races of *C. lindemuthianum*, including Race 3481, which infects the differential cultivar G2333 that carries three resistant genes, Co-4^2, Co-5 and Co-7 (Balardin and Kelly, 1997; Young and Kelly, 1997). Stable resistance to plant pathogens with extensive pathological variability, such as *C. lindemuthianum*, requires continual evaluation of germplasm and eventual introgression of diverse genetic resistance into commercial cultivars. The loss of resistance to a virulent race in some cultivars regarded as highly resistant (e.g., G2333) shows that stable resistance to *C. lindemuthianum* might not be found in the primary gene pool and, consequently, alternative sources of resistance must be sought (Mahuku *et al.*, 2002). In addition to their good disease resistance (Hunter *et al.*, 1982; CIAT, 2002), the secondary gene pool has been a source of many desirable agronomic traits, such as lodging resistance due to the thick stem at the base of the plant, the presence of a tuberous or fibrous root system that allows a perennial cycle, long epicotyls, and a high number of pods per inflorescence (Schmit and Baudoin, 1992). All these traits are important in the improvement of the agronomic characteristics of the common bean.

Races of *C. lindemuthianum* virulent to Middle American hosts show greater diversity in pathogenicity by attacking germplasm from both gene pools, whereas races virulent to Andean hosts are mostly pathogenic on Andean germplasm (Balardin *et al.*, 1997). In general, Middle American hosts are more resistant to Andean races and Andean hosts are resistant to Middle American races. For instance, the *Co-1* gene (Andean) conditions resistance to Race 73 (Middle American) and the *Co-2* gene (Middle American) conditions resistance to Race 7 (Andean). Therefore combining *Co-1* and *Co-2* genes in the same cultivar, has been shown as the best strategy to control these two highly virulent races currently present in North America (Young and Kelly, 1997). Broadly effective resistance to bean anthracnose, therefore, can be achieved if both Andean and Middle America genes are combined in the same cultivar (Young and Kelly, 1996a, 1996b). However, Andean sources of resistance to anthracnose have not been studied as extensively as Middle American sources (Melotto and Kelly, 2000). There is limited information available on the independence of the individual genes present in Andean cultivars or on the relationship of these genes to previously characterized anthracnose resistance genes. The only Andean anthracnose

resistance gene known is the *Co-1* gene, described by Burkholder in 1918 (Melotto and Kelly, 2000) and found in the Michigan Dark Red Kidney (MDRK) bean cultivar (Young and Kelly, 1997).

1.12 Resistance genes inheritance studies

The nature of inheritance of anthracnose resistance genes depends among other factors, on the tester genotype used as the susceptible parent, the isolate or race of the pathogen used for inoculation, and the stage at which the host plant is inoculated (Pastor-Corrales *et al.*, 1994). Also the resistance inherited from a source with two independent genes with equal effects and resistance, is likely to be more durable than the resistance controlled by the single gene (Muhalet *et al.*, 1981). According to Peloso *et al.* (1989), the dominant nature of inheritance makes transferring anthracnose resistance from such a durable source to susceptible cultivars relatively easy with any selection method. However, care should be taken to ensure that both dominant alleles are transferred into the desired cultivar. The latter process is the most crucial and determines the effectiveness of the transferred resistance genes. Without the use of genetic markers, the transfer of more than one gene into a susceptible cultivar becomes increasingly difficult because anthracnose resistance is controlled by major genes working independently or interacting in a different fashion (Mastenbroek, 1960; Cárdenas *et al.*, 1964; Muhalet *et al.*, 1981; Peloso *et al.*, 1989). Thus one has to rely on large crop populations and good screening techniques.

In the case of anthracnose, it has been reported that both dominant and recessive genes are responsible for its resistance in common bean crosses (Mastenbrock, 1960; Muhalet *et al.*, 1981; Peloso *et al.,* 1989; Pastor-Corrales *et al.*, 1994). As C. *lindemuthianum* is a variable pathogen, it is particularly important to select parents with good resistance. Successful development of a bean cultivar with durable resistance will depend on the sources of resistance genes and the number and type of pathotypes used to screen the beans (Pastor-Corrales *et al.*, 1994). The best method to identify resistant parents is to expose the potential sources of resistance to all the existing pathotypes over different production areas to eliminate susceptible genotypes (Beebe and Pastor-Corrales, 1991). Nevertheless, the stability and longevity of anthracnose resistant varieties is dependent upon the pathogenic variability displayed by the *C. lindemuthianum* population (Fernández *et al.*, 2000).

In order to develop an efficient breeding programme, there is a need to study the genetics and mode of inheritance and the magnitude of both the additive and non-additive genetic effects of anthracnose resistance in the dry bean crop. Estimation of these parameters requires use of an appropriate mating design. One of the best designs used for self-fertilizing crops is the diallel design (Christie and Shattuck, 1992).

1.12.1 The diallel cross analysis

The diallel cross is defined as the analysis of all possible crosses among a group of parents. A diallel cross with n parents would generate n^2 families (Jinks and Hayman, 1953). This is also called a complete diallel (Griffing, 1956a). Later generations, i.e., F_2 and backcrosses, can also be included in a diallel cross (Hayman, 1958). Since the advent of the diallel mating design, it has been widely used in plant breeding research to obtain genetic information. It is used in both self-pollinating and cross-pollinating species, as well as homozygous or inbred parents (Jinks and Hayman, 1953; Griffing 1956b) and non-inbred parents (Gardner and Eberhart, 1966). Christie and Shattuck (1992) concluded that diallel analysis is a sophisticated form of progeny testing from which information can be obtained that is not available from any other analysis, and can be used by plant breeders as an aid in selection.

According to Hallauer and Miranda (1981), the diallel mating design has been used and abused more extensively than any other mating design. However, they noted that it is very useful, if properly analyzed and interpreted. Sokol and Baker (1977) suggested that genetic interpretation of data from diallel experiments is valid only if the following assumptions about the parental material are true: diploid segregation, homozygous parents, gene frequencies are equal to one-half at all segregating loci, genes are independently distributed between parents, and no non-allelic interaction.

There are several methods of diallel analysis and modification but the basic methods have been described by Jinks and Hayman (1953), Griffing (1956b), Gilbert (1958), and Gardner and Eberhart (1966). Each analysis requires certain assumptions that may limit its use or interpretations of its results. Therefore, criticism of diallel analysis and perceptions of abuse may arise from the interpretations of results (Baker, 1978; Christie and Shattuck, 1992). Nevertheless diallel analyses are of great benefit to breeders and geneticists. Plant breeders and geneticists have used diallel mating designs extensively to investigate genetic properties

of plant cultivars and populations. Hayman's (1954) and Griffing's (1956b) analyses are frequently used together to complement interpretation of data.

Diallel analysis provides information on the average performance of individual lines in crosses known as general combining ability (GCA). It also gives information about the performance of each cross relative to the average performance of parents involved in the cross known as specific combining ability (SCA). Ghosh and Das (2003) explained that a cross between two lines has an expected value, which is the sum of the general combining abilities of its two parental lines. However, some crosses deviate from this expected value to a greater or lesser extent, and this deviation is what is known as the specific combining ability of the two parents in combination.

There are no genetic assumptions with Griffing's analysis on combining ability (Wright, 1985), and several scientists believe that this method conveys reliable information on the combining potential of parents (Gill *et al.*, 1977; Nienhuis and Singh, 1986). Griffing (1956b) proposed four methods of diallel analysis:

Method 1: Complete diallel. Includes parents, F1 and reciprocals (n^2 entries, where n is the number of parents).

Method 2: Half diallel. Parents and F1's without reciprocals [n(n+1)/2entries].

Method 3: F1's and reciprocals used but not parents [n(n-1) entries].

Method 4: Only F1's used, no reciprocals or parents [n(n-1)/2 entries].

Griffing (1956b) also postulated two models for analysis of variance of the diallel design. Model I (fixed effects) is used in assumption that the parents are the population, i.e., parents are a fixed set of lines. Estimates from this model apply only to the genotypes included and cannot be extended to some hypothetical reference population (Hallauer and Miranda, 1981). In this model, estimation of components of variance is not appropriate, but estimation of GCA and SCA effects is valid and informative. Model II (random effects) is used where parents are a sample of randomly chosen lines from a reference population and the estimates are interpreted relative to the reference population. With this model, estimates of variance components are the main interest.

According to Griffing (1956a) and Bhullar *et al.* (1979) a large GCA:SCA variance ratio suggests importance of additive gene effects, while a low ratio signifies presence of dominant and/or epistatic gene effects. Baker (1978) reported, in reference to Griffing's models, that for inbred parents, the closer the following equations are to unity, the greater the predictability based on general combining ability; Model I $2g_i^2/(2g_i^2 + s_i^2)$ and Model II $2\delta_g^2/(2\delta_g^2 + \delta_s^2)$, where g_i^2, δ_g^2 represent GCA mean square, and variance, respectively, and s_{ij}^2, δs^2 refers to SCA mean square and variance, respectively.

Therefore, performance of a single cross progeny can be predicted on the basis of the GCA of the parents if SCA is small relative to the GCA. Cockerham (1963) suggested partitioning of reciprocal effects into maternal and non-maternal effects. This is helpful in determining whether maternal or extranuclear factors are involved in the expression of a trait.

Wassami *et al.* (1986) noted that the GCA component contains additive effects in addition to additive x additive effects when present. Christie and Shattuck (1992) concluded that it is easy to select the appropriate analysis if the breeder decides on the purpose or level of the analysis desired and reference population before initiating a diallel cross. They also reported that diallels or other complicated designs do not ensure success in reaching plant breeding goals, but will increase the chances of success if properly utilized.

Together with the diallel analysis, use can be made of the generation mean analysis (GMA) to obtain information about a particular pair of lines. Means of different generations are used to estimate genetic effects in a cross. Several models of generation mean analysis have been developed (Hayman, 1958; Van der Veen, 1959; and Gardner and Eberhart, 1966).

According to Kearsey and Pooni (1996), there are very simple relationships between the expected means of different generations. However, these relationships hold only if the generation means depend solely on additive and dominance effects. In the presence of maternal effects, epistasis, and differential viability the expected relationships do not hold. In the presence of epistasis, the estimates of additive and dominance effects are biased by some of the epistatic effects.

Comparison among generation means provides a test for the presence of epistasis. Mather (1949) derived scaling tests (A, B, and C) to test the adequacy of the additive–dominance

model in explaining variation among generation means. Cavalli (1952) introduced a joint scaling test, which includes any combination of families simultaneously, following multiple linear regression.

In the case of a single gene, the generation mean analysis (GMA) allows computation of the dominance ratio [d]/[a], where [a] is the additive component and [d] is the dominance component. However, with two or more genes, this ratio is referred to as the potence ratio, indicating which parent has the most dominant alleles, hence the more potent in the cross (Kearsey and Pooni, 1996). The numerator of the ratio could be zero due to ambi-directional dominance while the denominator could be zero as a result of gene dispersion, and hence the ratio can take any value, irrespective of the true degree of dominance.

The errors in the estimate of GMA are smaller, because means (first degree statistics) are used instead of variances (second degree statistics). Also GMA is equally applicable to cross- and self-pollinating species, and requires smaller experiments to obtain a good degree of precision (Hallauer and Miranda, 1981). Different generations can be included in the analysis (Mather and Jinks, 1977; Kearsey and Pooni, 1996), and also extended to more complex models including epistasis, linkage and trigenic. GMA permits estimation of heterosis and inbreeding depression. Adequate sampling of segregating generations is necessary to produce a representative sample of genotypes and hence, better estimates of the generation means.

Generation mean analysis does not reveal opposing effects, i.e., cancellation of positive and negative gene effects. Also it does not permit estimation of heritability and genetic gain which are important in crop improvement. For a breeder the estimation of heritability is important because it is used to decide how effective selection might be.

In simple terms heritability is a ratio that describes the amount of phenotypic variation that can be attributed to the differences in the additive genetic merit of individuals in a population. Differences in additive genetic merit exist if individuals have different alleles at loci that contribute to measurable differences in performance (Falconer, 1989; Kearsey and Pooni, 1996). When heritability for a trait is high, phenotypic selection will be more effective, whereas when heritability is low, obtaining additional information on relatives is of less value. Heritability is basically graded into either narrow sense or broad sense heritability.

In the narrow sense, heritability is defined as the proportion of the total phenotypic variance in a trait that is due to the additive effects of genes, as opposed to dominance or environmental effects. In the broad sense, heritability is the proportion of the total phenotypic variance of a trait that is due to all genetic effects, including additive and dominance effects. Narrow sense heritability is the most useful parameter in designing appropriate breeding techniques, because it is a measure of genetic variation that is transferred unaltered from one generation to the next. Heritability in the narrow sense can be achieved by parent offspring regression if parental values are means of both parents (Dabholkar, 1992; Kearsey and Pooni, 1996) or from the expected mean squares from the analysis of variance of the progenies of interest (Fehr, 1987). The narrow sense heritability is of great practical importance to plant breeders, since low narrow sense heritability indicates that only a small fraction of the trait of interest will be reflected in the next generation. According to Dabholkar (1992) and Lavett (1993) the larger the narrow sense heritability value, the more the character will respond to selection.

1.13 Anthracnose inoculation methods

The success of any resistance breeding program highly depends on the development of a suitable and reliable inoculation and screening technique. Various inoculation methods can be used to introduce a given quantity of *C. lindemuthianum* inoculum to the bean plant and then subjecting the plant to suitable conditions for infection and disease progression. Different methods are used in different conditions but in all cases, enough inoculum has to be supplied to ensure even diseases development. In investigations to determine plant resistance, knowledge of biotypes and a standard inoculation method is necessary in order that the results obtained in various places and times may be comparable (Király *et al.*, 1974).

1.13.1 Laboratory inoculation

Under laboratory conditions, anthracnose inoculation methods used are normally very effective. According to Edgington and MacNeill (1978), the rate of success is about 95%. Several techniques have been developed with the aim of reducing the amount of inoculation required, while increasing its effectiveness. The difference between techniques is based on the plant organ to be inoculated and the method of transmitting spores from a spore suspension to the host. Techniques include dipping germinated seed into a spore suspension (Kruger *et al.*, 1977; Bigirimana and Höfte, 2001), spraying spores on seedlings (Mastenbroek, 1960; Schwartz *et al.*, 1982; Bigirimana and Höfte, 2001), brushing spores

onto seedlings (Ćardenas *et al.*, 1964; Bigirimana and Höfte, 2001) or onto detached leaves (Bigirimana and Höfte, 2001), and injecting inoculum into stems (Schwartz *et al.*, 1982). The choice of inoculation method is dependent on the availability of facilities.

With seed inoculation, because of the difficulties that may be encountered with coating bean seed with anthracnose spores, the seeds are partially germinated to ease the process of inoculation. The bean seeds are placed on a plate containing a moistened filter paper ($\geq$ 90% RH), at 25°C and covered with plastic sheets to maintain high humidity. After three days the radicle emerges and it is at this stage that seeds are then soaked for five minutes in an inoculum containing 10^6 spores ml^{-1}. Inoculated seeds are then replaced in humid plates and incubated in a dark room at 19-21°C for two days. The seeds are then transferred to trays and covered with a thin layer of soil or compost and incubated at 19-21°C in a moist chamber under at least 92% relative humidity (Tu, 1985; CIAT, 2000; 2002; Bigirimana and Höfte, 2001).

In the seedling inoculation method, bean seed is sown in a potting mixture in a greenhouse at 28±3°C and the pots containing the seed are covered with plastic sheets to maintain a relative humidity that is high enough for bean germination. Primary leaves of 10 day old plants are inoculated by spraying the lower and upper surfaces of the leaves until run-off with a spore suspension (10^6 spores ml^{-1}). Seedlings are then randomly put into a moist chamber and incubated at about 19-21°C for two days in darkness followed by a 12 h light: 12 h dark photoperiod (Tu 1983; Bigirimana and Höfte, 2001).

Excised leaves of *P. vulgaris* can be maintained in a lighted area at about 22°C and 100% RH for at least 10 days without noticeable deterioration (Tu, 1985). For the detached leaf inoculation technique, 10-14 day old plants are used and leaves are excised and placed on trays with the upper surface facing down on a humid surface. The leaves are then sprayed with a spore suspension until the lower surfaces are covered by the inoculum. The trays are then covered with transparent plastic sheets to maintain a minimum relative humidity of 90% and placed in a room at 20±2°C for two days in darkness, followed by a 12 h light: 12 h dark photoperiod (Tu, 1983; 1985; Bigirimana and Höfte, 2001).

Apart from available facilities, one has to consider a number of other factors before choosing an inoculation method. For example, the use of seedlings for inoculation may not be the best

option, depending on the experimental conditions. According to Tu (1986), there are many pitfalls in screening for anthracnose resistance using seedlings and these include;

(a) if the first screening fails due to inadequate spore concentration, improper incubation temperature or insufficient moisture period, re-screening is needed. By this time, the seedlings may be too big. In addition, prolonged covering with plastic tends to reduce their vigour and results in dwarfing and the seedlings may not be fit for re-screening.

(b) Seedling assay methods work well for single gene resistance for which each seedling is screened once. However, if resistance to different races involves two or more genes, seedling assay can be achieved only by applying an inoculum of a mixture of races. Hence, only progenies carrying dominant genes give resistance reactions. It is clear that the seedling assays methods cannot reveal the pattern of gene constitution in a segregating population.

(c) Seedlings that were inoculated with one race may produce phytoalexins against other races.

(d) For seed-borne diseases such as anthracnose, inoculated plants are unfit for seed production. It is always suggested that an alternative method to seedling inoculation be adapted to accommodate the above factors.

1.13.2 Field inoculation

Field inoculation is normally done under natural infestation and, because of the fact that the incidence of pathogens is erratic, field trials are often unsuccessful. The occurrence of anthracnose in the field grown with beans is in most cases as a result of two major sources, either the introduction of the disease through seed or through infected bean debris. Studies have shown that under natural conditions, conidia of bean anthracnose failed to overwinter in infected bean debris in wet conditions (Tu, 1983; Dillard, 1990). According to Nicholson and Moraes (1980), the rapid decrease in the viability in wet conditions may be attributed in part to the loss of the mucilaginous water-soluble matrix of the conidia which is essential for the fungus to survive. In such a situation, if one is to study the fungus under field conditions, there should be means and ways of ensuring the presence of the fungus in the field and this can only be achieved by artificially introducing the pathogen through controlled field inoculation techniques.

1.14 Conclusion

The literature reviewed has shown that anthracnose caused by the fungus *C. lindemuthianum* has the capacity of affecting both the quantity and quality of beans produced. The review has

also indicated that the fungus has the potential of genetically modifying itself into different pathotypes. The challenge for researchers is to find appropriate methods and techniques of acquiring and introgressing novel resistant genes into the preferred crop varieties.

References

Agrios, G. N. 1988. Plant Pathology, 3rd Ed. Academic Press, London.

Allen, D.J., Ampofo, J.K.O. and Wortman, C.S. 1996. Pest, disease, and nutritional disorders of the common bean in Africa: A Field Guide. *Centro International de Agricultura Tropical*, Cali Colombia. 132p.

Andrus, C.F and Wade, B.L. 1942. The factorial interpretation of anthracnose resistance in bean. *U.S. Department of Agriculture Technology Bulletin,* 8:10-29.

Bailey, J.A. 1991. Recognition events associated with specific interactions between plants and pathogenic fungi. In Smith, C.J. (ed.). Biochemistry and Molecular Biology of Plant-Pathogen Interactions. Pp. 210-224. Oxford University Press, UK.

Bailey, J.A. O'Connell, R.J. and Nash, C. 1992. Infection strategy of *Colletotrichum lindemuthiamun* species. In: Bailey, J.A. and Jeger, M.J. (eds.). *Colletotrichum: Biology, Pathology and Control* CAB International, Wallingford, UK. Pp. 88-120.

Baker, R.J. 1978. Issues in diallel analysis. *Crop Science*, 18:533-536.

Balardin, R.S. and Kelly, J.D. 1997. Re-characterisation of *Colletotrichum lindemuthianum* races. *Annual Report of the Bean Improvement Cooperative*, 40:126-127.

Balardin, R.S., Jarosz , A.M. and Kelly, J.D. 1997. Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central, and North America. *Phytopathology*, 87:1184-1191.

Balardin, R.S. and Kelly, J.D. 1998. Interaction between *Colletotrichum lindemuthianum* races and gene pool diversity in *Phaseolus vulgaris. Journal of American Society of Horticultural Sciences,* 123:1038 -1047.

Bannerot, H. 1965. Resultats de l'infection d'une collection de haricots par six races phyiologiques d'anthracnose. *Annual Amelior Plant*, 15:201-222.

Barrus, M.F. 1921. Bean anthracnose. *Cornell University Agriculture Experimental Station, Mere*, 42:101- 215.

Barrus, M.F. 1911. Variation in varieties of beans in their susceptibility to bean anthracnose. *Phytopathology*, 1:190-195.

Bassanezi, R.B., Martins, M.C., Godoy, C.V., Amorim, L. and Bergamin Filho A. 1997. Efeito da antracnose na eficiência fotossintética do feijoeiro. *Fitopatologia Brasileira,* 22:520-524.

Bechinger, C., Giebel, K.F., Schnell, M., Leiderer, P., Deising, H.B., and Bastmeyer, M. 1999. Optical measurements of invasive forces exerted by appressoria of a plant pathogenic fungus. *Science*, 285:1896-1899

Beckman, C.H. 1987. The Nature of Wilt diseases of plants. *The American Phytopathological Society*, St. Paul, Minnesota, USA. 175p.

Beebe, S.E. and Pastor-Corrales, M.A. 1991. Breeding for disease resistance. in: *Common beans: Research for crop Improvement*. Van Schoonhoven, A. and Voysest, O. (eds.). *Centro International de Agricultura Tropical*, Cali, Colombia. Pp. 562-617.

Berglund, B.O. and Brucher, H. 1976. The south American wild bean (*Phaseolus aborigineus* Burk) as ancestor of the common bean. *Economic Botany,* 30:257-272.

Bhullar, G.S., Gill, K.S. and Khehra, A.S. 1979. Combining ability analysis over F1-F2 generations in diallel crosses of bread wheat. *Theoretical Application of Genetics*, 55:77-80.

Bigirimana, J. and Höfte, M. 2001. Bean anthracnose: Inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. *Journal of Phytopathology*, 149:403-408.

Black, W., Mastenbrock, C., Mills, W.R., and Peterson, L.C. 1953. A proposal for an international nomenclature of races of *Phytophthora infestan* and of genes controlling immunity in *Solanum denissum* derivatives. *Euphytica*, 2:173-240.

Blondet, A. 1963. L'anthracnose du haricot: Etudé des races physiologiques du *Colletotrichum lindemuthianum*. Phd thesis, Faculté de Sciense, Paris.

Brown, G.E. 1977. Ultrastructure of penetration of ethylene degreened Robinson tangerines *Colletotrichum gloeosporioides*. *Phytopathology*, 67:315-320.

Bryson, R.J., Caten, C.E., Hollomon, D.W., and Bailey, J.A. 1992. Sexuality and genetics of *Colletotrichum*. In: Bailey, J.A and Jeger, M.J. (eds.). *Colletotrichum: Biology, Pathology and Control*. CAB International, Oxford, England, Pp 27-47.

Burkholder, W.H. 1923. The gamma strain of *Colletotrichum lindemuthianum* (Sacc. et Magn) B. and C. *Phytopathology*, 8:589-605.

Bushnell, W. R., and Gay, J. L. (1978). Accumulation of solutes in relation to the structure and function of haustoria in powdery mildews. In: Spencer, D.M. (ed.). The Powdery Mildews. Pp. 183-235. Academic Press, London.

Cárdenas, F., Adams, M.W. and Andersen, A. 1964. The genetic system for reaction of field beans (*Phaseolus vulgaris* L.) to infection by three physiological races of *Colletotrichum lindemuthianum*. *Euphytica*, 13:178-186.

Cavalli, L.L. 1952. An analysis of linkage in quantitative inheritance. Pp.135-144. In: Reeve, E.C.R. and Waddington, C.H. (eds.). Quantitative Inheritance. HMSO, London.

Christie, B.R. and Shattuck, V.I. 1992. The diallel cross: Design, analysis and use for plant breeders. *Plant Breeding Reviews*, 9:9-36.

CIAT. 2000. Annual Report of the Bean Program. Centro International de Agricultura Tropical, Cali, Colombia.

CIAT. 2002. Annual Report of the Bean Program. *Centro International de Agricultura Tropical*, Cali, Colombia.

Cockerham, C.C. 1963. Estimation of genetic variances. p. 53-94. In: Hanson, W.D. and Robinson, H.F. (eds.). Statistical Genetics and Plant Breeding. National Academy of Science-National Research Council, Washington, D.C.

Dabholkar, R.R. 1992. Elements of Biometrical Genetics. Ashok Kumar Mittal Concept Publishing Company, New Delhi, India.

Dillard, H.R. 1990. Overwintering of *Colletotrichum lindemuthianum* in dry bean debris in New York State. *Annual Report of Bean Improvement Cooperative*, 33: 53-54.

Drijfhout E. and Davis, J.H. 1989. Selection of new set of homogeneously reacting bean (*Phaseolus vulgaris*) differentials to differentiate races of *Colletotrichum lindemuthianum*. *Plant Pathology*, 38:391-396.

Dron, M. and Bailey, J.A. 1999. Improved control of bean anthracnose disease in Latin America and Africa through increased understanding of pathogen diversity. *Summary reports of European Commission supported STD-3 projects (1992-1995)*, Publisher, CTA.

Edgington, L.V. and MacNeill, B.H. 1978. Control of bean anthracnose by seed treatment with systemic fungicides. *Phytopathology News*, 12:235.

Esquerré-Tugayé, M.T., Mazau, D., Barthe, J.P., Lafitte, C. and Touzé, A. 1992. Mechanisms of resistance to *Colletotrichum* species. In: Bailey, J.A., and Jeger, M.J. (eds.). *Colletotrichum: Biology, Pathology and Control.* C. A. B. International, Wallingford. Pp. 121-186.

Fabre, J.V., Julien, J., Parisot, D., and Dron, M. 1995. Analysis of diverse isolates of *Colletotrichum lindemuthianum* infecting common bean using molecular markers. *Mycology Resource*, 99:429-435.

Falconer, D.S. 1989. Introduction to Quantitative Genetics. 3rd ed. Wiley, New York.

Fehr, W.R. 1987. Principles of Cultivar Development Vol. 1: Theory and Technique. Macmillan, New York.

Fernández, M.T., Fernández, M., Casares, A. Rodriguez, R. and Fueyo, M. 2000. Bean germplasm evaluation for anthracnose resistance and characterisation of agronomic traits: A new physiological strain of *Colletotrichum lindemuthianum* infecting *Phaseolus vulgaris* L. in Spain. *Euphytica*, 114:143-149.

Flor, H.H. 1971. Current status of the gene-for-gene concept. *Annual Review of Phytopathology*, 9:275-296.

Fouilloux, G. 1976. Bean anthracnose: new genes of resistance. *Annual Report of Bean Improvement Cooperative*, 19:36-37.

Fry, W.F. 1982. Principles of Plant Disease Management. Academic Press, London. Pp.196-234.

Gardner, C.O. and. Eberhart, S.A. 1966. Analysis and interpretation of the variety cross diallel and related populations. *Biometrics*, 22:439-452.

Gepts, P. 1988. Phaseolin as an evolutionary marker. In: P. Gepts. (ed.). *Genetic Resource of Phaseolus Beans*. Klumer Academic Publishers, Boston. Pp. 163-184.

Gepts, P., Osborn, T.C., Rashka, K., and Bliss, F.A. 1986. Phaseolin-protein variability in wild forms and land races of the common bean (*Phaseolus vulgaris*): Evidence for multiple centres of domestication. *Economic Botany*, 40:451-468.

Ghosh, H. and Das, A. 2003. Optimal diallel cross designs for estimation of heritability. *Journal of Statistics and Planning Inference*, 116:185-196.

Gilbert, N.E.G. 1958. Diallel cross in plant breeding. *Heredity,* 12:477-492.

Gill, K.S., Bhullar, G.S., Mahal, G.S. and Khehra, A.S. 1977. Combining ability for the sedimentation and pelshenke valve in F1-F5 diallel crosses in wheat (*Triticum aestivum* L.). *Zeitschrift fur Pflanzenzucht*. 78:73-78.

Godoy, C.V., Carneiro, S.M.T.P.G., Lamauti, M.T., Pria, M.D., Amorim, L., Berger, R.D. and Filho, A.B. 1997. Diagramatic scales for bean disease: development and validation. *Zeitschrift fÜr Pflanzenkrankheiten und Pflanzenschutz*, 104:336-345.

González, M., Rodríguez, R., Zavala, M.E., Jacobo, J.L., Hernández, F., Acosta, J., Martínez, O. and Simpson, J. 1998. Characterisation of Mexican Isolates of *Colletotrichum lindemuthianum* by using differentials and Molecular markers. *Phytopathology*, 88:4:292-299.

Goth, R.W. and Zaumeyer, W.J. 1965. Reaction of bean varieties to four races of anthracnose. *Plant Disease Reporter*, 49:815-818.

Griffing, B. 1956a. A generalized treatment of the use of diallel crosses in quantitative inheritance. *Heredity*, 10:31-50.

Griffing, B. 1956b. Concept of general combining and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences*, 9:463-493.

Griffiths, D.A., and Campbell, W.P. 1972. Fine structure of conidia development in *Colletotrichum atramentarium*. *Transactions of the British Mycological Society*, 59:483-489.

Guzmán, P., Gilbertson, R.L., Nodari, R., Johnson, W.C., Temple, S.R., Mandala, D., Mkandiwire, A.B.C. and Gepts, P. 1995. Characterisation of the fungus *Phaeoisariopsis griseola* suggests co-evolution in the common bean (*Phaseolus vulgaris*). *Phytopathology*, 85:600-607.

Hall, R. 1991. Compendium of Bean Disease. APS Press, St. Paul, MN.

Hallauer, A.R. and Miranda J.B. 1981. Quantitative Genetics in Maize Breeding. Iowa State University Press, USA.

Hamer, J.E., Howard, R.J., Chumley F.G., and Valent, B. 1988. A mechanism for surface attachment in spores of a plant pathogenic fungus. *Science*, 239:288-290

Hayman, B.I. 1954. The analysis of variance of diallel tables. *Biometrics*, 10:235-244.

Hayman, B.I. 1958. The separation of epistasis from additive and dominance variation in generation means. *Heredity*, 12:371-390.

Heath, M.C., 1981. Non-host resistance. In: Staples, R.C., and Toenniessen, G.H., (eds.). *Plant Disease Control: Resistance and Susceptibility.* John Wiley and Sons, New York. Pp. 201- 217.

Holiday, P. 1980. Fungus disease of tropical crops. Cambridge University Press; London 607p.

Hubbeling, N. 1957. New aspects of breeding for disease in beans *Phaseolus vulgaris. Euphytica*, 6:111-141.

Hubbeling, N. 1976. Selection for resistance to anthracnose, particularly in respect to the 'Ebnet" race of *Colletotrichum lindemuthianum. Annual Report of Bean Improvement Cooperative*, 19:49-50.

Hubbeling, N. 1977. The new iota race of *Colletotrichum lindemuthianum*. *Annual Report of Bean Improvement Cooperative*, 20:58.

Hughes, H.B., Carzaniga, R., Rawlings, S.L., Green, J.R. and O'Connell, R.J. 1999. Spore surface glycoproteins of *Colletotrichum lindemuthianum* are recognized by a monoclonal antibody which inhibits adhesion to polystyrene. *Microbiology*, 145:1927-1936.

Hunter, J.E., Dickson, M.H., Boettger, M.A. and Cigna, J.A. 1982. Evaluation of plant introductions of *Phaseolus* spp. for resistance to white mold. *Plant Disease*, 66:320-322

Jinks, J.L. and Hayman, B.I. 1953. The analysis of diallel crosses. Maize Genetics Cooperative Newsletter, 27:48-54.

Kearsey, M.J. and Pooni, H.S. 1996. The Genetic Analysis of Quantitative Traits. Chapman & Hall, London.

Kelly, J.D. Afanador, R.L., and Cameron, L.S. 1994. New races of *Colletotrichum lindemuthianum* in Michigan and implications in dry bean resistance breeding. *Plant Disease*, 78:892-894.

Király, Z., Klement, A., Solimosy, F. and Voros, J. 1970. Methods in Plant Pathology. Akademiai Kiadó, Budapest. 509p.

Király, Z., Klement, Z., Solymosy. F. and Vörös, J. 1974. Methods in Plant pathology: With Special Reference to Breeding for Disease Resistance. Elsevier Scientific Publishing Company, Amsterdam. 509p.

Krantz J., Schmutterer, H., and Koch, W. 1978. Diseases, Pests and Weeds in Tropical Crops. Wiley and Sons, Chichester.

Kruger, J., Hoffmann, G.M., and Hubbeeling, N. 1977. The Kappa race of *Colletotrichum lindemuthianum* and sources of resistance to anthracnose in *Phaseolus* beans. *Euphytica,* 26:23-25.

Kwon, Y.H. and Epstein, L. 1997. Involvement of the 90 kDa glycoprotein in adhesion of Nectria haematococca macroconidia. *Physiological and Molecular Plant Pathology*, 51:287-303.

Lavett, O.K. 1993. An Introduction to Genetic Analysis, 5th ed. W.H Freeman and Company. New York.

Leite, B. and Nicholson, R.L. 1992. Mycosporine-alanine: A self-inhibitor of germination from the conidial mucilage of *Colletotrichum graminicola. Experimental Mycology*, 16:76-86

Louis, I. and Cooke, R.C. 1985. Enzymes in the conidial matrix of *Colletotrichum gloeosporioides* and *Mycosphaerella pinodes. Transactions of the British Mycological Society*, 84:742-745.

Louis, I., Chew, A., and Lim, G. 1988. Influence of spore density and extracellular conidial matrix on spore germination in Colletotrichum capsici. *Transactions of the British Mycological Society*, 91:694-697.

Mahuku, G.S., Jara, C.E., Cajaiao, C., and Beebe, S. 2002. Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of Phaseolus vulgaris and in crosses of primary and secondary gene pool. *Plant Disease*, 86:1383-1387.

Mastenbroek, C. 1960. A breeding programme for resistance in dry shell haricots beans based on a new gene. *Euphytica*, 9:177-185.

Mather, K. 1949. Biometrical Genetics, 1st ed. Methuen, London.

Mather, K. and Jinks, J.L. 1977. Introduction to Biometrical Genetics. Cornell University Press, Ithaca, New York.

Mazau, D., and Esquerré-Tugayé, M.T. 1986. Hydroxyproline-rich glycoprotein accumulation in the cell walls of plants infected by various pathogens. *Physiological and Molecular Plant Pathology*, 29:147-157.

McDermott, J.M. 1993. Gene Flow in plant pathosystems. *Annual Review Phytopathology*, 31:353-357.

Melotto, M. and Kelly, J.D. 2000. Allelic series at the *Co-1* locus conditioning resistance in common bean of Andean origin. *Euphytica,* 116:143-149.

Menezes, J.R. and Dianese, J.C. 1988. Race characterization of Brazilian isolates of *Colletotrichum lindemuthianum* and detection of resistance of anthracnose in Phaseolus vulgaris. *Journal of Phytopathology*, 78:650-655.

Mercure, E.W., Kunoh, H. and Nicholson, R.L. 1995. Visualization of materials released from adhered, ungerminated conidia of *Colletotrichum graminicola*. *Physiological and Molecular Plant Pathology*, 46:121-135.

Mercure, E.W., Leite, B. and Nicholson, R.L. 1994. Adhesion of ungerminated conidia of *Colletotrichum graminicola* to artificial hydrophobic surfaces. *Physiological and Molecular Plant Pathology*, 45:421-440.

Meyer, S., Saccardy-Adji, K., Rizza, F. and Genty, B. 2001. Inhibition of photosynthesis by *Colletotrichum lindemuthianum* in bean leaves determined by chlorophyll fluorescence imaging. *Plant, Cell and Environment*, 24:947-955.

Mims, C.W., Richardson, E.A., Clay, R.P. and Nicholson, R.L. 1995. Ultrastructure of conidia and the conidium aging process in the plant pathogenic fungus *Colletotrichum graminicola*. *International Journal of Plant Science*, 156:9-18.

Mould, R.J.M., Boland, G.J. and Robb, J. 1991. Ultrastructure of the *Colletotrichum trifolii-Medicago sativa* pathosystem, I. Pre-penetration events. *Physiological and Molecular Plant Pathology*, 38:179-178.

Muhalet, C.S., Adams, M.W., Saettler, A.W. and Ghaderi, A. 1981. Genetic system for the reaction of field beans to beta, gamma, and delta races of *Colletotrichum lindemuthianum*. *Journal of American Society of Horticultural Science*, 106:601-604.

Neema, C., Siccard, D., Adam, A.F., Buchet, S., Catan, I. and Dron, M. 1994. Genetic variation within natural host-pathogen populations: *Phaseolus vulgaris-Colletotrichum lindemuthianum*. In: *CIAT Proceedings of the second international Scientific Meeting "Phaseolus' Beans Advanced Biotechnology Research Network*. 7-10 September 1993, pp 251-259. CIAT, Cali Colombia.

Nicholson, R.L. and Moraes, W.B.C. 1980. Survival of *Colletotrichum graminicola*: Importance of the spore matrix. *Phytopathology*, 70:255-261

Nicholson, R.L., Butler, L.G., and Asquith, T.N. 1986. Glycoproteins from *Colletotrichum graminicola* that bind phenols: Implications for survival and virulence of phytopathogenic fungi. *Phytopathology*, 76:1315-1318.

Nicholson, R.L. and Epstein, L. 1999. Adhesion of fungi to plant surface: Prerequisite for pathogenesis. In: Cole, G.T. and Hoch, H.C. (eds.). The fungal Spore and Disease Initiation in Plant and Animals. Pp. 3-23. New York, Plenum press.

Nienhuis, J. and Singh. S.P. 1986. Combining ability analyses and relationships among yield, yield components, and architectural traits in dry beans. *Crop Science*, 26:21-27.

O'Connell, R.J., Bailey, J.A. and Richmond, D.V. 1985. Cytology and physiology of infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. *Physiology Plant Pathology*, 27:75-98.

O'Connell, R. and Bailey, J.A. 1986. Cellular interactions between *Phaseolus vulgaris* and the hemibiotrophic fungus *Colletotrichum lindemuthianum*. In: Bailey, J. (ed.). NATO ASI Series, Biology and Molecular Biology of Plant-Pathogen Interactions. Pp. 39-48. Springer-Verlag, Berlin.

O'Connell, R.J. and Ride, J.P. 1990. Chemical detection and ultra-structural localization of chitin in cell walls of *Colletotrichum lindemuthianum*. *Physiology and Molecular Plant Pathology*, 37:39-53.

O'Connell, R.J., Nash, C. and Bailey, J.A. 1992. Lectin cytochemistry: a new approach to understand cell differentiation, pathogenesis and taxonomy in *Colletotrichum*. In: Teger, M.J. and Bailey, J.A. (eds.). *Colletotrichum*: B*iology, Pathology and Control*. CAB International.

O'Connell, R.J., Pain, N.A., Hutchinson, K.A., Jones, G.L and Green, J.R. 1996. Ultrastructure and composition of the cell infection structures formed by the fungal plant *Colletotrichum lindemuthianum*. *Journal of Microscopy*, 181:204-212.

O'Connell, R.J., Perfect, S.E., Hughes, H.B., Carzaniga, R., Bailey, J.A. and Green, J.R. 2000. Dissecting the cell biology of *Colletotrichum* infection processes. In: Prusky, D., Freeman S., and Dickman M. (eds). Host Specificity, Pathology, and Host-Pathogen Interaction of *Colletotrichum*. American Phytopathological Society Press. Pp. 57-77.

Oliari, L., Vieira, C., and Wilkinson, R.E. 1973. Physiological races of *Colletotrichum lindemuthianum* in the state of minas Gerais, Brazil. *Plant Disease Reporter*, 57:870-872.

Pain, N.A., Green, J.R., Jones, G.L. and O'Connell, R.J. 1996. Composition and organisation of extracellular matrices around germ-tubes and appressoria of *Colletotrichum lindemuthianum*. *Protoplasma*, 190:119-130.

Painter, R.H. 1951. Insect Resistance in Crop Plants. The MacMillan Company, New York, 520p.

Palmer, R. 1967. Bees aid bean pollination. *South African Bee Journal*, 39(4): 12-13.

Parbery, D.G. (1981) Biology of anthracnose on leaf surfaces. In: Blackman, J.P. (ed.). Microbial Ecology of the Phylloplane. Pp.135-154. Academic Press, London.

Pascholati, S.F., Deising, H., Leite, B., Anderson, D. and Nicholoson, R.L. 1993. Cutinase and non-specific esterase activities in the conidial mucilage of *Colletotrichum graminicola*. *Physiological and Molecular Plant Pathology*, 42:53-59.

Pastor-Corrales, M.A. Llano, G.A., Afanador, L. and Castellanos, G. 1985. Disease resistance mechanism in beans to the anthracnose pathogen. *Phytopathology*, 75:1176.

Pastor-Corrales, M.A., Erazo, O.A., Estrada, E.I. and Singh, S.P. 1994. Inheritance of anthracnose resistance in common bean accession G2333. *Plant Disease*, 78:959-962.

Pastor-Corrales, M.A., Otoya, M.M. and Molina, A. 1995. Resistance to *Colletotrichum lindemuthianum* isolates from Middle America and Andean South America in different common bean races. *Plant Disease*, 79:63-67.

Peloso, M.J., Cardoso, A.M., Vieira, C., Saraiva, L.S. and Zimmermann, M.J.O. 1989. Genetic system for the reaction of Phaseolus vulgaris to the BA-2 (alfa) race of *Colletotrichum lindemuthianum*. *Review of Brasil Genetics*, 12:313-318.

Prusky D. and Plumbley R.A. 1992. Quiescent infections of *Colletotrichum* in tropical and subtropical fruits. In: Bailey JA, Jeger MJ, (eds.). *Colletotrichum*: Biology, Pathology and Control. Pp. 289-307. Wallingford, UK: CAB International.

Ramadoss, C.S., Uhlig, J., Carlson, D.M., Butler, L.G. and Nicholson, R.L., 1985. Composition of mucilaginous spore matrix of Colletotrichum graminicola, a pathogen of corn, sorghum, and other grasses. *Journal of Agricultural Food Chemistry*, 33:728-732.

Rasmussen, J.B. and Hanau, R.M. 1989. Exogenous scytalone restores appressorial melanization and pathogenicity in albino mutants of *Colletotrichum graminicola*. *Canadian Journal of Plant Pathology*, 11:349-352.

Robinson, R.A. 1997. The acceptance of horizontal resistance in crops. *Biotechnology and Development Monitor*, No. 33, p. 24.

Rutger, J.N. and Beckham, I.S. 1970. Natural hybridization of *Phaseolus vulgaris* x p. Coccineus l. *American Society Horticultural Science Proceedings*, 95:659-661.

Schmit, V. and Baudoin, J.P. 1992. Screening for resistance to *Ascochyta* blight in populations of *Phaseolus coccineus* L. and *P. polyanthus* Greenman. *Field Crops Research*, 30:155-165.

Schwartz, H.F., Pastor-Corrales, M.A. and Singh, S.P. 1982. New sources of resistance to anthracnose and angular leaf spot of beans (*Phaseolus vulgaris* L.). *Euphytica*, 31:741-754.

Seebach, D., Adam, G., Zibuck, R., Simon, W., Rouilly, M., Meyer, W.L., Hinton, J.F., Privett, T.A., and Templeton, G.E., 1989. *Gloeosporone a macrolide* fungal germination self inhibitor; total synthesis and activity. *Liebigs Annalen der Chemie*, 12:1233-1240.

Sela-Buurlage, M., Epstein, L. and Rodriguez, R.J. 1991. Adhesion of ungerminated *Colletotrichum musae* conidia. *Physiological and Molecular Plant Pathology*, 39:345-352.

Sharman, P.N., Sharman, O.P. and Tyagi, P.D. 1994. Status and distribution of bean anthracnose. *Journal of Agriculture Resources*, 20:91 96.

Sharman, P.N., Kumar, A., Sharman, O.P., Sud, D., and Tyagi, P.D. 1999. Pathogenic variability in *Colletotrichum lindemuthianum* and evaluation of resistance in *Phaseolus vulgaris* in the north-western Himalayan region of India. *Journal of Phytopathology*, 147:41-45.

Sherwood, R.T. and Vance, C.P. 1980. Resistance to fungal penetration in *Gramineae*. *Phytopathology*, 70:273-279.

Sicards, D., Michalakis, Y., Dron, M. and Neema, C. 1997. Genetic diversity and pathogenic variation of *Colletotrichum lindemuthianum* in the three centres of diversity of its host, *Phaseolus vulgaris. Phytopathology, 87:807-813.*

Singh, S.P. 1992. Common bean improvement in the tropics. In: Janick, J. (ed.). *Plant Breeding Reviews.* 10:199-270. John Wiley and Sons Inc.

Sokol, M.J. and Baker, R.J. 1977. Evaluation of the assumptions required for the genetic analysis interpretation of diallel experiments in self-pollinated crops. *Canadian Journal of Plant Science*, 57:1185-1191.

Stakman, E.C. Stewart, P.M. and Loegering, W.Q. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. *Agricultural Research Service* E617. United States Department of Agriculture, Washington, D.C.

Sugui, J.A., Kunoh, H. and Nicholson, R.L. 1999. Detection of protein and carbohydrate in the extracellular matrix released by *Cochliobolus heterostrophus* during germination. *Mycoscience*, 40:11-19.

Tu, J.C. 1981. Epidemiology of anthracnose (*Colletotrichum lindemuthianum*) on white beans (*Phaseolus vulgaris* L.) in the Southern Ontario: Spread of the disease from an infection focus. *Plant Disease*, 65:477-480.

Tu, J.C. 1983. Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean (*Phaseolus vulgaris*) in Southern Ontario: Survival of the pathogen. *Plant Disease*, 67:402-404.

Tu, J.C. 1992. *Colletotrichum lindemuthianum* on beans: Population dynamics of the pathogen and breeding for resistance. In: Bailey J.A. and Jeger, M.J. (eds.). *Colletotrichum-Biology, Pathology and Control*. CAB International Wallingford, UK. Pp.204-224.

Tu, J.C. 1985. A detached leaf technique for screening bean (*Phaseolus vulgaris L*) in vitro against anthracnose (*Colletotrichum lindemuthianum*). *Canadian Journal of Plant Science*, 66:805-809.

Tu, J.C. 1994. Occurrence and characterization of the alpha-Brazil race of bean anthracnose (*Colletotrichum lindemuthianum*) in Ontario. *Canadian Journal of Plant Pathology*, 16:129-131.

Tu, J.C. 1995. Bean anthracnose. In: Chaube, H.S. Singh, S.P. Mukhopadhyay, A.N. and Kumar, J. (eds.). *Plant Diseases of International Importance*, 2:1-17. Prentice Hall, Englewood Cliffs, New Jersey, USA.

Tu, J.C. and Aylesworth, J.W. 1980. An effective method of screening white (pea) bean seedlings (*Phaseolus vulgaris* L.) for resistance to *Colletotrichum lindemuthianum*. *Phytopathology*, 99:131-139.

UEPB. 2005. Uganda Export Promotion Board: Dry beans. *Product Profile* No. 12. http://www.ugandaexportsonline.com

Vanderplank, J.E. 1968. Disease Resistance in Plants. Academic Press, NY, USA. 206 p

Vanderplank, J.E. 1975. Principles of Plant Infections. Academic Press, London. 195 p.

Van der Veen, J.H. 1959. Test of non-allelic interaction and linkage for quantitative characters in generation derived from two diploid pure lines. *Genetica*, 30:201-232.

Van Dyke, C.G. and Mims, C.W. 1991. Ultrastructure of conidia, conidium germination, and appressorium development in the plant pathogenic fungus *Colletotrichum truncatum*. *Canadian Journal of Botany*, 69:2455-2467.

Walters, K.J., Hosfield, G.L. Uebersax, M.A. and Kelly. J.D. 1997. Navy bean canning quality: correlations, heritability estimates and randomly amplified polymorphic DNA markers associated with component traits. *Journal of American Society of Horticulture Science*, 122:338-343.

Wassami, N.N., Isleib, T.G. and Hosfield, G.L. 1986. Fixed effects genetic analysis of a diallel cross in dry beans (*Phaseolus vulgaris* L.). *Theoretical Application of Genetics*, 72:449-454.

Wolkow, P.M., Sisler, H.D. and Vigil, E.L. 1983. Effect of inhibitors of melanin biosynthesis on structure and function of appressoria of *Colletotrichum lindemuthianum*. *Physiology of Plant Pathology*, 23:55-71.

Wright, A.J. 1985. Diallel designs, analyses, and reference populations. *Heredity*, 54:307-311.

Yerkes, W.D.Jr. and Ortiz, M.T. 1956. New races of *Colletotrichum lindemuthianum* in Mexico. *Phytopathology*, 46:564-467.

Young R.A. and Kelly J.D. 1996. A rapid marker for the Are anthracnose resistance gene in beans. In: *Annual Report of the Bean Improvement Cooperative,* 37:76-78.

Young, D.H. and Kauss, H. 1984. Adhesion of *Colletotrichum lindemuthianum* spores to *Phaseolus vulgaris* hypocotyls and to polystyrene. *Applied Environmental Microbiology*, 47:616-619.

Young, R.A. and Kelly, J.D. 1996a. Characterisation of the genetic resistance to *Colletotrichum lindemuthianum* in common bean differential. *Plant Disease,* 80:650-654.

Young, R.A and Kelly, J.D. 1996b. RAPD markers flanking the *Are* gene for anthracnose resistance in common bean. *Journal of American Society of Horticultural Science*, 121:37-41.

CHAPTER TWO

BREEDING FOR DRY BEAN ANTHRACNOSE DISEASE RESISTANCE: MATCHING RESEARCH INTERVENTIONS WITH LOCAL LIVELIHOODS THROUGH PARTICIPATORY RURAL APPRAISAL

Abstract
Dry beans play a very significant role in the diet and income of many resource poor farmers in Uganda. Any constraint hindering its production, directly affects the livelihoods of these families. Practical approaches are needed to assist resource poor farmers in reversing dry bean anthracnose disease, especially in cool highland areas of Western Uganda. In particular, methodologies are required to enable the choice of research interventions to be better targeted to specific crop stresses and client farmers through participatory approaches. In the case of anthracnose, research has to provide recommendations on cultivars and agronomic practices that offer a series of options in the management of the disease. For this to happen, a study was implemented to explore farmers' knowledge, experience, problems and cultivar preferences in association with managing anthracnose. Using participatory methods, a survey was conducted among 120 farmers in two sub-counties of Kisoro district. Results indicate that farmers in this area have different dry bean cultivar preferences and also the uses vary. The seed used to grow these cultivars is acquired from various sources, the most common is home saved seed (54%) and the least common from seed companies (1%). When it comes to management of the crop, women and children play the biggest role and yet they share the least of the profits. Further data gathered implicated anthracnose as an important limiting factor to dry bean production, although farmers do not refer to it as a disease but relate its symptoms to spoilage brought about by heavy rains. In this part of the world, disease management and control is not implemented, despite substantial yield losses as a result of the numerous pests and diseases prevalent in the area. This study acts as an entry point into the community where practical approaches and methodologies are being sought to breed for anthracnose resistant dry bean cultivars in consideration of farmers' preferences and the dynamics of the rural society.

2.1 Introduction

Bean research in Uganda is under the Uganda National Bean Program (UNBP), which has a broad goal of contributing to poverty alleviation and the income improvement of rural people (Anon., 2000). As a way of trying to achieve its goal, UNBP has released a number of bean varieties to different communities country-wide, in the hope of increasing productivity and commercialization of beans (David and Sperling, 1999). It is also assumed that with the adoption of suitable production technologies, higher production of beans would be achieved by farmers and this will directly translate into higher incomes, increased food security and alleviation of poverty. In this context, the definition of poverty is limited to a rather narrow focus of inadequate incomes and consumption compared with the more comprehensive perspective which seeks to define poverty as "the absence of a secure and sustainable livelihood" (Lipton and Maxwell, 1992).

In the sustainable rural livelihood framework, the livelihood assets are categorized into five categories, namely; natural, physical, financial, human and social (Scoones, 1998). Most technical options for disease and pest management involve expending these resources in some way (e.g., labour, capital and techniques). Thus, there is usually a relationship between farmers' access to resources and their pest and disease management practices. Therefore, the diversity of local livelihood, the ways farmers access livelihood assets, their perceptions of losses caused by pest and disease damage versus the expected technical options for pest and disease management, are all aspects that need to be determined.

Adoption of existing and newly generated technologies continues to be low, despite the time and resources committed by researchers in an effort to alleviate this problem (Chambers *et al.*, 1989). This has been attributed in part to low farmer involvement in the whole process of technology development. New technology options for pest and disease management will be adopted by farmers only if they are perceived to contribute to a significant increase in the production of the crop (Carney, 1998). For example, in eastern Uganda, different dry bean cultivars fulfill different livelihood functions and farmers respond to the multiplicity of needs (e.g., food, income, etc.) by growing a range of varieties (David *et al.*, 2000). In this case, if one is to introduce a superior variety in this locality, it must be able to fulfill all, or most of, these needs. To breed for such a variety, a breeder requires the participation of the grass-root stakeholders from the onset to the end of the breeding cycle.

Participatory approaches are increasingly being used, as part of the general trend towards involving target groups in development and research technologies (Mellis *et al.*, 1997). This strategy therefore, enhances acceptability and sustainability of technology after the initial stages. Encouraging farmers to share in knowledge development with researchers, leads to faster adoption of the new technologies. For example, Lightfoot and Ocado (1988) reported success in using participatory methods in jointly developing new ways to control a crucial weed problem in the Philippines. In Africa, successful farmer participatory breeding stories include: the varietal selection conducted by the West African Rice Development Association (WARDA) in West and Central Africa (Gridley, 2001), cassava variety selections in Tanzania (Kapinga *et al.*, 1997), sorghum breeding in Mali (Rattunde, 1997) and participatory improvement of maize varieties in Southern Nigeria (Ajala, unpublished). In all these areas attempts to involve farmers have been successful and farmers have increased their technical skills, production capacities, and have shown their willingness to work together with researchers.

The need to involve farmers arises from the fact that although technological systems undergo continuous changes as a result of physical and economic environments, farmers do not change as fast but are always aware of the direction of change in their production systems. These include changes in pest and disease infestation, soil fertility levels, and prices they receive for their crop. On the other hand, researchers being away from farmers, are less aware of the speed of change and generally know less than farmers about representative farming systems and their essential properties. In this case, farmers have a great capacity for responding to diverse ecological conditions and for adjusting their continuously evolving farming systems. They are concerned with minimising risks and to maximise at each step net returns to scarce resources (Balcett and Candler, 1982). Therefore, the probability for sustainable adoption increases when new technologies are integrated into practices and skills that are already known to farmers.

Farmers need recommendations on cultivars and agronomic practices that offer a series of options and which reflect their sequential decision making processes during and across seasons. There is a growing acceptance that the starting point in improving traditional small-holder agriculture needs to be knowledge, problems analysis and priorities of farmers and farm families (Eklund, 1990). Instead of viewing the research station and the extension system as the main locus for action, this new approach emphasizes the farm household and

its experimentation capacity. This method has been called the Farmer First Approach (Chambers *et al*., 1989). In parallel, the key to the development of new bean cultivars resistant to anthracnose in Uganda lies in participation of farmers through the whole process, from germplasm collection through to cultivar release. This encourages integration of indigenous knowledge of farmers with modern science to clearly identify those crop characteristics that may be ideal for their requirements. In this respect, therefore, the thrust of this study was to get farmers to share their experience, perceptions and problems associated with anthracnose management in dry bean production, with a view to breeding resistant cultivars which meet farmers' requirements.

2.2 Objectives

The objectives of this study were to explore farmers' knowledge and experience in the following aspects;

- Dry bean cultivar preferences;
- Seed acquisition and management;
- Gender considerations in bean production;
- Disease and pest management options;
- Impact of anthracnose disease on dry bean production

2.3 Materials and methods

2.3.1 The study area

Kisoro district is located about 500 km southwest of Kampala city and lies between longitudes 29°35' east and 29°50' east and latitudes 1°44' south and 1°23' south. The district has an area of 620 km^2 (Appleton, 2001). It is bordered by the Republic of Zaire in the west, Rukungiri District in the north, Kabale in the east and the Republic of Rwanda in the south.

The mean annual rainfall total of Kisoro district is about 1600 mm and is received in two seasons. The first rains are heavy and intense and stretches from April to July, while the second rains begin in August through to October. The rest of the period between the two rainy seasons is mainly dry. Due to the high altitude, Kisoro district has a relatively low temperatures with a mean annual maximum temperature of 24.4°C and a minimum of 10.9°C (UNFCCC, 1995; NEMA, 2001; Forest Department, 2002).

Agriculture is the main economic activity in Kisoro District, undertaken by over 90% of the population, mainly on small subsistence farms (UBOS, 2003). Land fragmentation is high and small plots of usually about 0.2 – 0.4 ha are seen on the hill slopes and valley bottoms (Anon., 1996; Raussen *et al.*, 2001). Most of the soils in the district are volcanic, although ferralitic soil at an advanced weathering stage can also be found in some areas. The latter has little or no mineral resources. In most parts of the district, the soils are characterized by clay and organic materials which are the main sources of fertility in this region (Bagora, 1988; Tumuhairwe *et al.*, 1988).

Many of the farmers practice mixed farming. Most crop husbandry practices are carried out manually. Most of the crops grown are consumed locally and these include; bananas, sweet potatoes, maize, beans, sorghum, tomatoes and many other minor crops (Tenywa, 1998; Anon., 2003). Beans occupy an important niche in both the farm household and general economy of the district because they are both a food and a cash crop (Wortman *et al.*, 1992; Kisoro District Administration, 1999). Due to the importance attached to this particular crop, it is grown by each and every household in the farming community and this leads to a rapid spread of bean diseases. The leading diseases include anthracnose, bean root rot, ascochyta leaf blight, angular leaf spot and rust. Anthracnose disease is on the increase and is thought to be taking centre stage because of the suitable weather conditions in this area and the predominant use of diseased seed. This study site was selected because it is a major production area, with a high incidence of bean anthracnose disease.

2.3.2 The study

The objective of the study was to get a deeper insight of the bean anthracnose disease and its related production problems for this area. A number of participatory methods were used in data collection, including semi-structured interviews using a questionnaire (Plate 2.1), focused group discussions (Plate 2.2), informative interviews and guided tours of farmer fields. With the help of the district administration and agricultural extension system, two sub-counties (Muramba and Murora) with the highest anthracnose incidences were selected and two parishes identified in each sub-county. From each parish, two villages were selected in which to conduct the household survey. Since all farmers in this area grow beans, 15 farmers were randomly selected from each village for the household interviews. The focused group discussions were only held at sub-county level. The household questionnaire survey included questions on household family data, different cultivars grown, seed acquisition, constraints to

bean production and problems associated with anthracnose. The focus group discussions concentrated on ranking in importance the available bean cultivars in the area, bean seed sources, ranking of important diseases and whether or not anthracnose was a known bean disease to farmers. Data collected from the different villages within the two sub-counties was compiled together and analysed using the Statistical Package for Social Scientists (SPSS). Percentages and graphic representations have been used to summarise and interpret the findings.

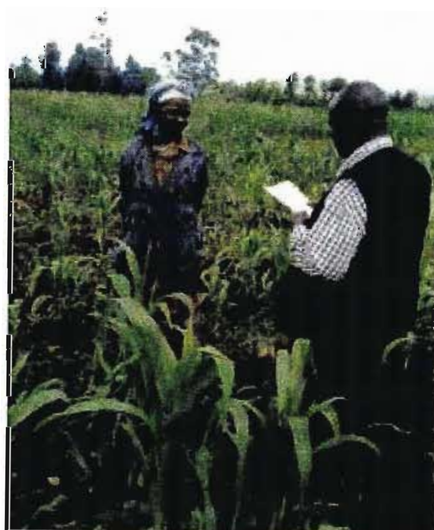


Plate 2.1: Scientists conducting interviews with farmers as a way of assessing problems related to bean production in different areas of Kisoro district.

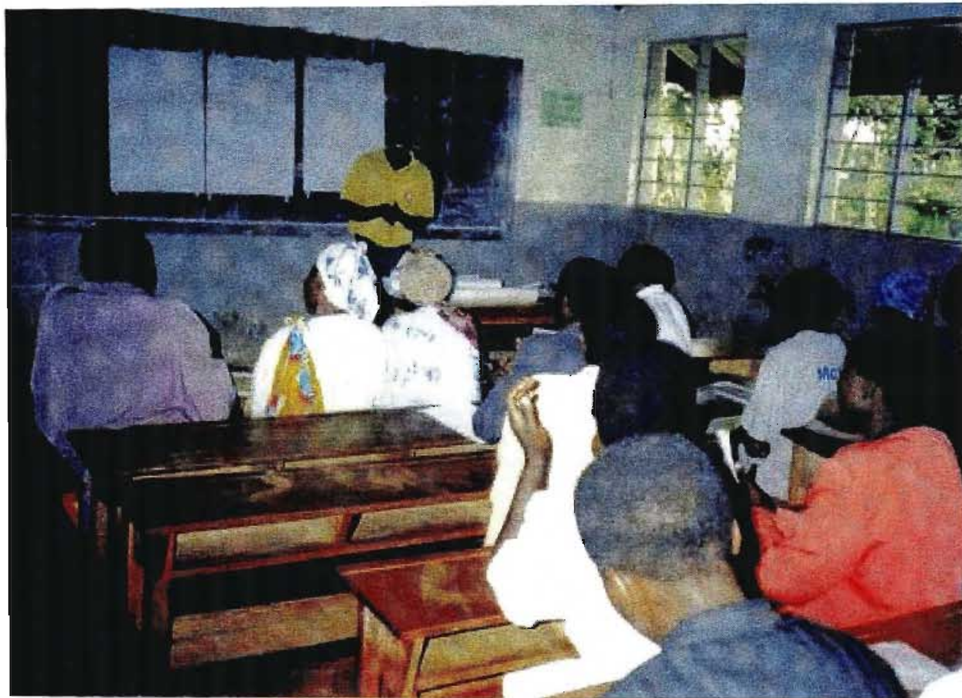


Plate 2.2: Scientists conducting focus group discussions with farmers regarding their experience, perceptions and problems associated with anthracnose disease management and dry bean production in general.

2.4 Results

2.4.1 Respondents

The results reported here were collected from 120 respondents from eight villages of which 64.2% were female and 35.8% were male. This difference in the gender of respondents was due to the fact that some of the households were run by women and in other cases, the men were not home to respond to questions. Besides, in homesteads, women are traditionally responsible for the production of food crops and as such, were better placed to respond to the questions.

The results indicate that data were gathered from a rather mature population, most of whom were household heads. Results show only 4% (5) of the respondents being below 20 years of age, the majority (27%) were in the 41-50 year age bracket and a good number (23%) were above 50 years of age (Figure 1).

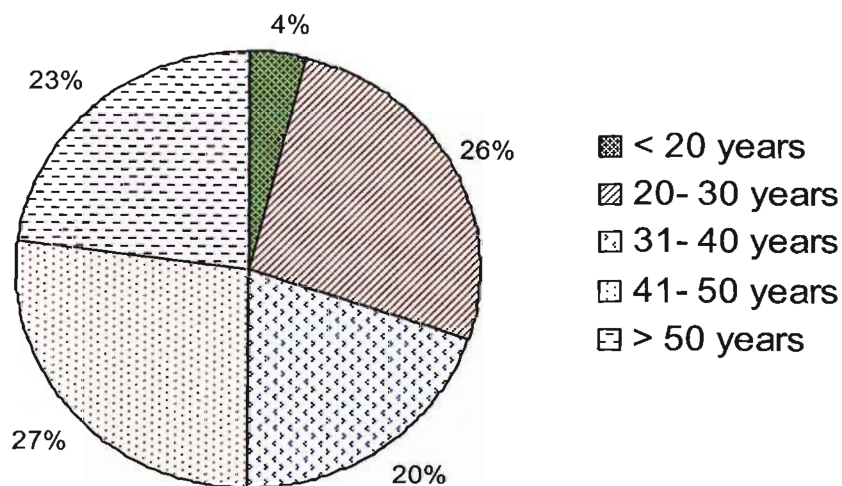


Figure 1: Age bracket of survey respondent farmers

2.4.2 Labour requirements of a typical farm household

The size of any household determines the kind and amount of labour available for that particular household. From the survey data, a typical household averaged between 6-10 people of varying ages but in almost equal proportions of men and women (Figure 2). The numbers in each household provide the basic labour to work the household's farm land. The amount of labour required depends on the workable farm size available to the household and is predominantly provided by all persons between the 10-45 age bracket. A household with large farms tends to hire labour whenever it is required.

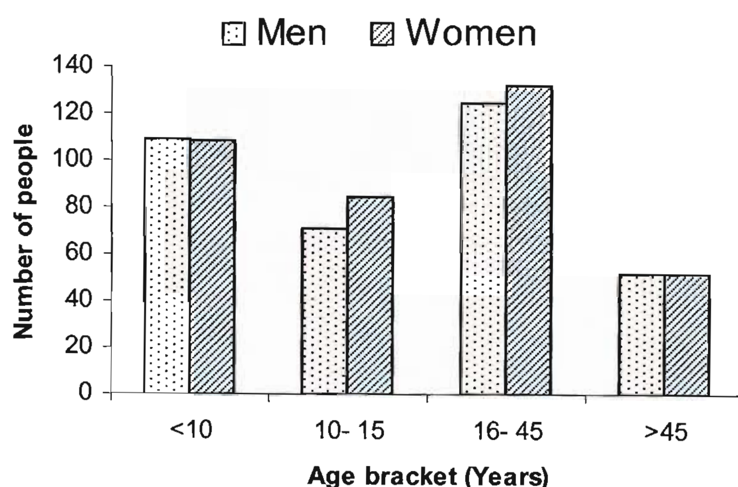


Figure 2: Number of people per age bracket from the surveyed households

The land available for the production of beans varies from household to household but the majority grow less than one hectare and very few plant more than 1.5 hectares (Table 2)

Table 2: Household (Hh) sizes and average farmer hectareage under bean cultivation

Hh size	No. of Hhs	Land allocated to bean production (hectares)	No. of Hhs
<2	01	<1	73
2-5	49	1-1.5	33
6-10	64	1.6-2.5	08
11-15	04	2.6-3	03
>15	02	>3	03

Although data shows equal proportions of women and men per household, women provide more than 60% of the labour requirement throughout the bean growing season. Figure 3 shows the different categories of

labour available for utilisation at the household level and the contribution of each kind of labour for each activity. It was noted though that female labour requirement decreases as the season progresses yet male labour increase progressively. There is a school of thought which suggests that the increase in the male labour towards harvesting time is because of the fact that they are the chief controllers of all the household income and expenditure. Children and hired labour are also employed depending on the amount of work at hand and size of the farm.

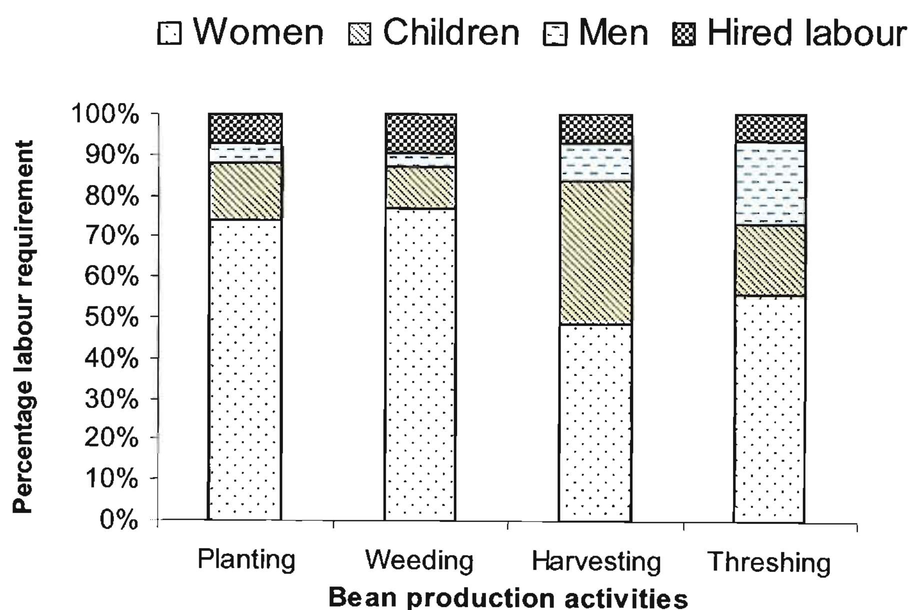


Figure 3: Household labour options used by surveyed farmers in bean production activities

2.4.3 Dry bean cultivars grown on household farms

Farmers grow many different cultivars of beans, the major ones included 10 non-climbers[1] and 12 climbers[2] types. Of these, only three of each, are popularly grown by the majority of farmers and they include *Nyagacecuru* (48.3%), *Biganza* (25.2%) and *Nyirakabonobono* (10.6%) for the non-climber beans and *Umwizarahenda* (26.5%), *Nyirakanada* (17.5%) and *Nyirasalayl* (18.4%) for climbers. The most common cultivar planted by nearly all farmers was the non-climber type called *Nyagacecuru* (Table 3).

[1] Growth types I, II and III (Singh *et al.*, 1992)
[2] Growth types IV (Singh *et al.*, 1992)

Table 3: Proportions of the different growth types of beans grown by the surveyed farmers

Non-climbers	Farmers growing non-climbers type (%)	Climbers	Farmers growing climber type (%)
Nyagacecuru	48.3	Nyiramwigodore	9.0
Biganza	25.2	Umwiza arahenda	26.5
Karorina	2.6	Nyirakanada	17.5
Bwanarensi	4.0	Impuramugabo	2.2
Nyirakabonobono	10.6	Urushari	4.0
Uruzara inyanza	2.6	Nyiragikoti	9.0
Nyiramuka	0.7	Inkunge	0.4
Amaharare	2.0	Nyiragihura	1.8
Shorongi	4.0	Nyirasalayi	18.4
		Famingi	9.4
		Sugar 31	1.8

Of the two types of beans, more farmers preferred to plant climber (60.8%) than the non-climber types (33.3%). The rest of the farmers (5.8%) did not have a preference and were happy to grow any type. The reason given for the difference in preference included among others, the ability of the bean type to yield highly (86.4%), resistance to pest and diseases (8%) and the level of labour required to manage that bean type (5.6%).

2.4.4 Cropping systems used in bean production

Farmers in this region use both the sole and mixed cropping systems. Most farmers (85%) interviewed, grow beans in a mixed cropping system with various crops and the rest (15%) grow it as a sole crop. In the mixed cropping systems, various crops are grown together with dry beans. The most popular crops with which beans are intercropped include maize (50%), sorghum (26.3%) and Irish potatoes (17.7%). Occasionally banana and sweet potatoes are also intercropped with beans (Table 4). Farmers prefer to grow more non-climbers to climber types because of the compatibility of the non-climber types in the mixed cropping system.

Table 4: Different types of crops farmers intercropped with beans

Crop	Respondents (%)
Maize	50.0
Sorghum	26.3
Irish potatoes	17.7
Bananas	4.0
Sweet potatoes	2.0

This use of non-climbing bean types allows for a more efficient use of land for food crops. In addition, climbers require much more resources like buying stakes and labour involved in the actual staking of the beans in the fields. The availability of stakes and the labour that goes with it, is often not affordable by the already resource constrained farmers. The lower input requirement, explains the preference of growing bush to the normally higher yielding climber types.

Concerning seed sizes, farmers have similar preferences for both home consumption and for marketing purposes, although the reasons for the preference in these two categories may be different. Table 5 shows that the percentage of farmers preferring the same seed sizes for both end uses are almost the same. There is a strong correlation (r = 0.995) between the size of bean seed preferred for marketing purposes and that consumed at home although the reason for which these seed sizes are preferred are quite different for the two uses. The reasons for consumption preferences included palatability (55%), the ease with which that bean size cooks (16.7%), the amount of seed required to make a meal (2.5%) and the ability of the size to expand on cooking (3.3%). Those for marketing were the ability of a seed size to fetch a high price (48.3%), ease with which it can be marketed (15.8%) and the market demand of that particular bean size (13.3%).

Table 5: The type of bean size preferred for consumption and marketing

Bean size	**Respondents (%)**	
	Home consumption	**Marketing**
Small size	1.7	0.8
Medium size	6.7	5.0
Large size	86.6	82.5
No preference	5.0	11.7

Table 6 shows seed colours most preferred by farmers irrespective of cultivar type. These include red (60%), white (18.3%), yellow (3.3%), black (1.7%), and cream (1.7%). The main reasons given for the preference was that those colours are associated with palatability, high yields, ease to cook and the formation of a nice soup on cooking. Some farmers reported to prefer a certain colour but with no apparent reason.

Table 6: Dry bean seed colours most preferred by respondent farmers

Seed colour	Respondents (%)
Red	60.0
White	18.3
Yellow	3.3
Black	1.7
Cream	1.7
No colour preference	15.0

2.4.5 Seed sources

From the survey it is clear that the larger portion of farmers (54%) plant home saved seed while 34% acquire seed from different markets. A few farmers reported to obtain their seed from research institutes (6%), seed companies (1%) and the remaining 4% acquire seed from other farmers (Figure 4).

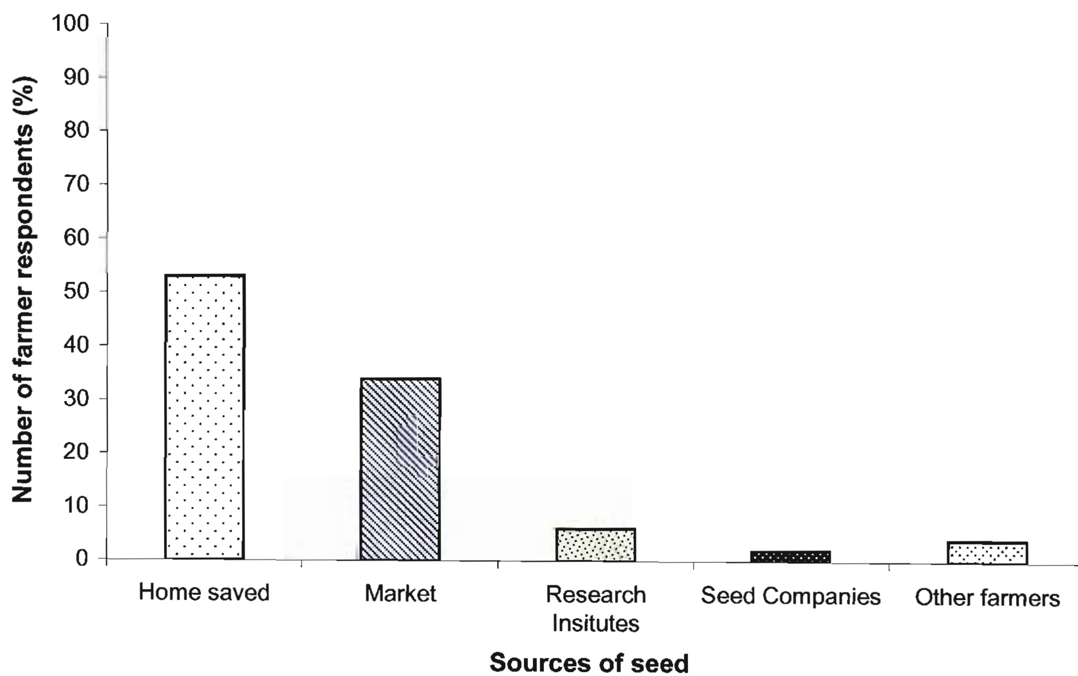


Figure 4: Sources of dry bean seed available to surveyed farmers

2.4.6 Constraints to dry bean production

Table 7 shows the outcome of the constraints analysis. During the constraints analysis exercise, 95.8% reported the presence of diseases in their field, although some could not tell the type of disease, 1.7% reported absence of disease and 2.5% were not sure whether they had diseases or not in their bean crop. Additionally, eight other major constraints were short-listed. These included pests, (36.5%), heavy rains (31.7%), land shortage (8%), lack of certified seed (7%), lack of stakes (6%), lack of labour (4%), lack of funds/money (3.5%) and low soil fertility (3%).

During the focus group discussion the ranking of the constraints changed and farmers ranked the lack of certified seed as their number one constraint followed by the lack of money. This was the same for both sub-counties (Table 7). Other constraints then followed but the ranking slightly differed between the two sub-counties. While the diseases and pests destroy the plant parts and as such cause physical injury to the plant, other constraints, although minor, provide an unsuitable environment for crop growth. All these have a negative effect on the yield expected from the crop.

Table 7: Farmers' bean production constraints

Results from individual household survey		**Results from focus group discussion**		
Constraint	Respondents (%)	Constraint	$Rank_1$	$Rank_2$
Pests	36.5	Pests	3	4
Heavy rainfall	32.0	Heavy rainfall	5	5
Land shortage	8.0	Land shortage	7	7
Lack of certified seed	7.0	Lack of certified seed	1	1
Lack of stakes	6.0	Lack of stakes	4	6
Lack of labour	4.0	Lack of labour	6	3
Lack of money	3.5	Lack of money	2	2
Soil fertility	3.0	Soil fertility	8	8

1= Murora sub-county; 2= Muramba sub-county

2.4.7 Disease control options utilised by farmers in dry bean management

Disease control is an important activity in combating spread of disease from one locality to another. Farmers in this region have limited control methods for the various pests and diseases they encounter in their fields. In most cases farmers may know the control methods that need to be taken but are limited by funds especially where purchasing is involved. The data collected gives an insight into methods used to by farmers to control diseases. From Figure 5, it can be seen that 92% of the farmers do not practice any form of disease control. Only a few reported the use of disease free seed (2%). A minority of 3% reported the use of chemicals and another 3% use crop rotation. It may be possible that the 3% using chemicals are the same ones practicing crop rotation.

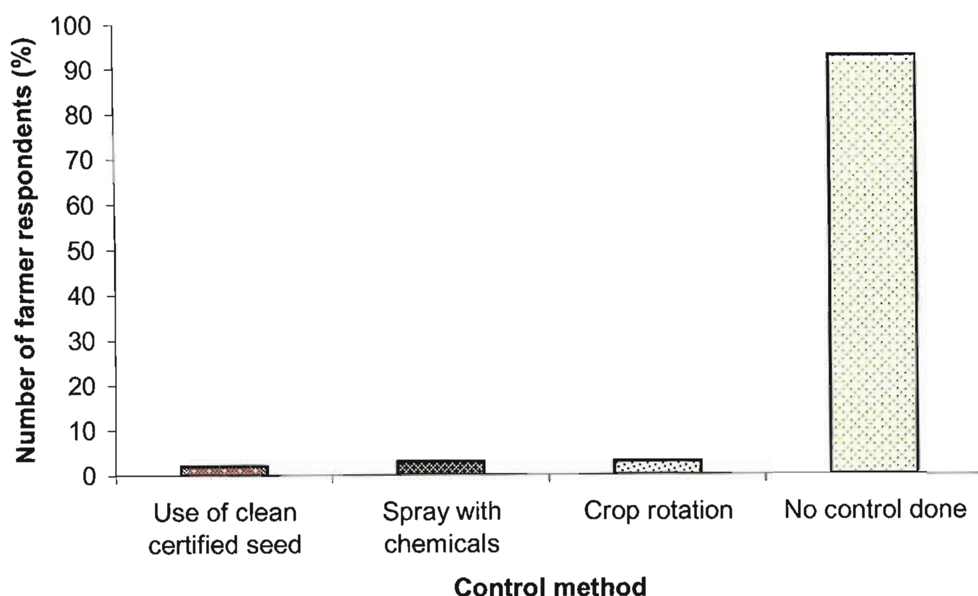


Figure 5: Different disease control methods used by the surveyed farmers

Considering that field sanitation plays a major role in the control of diseases like anthracnose, farmers were also requested to report on their bean residue disposal methods after harvesting and threshing. The results show that farmers manage their bean residues differently. Table 8 shows that 48.1% of the farmers in Kisoro district return the residues to the field and incorporate it in the soil, 24% use it for making compost, 15.6% use it for fuel purposes, 5.8% use it to mulch other crops, 2.6% dispose it off as garbage, 1.9% feed it to livestock and another 1.9% burn it in their fields.

Table 8: Respondent farmers' management of bean residues after harvesting

Bean residues management options	Respondents (%)
Return residues to field	48.1
Used to make compost	24.0
Fuel for cooking	15.6
Used as mulch in other crops	5.8
Just thrown away as garbage	2.6
Feed to livestock	1.9
Burn in field	1.9

The ability of diseases like anthracnose to overwinter in crop residues (Dillard, 1990), makes the disposal of its residues an important aspect in the control of this disease. This is important in this region where there is limited land for crop rotation. The return of bean residues to fields employed by nearly half of the respondent farmers and the favourable environment for the pathogen, explains the magnitude with which anthracnose has managed to serious hinder bean production in this region.

2.4.8 Impact of anthracnose on dry bean production

Having been familiarised with the symptoms of bean anthracnose, farmers were requested to indicate when such symptoms were first seen in their bean fields. The majority reported this to be at the pod formation and pod filling growth stages (48% and 35% respectively). These two stages were reported to coincide with the period when rainfall was at its peak and as a result farmers believed that the damage was merely caused by excessive rainfall. Farmers' identification of anthracnose symptoms at the other bean growth stages was minimal (Figure 6).

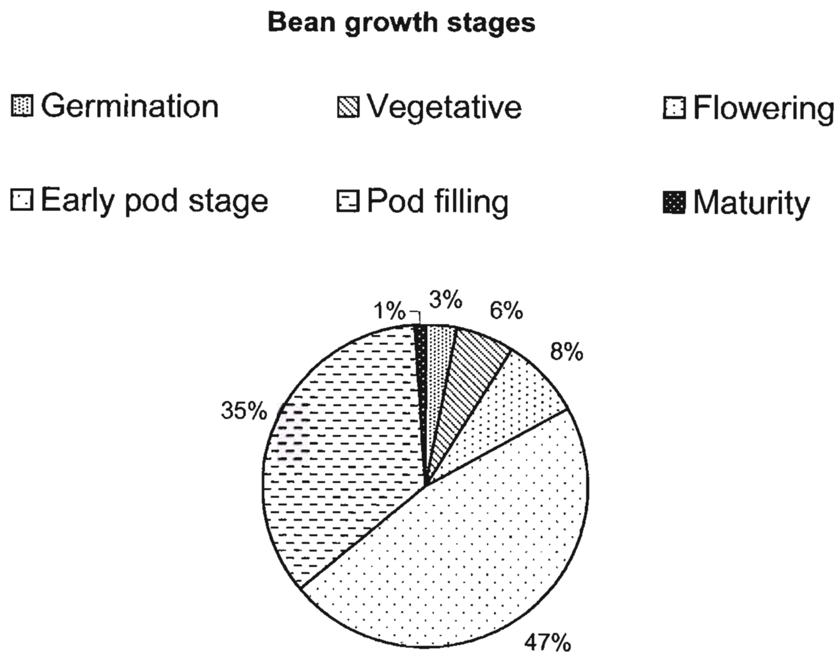


Figure 6: Bean growth stage at which anthracnose symptoms are first observed in farmer fields

Anthracnose was reported as very destructive by 61.7% of respondents, moderately destructive by 34.2% and the rest of the farmers (4.2%) did not know whether the disease was destructive to their crop or not. Although farmers were aware that a significant percentage of yield would be lost once such symptoms were noticed in the field, they could not put a particular name to these symptoms. The results presented in Table 9 show that only a handful of farmers were able to give local names, the meanings of which are all related to the fact that there is visible destruction of the plant by the pathogen. The majority (92.5%) did not know or had not heard of any name given for such symptoms.

Table 9: Local names given by the respondent farmers to anthracnose

Response	Meaning	Number of respondents	Percentage
I do not know/none	-	111	92.5
Chumia	Dried leaves	1	0.8
Iborwe	The big one	1	0.8
Nyiragakyetsi	-	2	1.7
Gahongo	Pod borer	1	0.8
Urusimba	Seedling killer	3	2.5
Kucecuka	Shredded leaves	1	0.8

2.5 Discussion and conclusion

The mandate of this study was to explore farmers' knowledge on bean anthracnose management as it impacts on their cultivar preferences and seed acquisition in dry bean production. The PRA as a tool used in this study was helpful in giving pointers in the right direction and also aided in suggesting the course of action.

It is well documented that farm families' livelihood securities can be improved by enhancing sustainable production through proper management of the available resources using appropriate technologies (Chambers *et al.*, 1989). However, this depends on the type of approaches that encourage personal and social learning, as was observed in the PRA study of two sub-counties of Muramba and Murora in the Ugandan district of Kisoro. Through guided field tour observations, focus group discussions and analyses of questionnaires, it was established that the majority of Kisoro farmers are very knowledgeable about dry bean production and were aware of the problems associated with production. The survey was conducted among mostly elderly farmers who had been growing dry beans for a long period of time and were therefore presumed knowledgeable about the different agronomic aspects of the crop.

The study was able to establish that farmers in this part of Uganda grow a range of dry bean cultivars predominately in a mixed cropping system because of limited acreage per household. Cultivar preference depends on its ability to be utilised for either home consumption or marketing. Farmers preferred large seeded, red coloured cultivars which are palatable, take a short time to cook and expand easily on cooking. This confirms earlier reports by David *et al.* (2000), which indicated that large seeded, red coloured or mottled bean cultivars are preferred in central, south and western Uganda. Most importantly, the cultivar should be able to withstand pests and disease in the field and yield highly. Furthermore, preferences are given to cultivars with the above agronomic traits and are able to compliment the farmers' mixed cropping system. Consequently it is essential that these factors be considered during the breeding process for acceptability and cultivar adoption purposes.

Over and above dry bean diseases like anthracnose, farmers identified other constraints, the most important being the lack of certified seed. Farmers lack access to improved dry bean variety seeds and as such depend heavily on home saved seed and whatever seed they purchase from local markets. The fact that only a small number of farmers obtain seed from companies shows either the unavailability or unawareness of seed companies in the area. In both cases the situation is worrying and needs addressing. It is suggested that the intervention of research organisations and seed companies be sought to sell certified seed to help reduce the spread of seed borne disease. In their findings, David *et al.* (2002) reported seed availability as the main factor limiting adoption of new seed technologies.

The study further established that farmers were familiar with anthracnose disease symptoms but were neither knowledgeable about the disease, its causes nor its methods of transmission. They attributed different names to the disease depending on either the type of damage caused or by that part of the crop most severely damaged. Anthracnose control measures are non-existent in the region, mainly because of limited knowledge of the disease along with the farmers' belief that it is just a result of the heavy rainfall. In general, disease control, especially in non-tradition market crops like beans, is still a grey area in these low income small-scale farming communities. It is the recommendation of this study that this problem needs addressing using appropriate low cost technologies such as the provision of cultivars resistant to anthracnose.

Finally, as noted from the study, members of any household have an important role in the livelihood of that particular household especially where farming activities are concerned. From the data gathered, it can be deduced that the role of males and females are divided according to the labour requirement at the particular phase in the bean growing season. While women and children are responsible for major labour operations in the production process, the men are the decision makers in a household (Anon., 2003). In this respect, and in consideration of earlier findings by David (1997), for any new cultivars to succeed in a community, it is important that the men's opinions be continuously sought. But as noted earlier, the key to the development of new bean cultivars resistant to anthracnose in this area will depend largely on the involvement of these farmers through the whole breeding process, from germplasm collection through to cultivar release. This encourages integration of indigenous knowledge of farmers with modem science to clearly identify those crop characteristics that may be ideal for their requirements and can easily be adopted into their farming systems.

References

Ajala. S.O. Unpublished. Participatory improvement and dissemination of maize varieties with resistance to stem borer in Southern Nigeria. IITA.

Anonymous 1996. Department of Agriculture Report by Kisoro

Anonymous 2000. Marketability and new bean varieties in Uganda. *ASARECA AGRICFORUM.* Vol. 2.

Anonymous 2003. Promoting Community Empowerment for Sustainable Livelihoods. Africa 2000 Network-Uganda.

Appleton, S. 2001. What can we expect from Universal Primary Education? In: Reinikka, R. and Collier, P. (eds.). *Uganda's Recovery: The Role of Farms, Firms, and Government.* The World Bank, Washington, D.C. Pp. 371-406.

Bagora, F.D.K 1988. Soil erosion and mass wasting risk in the highland areas of Uganda. *Mountain Research and Development*, 8:173-182.

Balcett, J.C. and Candler, W. 1982. Farmer technology adoption in Northern Nigeria. Vol. 1, World Bank Paper.

Carney, D., 1998. Implementing the sustainable rural livelihoods approach, In: Carney, D. (ed). Sustainable Rural Livelihoods: What Contribution Can We Make? DFID, London.

Chambers, R., Pacey R.A. and Thrupp, L.A. 1989. Farmers First: Farmer Innovation and Agricultural Research. Intermediate Technology Publication. London. U.K.

David, S. 1997. Dissemination and adoption of new technology: A review of experiences in bean research in Eastern and central Africa, 1992-1996, *Network on Bean Research in Africa, occasional Publication Series*, No. 21, CIAT, Kampala, Uganda.

David, S. and Sperling L., 1999. Improving technology delivery mechanism: Lessons from bean seed systems research in eastern and central Africa. *Agriculture and Human Values,* 16:381-388.

David, S., Kirkby R., and Kasozi S., 2000. Assessing the impact of bush bean varieties on poverty reduction in Sub-Saharan Africa: Evidence from Uganda. *Network on Bean Research in Africa, Occasional Publication Series,* No. 31, CIAT, Kampala, Uganda.

David, S., Mukandala L., and Mafuru J., 2002. Seed availability, an ignored factor in crop varietal adoption studies: a case study of beans in Tanzania. CIAT, Kampala (Unpublished).

Dillard, H.R. 1990. Overwintering of *Colletotrichum lindemuthianum* in dry bean debris in New York State. *Annual Report of Bean Improvement Cooperative,* 33:53-54

Eklund, P. 1990. Rapid rural assessments for Sub-Saharan Africa: Two case studies. EDI Working Papers. The Economic Development Institute of The World Bank.

Forest Department, 2002. Forest Nature Conservation Master Plan. Vol.1. Forest Department, Kampala, Uganda.

Gridley, H. 2001. Participatory varietal selection in West and Central Africa. WARDA Annual Report 2001-02.

Kapinga, R., Bart de Steenhuijsen, P., Kajiru, S., Chirimi, J., Rugutu, C. and Mahungu, N.M. 1997. Selection of cassava varities by farmers in the Lake Zone of Tanzania. *African Journal of Root and Tuber Crops,* 2:248-253.

Kisoro District Administration. 1999. Kisoro District Three-Year Development Plan, Kisoro District.

Lightfoot, C. and Ocado, A. 1988. A participatory method for systems problem research: rehabilitating marginal uplands in the Philippines. *Experimental Agriculture*, 112:3.

Lipton, M. and Maxwell, S. 1992. The new poverty agenda. Institute of Development Studies Discussion Paper 306. Institute of Development Studies, Brighton, U.K.

Mellis, D., Matsaert, H.S., and Wmaniki, B. 1997. Tillage research challenges toolmakers in Kenya. In: Van Veldhuizen, L., Waters-Bayer, A., Ramirez, R and Thompsom, J. (eds.). *Farmers' Research in Practice*, pp. 127-138. Intermediate Technology Publication. London, U.K.

NEMA. 2001. State of the Environment Report for Uganda 2000. *National Environment Management Authority*, Kampala, Uganda.

Rattunde, E.W. 1997. Participatory breeding with sorghum in Mali: Statistical and analytical aspects. INTSORMIL/ICRISAT.

Scoones, I. 1998. Sustainable rural livelihoods: A framework for analysis, IDS working paper No. 72. IDS, Sussex, UK.

Raussen T., Ebong G. and Musiime, J. 2001. More effective natural resource management through democratically elected decentralised government structures in Uganda. *Development Practice,* 11 (4):123-139.

Singh, S.P. 1992. Common bean improvement in the tropics. In: Janick, J. (ed.). *Plant Breeding Reviews,* 10:199-270. John Wiley and Sons Inc.

Tenywa, M.M. 1998. Agricultural potential in the Rwenzori Mountains. With special reference to lower slopes in Bwamba, pp. 180-189. In: Osmaston H., Tukahirwa J., Basalirwa C. and Nyakaana J. (eds). *The Rwenzori Mountains Natural Parks, Uganda.* Fountain Publishers Ltd. Kampala, Uganda.

Tumuhairwe, J.K., Nsubuga, E.N.B., Kahembwe, F. 1998. Acceptance and impact of soil and biodiversity conservation approaches in southwestern Uganda. A report on production of the Ugandan subcluster of the EAPLEC project to the United Nations University. Makerere University.

UBOS. 2003. Uganda National Household Survey 2002/2003. Report on the Socio-economic Survey. *Uganda Bureau of Statistics*, Entebbe, Uganda.

UNFCCC. 1995. United National Framework Convention on Climate Change (UNFCCC), Intergovernmental Panel on Climate Change (IPCC), Second Assessment 1995.

Wortmann, C.S. Sengooba, T. and Kyamanywa, S. 1992. Banana and bean intercropping: Factors affecting bean yield and land use efficiency. *Experimental Agriculture,* 28:287-294.

CHAPTER THREE

PHYSIOLOGICAL RACES OF DRY BEAN ANTHRACNOSE (*Colletotrichum lindemuthianum*) IN UGANDA

Abstract

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. et Magn.) Lams.Scrib.,, is a highly variable disease found in most of the major bean growing areas of Uganda. In an effort to breed for resistance to anthracnose in the Ugandan market class bean cultivars, a study on the identification and classification of the physiological races of C. *lindemuthianum* was undertaken. Samples of anthracnose were collected and isolated on potato dextrose agar (PDA) media. A set of 12 internationally accepted anthracnose differential cultivars were inoculated with anthracnose isolates collected from various regions. These were then incubated in conditions of a controlled environment. This experiment was replicated three times for each isolate. Disease evaluation was done after 10-14 days using disease severity based on a 1-5 point scale. The differential series identified eight physiological anthracnose races with varying growth characteristics, both on growth media and on the differential cultivars. Race 767 was found to be the most widely spread and most aggressive of the eight identified races. Differential Cultivars AB 136 and G2333 were resistant to all eight races and it is suggested that these cultivars could be utilised in the introgression of resistance genes into susceptible local cultivars.

3.1 Introduction

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. et Magn.) Lams.Scrib., was the first pathogen for which physiological races were described, back in 1918, by Barrus (Beebe and Pastor-Corrales, 1991). Although pathogenic variation was initially thought to be minimal, recent studies by various researchers have indicated wide pathogenic variation of this particular pathogen. This variability has been found in most of the major bean growing areas, such as Mexico and Brazil, where various races have been identified (Menezes and Dianese, 1988; Pastor-Corrales, 1986; 1991; Balardin and Kelly, 1998;).

Colletotrichum lindemuthianum variability on its host plant has been studied by various researchers (Young and Kelly, 1996; Sharman *et al.*, 1999; Thomazella *et al.*, 2000), who over time have identified the existence of different races. The first races, alpha and beta, were identified by Barrus in 1918 and these were followed with studies by Burkholder in 1932, Andrus and Wade in 1934, Blondet in 1963, Fouiloux in 1975 and Kruger *et al.* in 1977, who were able to identify the gama, delta, epsilon, kappa and alfa-Brasil races, respectively (Thomazella *et al.*, 2000). The pathogen has been reported to posses a high degree of pathogenic variability in different parts of the world (Menezes and Dianese, 1998; Drijfhout and Davis, 1989; Tu, 1992; Young and Kelly, 1994; Pastor-Corrales *et al.*, 1995; Sharman *et al.*, 1999). Numerous races have been identified in different bean growing regions using traditional differential cultivars, but these have been reclassified using the international set of 12 differential cultivars (Menezes and Dianese, 1988; Drijfhout and Davis, 1989; Pastor-Corrales, 1991; Tu, 1992; Balardin and Kelly, 1997). This set of cultivars, referred to as differentials, is normally inoculated with the different pathogen isolates and the races are designated according to susceptible differentials. The characterized races can then be used to identify sources of resistance by testing them on local and imported germplasm (Bigirimana and Höfte, 2001).

In Uganda, the anthracnose disease was the first focus of research on beans because of the severe epidemics it was causing. The existence of different *C. lindemuthianum* races in Uganda was first reported by Leakey and Simbwa-Bunnya (1971). In their study, they collected fungal isolates in different regions within Uganda and used differential cultivars from Schreiber and Hubberling to distinguish these isolates (Leakey and Simbwa-Bunnya, 1971). Although no conclusive verifications were made, the races identified at that time appeared to conform to the alpha, delta, beta and gamma races and were shown to severely attack mainly the bush bean cultivars, both small and large seeds (Leakey and Simbwa-Bunnya 1971).

It is almost four decades after Leakey and Simbwa-Bunnya's report on the *Colletotrichum* race status in Uganda, and during this period, substantial movement of dry bean seed has taken place within and outside the country. It is most likely that new and more destructive races have emerged or evolved from those that had earlier been identified by Leakey and Simbwa-Bunnya. This arises from the fact that the C. *lindemuthianum* pathogen is known to have a high level of genetic variability (Menezes and Dianese, 1988; Drijfhout and Davis, 1989; Tu, 1992; Sharman, *et al.*, 1999), which leads to the frequent formation of new races and as such increases the potential loss in resistance of originally resistant cultivars. It is for this reason, that this study was aimed at identifying the *C. lindemuthianum* races in the different bean producing regions of Uganda. Also this study would allow for a better understanding of the pathogenic variability within the country so as to enable the development of a breeding strategy for anthracnose resistance in Uganda, using vertical resistance genes.

3.2 Objective

The objectives of this study were to;

- Identify the different anthracnose races in Uganda
- To demostrate the variability of *C. lindemuthianum* races within major bean growing regions within Uganda

3.3 Materials and methods

3.3.1 Collection of *Colletotrichum lindemuthianum* samples

The identification and classification of the different *C. lindemuthianum* races begun with the collection of anthracnose samples in the various disease prone bean growing areas of Uganda. Collection of pods showing symptoms of anthracnose infection took place during the second bean growing season, in regions with high potential for anthracnose infestation. Anthracnose samples were collected in the western (Kabale, Bushenyi and Kisoro districts) and central (Mpigi district) regions. In a single region, samples of the fungus were collected from several farmers' fields. Data collected per field included the area where different anthracnose samples were collected, the bean growth type (i.e., bush, climber or semi-climber) and, where applicable, the bean variety name. Anthracnose infected samples were transferred to the laboratory for isolation, multiplication, storage and characterization.

3.3.3 Isolation, multiplication and storage of Colletotrichum lindemuthianum

Isolations were made from infected pods and seeds showing typical anthracnose symptoms and each sample constituted a separate isolate. Pods or seeds were surface sterilized by submerging them for three minutes in a solution containing 30 ml sodium hypochlorate, 1 ml alcohol, 270 ml water and 2 drops of Tween 80. The selected bean tissues were then rinsed twice in distilled water for five minutes each time. After thorough rinsing, the tissues were allowed to drain free of excess water, cut into appropriate pieces, placed on solidified potato dextrose agar (PDA) in Petri dishes and incubated between 22-25° C. After 4-7 days, portions of the mycelial growth of *C. lindemuthianum* were transferred to fresh PDA medium to purify the fungus (Plate 3.1).

Due to poor sporulation of *C. lindemuthianum* on the PDA medium, the fungus was later transferred to a modified Mathur's agar medium as utilised by Tu (1983) (glucose 2.8 g; $MgSO_4$ 13 g; KH_2PO_4 2.72 g; bacto peptone 1.0 g; yeast extract 0.5 g; agar 20.0 g, and distilled water, 1,000 ml supplemented by 0.04 g of tetracycline). This medium was also utilized in the production and multiplication of inoculum. The cultures were then identified using a microscope to verify whether they were true *C. lindemuthianum* pathotypes. Single spore isolation technique was utilized to produce pure isolates, which were later used for inoculum production, characterization of different races and storage purposes.

For conservation purposes, the *C. lindemuthianum* fungus was stored on PDA media and transferred to fresh medium every two months. However, with this method there is a high risk of losing pathogenicity (Tu, 1992; Pastor-Corrales *et al.*, 1995). To ensure continued pathogenicity, the fungus was re-inoculated onto susceptible bean plants every six months and then re-isolated afresh. For those isolates which were not required for constant use, the conservation period was lengthened by storing cultures on PDA in tubes (slants) at 4° C and these were kept for longer periods without re-isolation (González *et al.*, 1997).

3.3.4 Preparation of inoculum and inoculation method used for *Colletotrichum lindemuthianum*

Each multiplied single spore isolate was placed on fresh Mathur's agar medium in a Petri dish and incubated at a controlled temperature of between 22-25° C for 4-7 days to allow the fungus enough time to produce conidial spores. For inoculation purposes, conidial spores were scraped off the growth medium into a small amount of water to make a suspension. This was then

passed through a cheese cloth (Sharman *et al.*, 1999) to obtain a clear spore suspension. The concentration of the suspension was determined using a haemocytometer. The dilution factor was calculated with the formula shown below.

$V_0 \times C_0 = V_f \times C_f$ where: V_0 = initial volume

C_0 = initial concentration

V_f = final volume

C_f = final concentration = 1.2×10^6

Spore density of 1.2×10^6 conidia ml^{-1} (Tu, 1982; Balardin and Kelly, 1997; Bigirimana and Höfte, 2001) obtained was then used for plant inoculations.

3.3.5 Identification and characterization of *C. lindemuthianum* races

Pathogenicity and race identification of the different pathotypes was determined and confirmed by inoculation onto a set of 12 differential cultivars (Michelite, MDRK, Perry marrow, Cornell 49.242, Widusa, Kaboon, Mexico 222, PI 207262, TO, TU, AB 136, G2333) internationally recommended for identification of classification of unknown *C. lindemuthianum* isolates into races (CIAT, 1988; Drijfhout and Davis, 1989; Pastor-Corrales, 1991; Sharman, *et al.*, 1999). Using the prepared inoculum, the different isolates were suspended in distilled water with a few drops of Tween 80 (0.1% v/v) and the concentration adjusted to 1.2×10^6 conidia ml^{-1}.

For each differential cultivar and for each isolate, five seeds were pre-geminated for three days and then soaked for 5 minutes in 50 ml of *C. lindemuthianum* spore concentration of 1.2×10^6 conidia ml^{-1}. Inoculated seeds were later sown in (84 x 58 x 12 cm) plastic trays (Plate 3.2), half filled with potting compost and placed in the controlled environment to germinate (each box contained 6 differentials). The temperature was maintained between 18-22°C and the humidity at above 90% using water mist sprays up to the time when disease evaluation was done. The experiments were repeated at three different times to confirm the isolate to differential cultivar reactions.

3.3.6 Disease evaluation, race identification and data analysis

Ten days after inoculation anthracnose symptoms were evaluated using a 1-5 point scale (Drijhout and Davis, 1989) where 1 = no disease; 2= pin point lesions on lower part of stem; 3 = larger lesions covering stem; 4 = very large, deep lesions up to stem centre and 5 = seedling killed by pathogen. Data from all three trials were entered into an Excel worksheet and using

simple means and standard mean error, isolates were accorded varying disease scores for each differential cultivar. Plants showing Reaction Types 1 and 2 were graded as resistant (R) while those showing Reaction Types 3, 4 and 5 were graded as susceptible (S) (Plates 3.3 and 3.4). The race or pathotype designation given to an isolate was determined by the cultivars of the differential set that were infected by that isolate. The race or pathotype number was distinguished numerically as the sum of the binary values assigned to differential cultivars on which the isolate was pathogenic (CIAT, 1988; Drijfhout and Davis, 1989; Pastor-Corrales, 1991; Sharman *et al.*, 1999).

The races obtained were then placed in triplicates on PDA medium and their rates of growth determined by measuring the diameter of the colony. The data obtained were statistically analysed using ANOVA, with the help of the Genstat statistical package (Lawes Agricultural Trust. 2005) and this was used to obtain growth curves for each pathotype.

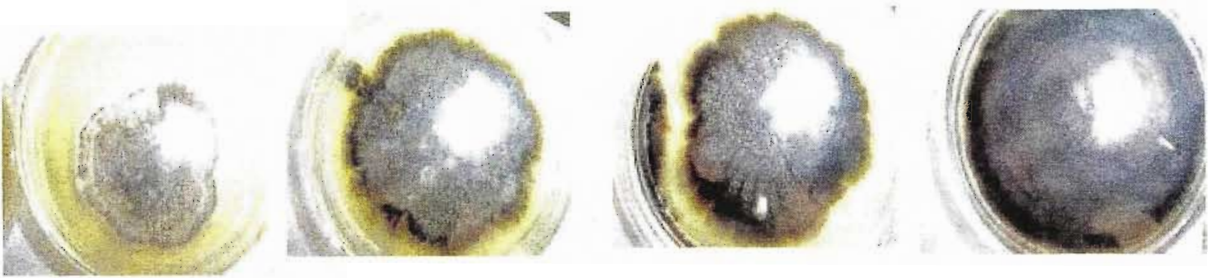


Plate 3.1: Different isolates of anthracnose on potato dextrose agar (PDA)

Plate 3.2: Trays used in the identification of anthracnose pathotypes using differential cultivars

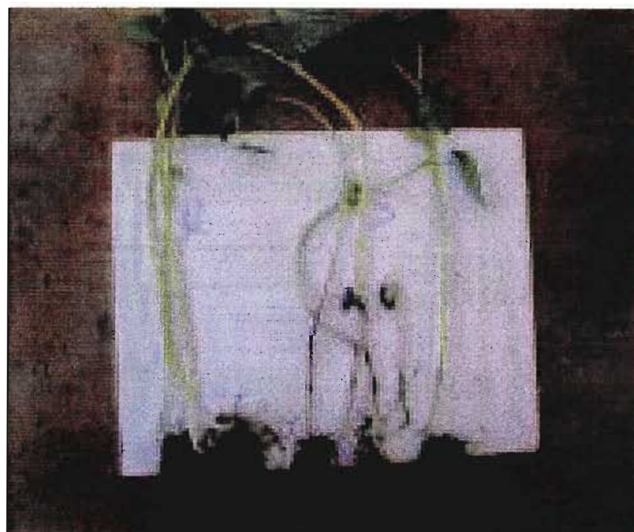


Plate 3.3: Dry bean seedlings showing the resistant (R) and susceptible (S) reaction types

Plate 3.4: Sample of seedlings showing the 1-5 point scale used in the evaluation of the reactions of different isolates on differential cultivars

3.4 Results

The reactions of the 12 differential cultivars to the 52 isolates of C. lindemuthianum identified and confirmed the presence of eight physiological races (23, 55, 102, 130, 227, 375, 511 and 767) of C. *lindemuthianum* on *Phaseolus vulgaris* in the east and central dry bean growing regions of Uganda. None of the races were pathogenic on all 12 differentials and two differentials AB 136 and G2333 were resistant to all the isolates while Michigan Dark Red Kidney (MDRK) was susceptible to all the isolates (Table 10). The majority of the isolates were more susceptible to the differential cultivars of the Andean gene pool (Guzmán *et al.*, 1995; Kelly and Vallejo, 2004), yet those differential cultivars from the Mesoamerican gene pool showed good resistance to the different *C. lindemuthianum* isolates with the exception of Michelite, normally referred to as the universal susceptible cultivar, and Widusa cultivars. These two cultivars were only resistant to races 102 and 130.

Table 10: Disease reaction of 52 isolates of *Colletotrichum lindemuthianum* on CIAT differential bean cultivars

Race No.	Differential hosts												Race Designation
	A	B	C	D	E	F	G	H	I	J	K	L	
1	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	23
2	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	55
3	**R**	**S**	**S**	**R**	**R**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	102
4	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	130
5	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	227
6	**S**	**S**	**S**	**R**	**S**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	375
7	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	511
8	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**S**	**R**	**R**	767
Gene pool	M	A	A	M	M	A	M	M	M	M	M	M	

S = Susceptible reaction; R = Resistance reaction; Differential cultivars: A-Michelite; B-Michigan dark red kidney; C-Perry marrow; D-Cornell49-242; E-Windusa; F-Kaboon; G-Mexico 222; H-PI 207262; I-TO; J-TU; K-AB 136; L-G 2333; M = Mesoamerican; A = Andean.

Of the eight identified races, Race 767 was most common (32%), which was followed by Races 130[3], 55 and 375 with 19%, 14% and 12% occurrence respectively. Three races (23[3], 102[3] and 227 had the same occurrence of 8%. The least prevalent race was 511 at only 2% frequency (Figure 7).

[3]Races previously identified (Leakey and Simbwa-Bunnya, 1971)

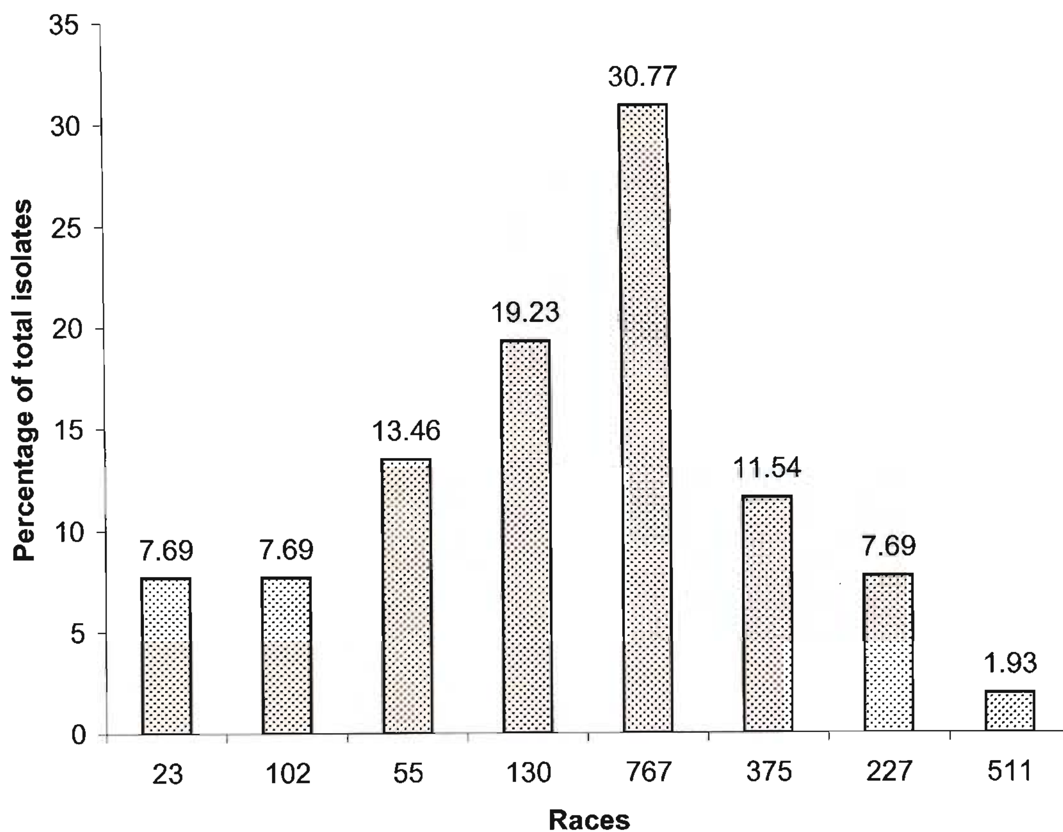


Figure 7: Physiological races of anthracnose occurring on dry beans in central and western Uganda

Race 767 occurred in all sampled localities whereas race 511 was only found in one locality. Races 375 and 23 were restricted to climber and semi-climber host types respectively. The remender of the races were found on either bush or climber host types (Table 11). Whereas seven different races were found in the eastern region, which includes the districts of Kisoro, Kabale and Bushenyi, only three races were identified in the central bean growing region of Uganda. Although the prevalence of Races 55 and 375 was not as high as that of Race 767, the former two races were almost as widely spread throughout the different bean growing areas as the latter Race (Table 11).

Table 1: Origin of *Colletotrichum lindemuthianum* isolates selected for race identification

Locality	Host type	Number of isolates	Races detected
Kisoro	Climber[4]	13	767, 375, 55, 102
	Semi-climber[5]	2	23
	Bush[6]	10	767, 130, 227, 55
Kabale	Climber	2	375
	Semi-climber	-	
	Bush	10	767, 102, 55
Bushenyi	Climber	1	375
	Semi-climber	-	
	Bush	8	767, 130, 227
Mpigi	Climber	-	
	Semi-climber	-	
	Bush	6	767, 511, 55

Most of the isolates and thus races were obtained from the bush growth type (67%), followed by the climber (29%) and lastly the semi-climbers (3%). Isolates from the semi-climber bean type were obtained in only one location, the climber type in three locations but the isolates from the bush where obtained from all locations (Table 11).

In addition to the differential cultivar classification process, all identified races were grown on a uniform growth medium to determine their growth patterns. Based on the growth patterns, (Figure 8) the eight races were categorized into three groups: very steep angled growth curve [767], medium steep angled growth curve [511, 375, 227, 130, 55 and 23] and shallow angled growth curve [102]. The growth curve of Race 767 was significantly higher ($P \leq 0.05$) than all the other races by the second week of growth. It was followed by Race 511. Races 130, 227 and 375 had almost similar growth curves, although Race 130 had a significantly higher growth curve at Week Three and an almost constant growth in the fourth week of growth. Races 23 and 55 had growth patterns which were almost identical and were significantly less vigorous than the former category of races. Race 102 was the least aggressive and had a significantly slower ($P \leq 0.05$) growth rate than the rest of the races.

[4] Growth Type IV (Singh, 1992)
[5] Growth Type II and III (Singh, 1992)
[6] Growth Type I (Singh, 1992)

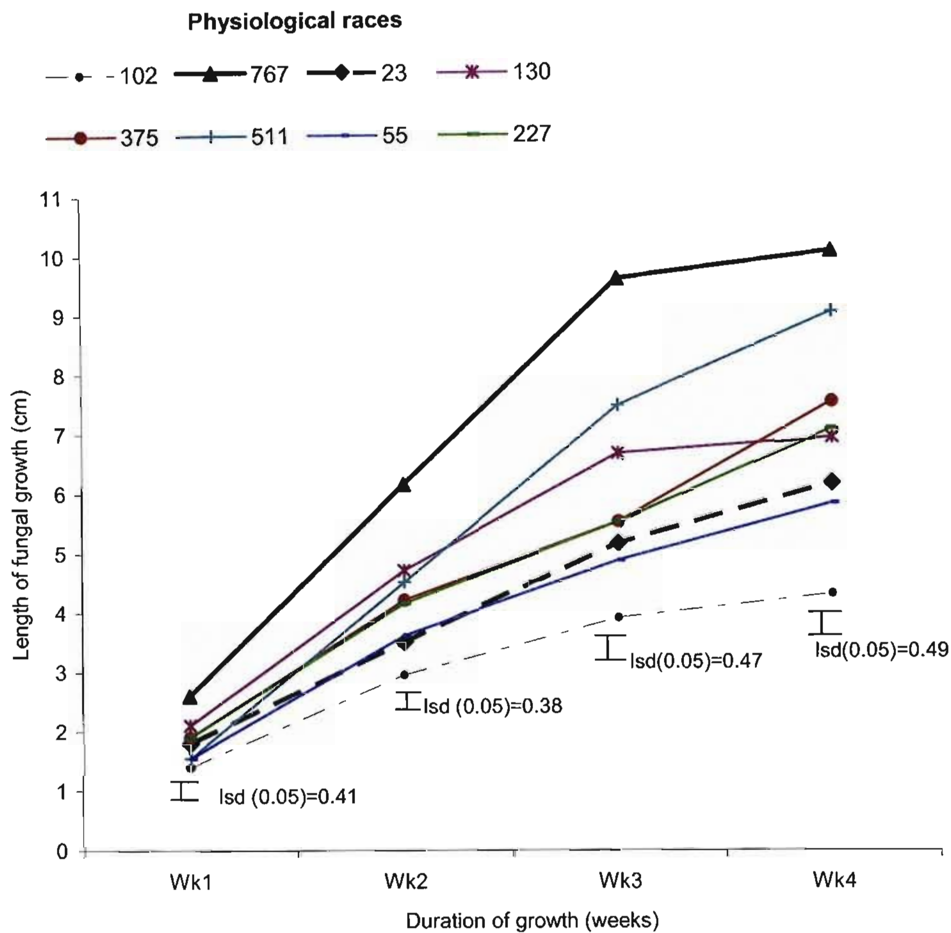


Figure 8: Saprophytic growth curves of Ugandan races of *C. lindemuthianum* on PDA media

3.5 Discussion and conclusion

This study was aimed at identifying *C. lindemuthianum* pathotypes prevailing in the dry bean growing areas of Uganda as the first step in establishment of an anthracnose resistance breeding programme strategy. From the participatory rural appraisal study of farmers in Kisoro district, it was noted that anthracnose was a serious constraint to dry bean production and there was need for scientific interventional strategies.

The first strategy and the core objective of this study, was to make an inventory of the existing physiological races within some of these regions and our emphasis was in the highland production areas where dry bean production is considered to be the highest. It is also in these

areas where anthracnose is most prevalent because of existing climatic conditions which favour its development.

This study confirmed the existence of the delta (23), beta (130) and gamma (102) Races and five other races, previously undocumented in Uganda. The existence of new races could be as a result of introduction into the country of already infected seed or due to genetic variability of the fungus. *Colletotrichum lindemuthianum* has been reported to posses a high degree of genetic variability in different bean growing regions of the world (Menezes and Dianese, 1988; Drijfhout and Davis, 1989; Tu, 1992; Sharman *et al.*, 1999). It may also be possible that the differential cultivars used by Leakey and Simbwa-Bunya in 1971 to identify the races may not have been as comprehensive as the internationally accepted differential cultivars used in the present study.

Out of the eight physiological races identified, Race 767 was most widespread and was present throughout the regions where the isolates were collected. Additionally its growth, as depicted by the growth curve patterns, was the fastest. Although this is saprophytic growth which may not be proportional to pathogenic ability, similar studies done with anthracnose on mangoes and avocado by Sanders and Korsten (2003) and Duarte *et al.* (2006), indicated a direct positive relationship between saprophytic growth and the virulence of the fungus on the host. It is thus thought that in actual host-pathogen field situations, *C. lindemuthianum* Pathotype 767 would be expected to out-compete the other less aggressive pathotypes leading to its dominance. Such situations cause populations of one pathotype to increase over and above that of other pathotypes, in some cases masking them completely (Nasir and Bretag, 1997; Crute, 1998). The benefit of the dominant race would then be its use as a sole pathotype in the germplasm screening process.

The results further indicate that the anthracnose pathotypes identified were pathogenic to common beans from the two major gene pools (i.e., Andean and Mesoamerican). The assessment of the resistance genes within the differential cultivars against the pathotypes shows that none was pathogenic to differential Cultivars AB 136 [*Co-6*; *Co-8*] and G2333 [*Co-4*^{2}*, Co-5, Co-7*] (Pastor-Corrales *et al.*, 1995; Young and Kelly, 1996; Kelly and Vallejo, 2004). The indication here is that resistance genes *Co-4 to Co-8* are suspected to be responsible for resistance. However, additional data show that differential Mexico 222, which contains resistance gene *Co-4*, was highly susceptible to nearly all races. This eliminates this gene from

those which are thought to have provided resistance to the Ugandan anthracnose races. At the same time, differential cultivar TU was also expected to be resistant to all identified races considering that it contains resistance genes *Co-5* and *Co-7* both of which are contained in differential cultivar G2333. It could be possible that the absence of resistance gene *Co-4*^{2} in differential cultivar TU, which is present in G2333, could have been responsible for observed resistance in the latter cultivar.

This is important information which is required for the establishment of an effective anthracnose resistance breeding programme for Uganda's dry bean cultivars. It is suggested that resistant cultivars AB 136 and G2333 be used in the introgression of resistance genes into susceptible local cultivars.

References

Balardin, R.S. and Kelly, J.D. 1997. Re-characterisation of *Colletotrichum lindemuthianum* races. *Annual Report of the Bean Improvement Cooperative*, 40:126-127.

Balardin, R.S and Kelly, J.D. 1998. Interaction between races of *Colletotrichum lindemuthianum* and gene pool diversity in *Phaseolus vulgaris*. *Journal of American Society of Horticultural Science*, 123:1038-1047.

Barrus, M.F. 1918. Varietal susceptibility of beans to strains of *Colletotrichum lindemuthianum* (Sacc.& Magn.) B. and C. *Phytopathology*, 8:589-614.

Beebe, S.E. and Pastor-Corrales, M. 1991. Breeding for disease resistance. In: Van Schoonhoven, A. and Voysest, O. (eds.). Common Beans: Research for Crop Improvenment. C.A.B. International, Centro Internacional de Agricultura Tropical (CIAT), Pp. 561-617.

Bigirimana, J. and Höfte, M. 2001. Bean anthracnose: Inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. *Journal of Phytopathology*, 149:403-408.

CIAT. 1988. Annual Report of Bean Program. Centro International de Agricultura Tropical, Cali, Colombia (CIAT), Pp. 173-175.

Crute, I.R. 1998. The elucidation and exploitation of gene-for-gene recognition. *Plant Pathology*, 47:107-113

Duarte, I., Thon, M.R. and Sukno, S.A. 2006. Characterization of a pathogenicity mutant of *Colletotrichum graminicola*. MANRRS 21st Annual Career Fair and Training Conference, St. Louis, Missouri. March 30- April 1, 2006.

Drijfhout E. and Davis, J.H. 1989. Selection of new set of homogeneously reacting bean (*Phaseolus vulgaris*) differentials to differentiate races of *Colletotrichum lindemuthianum. Plant Pathology*, 38:391-396.

González, M., Rodríguez, R., Zavala, M.E., Jacobo, J.L., Hernández, F., Acosta, J., Martínez, O. and Simpson, J. 1997. Characterisation of Mexican Isolates of *Colletotrichum lindemuthianum* by using differentials and Molecular markers. *American Phytopathological Society*, 88:4:292-299.

Guzmán, P., Gilbertson, R.L., Nodari, R., Johnson, W.C., Temple, S.R., Mandala, D., Mkandiwire, A.B.C., and Gepts, P. 1995. Characterisation of the fungus *Phaeoisariopsis griseola* suggests co-evolution in the common bean (*Phaseolus vulgaris*). *Phytopathology*, 85:600-607.

Kelly, J.D. and Vallejo, V.A. 2004. A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. *Horticultural Sciences,* 39:1196-1207.

Lawes Agricultural Trust. 2005. GenStat Release 8.1. PC/Windows XP. Rothamsted Experimental Station.

Leakey, C.L.A. and Simbwa-Bunnya, M. 1971. Races of *Colletotrichum lindemuthianum* and implications for bean breeding in Uganda. *Annual Application Biology*, 70:25-34.

Menezes, J.R. and Dianese, J.C. 1988. Race characterisation of Brazilian isolates of *Colletotrichum lindemuthianum* and detection of resistance of anthracnose in *Phaseolus vulgaris. Journal of Phytopathology*, 78:650-655.

Nasir, M. and Bretag, T.W. 1997. Pathogenic variability in Australian isolates of *Ascochyta fabae* f. sp. *lentis*. *Australian Plant Pathology*, *26:* 217-220.

Pastor-Corrales, M.A. 1991. Estandarizacion de variedades differenciales y de desginacion de razas de *Colletotrichum lindemuthianum*. *Phytopatholog*, 81:694. (abstr.).

Pastor-Corrales, M.A., Otoya, M.M., Molina, A., and Singh, S.P. 1995. Resistance to *Colletotrichum lindemuthianum* isolates from Middle America and Andean South America in different common bean races. *Plant Disease*, 79:63-67.

Sanders G.M. and Korsten L. 2003. A comparative morphological study of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. *Canadian Journal of Botany,* 81:77-885.

Sharman, P.N., Kumar, A., Sharmann, O.P., Sud, D. and Tyagi, P.D. 1999. Pathogenic variability in *Colletotrichum lindemuthianum* and evaluation of resistance in *Phaseolus vulgaris* in the north-western Himalayan region of Indian. *Journal of Phytopathology,* 147:41-45.

Thomazella, C., Goncalves-Vidigal, M.C., Vida, J.B., Filho, P.S.V. and Rimoldi, F. 2000. Identification of *Colletotrichum lindemuthianum* races in *Phaseolus vulgaris* L. *Annual Report of the Bean Improvement Cooperative*, 43:82-83.

Tu, J.C. 1983. Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean *(Phaseolus vulgaris*) in Southern Ontario: Survival of the pathogen. *Plant Disease*, 67:402-404.

Tu, J.C. 1992. *Colletotrichum lindemuthianum* on beans: Population dynamics of the pathogen and breeding for resistance. In: Bailey J.A. and Jeger, M.J. (eds.). *Colletotrichum Biology, Pathology and Control*. Pp. 204-224. CAB International Wallingford, UK.

Young, R.A. and Kelly J.D. 1994. A rapid marker for the Are anthracnose resistance gene in beans. In: *Annual Report of the Bean Improvement Cooperative*, 37:78-79.

Young, R.A and Kelly, J.D. 1996. Characterisation of the genetic resistance to *Colletotrichum lindemuthianum* in common bean differential cultivars. *Plant Disease,* 80:650-654.

CHAPTER FOUR

GERMPLASM EVALUATION AND THE RELATIONSHIP BETWEEN ANTHRACNOSE RESISTANCE AND THE PHENOTYPIC AND AGRONOMIC CHARACTERISTIC OF UGANDAN DRY BEAN CULTIVARS

Abstract

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et. Magn.) Lams. Scrib., is a serious constraint in the cultivation of the common bean (*Phaseolus vulgaris* L.) in Uganda, particularly in the cool and wet highland production areas. In order to obtain resistant cultivars, a germplasm evaluation was conducted to quantify the impact of the disease on phenotypic and agronomic traits under natural conditions. Field screening trials of 120 accessions from different localities within and outside the country were conducted at Kachwekano Research Institute in Kabale district for two seasons. Area under the disease progress curve (AUDPC) based on evaluations of disease severity (percentage leaf area affected) was used to screen the accession lines. From the data obtained, eleven accessions were identified with some level of anthracnose resistance. Additional results showed a negative relationship ($r = -0.42$ and -0.51 for seasons A and B, respectively) between anthracnose development and yield. Further relationships were identified between the plant flower colour, seed size and growth habit with accessions exhibiting anthracnose resistance. Some of the resistant lines identified are being used to introgress anthracnose resistance to susceptible Ugandan market class dry bean cultivars. It is suggested that a further study be done to determine the quality of resistance exhibited by these accessions.

4.1 Introduction

Dry beans (*Phaseolus vulgaris* L.) are an important food legume crop and provide an essential component of the daily diet for millions of people in Uganda and many other parts of the world. According to Sanders and Schwartz (1980), in most areas, the bean yields are about 500 kg ha^{-1}, which is less than 25% of the actual yield potential achieved by most commercial dry bean varieties. For the poor farmers, these low yields result from the combined effects of various constraints such as adverse climate, low soil fertility, damaging insect pests and diseases. The major diseases include anthracnose, angular leaf spot, root rot, ascochyta blight and many others, all leading to severe yield losses.

Anthracnose is a fungal disease caused by *Colletotrichum lindemuthianum* (Sacc.& Magn.) Scrib.,) and is very destructive to common dry beans grown at moderate temperatures (13-26°C) and high relative humidity regions (Chaves, 1980; Schwartz *et al.*, 1982; Bailey *et al.*, 1992; Pastor-Corrales *et al.*, 1985; Sicard *et al.*, 1997). It is one of the most important bean diseases worldwide and yield losses can reach 100% especially when infected seeds are used (Allen *et al.*, 1996). In Uganda, anthracnose is most important in the high altitude, low temperature areas (Opio *et al.*, 2006). It is very severe in these areas firstly, because of the favourable weather conditions which allow disease development and secondly, because of the predominance of susceptible bean cultivars in most of the farmers' fields (Opio *et al.*, 2001). Anthracnose often causes damage which affects seed quantity, quality and marketability of beans, leading to losses in income. It has been estimated that each 1% increase in anthracnose incidence manifests into dry bean grain yield losses of 9 kg ha^{-1} (Wortman and Allen, 1994).

Various control measures have commonly been advocated in attempts to reduce losses caused by anthracnose. These include: planting pathogen-free seed, field sanitation, crop rotation, shifting planting dates, fungicides and plant resistance (Chaves, 1980; Ferraz, 1980; Schwartz, 1989). However, farmer acceptance and utilization of many of these strategies are not always possible especially in the case of subsistence farmers in developing countries (Schwartz *et al.*, 1982). These farmers possess few land-holdings and resources, and are often unable to readily obtain or adopt the recommended practices for their region. Farmers in Uganda rely heavily on home saved seed (David *et al.*, 2000) and lack the equipment for seed cleaning and cannot afford the cost of seed dressing. In such instances, seed borne diseases are easily spread from one season to the other and one farmers' field to the next, occasionally causing epidemics. According to various

research findings (Silbernagel and Zaumeyer, 1973; Zaumeyer and Meiners, 1975; Chaves, 1980), the most practical and desirable strategy to utilize in this case as the sole management practice is plant resistance. Therefore, this study was designed to analyse the reactions of several accessions of dry beans to the predominant *C. lindemuthianum* pathotype prevailing in the central and east southern Uganda. This would act as the initial step in the breeding of Ugandan dry bean cultivars resistant to anthracnose.

4.2 Objective

The objectives of this study were to;

- Screen germplasm accessions for effective sources of anthracnose resistance
- Identify any plant phenotypic and agronomic traits related to anthracnose resistance

4.3 Materials and methods

The reaction to anthracnose caused by *C. lindemuthianum* was evaluated in the field using 120 *P. vulgaris* germplasm collections. The 120 accessions were collected from different areas within and outside Uganda. The accessions were then screened in the field for anthracnose resistance for two growing seasons in year 2004 at Kachwekano Research Station where conditions are favourable for disease development and establishment.

4.3.1 Screening of germplasm for resistance to anthracnose

The 120 accession lines (Appendix 1) comprising of 12 differential lines acquired from the Agricultural Research Council (ARC), Grain Crop Institute, Potchefstroom in South Africa, 10 lines from different seed centres in South Africa (Dept. of Agric & Env. Affairs, Cedara, Pannar and Pro-Seed), 19 CIAT bean accessions and 79 Ugandan local bean varieties, were tested for resistance to anthracnose. Screening was done in the field using inoculum from one dominating anthracnose race type Race 767 (see Chapter 3) which had been previously obtained from the same region. For easier identification, the germplasm lines were given accession numbers (NAT001-NAT120) and from hereon will be referred to as such.

4.3.2 Study area

The study was conducted at Kachwekano Research Station which is found in Kabale district and is located in south western Uganda (01° 15'S, 029° 59'E), at an altitude of 2200 m above sea level (Lindblade *et al.*, 1998). The climate at the research institute is comprised of moderate temperatures (10.9°C-24.4°C) and high relative humidity conditions (Lindblade *et al.*, 1998), all of which favour the development of the anthracnose pathogen. Rainfall in Kabale district is bimodally distributed with the long heavy rains from March to July and shorter rains from September to November. Kabale district has been observed to have had a rise in average minimum temperatures of 0.7° C since 1995 (NEMA, 2001) and this results in many changes in microclimate in the district's valleys and hills.

In the district, agriculture and agricultural related activities are the main occupation of the people and it is estimated that over 90% of the population is engaged in agriculture. Most of the crops produced are consumed at household level and it is only the surplus that is sold in local markets, although some of the crop eventually finds its way to urban centres (NEMA, 2001).

4.3.3 Field experimental design

Two field trial experiments were carried out in 2004 with a germplasm collection of 120 accessions, one in the first planting season (March-July) and the other in the second season (September-December). For each trial, the experimental design was a randomized block design with three replications and 1.5 m long rows per plot. Between rows, the spacing was 0.5 m and within rows spacing was 0.15 m. A basal application of 10.5 kg NPK (17% 1:1:1) fertilizer per block was applied at planting (Appendix 2). Agronomically the trials were maintained with the conventional cultural practices, including planting and weeding. Irrigation was only done in the first season to supplement rainfall.

To avoid inconsistencies arising from natural infestation, disease evaluation relied upon artificial inoculation, which was done 25 days after planting, by spraying leaves with an aqueous spore suspension (1.2×10^{6} spores ml^{-1}, containing 0.1 % Tween 20 as surfactant). This helped establish uniform anthracnose infection. The inoculum was made from pure cultures of Race 767.

4.3.3.1 Inoculum preparation

A pure culture of anthracnose Race 767 previously obtained (see Chapter 3) was used to prepare the inoculum used in the field inoculations. Because of the large quantities of inoculum needed, mass production was done in an Erlenmeyer flask using sterile premature french bean pods (Cardoso de Arruda *et al.*, 2000). Pods used were rinsed in clean tap water and chopped into 0.04-0.05 m long pieces and placed in 1000 ml Erlenmeyer flask until it was 2/3 full. The flask was plugged with cotton wool and then aluminum foil paper was used to wrap the mouth of the flask and contents before sterilizing by autoclaving. After sterilization, the remaining water from the pods was removed from the Erlenmeyer flask. A suspension of spores was dissolved in sterilized water and, using a sterile pipette, 5 ml of the suspension, was added to the french bean pods in the 1000 ml flask. The flask was then incubated between 22-25° C for 7 days to allow the fungus to sporulate well. Four flasks were used in order to prepare enough inoculum required to cover the entire experimental field.

4.3.3.2 Field inoculation

After 7 days of incubation, bean pods from the flask were transferred to an electric blender and macerated so as to free the pods of all spores. The contents from the blender were then strained through a double layer of cheesecloth and the anthracnose spore concentration from each flask determined using a haemocytometer. The inoculum concentration was adjusted to approximately 1.2×10^6 conidia ml^{-1} in a 20 litre back pack knapsack sprayer to which 5 ml of Tween 20 (0.01% v/v) was added to act as a surfactant. This concentration was later used to spray and inoculate the 120 different bean accessions in all the blocks. Inoculation was made at the third trifoliate leaf growth stage. Two inoculations were made each season, the second inoculation made four days after the first one to ensure good infection.

4.3.3.3 Disease evaluation

The first disease evaluations were made 14 days after inoculation and the subsequent evaluations done every 14-16 days in both seasons. Percentage diseased foliage of five individual plants was estimated. The five-plant assessments were converted to a single value for each plot (Redman *et al.*, 1969). Growth habit and flower colour for each accession was noted and at the end of the growing season the plants were harvested and dry seed yields for each accession (kg ha^{-1}) recorded.

4.4 Data analysis

For statistical data analysis, the area under the disease progress curve (AUDPC) was calculated for each accession using the midpoint rule method (Campbell and Madden, 1990) as shown in Eq.3.1 below was used.

$$AUDPC = \sum_{i=1}^{n-1} [(y_i + y_{i-1})/2](t_{i+1} - t_i) \quad (3.1)$$

Where "t" is time in days of each evaluation, "y" is the disease percentage representing the affected foliage at each evaluation and "n" is the number of evaluations.

Means were separated by least significant difference at P=0.05. Also the correlations between AUDPC, yield, mean AUDPC and mean yield for seasons A and B were determined. The AUDPC was used to evaluate and select accession lines to be used in the breeding experiment.

4.5 Results

4.5.1 Weather

Variations in rainfall and temperature are shown in Figure 9 for Kachwekano Research Station during the year of experimentation. The monthly mean temperatures were low, ranging from 8.6 to 25.6°C, while rainfall ranged from 0-183 mm. The lowest temperatures were experienced in Season A during the months of June and July but these months also experienced the lowest amount of rainfall. Season A had the month with the highest rainfall. More rainfall was received in Season B than season A.

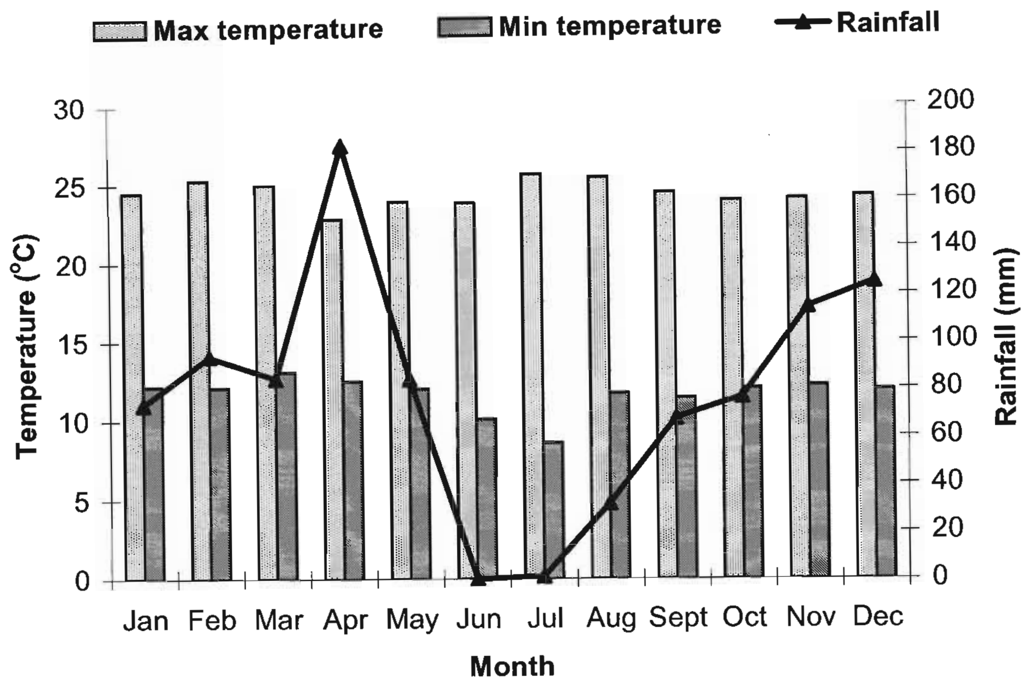


Figure 9: Temperature and rainfall data for Kachwekano Research Station for the year 2004

4.5.2 Disease severity

The analysis of variance for AUDPC for beans planted in Seasons A and B showed that the sources of variation associated with variance in the different accessions for anthracnose resistance were highly significant (P<0.001). Results of the screening for anthracnose in all the 120 accessions for both seasons are given as the area under disease progress curve (AUDPC) in Table 12. The severity of anthracnose measured as AUDPC had significantly higher values in the second season than in the first season but the reaction of the accessions to the pathogen were similar. This is illustrated by the highly positive correlation between AUDPC of Season A and B (r = 0.80; P< 0.001). The AUDPC values in Season A ranged from 556-3982 with accessions having an AUDPC value ≤ 1500 being considered resistant, 1500-3000 as having intermediate resistance and those having AUDPC > 3000 being considered susceptible. For Season B the AUDPC values ranged from 1170-5413 with an accession having an AUDPC value of ≤ 1800 being considered resistant, 1800-3600 as having intermediate resistance and those having AUDPC > 3600 being considered susceptible.

Accessions showed significantly varying resistance (P=0.026) to anthracnose (Table 12). The majority of them (53.3%) gave a susceptible reaction to the pathogen, with heavy symptoms on leaves, stems and pods. 37.5 % gave an intermediate reaction with disease symptoms limited

to small lesions and only 11 accessions (9.2%) showed resistance to the pathogen. In some resistant plants, a few symptoms were identified, mainly on primary leaves. The most resistant accessions are indicated by the lowest AUDPC values (Table 12).

Table 2: Response of 120 germplasm lines to anthracnose pathotype 767

Accession No.	AUDPC (Season A)	Classification (Season A)	AUDPC (Season B)	Classification (Season B)	Mean	Final Classification
NAT001	3658	S	4338	S	3998	S
NAT002	977	R	1670	R	1323	R
NAT003	1047	R	1623	R	1335	R
NAT004	1307	R	1425	R	1366	R
NAT005	3823	S	4098	S	3961	S
NAT006	1723	I	2087	I	1905	I
NAT007	2429	I	2709	I	2569	I
NAT008	976	R	1212	R	1094	R
NAT009	3519	S	1987	I	2753	I
NAT010	2284	I	3744	I	3014	I
NAT011	2404	S	3080	S	2742	S
NAT012	2517	I	3530	I	3023	I
NAT013	2612	I	3563	I	3087	I
NAT014	3400	S	4224	S	3812	S
NAT015	2407	I	3465	I	2936	I
NAT016	2495	I	3668	I	3081	I
NAT017	2283	I	3706	I	2995	I
NAT018	3820	S	4982	S	4401	S
NAT019	3377	S	4991	S	4184	S
NAT020	1682	I	2879	S	2281	S
NAT021	2403	I	3447	I	2925	I
NAT022	2518	I	3561	I	3040	I
NAT023	1377	R	1946	I	1662	I
NAT024	1613	I	2496	I	2055	I
NAT025	2353	I	2971	I	2662	I
NAT026	1587	I	2374	I	1981	I
NAT027	3578	S	4279	S	3929	S
NAT028	1726	I	2170	I	1948	I
NAT029	2541	S	3907	S	3224	S
NAT030	3517	S	5413	S	4465	S
NAT031	1677	I	3210	S	2443	S
NAT032	1777	I	3540	I	2658	I
NAT033	1587	I	2541	I	2064	I
NAT034	2625	I	3417	I	3021	I
NAT035	3683	S	4618	S	4151	S
NAT036	1168	R	1527	R	1348	R
NAT037	1854	I	2618	I	2236	I
NAT038	3683	S	4898	S	4291	S
NAT039	1798	I	2072	I	1935	I
NAT040	3540	S	4681	S	4111	S
NAT041	1544	I	2619	I	2082	I

Accession No.	AUDPC (Season A)	Classification (Season A)	AUDPC (Season B)	Classification (Season B)	Mean	Final Classification
NAT042	1537	I	2770	S	2153	S
NAT043	1647	I	2713	I	2180	I
NAT044	3982	S	4478	S	4230	S
NAT045	3110	S	4864	S	3987	S
NAT046	1842	I	2160	I	2001	I
NAT047	3610	S	4640	S	4125	S
NAT048	3448	S	5129	S	4288	S
NAT049	3287	S	5128	S	4207	S
NAT050	3543	S	4883	S	4213	S
NAT051	3470	S	5136	S	4303	S
NAT052	1705	I	5251	S	3478	S
NAT053	3747	S	4736	S	4241	S
NAT054	3823	S	4670	S	4247	S
NAT055	3541	S	5129	S	4335	S
NAT056	3284	S	5222	S	4253	S
NAT057	3264	S	5349	S	4306	S
NAT058	3495	S	5381	S	4438	S
NAT059	3425	S	5183	S	4304	S
NAT060	2471	I	3567	I	3019	I
NAT061	3520	S	3023	S	3272	S
NAT062	3543	S	4726	S	4135	S
NAT063	3954	S	5412	S	4683	S
NAT064	1961	I	5257	S	3609	S
NAT065	1737	I	4762	S	3249	S
NAT066	3541	S	5051	S	4296	S
NAT067	920	R	1298	R	1109	R
NAT068	1463	R	1678	R	1571	S
NAT069	2354	I	3742	I	3048	I
NAT070	1662	I	2828	I	2245	I
NAT071	1485	R	1885	I	1685	I
NAT072	3448	S	3930	S	3689	S
NAT073	1634	I	4570	S	3102	S
NAT074	1381	R	1708	R	1545	R
NAT075	3451	S	5295	S	4373	S
NAT076	1884	I	3733	I	2809	I
NAT077	1728	I	2425	I	2077	I
NAT078	1543	I	3694	S	2619	S
NAT079	3595	S	4715	S	4155	S
NAT080	3489	S	4735	S	4112	S
NAT081	1567	I	2170	I	1868	I
NAT082	1563	I	3675	S	2619	S
NAT083	1627	I	2610	I	2119	I
NAT084	3517	S	4786	S	4152	S
NAT085	1590	I	2482	I	2036	I
NAT086	3562	S	4377	S	3969	S
NAT087	3567	S	4272	S	3920	S
NAT088	1810	I	4091	S	2951	S
NAT089	3502	S	4881	S	4192	S
NAT090	1253	R	1708	I	1481	R

Accession No.	AUDPC (Season A)	Classification (Season A)	AUDPC (Season B)	Classification (Season B)	Mean	Final Classification
NAT091	3332	S	4071	S	3702	S
NAT092	1661	I	4769	S	3215	S
NAT093	3489	S	4860	S	4175	S
NAT094	3008	S	5193	S	4101	S
NAT095	1729	I	2448	I	2088	I
NAT096	3730	S	4829	S	4280	S
NAT097	3311	S	5065	S	4188	S
NAT098	1340	R	1985	I	1663	I
NAT099	1522	R	1875	I	1699	I
NAT100	3429	S	4868	S	4148	S
NAT101	1625	I	2678	I	2152	I
NAT102	1874	I	2285	I	2080	I
NAT103	1887	I	3029	I	2458	I
NAT104	1833	I	2563	I	2198	I
NAT105	556	R	1176	R	866	R
NAT106	559	R	1170	R	865	R
NAT107	1620	I	2427	I	2024	I
NAT108	1710	I	2221	I	1965	I
NAT109	3335	S	4788	S	4062	S
NAT110	1548	I	4771	S	3160	S
NAT111	3103	S	4945	S	4024	S
NAT112	3508	S	4988	S	4248	S
NAT113	3057	S	4689	S	3873	S
NAT114	3124	S	4949	S	4037	S
NAT115	1797	I	4680	S	3239	S
NAT116	1301	R	1725	R	1513	R
NAT117	3615	S	4660	S	4138	S
NAT118	1969	I	3514	I	2742	I
NAT119	1729	I	2562	I	2146	I
NAT120	1485	I	2394	I	1940	I
s.e.d	**78.7**		**485.5**			
Lsd (0.05)	**155**		**947.4**			
%CV	**20.6**		**28.3**			

AUDPC= Area under disease progress curve; R= Resistant; S=Susceptible; I= Intermediate Resistance; Season A classification: AUDPC values ≤1500 = R; ≤3000 = I; and >3000 = S; Season B classification: AUDPC values ≤1800 = R; ≤3600 = I and > 3600 = S; Final classification: AUDPC values ≤1650 = R; ≤3300 = I and ≥3300 = S.

4.5.3 Relationship between anthracnose and phenotypic traits

Using the AUDPC values obtained in the trials, the accessions conforming to the Growth Type III (indeterminate, weak stemmed semi-climbers) had the highest number of resistant lines followed by Growth Type IV (indeterminate weak stemmed climbers). None of the Type I (determinate upright growth type) cultivars in the germplasm collection was resistant. There were few Type II (indeterminate upright growth type) accessions and they had varying reactions to the pathogen (Table 13).

The data obtained shows that all the accessions that gave a resistant reaction to the anthracnose, possessed pink or dark pink flowers with the exception of accession NAT 008 which had white flowers. Findings further revealed that all resistant accessions were small seeded but with varying seed colours (Table 13). In resistant accession lines, the lesions caused by the pathogen were darker tiny spots with a sharply defined border between the healthy and affected tissues and in most cases were limited to primary leaves. In indeterminate and susceptible accession lines, symptoms were scattered throughout the canopy leaves, stems and pod. Lesions caused by the pathogen gave a brick red colouration to the leaf veins with brown margins and veins, while the pods had sunken cankerous centres. These symptoms varied in intensity depending on the strength of resistance resident in the different accessions.

Table 3: Phenotypic characteristics of accessions evaluated for anthracnose resistance

Accession No.	Place of origin	Flower colour	Growth habit	Seed		Class
				Size	Colour	
NAT001	Iganga	White	I	M	Brown	S
NAT002	Iganga	Pink	II	S	Light brown	R
NAT003	Arua	Dark pink	III	S	Brown + black speckles	R
NAT004	Arua	Pink	III	S	Black	R
NAT005	Mbarara	White	III	S	Brick-red	S
NAT006	Arua	White	III	S	White	I
NAT007	Masaka	White	I	L	Red mottled	I
NAT008	Lira	White	II	S	Cream+ brown speckles	R
NAT009	Iganga	Pink	I	S	Brown	S
NAT010	CIAT	Pink	I	L	Maroon speckles	I
NAT011	CIAT	White	IV	S	Maroon	S
NAT012	Masaka	Dark pink	III	S	White	I
NAT013	Arua	Dark pink	I	S	Brown	I
NAT014	Mubende	White	IV	M	Brown	S
NAT015	Arua	Pink	I	L	Brick-red	I
NAT016	CIAT	Pink	I	L	Brown	I
NAT017	Wakiso	White	I	L	Red mottled	I
NAT018	Nebbi	Pink	II	S	Maroon	S
NAT019	Nebbi	Pink	I	M	Brick-red	S
NAT020	CIAT	Pink	III	S	Dirty white	S
NAT021	Arua	Pink	I	M	Brick-red	I
NAT022	CIAT	Pink	I	M	Brown	I
NAT023	CIAT	White	III	S	White	R
NAT024	Nebbi	Dark pink	III	S	Maroon	I
NAT025	Wakiso	Pink	I	L	Maroon	I
NAT026	Mbarara	Pink	III	L	Red	I
NAT027	Nebbi	Pink	III	S	Purple	S
NAT028	CIAT	Pink	III	S	Black	I
NAT029	Mbarara	Pink	I	M	Yellow + maroon speckles	S
NAT030	Iganga	White	I	S	Dark brown	S

Accession No.	Place of origin	Flower colour	Growth habit	Seed		Class
				Size	Colour	
NAT031	CIAT	Pink	I	L	Pink + maroon Speckles	S
NAT032	Iganga	Pink	III	L	Red mottled	I
NAT033	Arua	Dark pink	II	S	Brown	I
NAT034	CIAT	Pink	I	M	Red mottled	I
NAT035	Kabale	Pink	I	L	Brick-red	S
NAT036	Masindi	White	IV	M	White	R
NAT037	Kamuli	White	I	L	Red mottled	I
NAT038	Arua	White	III	S	Pink	S
NAT039	Arua	White	III	S	Dark brown	I
NAT040	CIAT	Dark pink	III	S	Brown	S
NAT041	Iganga	White	III	S	Orange	I
NAT042	Masindi	Pink	I	M	Brick-red	S
NAT043	Kabale	Pink	I	L	Brick-red	I
NAT044	Masaka	White	I	S	White	S
NAT045	CIAT	Pink	III	M	Red	S
NAT046	Nebbi	White	I	M	Brown + black speckles	I
NAT047	Iganga	Pink	III	S	Brick-red	S
NAT048	Mbarara	Pink	III	M	Brick red speckles	S
NAT049	Kamuli	White	I	S	White	S
NAT050	Iganga	Pink	I	M	Brick red speckles	S
NAT051	CIAT	Pink	I	M	Brick red	S
NAT052	Lira	White	III	S	Grey	S
NAT053	Lira	White	IV	M	Brown	S
NAT054	Iganga	White	IV	M	White	S
NAT055	Nebbi	Pink	I	M	Red mottled	S
NAT056	Masaka	White	III	L	Pink	S
NAT057	CIAT	White	IV	S	Red	S
NAT058	Iganga	Pink	I	S	Dark brown	S
NAT059	Kamuli	Dark pink	III	L	Red mottled	S
NAT060	Masindi	Pink	III	S	Purple	I
NAT061	Arua	Pink	I	M	Black	S
NAT062	Bushenyi	White	IV	M	Yellow+ maroon speckles	S
NAT063	Lira	White	I	S	Brown	S
NAT064	Arua	White	III	S	Light brown	S
NAT065	Iganga	White	I	S	Black	S
NAT066	Arua	White	III	S	Light brown	S
NAT067	Iganga	Dark pink	III	S	Black	R
NAT068	Iganga	Dark pink	I	S	Dirty white	S
NAT069	Wakiso	Pink	III	L	Red mottled	I
NAT070	Masaka	Pink	III	M	Maroon	I
NAT071	Wakiso	Pink	III	L	Black + yellow speckles	R
NAT072	Iganga	White	II	M	Cream + brown speckles	S
NAT073	Arua	Pink	I	L	Red mottled	S
NAT074	Iganga	Pink	III	M	Khaki	R
NAT075	CIAT	Pink	I	M	Brick red speckles	S
NAT076	Masaka	Pink	I	M	Red mottled	I
NAT077	CIAT	Dark pink	II	S	Brown	I
NAT078	Nebbi	Dark pink	II	S	Brown + black speckles	S

Accession No.	Place of origin	Flower colour	Growth habit	Seed		Class
				Size	Colour	
NAT079	Nebbi	Pink	III	M	Brown	S
NAT080	Mubende	White	IV	M	Black	S
NAT081	Arua	Pink	III	L	Red mottled	I
NAT082	Arua	Dark pink	IV	M	Brown + maroon speckles	S
NAT083	CIAT	White	I	M	Black	I
NAT084	CIAT	White	I	L	Red mottled	S
NAT085	CIAT	Yellow	III	M	Brown	I
NAT086	Iganga	White	III	S	Khaki	S
NAT087	Mayuge	Pink	I		Pink + maroon speckles	S
NAT088	Masaka	Pink	III	S	White	S
NAT089	Arua	Dark pink	I	S	Brick-red	S
NAT090	Arua	Pink	II	L	Pink + maroon speckles	R
NAT091	CIAT	White	IV	S	Pink	S
NAT092	Iganga	Pink	I	S	Brown	S
NAT093	Wakiso	Pink	I	L	Maroon	S
NAT094	Namulonge	White	I	L	Red mottled	S
NAT095	Potchefstroom (SA)	White	III	S	White	I
NAT096	Potchefstroom (SA)	Pink	I	L	Maroon	S
NAT097	Potchefstroom (SA)	White	III	L	White	S
NAT098	Potchefstroom (SA)	Dark pink	III	S	Black	R
NAT099	Potchefstroom (SA)	White	I	M	White	I
NAT100	Potchefstroom (SA)	White	I	L	White	S
NAT101	Potchefstroom (SA)	Pink	I	S	White	I
NAT102	Potchefstroom (SA)	White	III	S	Cream	I
NAT103	Potchefstroom (SA)	Pink	II	S	Cream + brown speckles	I
NAT104	Potchefstroom (SA)	Pink	I	S	Black	I
NAT105	Potchefstroom (SA)	Pink	IV	S	Maroon	R
NAT106	Potchefstroom (SA)	Pink	IV	S	Maroon	R
NAT107	Potchefstroom (SA)	White	IV	M	White	I
NAT108	Pannar (SA)	White	I	M	Brown + maroon speckles	I
NAT109	Namulonge	White	I	M	Red Mottled	S
NAT110	Ukulinga(SA)	White	I	M	Brown + maroon speckles	S
NAT111	Pannar (SA)	White	I	L	Yellow + red speckles	S
NAT112	Cedara (SA)	Pink	I	M	Cream	S
NAT113	Cedara (SA)	Pink	I	M	Brown + maroon speckles	S
NAT114	Pro-seed(SA)	White	I	M	Brown + black speckles	S
NAT115	Pro-seedSA)	White	IV	L	Brick red	S
NAT116	Pannar (SA)	Pink	I	M	Maroon speckles	I
NAT117	Pro-seed(SA)	White	II	M	Brown + maroon speckles	S
NAT118	Namulonge	White	IV	L	Black speckles	I
NAT119	Namulonge	Pink	IV	L	Red mottled	I
NAT120	Namulonge	White	I	L	Red mottled	I

Growth habit classification: I = determinate, II= indeterminate prostrate, III = indeterminate weak climber, and IV = indeterminate strong climber. S= small sized seed (< 25g/100 seed); M = medium sized seed (25-40 g/100 seed); L = large sized seed (> 40 g/100 seed)

4.5.4 Relationship between anthracnose and yield

Significant differences (P<0.001) were observed in the yield obtained from different accessions. Dry seed yields of the different accessions ranged from 12 - 1,349 kg ha^{-1} in the first season and from 86 - 3,253 kg ha^{-1} in the second season. Considering the reaction to anthracnose, the mean yield varied widely i.e., the yield from 463 - 1,970 kg ha^{-1} for resistant accessions, 57 - 1,904 kg ha^{-1} for the intermediate resistant and from 54 - 339 kg ha^{-1} for the susceptible accessions (Table 14). Differences in yield varied significantly (P<0.001) between different accession and not between resistant classifications. There were accessions with an intermediate resistant reaction that yielded significantly higher (P<0.001) than accessions with a resistant reaction. This observation was also true for susceptible versus intermediate resistant accessions (Table 14). Although there was a strong correlation (r = 0.68; P< 0.05) between yields of the two season, mean yields obtained in the second season was significantly higher (data not shown) than those obtained in the first season. The results also indicate a significant negative correlation between the AUDPC and yield (r =-0.53, P = 0.05).

Table 4: Yield of 120 germplasm accessions in the presence of anthracnose pathogen

Accession No.	Season A (kg ha^{-1})	Season B (kg ha^{-1})	Mean yield (Kg ha^{-1})	Classification
NAT001	104	311	208	S
NAT002	498	793	645	R
NAT003	441	1,012	727	R
NAT004	485	1,430	958	R
NAT005	192	255	224	S
NAT006	442	655	548	I
NAT007	231	639	435	I
NAT008	560	911	735	R
NAT009	140	214	177	S
NAT010	163	276	220	I
NAT011	150	267	208	S
NAT012	86	135	110	I
NAT013	270	401	336	I
NAT014	194	242	218	S
NAT015	100	515	308	I
NAT016	64	254	159	I
NAT017	111	789	450	I
NAT018	253	313	283	S
NAT019	143	242	193	S
NAT020	165	183	174	S
NAT021	131	498	315	I
NAT022	157	700	429	I
NAT023	424	590	507	R

Accession No.	Season A (kg ha^{-1})	Season B (kg ha^{-1})	Mean yield (Kg ha^{-1})	Classification
NAT024	156	737	446	I
NAT025	120	694	407	I
NAT026	57	699	378	I
NAT027	158	321	239	S
NAT028	28	436	232	I
NAT029	140	209	175	S
NAT030	148	131	139	S
NAT031	85	161	123	S
NAT032	105	522	313	I
NAT033	78	753	415	I
NAT034	96	150	123	I
NAT035	96	187	141	S
NAT036	442	795	619	R
NAT037	27	86	57	I
NAT038	102	185	143	S
NAT039	87	765	426	I
NAT040	124	441	282	S
NAT041	73	632	353	I
NAT042	118	268	193	S
NAT043	116	247	182	I
NAT044	59	155	107	S
NAT045	111	132	122	S
NAT046	93	251	172	I
NAT047	150	171	160	S
NAT048	67	173	120	S
NAT049	170	339	255	S
NAT050	115	166	141	S
NAT051	196	309	252	S
NAT052	57	374	215	S
NAT053	163	408	286	S
NAT054	184	405	295	S
NAT055	155	358	256	S
NAT056	82	179	131	S
NAT057	147	358	252	S
NAT058	187	135	161	S
NAT059	140	353	246	S
NAT060	194	440	317	I
NAT061	93	169	131	S
NAT062	27	93	60	S
NAT063	83	154	118	S
NAT064	127	195	161	S
NAT065	275	169	222	S
NAT066	59	247	153	S
NAT067	561	2,009	1,285	R
NAT068	59	181	120	S
NAT069	224	286	255	I
NAT070	100	135	117	I
NAT071	356	633	494	R

Accession No.	Season A (kg ha^{-1})	Season B (kg ha^{-1})	Mean yield (Kg ha^{-1})	Classification
NAT072	67	301	184	S
NAT073	21	118	69	S
NAT074	244	712	478	R
NAT075	51	205	128	S
NAT076	81	787	434	I
NAT077	97	300	199	I
NAT078	27	208	118	S
NAT079	95	314	205	S
NAT080	253	312	283	S
NAT081	217	664	441	I
NAT082	230	229	229	S
NAT083	350	670	510	I
NAT084	237	288	263	S
NAT085	262	594	428	I
NAT086	110	195	153	S
NAT087	125	215	170	S
NAT088	110	212	161	S
NAT089	209	444	326	S
NAT090	344	915	630	R
NAT091	75	326	201	S
NAT092	35	150	93	S
NAT093	184	119	152	S
NAT094	245	307	276	S
NAT095	20	202	111	I
NAT096	284	395	339	S
NAT097	125	426	276	S
NAT098	277	650	463	R
NAT099	262	673	468	R
NAT100	125	443	284	S
NAT101	103	639	371	I
NAT102	404	301	352	I
NAT103	199	474	337	I
NAT104	81	659	370	I
NAT105	704	1,513	1,108	R
NAT106	1,348	2,592	1,970	R
NAT107	100	428	264	I
NAT108	118	539	329	I
NAT109	43	212	128	S
NAT110	158	109	133	S
NAT111	230	334	282	S
NAT112	12	97	54	S
NAT113	27	123	75	S
NAT114	47	100	74	S
NAT115	59	199	129	S
NAT116	373	714	544	R
NAT117	101	229	165	S
NAT118	554	3,253	1,904	I
NAT119	89	776	432	I

Accession No.	Season A (kg ha^{-1})	Season B (kg ha^{-1})	Mean yield (Kg ha^{-1})	Classification
NAT120	199	588	394	I
S.e.d	**23.42**	**55.38**		
Lsd ($P \leq 0.05$)	**46.15**	**109.21**		
CV (%)	**18.2**	**13.8**		

R= Resistant; S=Susceptible; I= Intermediate Resistance

4.6 Discussion and conclusion

Sources of good resistance are an important tool to pursue as the principal component in a pedigree or backcross breeding programme. The best possible method of identifying resistant sources is to expose the potential sources of resistance to all dominant pathotypes over different production areas to eliminate highly susceptible genotypes (Beebe and Pastor-Corrales, 1991). In this study a germplasm collection of 120 dry bean accessions was screened to establish whether there are any accession lines which could be used as effective sources of anthracnose resistance.

The use of the area under disease progress curve (AUDPC) as a disease severity measure and as a tool for plant resistance evaluation helps to reflect disease progress throughout the whole growing season (Campbell and Madden, 1990). In this study, the highest AUDPC values represented accessions with the highest disease infection. There were differences in the AUDPC values between accessions within seasons and between seasons. The differences observed between AUDPC values of germplasm accessions within seasons suggest differences in resistance of individual accessions. On the other hand, the difference observed between seasons could be explained by the differences in the climatical conditions. Related studies done by Bailey *et al.* (1992), show that climatic conditions have a strong influence on the severity of anthracnose.

According to Evans (1993), disease is one of the major factors affecting crop yield as it disrupts the balance between the source and sink activities of the plant. In this study anthracnose had a negative effect on yield, especially with the most susceptible accessions. The season with the highest disease severity, was also observed to have better yield performance. The inconsistency in results could be explained by Gaunt's (1995) theory which states that the green leaf area and the green area duration is directly correlated to yield in both healthy and diseased crop species. The observations from the study show that Season B which had higher disease severity also had better climatical conditions resulting in longer green leaf area duration which culminated in higher yields. Also root rot disease

was observed in the field during the first season trials, and caused many plant deaths. This could also explain the significantly lower yield observed in the first season.

In addition, a strong negative correlation between disease and yield would be expected but as reported in this study, the correlation between these two factors, although negative, was only moderately strong and can not give a full explanation to all the yield variations. A partial explanation could be offered by the tolerance observed in some accessions, resulting in high yields despite high disease incidences. According to Gaunt's (1995) and Gaunt and Bryson's (1995) findings, the absence of a strong negative relationship between yield and AUDPC is more common when data from different seasons is used, as was the case in this study. Also the analysis of data for the individual seasons resulted in moderate correlations (r = -0.42 and r = -0.51 for Seasons A and B, respectively). This moderate correlation between yield and AUDPC could partially be explained by the lack of an estimate of defoliation in the disease assessment method, the variation in growth habits and differences in the yield potential exhibited by the different accessions. Additionally, there was a lack of precision and accuracy in measuring disease by visual rating. As revealed by O'Brien and van Bruggen's (1992) study, the inaccuracies made while measuring disease in the field are a major constraint in relating disease to yield and in some cases, there may be no relationship between these two variables. Also similar studies by Waggoner and Berger (1987); Gaunt (1995); and Bergamin-Filho *et al.* (1997), have indicated that the measurement of disease severity based on lesion number or leaf area may be less related to yield.

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

The study further indicates relationships between crop resistance and some phenotypic traits. Results show that all resistant accessions were either of Type III or IV growth habit, had either pink or red flowers and were small seeded (< 25 g per 100 seeds) (Singh, 1989). The germplasm collection used in this study had few Type II growth habit accessions and all Type I

growth habit accessions evaluated were susceptible. It was also observed that most of the accessions showing resistant, yielded far better than those showing an intermediate and susceptible disease reaction, apart from accessions NAT118, NAT006 and NAT083, which gave intermediate reactions but yielded better than some resistant accessions. These three accessions should in this case be described as being tolerant to *C. lindemuthianum*. Also, with the exception of the three accessions obtained from outside Uganda, the rest of the resistant accessions originated from Ugandan bean growing regions (Lira and Apac) which had previously been reported to have severe anthracnose problems (Fina Opio, Pers. Com.). It is possible that resistant varieties may have been selected by farmers as a result of the high disease pressure that initially existed but this is a hypothesis which needs further investigation.

Finally, the use of AUDPC as the measure of resistance was very useful in this study as it was able to show that out of the 120 germplasm accessions, 11 gave a consistent resistant reaction to the predominant anthracnose pathotype in this region; 40 gave an intermediate resistance reaction and 69 were susceptible. It is therefore suggested that use should be made of the identified resistant accession lines in the development of an anthracnose breeding programme for Ugandan dry bean cultivars. There is also a need for a further study to determine the quality of resistance exhibited by the resistant accessions.

References

Allen, D.J., Ampofo, J.K.O., and Wortman, C.S. 1996. Pest, disease, and nutritional disorders of the common bean in Africa: A Field Guide. CIAT, Cali Colombia. 132p.

Bailey, J.A. O'Connell, R.J. and Nash, C. 1992. Infection strategy of *Colletotrichum lindemuthiamun* species. In: Bailey, J.A. and Jeger, M.J. (eds.). *Colletotrichum: Biology, Pathology and Control.* Pp. 88-120. CAB International, Wallingford, UK.

Beebe, S.E. and Pastor-Corrales, M.A. 1991. Breeding for disease resistance. In: Van Schoonhoven, A. and Voysest, O. (eds.). *Common Beans: Research for Crop Improvement.* Pp. 562-617. Centro International de Agricultura Tropical, (CIAT). Cali, Colombia.

Bergamin-Filho, A., Carneiro, S.M.T.P.G., Godoy, C.V., Amorim, L., Berger, R.D., and Hau, B. 1997. Angular leaf spot of *Phaseolus* beans: Relationships between disease, healthy leaf area, and yield. *Phytopathology*, 87:506-515.

Campbell, C.L. and Madden, L.V. 1990. Introduction to Plant Epidemiology. John Wiley and sons, NY, USA. 532p.

Chaves, G. 1980. Anthracnose. In: Schwartz H.F. and Galvez, G.E. (eds.), Bean Production Problems: Disease, Insect, Soil and Climatic Constraints of *Phaseolus vulgaris.* Pp. 37-54. Centra Internacional de Agricultura Tropical (CIAT), Series 09EB-1, Cali, Colombia.

Cardoso de Arruda, M.C., Alzate-Marin, A.N., Chagas, J.M., Moreira, M.A. and Gonzales de Barros, E. 2000. Identification of random amplified polymorphic DNA markers linked to the *Co-4* resistance gene to *Colletotrichum lindemuthianum* in common bean. *Phytopathology,* 90:758-761.

David, S., Kirkby, R., and Kasozi, S. 2000. Assessing the impact of bush beans varieties on poverty reduction in Sub-Saharan Africa: Evidence from Uganda. Paper presented at the workshop *"Assessing the impact of Agriculture Research on Poverty Alleviation", 14-16 September, 1999*, San Jose, Costa Rica.

Evans, L.T. 1993. Crop Evolution, Adaptation and Yield. Cambridge, UK: Cambridge Univ. Press. 500p.

Ferraz, S. 1980. Angular leaf spot. In: *Schwartz,* H.F. and Galvez G.E. (eds.)*, Bean Production Problems: Disease, Insect, Soil and Climatic Constraints of Phaseolus vulgaris.* Pp. 55-65. Centra Internacional de Agricultura Tropical (CIAT), Series 09EB-1, Cali, Colombia,

Gaunt, R.E. 1995. The Relationship between plant disease severity and yield. *Annual Review Phytopathology*, 33:119-44.

Gaunt, R.E., and Bryson, R.J. 1995. Plant and crop yield potential and response to disease. In: Walters, R.D., Scholes, J.D., Bryson., R.J., Paul, N.D., and McRoberts, N. (eds.). Physiological Responses of Plant to Pathogens. *Association of Applied Biologists.* Pp. 1-7. Wellesbourne, Warwick, England.

Lindblade, K.A, Carswell, G. and Tumuhairwe, J.K. 1998. Mitigating the relationship between population growth and land degradation: Land-use change and farm management in Southwestern Uganda. *Ambio* 27:565-571.

NEMA. 2001. Republic of Uganda, 2001. State of the Environment Report: Kabale 2000/2001. National Environment Management Authority. Kampala, Uganda.

O' Brien, R.D. and Van Bruggen, A.H.C. 1992. Accuracy, precision, and correlation to yield loss of disease severity scales for corky root of lettuce. *Phytopathology*, 82(1): 91-96.

Opio, F., Ugen, M.A., Kyamanywa, S., David, S., and Mugisha-mutetikka, M. 2001. Beans. In: Mukiibi, J.K, Esete, J.P., Musoli, P.C., Oryokot, J.O.E., Twinamasiko, E., Odongkan, O.K., Esegu, J.F.O., Otim-Nape, G.W., Ssali, H., and Odogola, W. (eds.). *Agriculture in Uganda. Volume II Crops*. NARO. Kampala, Uganda.

Opio, F., Mugagga., Mawejje, D., and Nkalubo, S. (2006). Progress report on bean anthracnose research in Uganda. *MUARIK bulletin (in press)*.

Pastor-Corrales, M.A. Llano, G.A. Afanador, L. and Castellanos, G. 1985. Disease resistance mechanism in beans to the anthracnose pathogen. *Phytopathology,* 75:1176.

Redman, C.E, King, E.P. and Brown, I.F.Jr., 1969. Tables for converting Barratt and Horsfall rating scores to estimated mean percentages. Eli Lilly and Company Research Laboratories. Indianapolis, IN. Mimeo.

Sanders, J.H., Schwartz, H.F. 1980. Bean production problems and pest constraints in Latin America. In: Schwartz, H.F., Galvez, G.E., (eds). *Bean Production Problems*. Pp. 1-14. Centro International de Agricultura Tropical (CIAT). Cali, Columbia.

Schwartz, H.F. 1989. Additional fungal pathogens. In: Pastor Corrales, M.A. and Schwartz, H.F. (eds.). *Bean Production Problems in the Tropics*. Pp. 231-259. Centro International de Agricultura Tropical (CIAT). Cali, Columbia.

Schwartz, H.F., Pastor Corrales, M.A. and Singh, S.P. 1982. New sources of resistance to anthracnose and angular leaf spot of beans (*Phaseolus vulgaris* L.). *Euphytica,* 31:741-754

Sicard, D., Michalakis, Y., Dron, M., and Neema, C. 1997. Genetic diversity and pathogenic variation of *Colletotrichum lindemuthianum* in the three centres of diversity of its host, *Phaseolus vulgaris*. *Phytopathology*, 87:807-813.

Silbernagel, M.J. and Zaumeyer, W.J. 1973. Beans. In: Nelson R.R. (ed.), Breeding Plants for Disease Resistance: Concepts and Applications. Pp. 253-269. The Penn. State University Press, London.

Singh, S.P. 1989. Patterns of variation in cultivated common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany*, 43:1:39-57.

Waggoner, P.E and Berger, R.D. 1987. Defoliation, disease and growth. *Phytopathology*, 77:393-398.

Wortman, C.S. and Allen, D.J. 1994. African bean production environments: their definition, characteristics and constraints. Network on Bean Research in Africa. *Occasional Paper Series No.* 11, Dar Salaam, Tanzania.

Zaumeyer, W.J. and Meiners, J.P. 1975. Disease resistance in beans. *Annual Review Phytopathology* 13:318-320.

Appendices

Appendix 1: Germplasm collection

Collection No./ Name	Description	Place of Origin	Accession No.
26	Khaki	Iganga	NAT001
33-5	Cream	Iganga	NAT002
39-2	Brown + black speckles	Arua	NAT003
8	Black	Arua	NAT004
66-2	Pink	Mbarara	NAT005
15	White	Arua	NAT006
46	Calima	Masaka	NAT007
51	Cream stripped	Lira	NAT008
33-3	Khaki	Iganga	NAT009
1-1	Kanyembwa	CIAT	NAT010
3-1	Red	CIAT	NAT011
24	White	Masaka	NAT012
16-1	Cream	Arua	NAT013
64	Khaki	Mubende	NAT014
29-4	Kayembwa	Arua	NAT015
73-2	Pink + white speckles	CIAT	NAT016
20	Calima	Wakiso	NAT017
54-4	Pink	Nebbi	NAT018
54-2	Kanyembwa	Nebbi	NAT019
21-6	Cream	CIAT	NAT020
29-3	Kanyembwa	Arua	NAT021
11-5	Grey speckles	CIAT	NAT022
35-6	White	CIAT	NAT023
54-3	Red	Nebbi	NAT024
Manyigamulimi	Maroon	Wakiso	NAT025
66-1	Red	Mbarara	NAT026
54-7	Purple (light)	Nebbi	NAT027
29	Black	CIAT	NAT028
66-4	Kanyembwa	Mbarara	NAT029
21-5	Khaki	Iganga	NAT030
61-2	Kanyembwa	CIAT	NAT031
26-3	Kanyembwa	Iganga	NAT032
16-3	Cream	Arua	NAT033
14-3	Kanyembwa	CIAT	NAT034
71-1	Kanyembwa	Kabale	NAT035
V90	white	Masindi	NAT036
75-1	Calima	Kamuli	NAT037
29-1	Red	Arua	NAT038
6-3	Khaki	Arua	NAT039
16-2	cream	CIAT	NAT040
38-3	cream	Iganga	NAT041
60-1	Kanyembwa	Masindi	NAT042
71-2	Kanyembwa	Kabale	NAT043
22	White	Masaka	NAT044
13-1	Red	CIAT	NAT045

Collection No./ Name	Description	Place of Origin	Accession No.
52-2	Brown speckled black	Nebbi	NAT046
33-2	Cream	Iganga	NAT047
14-1	Kanyembwa	Mbarara	NAT048
42	White	Kamuli	NAT049
26-1	Kanyembwa	Iganga	NAT050
26-4	Kanyembwa	CIAT	NAT051
17-1	Cream	Lira	NAT052
28	Khaki	Lira	NAT053
4	white	Iganga	NAT054
52-1	Kanyembwa	Nebbi	NAT055
36	Kanyembwa	Masaka	NAT056
3-3	Red	CIAT	NAT057
21-6	Cream	Iganga	NAT058
48	Calima	Kamuli	NAT059
60-2	Red	Masindi	NAT060
61-5	Kanyembwa	Arua	NAT061
V77	Sugar bean	Bushenyi	NAT062
17-2	Cream	Lira	NAT063
6-2	Pink	Arua	NAT064
33-7	Black	Iganga	NAT065
13-10	Khaki	Arua	NAT066
7-1	Black	Iganga	NAT067
21-3	Cream	Iganga	NAT068
Carolina	Calima	Wakiso	NAT069
Kayinja	Maroon	Masaka	NAT070
Nakawunde	Black stripped	Wakiso	NAT071
5	Cream stripped	Iganga	NAT072
7-2	Kanyembwa	Arua	NAT073
21-7	Khaki	Iganga	NAT074
14-5	Kanyembwa	CIAT	NAT075
55	Kanyembwa	Masaka	NAT076
18-1	Cream	CIAT	NAT077
52-3	Cream + black speckles	Nebbi	NAT078
50-3	Cream	Nebbi	NAT079
63	Black	Mubende	NAT080
11-6	Calima	Arua	NAT081
13-6	Brown + maroon speckles	Arua	NAT082
6	Black	CIAT	NAT083
74-1	Calima	CIAT	NAT084
65	Khaki	CIAT	NAT085
21-4	Khaki	Iganga	NAT086
Kanyembwa	Kanyembwa	Mayuge	NAT087
19	White	Masaka	NAT088
39-1	Khaki	Arua	NAT089
7-3	Kanyembwa	Arua	NAT090
29-2	Pink	CIAT	NAT091
21-8	Khaki	Iganga	NAT092
40	Maroon	Wakiso	NAT093
K132	Red mottled	Namulonge	NAT094

Collection No./ Name	Description	Place of Origin	Accession No.
Michelite	White	Potchefstroom (SA)	NAT095
MDRK)	Dark red	Potchefstroom (SA)	NAT096
Perry Marrow	white (large)	Potchefstroom (SA)	NAT097
Cornell 49.242	Black	Potchefstroom (SA)	NAT098
Widusa	White (medium)	Potchefstroom (SA)	NAT099
Kaboon	White	Potchefstroom (SA)	NAT100
Mexico 222	White	Potchefstroom (SA)	NAT101
PI 207262	Khaki	Potchefstroom (SA)	NAT102
TO	Cream + brown speckles	Potchefstroom (SA)	NAT103
TU	Black	Potchefstroom (SA)	NAT104
AB 136	Red-Maroon	Potchefstroom (SA)	NAT105
G2333	Maroon	Potchefstroom (SA)	NAT106
Mexico 142	White (small)	Potchefstroom (SA)	NAT107
Pan 178	Brown + Maroon speckles	Pannar (SA)	NAT108
K20	Red mottled	Namulonge	NAT109
RS4	Brown + Maroon speckles	Pro-seed (SA)	NAT110
Pan 109	Brown + Maroon speckles	Pannar (SA)	NAT111
Cerillos	Whitish cream	Cedara (SA)	NAT112
OPS-RS1	Brown + Maroon speckles	Cedara (SA)	NAT113
Mkuzi	Brown speckles	Pro-seed (SA)	NAT114
Pan 117	Brown + Maroon speckles	Pro-seed (SA)	NAT115
Pan 146	Brown + Maroon speckles	Pannar (SA)	NAT116
Bonus	Brown + Maroon speckles	Pro-seed (SA)	NAT117
NABE 12C	Light brown + speckles	Namulonge	NAT118
NABE 4(POA2)	Brick red + black speckles	Namulonge	NAT119
NABE 1(OBA1)		Namulonge	NAT120

Appendix 2: Experimental design for bean germplasm screening against anthracnose in Kachwekano

Block I (Rep 1)

29.5m

7.5m

NAT046	NAT070	NAT114	NAT 52	NAT 28	NAT111	NAT083	NAT116	NAT035	NAT040	NAT082	NAT036	NAT019	NAT066	NAT011
NAT016	NAT113	NAT089	NAT117	NAT043	NAT008	NAT103	NAT072	NAT045	NAT025	NAT015	NAT119	NAT062	NAT085	NAT005
NAT007	NAT027	NAT098	NAT018	NAT115	NAT039	NAT099	NAT044	NAT078	NAT020	NAT058	NAT060	NAT102	NAT071	NAT021
NAT023	NAT056	NAT108	NAT041	NAT055	NAT029	NAT026	NAT107	NAT095	NAT003	NAT010	NAT031	NAT004	NAT017	NAT094
NAT014	NAT084	NAT054	NAT048	NAT073	NAT022	NAT069	NAT118	NAT059	NAT079	NAT076	NAT101	NAT097	NAT037	NAT057
NAT081	NAT065	NAT024	NAT051	NAT033	NAT064	NAT034	NAT030	NAT112	NAT077	NAT53	NAT104	NAT093	NAT100	NAT032
NAT067	NAT047	NAT013	NAT105	NAT068	NAT006	NAT063	NAT109	NAT092	NAT050	NAT042	NAT074	NAT002	NAT080	NAT038
NAT086	NAT096	NAT012	NAT091	NAT075	NAT088	NAT120	NAT090	NAT001	NAT009	NAT061	NAT106	NAT087	NAT110	NAT049

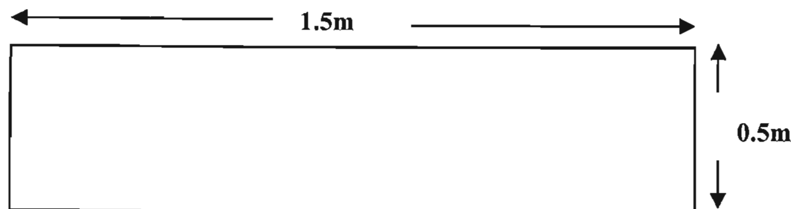
Block II (Rep 2)

NAT104	NAT038	NAT005	NAT074	NAT114	NAT087	NAT016	NAT062	NAT017	NAT050	NAT053	NAT113	NAT099	NAT047	NAT089
NAT001	NAT018	NAT049	NAT080	NAT109	NAT065	NAT095	NAT012	NAT081	NAT105	NAT023	NAT054	NAT045	NAT009	NAT028
NAT040	NAT112	NAT025	NAT010	NAT117	NAT042	NAT097	NAT110	NAT094	NAT069	NAT052	NAT060	NAT048	NAT100	NAT046
NAT004	NAT007	NAT033	NAT003	NAT044	NAT067	NAT066	NAT088	NAT061	NAT063	NAT096	NAT103	NAT106	NAT022	NAT115
NAT102	NAT037	NAT029	NAT086	NAT034	NAT076	NAT021	NAT108	NAT073	NAT006	NAT036	NAT083	NAT071	NAT116	NAT068
NAT014	NAT056	NAT039	NAT013	NAT091	NAT079	NAT058	NAT035	NAT120	NAT118	NAT041	NAT107	NAT024	NAT111	NAT077
NAT026	NAT085	NAT064	NAT072	NAT055	NAT059	NAT051	NAT002	NAT027	NAT070	NAT101	NAT011	NAT119	NAT015	NAT075
NAT093	NAT098	NAT078	NAT031	NAT030	NAT019	NAT090	NAT057	NAT020	NAT092	NAT008	NAT043	NAT084	NAT032	NAT082

Block II (Rep 3)

NAT026	NAT117	NAT022	NAT030	NAT007	NAT014	NAT004	NAT075	NAT092	NAT080	NAT073	NAT038	NAT072	NAT109	NAT120
NAT076	NAT024	NAT029	NAT079	NAT009	NAT031	NAT043	NAT027	NAT093	NAT036	NAT106	NAT102	NAT053	NAT089	NAT006
NAT010	NAT008	NAT037	NAT033	NAT086	NAT056	NAT081	NAT064	NAT041	NAT035	NAT111	NAT039	NAT12	NAT005	NAT115
NAT114	NAT063	NAT044	NAT083	NAT054	NAT016	NAT112	NAT050	NAT001	NAT088	NAT108	NAT084	NAT013	NAT119	NAT055
NAT034	NAT047	NAT067	NAT059	NAT058	NAT049	NAT032	NAT071	NAT015	NAT062	NAT099	NAT095	NAT025	NAT021	NAT097
NAT077	NAT110	NAT017	NAT118	NAT069	NAT018	NAT042	NAT052	NAT068	NAT104	NAT103	NAT046	NAT051	NAT028	NAT100
NAT074	NAT003	NAT113	NAT020	NAT065	NAT070	NAT082	NAT116	NAT040	NAT023	NAT048	NAT094	NAT090	NAT096	NAT066
NAT019	NAT011	NAT098	NAT101	NAT087	NAT105	NAT061	NAT002	NAT045	NAT060	NAT091	NAT107	NAT057	NAT085	NAT078

Plot size



CHAPTER FIVE

YIELD LOSS ASSOCIATED WITH ANTHRACNOSE DISEASE ON UGANDAN MARKET-CLASS DRY BEAN CULTIVARS

Abstract

On dry beans (*Phaseolus vulgaris* L.), the anthracnose (Sacc. et. Magn.) Lams. Scrib., causes a severe, rapidly developing disease that can bring about complete plant defoliation and extensive yield loss. Studies were conducted to quantify the yield loss attributed to dry bean anthracnose on three Ugandan market class dry bean cultivars. Using a split plot design, trials were conducted for three consecutive seasons in a bean growing region of Uganda, where anthracnose disease is very prevalent, using three susceptible market-class dry bean cultivars (K132, K20 and Kanyebwa) and comparison made with resistant cultivars G2333 and K131 in the third season. The results obtained showed that market class cultivars K132 and Kanyebwa recorded higher disease incidences and higher yield losses (39% and 44%, respectively). There was a strong positive correlation (r=0.78) between the AUDPC values and yield losses. Percentage marketable yield loss for susceptible cultivars was found to be almost double the percentage total yield loss. It was also established that as a result of the anthracnose, the yield potential of a susceptible cultivar is reduced by about 30-45%. The study suggests the use of desirable resistant cultivars as the best way of reducing yield loss caused by anthracnose.

5.1 Introduction

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. et. Magn.) Lams. Scrib., is a very destructive disease of common dry bean in cool and wet production regions of the world (Pastor-Corrales, 1995; Allen *et al.*, 1996). Anthracnose causes a severe, rapidly developing disease that can bring about complete plant defoliation and extensive yield loss (Plate 5.1 and 5.2) if plants become diseased prior to and/or during pod filling (Tu, 1981; Bailey et al., 1992; Pastor-Corrales, 1995; Young and Kelly, 1996; Bassanezi *et al.*, 2001).

The disease can easily be identified by its symptoms, which initially appear on the lower surface of the leaf, and forms large reddish or dark brown lesions along the veins. The disease is widely spread in all bean growing regions and often causes severe damage, which affects yield, seed quality, and marketability of beans. It is one of the most important bean diseases worldwide and yield losses are very extensive especially when infected seeds are used (Allen *et al.*, 1996; Bailey *et* al., 1992). In Uganda anthracnose is most important in the high altitude, low temperature areas (Opio *et al.*, 2006). In these areas the disease is known to cause severe losses because of the favourable climatical conditions which enhance disease development.

Being mainly a seed borne disease (Thomas and Sweetingham, 2003; del Rio and Bradley, 2004), anthracnose is easily spread in Uganda as farmers depend highly on farm saved seed and exchange of seed is common (Rubaihayo *et al.*, 1981; David *et al.*, 2000; Opio *et al.*, 2001), and as such disease severity on farmer fields is perceived to be quite high. With the sharing of farm saved seed between farmers, there is an increase in the chances of disease transmission between farmer fields, and between different agro-ecological zones where beans are grown.

Anthracnose has been observed to develop and spread quickly during the rainy seasons when night temperatures are cool and dew forms on bean plants (Pastor-Corrales, 1995). Significant disease development and yield loss occur if these weather conditions are prolonged throughout the pod formation and pod filling stages of the crop (Peloso, 1992). Yield loss is due to early leaf senescence and plant death, shrunken seed and an increase in the amount of seed that has disease lesions on the seed coat (Pastor-Corrales and Tu, 1989). Such beans have a repulsive appearance and are not popular to consumers (Plate 5.3). This lowers the marketability and thus the income arising from their sale. It has been observed that higher lesion density is associated with premature yellowing and defoliation of leaves (Bassanezi *et al.*, 2001) resulting

in lower yields. Also the extent of the yield loss depends very much on the climatical conditions and the crop growth stage at which the disease starts (Peloso, 1992).

In areas where the disease is endemic, yield losses have been known to be very high, especially on susceptible cultivars (Singh *et al.*, 1992). But whether the disease causes economic loss or not in the different Ugandan market class varieties is not known. However, before developing and/or implementing any research interventions against any pathogen or disease, it is necessary to quantify the extent and nature of damage attributed to that disease. It is for this reason that a study was conducted to quantify yield loss due to anthracnose for three Ugandan market-class dry beans.

5.2 Objectives

The objectives of the study were to;

- Determine the yield loss caused by anthracnose on dry beans
- Determine the variation in resistance of three market class varieties of dry beans

5.3 Materials and methods

Yield loss was conducted for the three susceptible Ugandan market class bean cultivars namely; K132, K20 and Kanyebwa. Also two other resistant cultivars, G2333 and K131, were also assessed.

5.3.1 Study area

The study was conducted at Kachwekano Research Station which is in Kabale district, located in south western Uganda (01° 15'S, 029° 59'E), at an altitude of 2200 m. Climatically, the Research Station has a moderate temperature (10.9°C-24.4°C) and high moisture conditions (Lindablade *et al.*, 1998), all of which favour the development of *C. lindemuthianum*. Rainfall in Kabale district is bimodally distributed with the long heavy rains from March - May and shorter rains from October - November.

In Kabale, agriculture and agricultural related activities are the main occupation of the people and it is estimated that over 90% of the population is engaged in agriculture (Niringiye *et al.*, 2005). Most of the crops produced are consumed at the household level and it is only the surplus that is put to market (NEMA, 2001). Of the crops produced, dry beans rank among the

most important food and cash crops within this region (Grisley *et al.*, 1993; Niringiye *et al.*, 2005).

5.3.2 Field experimental design and treatments

Two experiments were conducted in 2005 using three susceptible market class varieties (K132, K20 and Kanyebwa). The experiments were run for the two consecutive seasons: March-July 2005 and September-December 2005. Each main plot was subjected to either a Benlate fungicide spray at a rate of 2.5 g l^{-1} or was inoculated with anthracnose fungus. The experiment was arranged in a split-plot design with three replicates. The main plots were those with either fungicide application or anthracnose fungus inoculation, while the subplots were the varieties. Each subplot measured 5 x 5 m and consisted of eleven rows of beans planted at a spacing of 0.5 m between rows and 0.1 m within rows. There were 2 m alleys planted with maize between replicates to avoid inter-block interference.

To avoid inconsistencies arising from natural infestation, uniform disease infestation was obtained by the use of artificial inoculation using inoculum prepared and stored in the laboratory. Inoculation was done 25 days after planting by run-off spraying of leaves with an aqueous spore suspension (1.2 x 10^6 spores ml^{-1}, containing 0.1% Tween 20 as surfactant). The inoculum was made from isolates obtained from the same region and multiplied in the laboratory (see chapter 4). For the fungicide-protected plots, spraying commenced one week after the fungus inoculation and continued at two week intervals until physiological maturity.

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

Disease incidence, defined as the number of the dry bean plants with 10% or more necrosis of the surface area caused by anthracnose in a 1 m^2 quadrant, was recorded at four different plant growth stages (V3, V6, R1 and R6).

At maturity all plants within the inner 4 x 4 m were harvested for each sub-plot, sun-dried and then threshed and yield loss determined using the formula:

$$\% \text{ Yield loss} = \frac{(PPY - IPY)}{PPY} \times 100 \qquad (5.1)$$

Where;

PPY = Protected plot yield

IPY = Inoculated plot yield

Anthracnose tolerance index (AT I) was computed as follows:

$$AT\ I = \frac{IPY}{PPY} \qquad (5.2)$$

To substantiate the results obtained in the first two seasons, the above experiment was repeated for a third season (III) with the inclusion of two extra varieties. One was resistant (G2333) and the other tolerant (K 131) to anthracnose Pathotype 767. In Season III data were collected on the progressive development of the disease at V3, V6, R1 and R6 for all varieties. Data were evaluated using the method of Redman *et al.* (1969), where the diseased foliage of several plants was converted to a single value for each plot (Plate 5.1). This was later used to calculate the area under the disease progress curve (AUDPC) following the Campbell and Madden (1990) method.

Yields obtained in this season were used to determine the percentage marketable yield loss (PMYL). In this case, beans were sorted to remove those that had been damaged by anthracnose and could not be sold on the open market (Plate 5.3).

PMYL was calculated as follows;

$$PMYL = \frac{[(PPY - IPY) + WADS]}{PPY} \times 100 \qquad (5.3)$$

Where;

PMYL = Percentage marketable yield loss

PPY = Protected plot yield

IPY = Inoculated plot yield

WADS = Weight of anthracnose damaged seed per plot

All the data obtained were subjected to analysis of variance using GenStat Computer Package Release 8.1 (Lawes Agricultural Trust, 2005). Mean values were separated using the least significant difference (lsd) at the 5% level of probability and the two mean values were declared significantly different when the difference between them was greater than the lsd.

Plate 5.1: Dry bean foliage destroyed by anthracnose disease leading to a reduction in the photosynthesising area and thus reducing potential yield

Plate 5.2: Anthracnose disease attacks dry bean pods and eventually destroys the seed

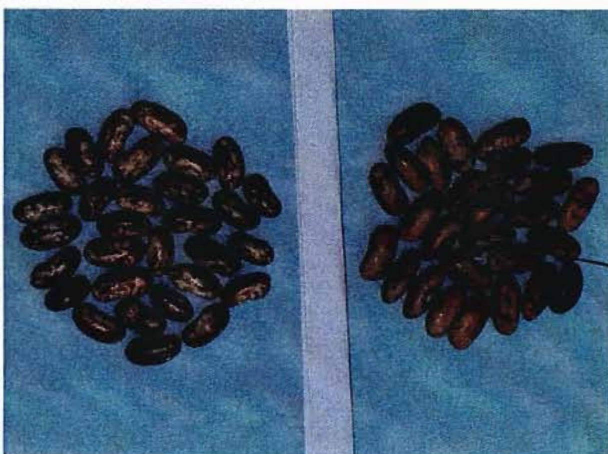


Plate 5.3: Sorted seeds showing marketable and unmarketable seed/grain

5.4 Results

5.4.1 Anthracnose incidences on bean cultivars at different growth stages and seasons

Results of anthracnose incidence are presented in Table 15. They indicate significant differences in anthracnose incidence between the bean cultivars (P<0.001), seasons (P<0.001) and stage of growth (P<0.001). Also the interaction of cultivar plus stage of growth and season plus stage of growth were significantly different at (P<0.001) and (P=0.002), respectively. There were no significant differences of anthracnose incidences for the same cultivars between different seasons and at the same stage of growth between seasons for the same cultivar. Dry bean Cultivar K20 consistently had lower anthracnose incidence levels throughout the two seasons while Cultivar K132 consistently had highest incidence levels of all the three cultivars (Table 15).

Table 5: Incidences of anthracnose disease on the three bean cultivars at various growth stages and seasons

Cultivar	Season	Stage of growth			
		V3	V6	R 1	R 6
K132	I	2.8	7.2	13.9	16.2
	II	4.7	13.0	16.6	17.1
K20	I	2.2	4.8	8.3	12.7
	II	4.2	5.1	10.1	14.7
Kanyebwa	I	2.3	8.3	13.3	15.2
	II	4.5	8.6	14.9	17.3
lsd (0.05)[1]	0.656				
lsd (0.05)[2]	0.535				
lsd (0.05)[3]	0.757				
lsd (0.05)[4]	ns				
lsd (0.05)[5]	1.311				
lsd (0.05)[6]	1.071				
lsd (0.05)[7]	ns				
CV (%)	11.4				

lsd (0.05) = Least significant difference of means at 5% level of probability; ns= not significant; [1]cultivar; [2]Season; [3]Stage of Growth; [4]Cultivar.Season; [5]Cultivar.Stage of Growth; [6]Season.Stage of Growth; [7]Cultivar.Season.Stage of Growth

5.4.2 Effect of anthracnose on bean yield

Results presented in Table 16 show the yields obtained from the protected and inoculated dry bean cultivars tested. The data obtained showed that there were significant differences between

treatments (P<0.001), between cultivars (P<0.001) for both seasons and significant differences in the combination of treatment and cultivar for Season I and II (P=0.002). The results further show that for all cultivars, protected plots yielded significantly higher than inoculated plots for both seasons.

Apart from the inoculated treatment where Cultivar K20 did not yield significantly differently from Kayebwa, in all other cases, all three cultivars yielded significantly differently from each other. Cultivar K132 yielded the highest followed by Kanyebwa, with K20 yielding lowest for all treatments and in both seasons. Additionally, for both seasons, Cultivar Kanyebwa yielded significantly higher that Cultivar K20 in the anthracnose-protected treatment (Table 16).

Table 6: Comparison of yield (kg ha^{-1}) of three bean cultivars grown under anthracnose inoculated and protected condition for seasons I and II

	Treatment			
Variety	**Inoculated**	**Protected**	**Inoculated**	**Protected**
	Season I		**Season II**	
K132	1069	1756	1283	2066
K20	742	1133	941	1220
Kanyebwa	710	1387	939	1564
lsd (0.05)[1]	93.4		92.4	
lsd (0.05)[2]	114.3		113.2	
lsd (0.05)[3]	161.7		160.1	
CV (%)	7.8		6.6	

[1]Treatment; [2]Cultivar; [3]Treatment.Cultivar

Irrespective of treatment, Cultivar K132 yielded significantly higher (P<0.05) than either K20 or Kanyebwa whereas Cultivar K20 and Kanyebwa yielded significantly the same in both seasons (Table 17). This suggests that Cultivar K132 has a higher yield potential than either Cultivar K20 or Kanyebwa.

Table 7: Mean yield obtained per cultivar irrespective of treatment

Cultivar	Average yield per cultivar (Kg ha^{-1})	
	Season I	Season II
K132	1412	1674
K20	937	1081
Kanyebwa	1048	1251
Lsd (0.05)	114.3	113.2
CV (%)	7.8	6.6

5.4.3 Yield loss

Yield loss (%) and anthracnose tolerance indices (ATI) are shown in Table 18. Using equation 5.1, the pooled analyses show significant differences in yield loss only for Cultivars K120 and Kanyebwa (P=0.032). When the ATI was considered, Cultivar K20 recorded a significantly higher index (P=0.011) than the other two cultivars. It was further observed that Kanyebwa recorded the highest yield loss (44.4%) followed by K132 (38.6%) and K20 had the lowest (29.2%) yield. Using equation 5.2, K20 had the highest ATI (0.72) while Kanyebwa had the lowest (0.56) (Table 18). This could be indicative of some level of anthracnose tolerance resident in Cultivar K20.

Table 8: Mean yield loss (%) and ATI of all cultivars across both seasons

Cultivar	Yield loss (%)	Anthracnose tolerance index (ATI)
K132	38.6	0.61
K20	29.2	0.72
Kanyebwa	44.4	0.56
lsd (0.05)	11.1	0.10
CV (%)	23.7	12.9

Yield comparison trials were made between anthracnose susceptible and resistant cultivars in Season III. The data obtained showed significant differences (P<0.001) in yields obtained between treatments, cultivars and the interaction between treatment and cultivars. In all cases the resistant cultivars performed seemingly the same but yielded significantly better than the susceptible cultivars (Table 19).

Table 9: Yield obtained per treatment in Season III

Cultivar	Inoculated (kg ha^{-1})		Fungicide Protected (kg ha^{-1})
K132	999		1624
K20	1072		1533
Kanyebwa	701		1354
G2333	2072		2086
K 131	2073		2210
lsd (0.05)[1]		52.7	
sd (0.05)[2]		83.3	
lsd (0.05)[3]		117.8	
% CV		4.4	

[1]Treatment; [2]Cultivar; [3]Treatment.Cultivar; CV= Coefficient of Variation

Disease progression rates in the field were compared between cultivars at different stages of growth. The data showed significant variations (P<0.001) of disease between cultivars at the different growth

stages. Cultivar G2333 showed the lowest disease development at all stages. The disease development on resistant Cultivar K131 was significantly similar to that of susceptible Cultivars K20 and K132 at only the V3 and V6 growth stages. By the R1 and R6 growth stages, disease development on all susceptible cultivars was significantly higher than that of the resistant cultivars (Figure 10).

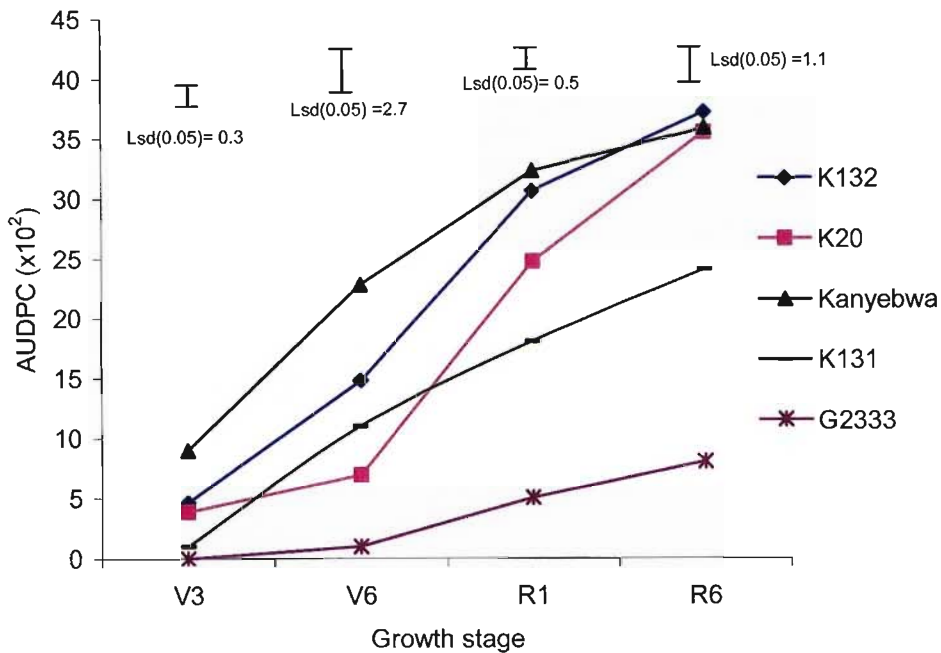


Figure 10: Anthracnose disease progression among cultivars at four growth stages

Results of yield losses associated with anthracnose in Season III are presented in Table 20. Susceptible Cultivars K132 and Kanyebwa showed significantly similar ($P<0.001$) yield losses. When all cultivars are considered, Kanyebwa had the greatest yield loss and G2333 had the lowest. This trend was the same for the AUDPC values. Additionally there was a strong positive correlation ($r = 0.78$) between the AUDPC values and yield loss. The results further show that, although Cultivars K132, K20 and Kanyebwa had statistically similar AUDPC values, the yield loss observed in Cultivar K20 was significantly lower (Table 20).

Table 10: Yield loss obtained in Season III per cultivar and the disease assessment

Cultivar	Yield loss (kg ha^{-1})	AUDPC
K132	625	3698
K20	461	3531
Kanyebwa	653	3565
G2333	14	754
K 131	137	2383
lsd (0.05)	73.7	833.4
CV (%)	11	15.9
R (P<0.001)	0.78	

AUDPC = Area under disease progression curve; r = Correlation

5.4.4 Percentage marketable yield loss

Percentage yield and marketable yield losses are indicated in Table 21. Using equation 5.3 it is shown that there are high significant (P<0.001) differences between G2333 and K131, and the rest of the cultivars. These results further show an almost double loss in the marketable yield for each increase in total yield loss.

Table 11: Comparison of percentage total yield and marketable yield losses

Cultivar	Percentage total yield loss (%)	Percentage marketable yield loss (PMYL) (%)
K132	38.5	70.7
K20	30.0	62.6
Kanyebwa	44.1	72.9
G2333	0.7	9.4
K 131	6.0	21.6
lsd (0.05)	5.0	19.7
CV (%)	11.0	21.1

5.5 Discussion and conclusion

Anthracnose has been reported on beans in Uganda since the late 1960s, but no quantification has been done on the yield loss it causes. This study was conducted to establish the difference in anthracnose damage levels between three Ugandan market class cultivars and the relationship of this damage to yield losses.

Being an aggressive foliar disease, anthracnose destroys plant photosynthetic tissue (Plate 5.1) causing premature defoliation and early maturation thus lowering yields (Gaunt, 1995; Hughes and Madden, 1997). Although susceptible bean cultivars can be infected at all growth stages,

yield loss will depend on the crop stage when infection occurs (Bassanezi *et al.*, 2001). The most important crop stages determining yield loss are between early flower stage (R1) through pod fill to the end of seed development stage (R6) (Bassanezi *et al.*, 2001).

From the results, the significant differences in anthracnose incidences and damage levels between cultivars could be indicative of differences in susceptibility levels. Susceptibility differences were also shown by the differences in the anthracnose tolerance index (ATI). Although all three Ugandan market-class cultivars are known to be highly susceptible to anthracnose, Cultivar K20 exhibited a significantly higher ATI indicating that it is less susceptible than Cultivars K132 and Kanyebwa. The higher ATI shown by Cultivar K20 could be explained by the fact that it was initially bred for resistance to anthracnose (Leakey and Simbwa-Bunya, 1971). In addition, it has been observed that different cultivars differ in susceptibility to the same pathotype depending of the type and number of resistance genes present in each cultivar (Fisher *et al.*, 1973; Durango *et al.*, 2002; Ombiri *et al.*, 2003). Cultivars K132 and Kanyebwa are not known to possess any anthracnose resistance genes. This could explain the observed differences in disease severity and yield losses for the three cultivars.

Significantly different AUDPC results were obtained, indicating that anthracnose progressed differently among cultivars. The study showed that *C. lindemuthianum* grows faster on the more susceptible cultivars. These findings are in agreement with earlier studies that showed that susceptible dry bean varieties succumbed to anthracnose earlier than the resistant varieties (Bassanezi *et al.*, 2001; Lopes and Berger, 2001; Meyer *et al.*, 2001). The data obtained further show a high positive correlation between the AUDPC and the cultivar yield losses. Accordingly, the differences in AUDPC values explain the observed differences in yield loss among cultivars.

In comparison with the resistant Cultivars G2333 and K131, severe yield losses were observed in all the three market-class cultivars for three consecutive seasons. The study took into consideration the yield lost as a result of the disease's interference with the photosynthetic areas of the plant (% yield loss) and the yield lost due to damaged seed (PMYL). While % yield loss ranged between 30-45%, the PMYL was almost double (63-73%). This loss in yields could be as a result of the loss in weight of the pods produced and an increase in the 'pick'[7].The yield losses observed in the study may even be on the low side, considering that clean seed was

[7] Seed that have been discoloured by the disease lesions on the seed coat and are thus unsightly and cannot be sold in an open market and have to be discarded.

used. It has been reported that total yield loss may occur when anthracnose contaminated seed is used and the climatic conditions are conducive for disease development (Pastor-Corrales and Tu, 1989; Hall, 1991; Schwartz, 1991; Peloso, 1992). This loss in marketable yield is normally a very big setback to the farmers in terms of household income, considering that over 90% of the dry bean producers in Uganda have very limited resources (Kiwanuka, 1996).

The significant low percentage yield losses and PMYL observed for the resistant cultivars justifies the importance of using resistance to manage anthracnose. Unfortunately the resistant cultivars (G2333 and K131) used in this study have poor marketability in Uganda (Kalyebara *et al.*, 2006) and as such, cannot easily replace the marketable susceptible cultivars.

It could therefore be suggested from the study that the use of suitable anthracnose resistant or tolerant cultivars would be the best option in the management of this disease. Additionally, it is recommended that a breeding programme be establishment to help in the introgression of anthracnose resistance genes into the susceptible market class cultivars. This would first and foremost help maintain the preferred market class dry bean cultivars. Secondly, it would reduce yield losses and eventually improve the net incomes of the farm families.

References

Allen, D.J., Ampofo, J.K.O. and Wortman C.S., 1996. Pests, diseases, and nutritional disorders of the common bean in Africa: A Field Guide. Cali *Colombia. International Centre for Tropical Agriculture*; Wageningen, The Netherlands: technical centre for Agricultural and Rural Cooperation, 132p.

Bailey, J.A. 1991. Recognition events associated with specific interactions between plants and patholgenic fungi. In Smith, C.J. (ed.). *Biochemistry and Molecular Biology of Plant-Pathogen Interactions.* Pp. 210-224. Oxford University Press, UK.

Bailey, J.A. O'Connell, R.J. and Nash, C. 1992. Infection strategy of *Colletotrichum lindemuthiamun* species. In: Bailey, J.A. and Jeger, M.J. (eds.). *Colletotrichum: Biology, Pathology and Control* CAB International, Wallingford, UK. Pp. 88-120.

Bassanezi R.B., Amorin L., Bergamin Filho A., Hau B. and Berger, R.D. 2001. Accounting for photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthracnose to assess crop damage. *Plant Pathology* 50:443-452.

Campbell, C.L. and Madden, L.V. 1990. Introduction to Plant Epidemiology. John Wiley & sons, NY, USA. 532p.

David, S., Kirkby, R., and Kasozi, S. 2000. Assessing the impact of bush beans varieties on poverty reduction in Sub-Saharan Africa: Evidence from Uganda. Paper presented at the workshop *"Assessing the Impact of Agriculture Research on Poverty Alleviation", 14-16 September, 1999*, San Jose, Costa Rica.

del Rio, L. and Bradley, C. 2004. Anthracnose of dry beans. North Dakota State University Extension Service, North Dakota State University, Fargo, North Dakota. 58105.

Durango D., Quiñones W., Torres F., Rosero Y., Gil J., and Echeverri F. 2002. Phytoalexin accumulation in colombian bean varieties and aminosugars as elicitors. *Molecules*, 7:817-832.

Fisher, M.L., Anderson A.J., and Albersheim P. 1973. Host-pathogen interactions VI. A single plant protein efficiently inhibits endopolygalacturonases secreted by *Colletotrichum lindemuthianum and Aspergillus niger*. *Plant Physiology,* 51:489-491.

Gaunt R.E. 1995. The relationship between plant disease severity and yield. *Annual Review of Phytopathology*, 33:119-144.

Grisley, W., Mwesigwa, D. and Kisakye, J. 1993. Adoption of climbing beans following the introduction of new varieties from on-farm trials in the Kabale District. In: *[S.P.I.]. Kawanda Agricultural Research Institute*. Pp. 174-189. Kampala, Uganda.

Hall, R. 1991. Compendium of Bean Diseases. American Phytopathological Society, Press, St. Paul, MN. 346p.

Hughes, G., and Madden, L.V. 1997. The determination of yield losses with aggregated patterns of diseased or missing plants. In: Francle, L.J. and Neher, D. (eds.). *Exercises in Plant Disease Epidemiology.* The American Phytopathological Society. Pp. 156-160. St. Paul, MN.

Kalyebara, R., Mugisha K.S., Andima D. and Nasirumbi, L. 2006. The impact of improved bush bean varieties and management technologies in Uganda. Draft No. 4. Centro Internacional de Agricultura Tropical. Kampala, Uganda. 94p.

Kiwanuka, M.C.G. 1996. Modernisation of Agriculture in decentralised administration. In: *Proceedings of the national workshop on modernisation of agriculture in Uganda. Challenges for Agricultural Extension.* Pp. 64-67.

Lawes Agricultural Trust. 2005.GenStat Release 8.1. PC/Windows XP. Rothamsted Experimental Station.

Leakey, C.L.A. 1970. The improvement of beans (*Phaseolus vulgaris*) in East Africa. In: Leakey, C.L.A (ed.). *Crop Improvement in East Africa.* Pp. 98-128. The Commonwealth Agricultural Bureaux, London.

Leakey, C.L.A. and Simbwa-Bunnya, M. 1971. Races of *Colletotrichum lindemuthianum* and implications for bean breeding in Uganda. *Annual Application Biology*, 70:25-34.

Lindblade, K.A, Carswell, G. and Tumuhairwe J.K. 1998. Mitigating the relationship between population growth and land degradation: Land-use change and farm management in Southwestern Uganda. *Ambio,* 27:565-571.

Lopes, D. B., and Berger, R.D. 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. *Phytopathology*, 91:212-220.

Meyer, S., Saccardy-Adji, K., Rizza, F. and Genty, B. 2001. Inhibition of photosynthesis by *Colletotrichum lindemuthianum* in bean leaves determined by chlorophyll fluorescence imaging. *Plant, Cell and Environment*, 24:947-955.

NEMA. 2001. Republic of Uganda, 2001. State of the Environment Report: Kabale 2000/2001. National Environment Management Authority.

Niringiye, C.S., Kyamanywa, S. and Ssekabembe, C.K. 2005. Effect of plant population on yield of maize and climbing beans grown in an intercropping system. *African Crop Science Journal,* 13:83-93.

Ombiri J., Zinkernagel, V., Gathuru, E.M., Achwanya, O., and Lebeda, A. 2003. Induction of post-infection ethylene and its role in resistance of bean (*Phaseolus vulgaris*) against *Colletotrichum lindemuthianum. Plant Protection Science*, 39:79–87.

Opio, F., Mugagga., Mawejje, D., and Nkalubo, S. (2006). Progress report on bean anthracnose research in Uganda. *MUARIK bulletin* (*in press*).

Opio, F., Ugen, M.A., Kyamanywa, S., David, S., and Mugisha-Mutetikka, M. 2001. Beans. In: Mukiibi, J.K, Esete, J.P., Musoli, P.C., Oryokot, J.O.E., Twinamasiko, E., Odongkan, O.K., Esegu, J.F.O., Otim-Nape, G.W., Ssali, H., and Odogola, W. (eds.). *Agriculture in Uganda. Volume II Crops.* NARO. Kampala, Uganda.

Pastor-Corrales, M.A. 1995. Enfermedades del frijol causadas por hongos. In: López, M., Fernández, F. and Schoonhoven, A. (eds.). FRIJOL: Investigación y Producción Pp. 172-180. PNUD- Centro Internacional de Agricultura Tropical, (CIAT). Cali, Colombia.

Pastor-Corrales, M. and Tu, J.C. 1989. Anthracnose. In: Schwartz, H.F. and Pastor-Corrales, M.A. (eds.). Bean Production Problems in the Tropics. Pp.77-93. Centro Intemacional de Agricultura Tropical, (CIAT). Cali. Colombia.

Peloso, M.J. 1992. Antracnose do feijoeiro no Estado de Minas Gerais-Brasil. In: Pastor-Corrales, M.A (ed.). La Antracnosis del Frijol Común, *Phaseolus vulgaris,* en América Latina. pp. 86-108. Doc. de trabajo No. 113. Centro Internacional de Agricultura Tropical, (CIAT). Cali, Colombia.

Redman, C.E, King, E.P. and Brown, I.F.Jr., 1969. Tables for converting Barratt and Horsfall rating scores to estimated mean percentages. Eli Lilly and Company Research Laboratories. Indianapolis, IN. Mimeo.

Rubaihayo, P.R., Milidwa, D, Songoba, T. and Kamugira, F. 1981. Bean production in Uganda. *In:* Regional workshop on Potential for Field Beans in Eastern Africa, Lilongwe, Malawi, 1980 proceedings. Centro Internacional de Agricultural Tropical, (CIAT). Cali, Colombia. 226p.

Schwartz, H.F. 1991. Anthracnose. In: Hall R. (ed.). Compendium of Bean Diseases. American Phytopathological Society Press. Pp. 16-7. St. Paul, MN.

Singh, S., Pastor-Corrales, M.A., Molina, A. and Otoya, M. 1992. Breeding common bean for resistance to anthracnose. In: Pastor-Corrales, M.A. (ed.). *La Antracnosis del Frijol Común, Phaseolus vulgaris, en América Latina*. Pp. 198-211. Doc. de trabajo No. 113. *Centro Internacional de Agricultural Tropical.* Cali, Colombia (CIAT). Cali, Colombia.

Thomas, G.J. and Sweetingham, M.W. 2003. Fungicide seed treatments reduce seed transmission and severity of lupin anthracnose caused by *Colletotrichum gloeosporioides. Australasian Plant Pathology,* 32:39-46

Tu, J.C. 1981. Epidemiology of anthracnose (*Colletotrichum lindemuthianum*) on white beans (*Phaseolus vulgaris* L.) in the Southern Ontario: Spread of the disease from an infection focus. *Plant Disease*, 65:477-480.

Young, R.A. and Kelly, J.D. 1996. Characterisation of the genetic resistance to *Colletotrichum lindemuthianum* in common bean differentials. *Plant Disease*, 80:650-654.

CHAPTER SIX

INHERITANCE OF RESISTANCE TO ANTHRACNOSE RACE 767 IN UGANDA'S MARKET CLASS DRY BEAN CULTIVARS

Abstract

Anthracnose is a serious disease affecting dry bean production in the cool highland areas of Uganda. The objective of this research was to study the inheritance of resistance to anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et. Magn.) Lams. Scrib., Pathotype 767. A complete 9x9 diallel set of crosses was produced from nine diverse parents comprising six resistant and three susceptible to anthracnose. Data were analysed using chi-square analysis, and Griffing's and Jinks-Hayman's methods of diallel analysis. Results from Griffing's analysis clearly indicated that additive genetic variance predominantly controlled the inheritance of resistance to anthracnose Race 767. Hayman's diallel analysis indicated significant variation of Wr+Vr and Wr-Vr over arrays suggesting that epistatic gene action was also important. Similarly results from a joint regression coefficient over replications were significantly different ($P<0.05$) from unity and zero which further indicated the presence of non-allelic interaction. In addition, the chi-square test displayed significant deviations from the expected 3:1 resistant to susceptible phenotypic ratio of F_2 and 1:1 ratio in BC_1F_1 populations confirming the importance of epitasis in conferring resistance to anthracnose. However, reciprocal effects were not significant indicating that maternal effects did not influence resistance to anthracnose. Results suggested that one to three genes displaying partial dominance were responsible for determining resistance. Parental lines with high negative GCA effects were G2333, AB136, NAT002 and NAT003 qualifying them as suitable parents for transferring anthracnose resistance to their progenies. Of the susceptible varieties, the parent Kanyebwa was the best general combiner for resistance, followed by K132. The cross G2333 x Kanyebwa, was found to be the best specific combination. All the crosses showing superior specific combining ability effects resulted from poor x good general combiners for disease resistance. Heritability estimates in narrow and broad senses were high. Predominance of additive genetic variance, coupled with high heritability, suggested that simple pedigree breeding procedures, such as backcrossing would be useful for improving the level of resistance in the Ugandan market class varieties. Additionally the low gene numbers suggest possible application of assisted marker selection for future breeding work.

6.1 Introduction

Anthracnose disease, caused by the fungus *Colletotrichum lindemuthianum* (Sacc.& Magn.) Lams. Scrib., is a serious disease affecting dry bean (*Phaseolus vulgaris* L.) production in the cool highland areas of Uganda (Opio *et al.*, 2006). The incidence of the diseased plants in affected fields can be very high, especially when infected seed is used under favourable conditions (Wortman and Allen, 1994; Allen *et al.*, 1996). In susceptible cultivars anthracnose symptoms may initially appear on the cotlyledons and then spread to the leaves through to the whole plant. With time, the fungus invades the pods and the lesions develop into sucken cankers which affect the seed. Infected seeds normally appear discoloured with dark brown to black cankers (Pastor-Corrales and Tu, 1989) thus reducing their marketability.

Anthracnose was first described in Uganda in the late 1960's, and at that time only four races were identified (Leakey, 1970). Other races have recently been reported according to the recent binary system nomenclature (see Chapter 3). Although the former are still prevalent, Race 767 has been found to be the mostly widely spread and overcomes more resistance genes than others in one of the major dry bean growing areas of Uganda (see Chapter 3). This pathotype is very destructive and more so to the market dry bean cultivars grown in this regions.

The area under dry bean production in Uganda is expanding but the yield still remains constant (Ugen *et al.*, 2002) in most of the high altitude production areas due to constraints such as anthracnose. As reported in the previous study (see Chapter 5) anthracnose causes large yield losses to farmers, especially when susceptible cultivars are used. It is envisaged that the use of resistant cultivars or the introgression of resistant genes into the desirable susceptible cultivars is the most viable option for controlling anthracnose, particularly for small-scale growers (Singh *et al.*, 1992). Resistant varieties, like G2333 and AB136 and the moderately resistant local variety K131, have poor marketability (David *et al.*, 1997) and as such, cannot be successfully used as alternatives. It is therefore important that a breeding program be established to create new anthracnose resistant cultivars which meet the market requirements. However, in order to initiate an effective anthracnose resistance breeding program, it is necessary to understand the genetics of resistance to anthracnose. The most efficient breeding procedure to enable selection of superior genotypes in self-pollinating crops like dry beans depends on the magnitude of additive genetic variance for the trait (Kearsey and Pooni, 1996).

Heritability studies are used to evaluate the genetic control of traits determined by many loci and can be used to effectively plan strategies for incorporating characters into new cultivars. Broad sense heritability (h^2_b) estimates include additive, dominance and epistatic sources of genetic variation, while narrow sense heritability (h^2_n) is a reflection of additive effects (Wyman, 1991). Thus, h^2_n results in a reliable genetic parameter on which to base a breeding procedure. It is therefore necessary to establish h^2_n of anthracnose resistance and the number of genes conditioning the expression of resistance to be able to come up with more efficient methods for selecting anthracnose resistant genotypes.

Findings from previous researchers show that anthracnose resistance is conditioned mainly by dominant genes (Peloso *et al.*, 1989; Alzate-Martin *et al.*, 1999) and that some cultivars can have more than one of such independent genes. Inheritance studies with the cultivars G2333 and AB 136 have shown that more than one independent dominant resistance gene is resident in each of these cultivars (Pastor-Corrales *et al.*, 1994; Young and Kelly, 1996; Poletine *et al.*, 2000). It has also been reported that resistance from these two cultivars is easily transferable to susceptible cultivars (Poletine *et al.*, 2000; Anju *et al.*, 2006). Additional findings show that although various sources of resistance have been discovered (Menezes and Dianese, 1988), breeding for resistance is complicated by the continuous variability in the physiological races of the anthracnose pathogen. Further studies show that the genetic base for resistance to anthracnose in common bean varies from simple to complex, depending on the varieties involved and the physiological races being considered (Peloso *et al.*, 1989).

According to Anju *et al.* (2006) there is wide genetic variability in most of the available germplasm and it is possible to develop cultivars resistant to anthracnose with the recombination of this variability. Diallel crossing (Kearsey and Pooni, 1996) is a technique that helps in the choice of parents based on their genetic traits. It takes into account their capacity to combine in hybrids and to produce promising segregant populations. However, this technique does not provide information about the segregant loci, which are important to determine the chances of obtaining superior performing lines (Christie and Shattuck, 1992; Kearsey and Pooni, 1996). Thus, besides diallel analysis, it is important to know the genetic variance of the segregating populations in order to be able to predict their true individual potential. It is further reported that the interaction between genotypes and environments complicates the selection and the estimation of the combining ability of the parents (Fernandez and Miller, 1985; Dabholkar, 1992). The assessment of the populations in several

environments and generations would allow for a more precise estimation of these interaction effects, resulting in more reliable estimates of the combining ability and permitting the correct choice of parents.

The size of the specific combining ability (SCA) estimates gives an idea of the degree of complementation of the parents in a cross (Dabholkar, 1992). Its significance indicates that the populations have heterogeneous performance and that their behaviour cannot be predicted based only on the general combining ability (GCA) as there are interactions among parents according to the degree of divergence of the dominant loci (Christie and Shattuck, 1992). Melo *et al.* (1997), however, used simulations to show that the SCA depends not only on the heterozygous loci, but also on the number of fixed loci with favourable alleles. It depends, therefore, on the proportion of homozygous loci with favourable and unfavourable alleles as it is a deviation from the mean. Thus, the greatest SCA value does not always mean a greater number of superior lines after complete homozygosis and neither a greater variance among the lines (Dabholkar, 1992; Melo *et al.*, 1997). Thus it will not always contribute to identifying lines with better performance.

A recent study (see Chapter 4) indicated possible existence of anthracnose resistant lines within the Ugandan germplasm collection. The study identified landrace lines NAT002, NAT003, NAT008 and NAT067 as resistant (see Chapter 4) but none of these cultivars belong to the known Ugandan market class cultivars. In addition, there is no recent information reported on the incorporation of resistance to these agronomically superior Ugandan cultivars. The purpose of this study therefore, was to try and understand the nature and mechanism of inheritance of anthracnose resistance in these market class cultivars from crosses made with the previously identified resistant lines. Additionally, the study will be used to facilitate planning of an efficient breeding programme for the improvement in the level of resistance to dry bean anthracnose in Uganda.

6.2 Objectives of the study

The objectives of the study were to:

- Determine the inheritance mechanism of anthracnose resistance in the Ugandan dry bean cultivars,
- Determine the type of gene action involved in the resistance,
- Determine the number of genes involved in anthracnose resistance,
- To test whether maternal effects play a role in determining anthracnose resistance,
- Estimate the combining ability effects of the different parent inbred lines, and
- Estimate narrow sense heritability for anthracnose resistance in dry bean.

6.3 Materials and Methods

6.3.1 Germplasm

The inheritance of resistance to anthracnose Race 767 was studied in three susceptible Ugandan cultivars (K132, K20 and Kanyebwa) and six resistant accessions (NAT002, NAT003, NAT008, NAT067, AB136 and G2333) of dry beans which were selected from germplasm previously screened for anthracnose resistance (Table 22). Parental lines G2333 and K132 were also used as resistant and susceptible controls, respectively.

Table 12: Characteristics and type of reactions to *Colletotrichum lindemuthianum* Race 767 of the different parental test lines

Parental line	Growth habits	Seed size	Seed colour	Reaction to Race 767
K132	I	Large	Red mottled	Susceptible
K20	I	Large	Red mottled	Susceptible
Kanyebwa	I	Large	Brick red	Susceptible
NAT002	II	Small	Cream	Resistant
NAT003	III	Small	Brown + black speckles	Resistant
NAT008	II	Small	Cream +white stripes	Resistant
NAT067	III	Small	Black	Resistant
AB 136	IV	Small	Red	Resistant
G2333	IV	Small	Maroon	Resistant

I = determinate; II = indeterminate prostrate; III = indeterminate prostrate weak climber; IV = indeterminate strong climber (Singh, 1992).

The nine dry bean accessions were used as parents in a 9x9 diallel mating design, according to Griffing's (1956) method. The parental accessions were crossed in all combinations, with reciprocals, and ignoring selfs to generate 72 families. This was done in order to determine the effect of the maternal parent on resistance and estimate the number of genes controlling anthracnose resistance. Emasculation was followed immediately by hand pollination. Before

handling a different cross, the forceps used to tease flower buds were sterilised in 70% alcohol to avoid contamination of the new cross with pollen from the previous parental line. Forty five F_1 seeds from each cross were grown in plastic pots at Namulonge Agriculture and Animal Research Institute to generate F2 progenies. Three seeds were planted in each pot. The BC_1F_1 seed was obtained only from progenies of crosses made with the six resistant and susceptible market class cultivars (K132, K20 and Kanyebwa) and not between susceptibles.

6.3.2 Pathogen

The *C. lindemuthianum* Race 767 was used in tests of evaluation of resistance. Isolates of this race were collected in the western and central bean growing areas of Uganda (see Chapter 3). Race 767 was characterised as the most pathogenic and abundant race among those identified in the major bean growing areas of Uganda. Prior to the resistance tests, the inoculum for this particular race was increased on Mathur's agar (Champion *et al.*, 1973).

6.3.3 Resistance testing and evaluation of F_1, BC_1F_1 and F_2 populations

Evaluations of the reactions of F_1, F_2 and BC_1F_1 dry bean generations were performed in a growth chamber, controlled at a temperature of 18 - 22°C and the humidity maintained at above 90% using water mist sprays. To conduct the test on anthracnose resistance, 160 F_2 seeds from each of the crosses, were surface-sterilized by dipping them in 10% sodium hypochlorite for 5-10 minutes, then thoroughly rinsed in distilled water, and incubated in the dark under humid conditions in a germinator set at 28°C for 3 days. Inoculations were performed by dipping whole pre-germinated seeds in a conidial suspension (1.2×10^6 ml^{-1}) for 10-15 minutes (Champion *et al.*, 1973).

Inoculated seeds were later sown in (84 x 58 x 12 cm) plastic trays, half filled with partially composted sawdust. Each tray was planted with 10 rows of 20 seeds each. Eight rows were sown with F_2 seed, the ninth row with seed of a resistant parent (G2333) and the last row with seed of a susceptible parent (K132). Each tray contained a maximum of 200 seedlings. Plants were watered daily for 10 days. For F_1 and BC_1F_1 seed, only two and three rows, respectively were sown for each population because of the limited amount of seed available. Trays were laid out in a growth room in a randomized complete block design and the experiment was repeated twice.

Ten days after leaf inoculation, anthracnose symptoms were evaluated using a 1 to 5 point scale (Drijfhout and Davis, 1989). In this scale 1 = no disease; 2 = pin point lesion on lower

part of stem; 3 = larger lesions covering stem; 4 = very large, deep lesion up to stem centre and 5 = seedling killed by pathogen. Plants showing Reactions 1 and 2 were graded as resistant while those showing Reactions 3, 4 and 5 were classified as susceptible.

6.3.4 Genetic analysis

Analysis of variance was performed with the help of the GenStat Computer Package Release 8.1 (Lawes Agricultural Trust, 2005). Parents and crosses were considered fixed effects, while replications were considered as random factors. Griffing's (1956) Method I of Model I was used to determine the value of general combining ability (GCA) and specific combining ability (SCA). The following model was used:

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + r_{ij} + e_{ijlk} \qquad (6.1)$$

Where; Y_{ij} is the mean phenotypic value; μ is general mean; gi and gj are the GCA effects of the ith and j^{th} parents, respectively; s_{ij} is SCA effects of the ij^{th} cross; r_{ij} is the reciprocal effect associated with the ij^{th} cross and e_{ijlk} is the residual effect.

The mid-parent heterosis (½[H-D]) and degrees of dominance (H/D) for each cross were calculated according to Mather and Jinks (1971). Also Hayman's (1954) method was utilised to generate variances (V_r=¼$[D+H]^2$) and parent-offspring covariances (W_r=½[D(D+H)]) of arrays for the estimation of genetic parameters and to detect the presence of epistasis among the crosses or to test the adequacy of the additive-dominance model in explaining resistance of beans to anthracnose. Using the generated variance components and assuming random chromosome segregation, no epistasis, no maternal effects, presence of homozygous parents and independent assortment, narrow sense heritability was estimated using the model:

$$h^2_n = \sigma^2_A / (\sigma^2_A + \sigma^2_D + \sigma^2_E) \qquad (6.2)$$

Where h^2_n is the narrow sense heritability; σ^2_A is the variance due to additive gene effects; σ^2_D is the variance due to dominance gene effects and σ^2_E is the environmental error variance. The values for the measure of the additive effects (D) and dominance effects (H_1 and H_2) were computed from the covariances in accordance with Hayman's (1954) method as follows;

$$D = V_p - E \quad (6.3)$$

$$H_1 = 4V_m + V_p - 4_f - (3n-2)E/n \quad (6.4)$$

$$H_2 = 4\,V_m - 4V_f - 2E \quad (6.5)$$

Where V_p is variance among the parents; V_m is mean value of V_r over all arrays; V_f is the variance among family means within an array; E is the environmental variance and n being the number of parents

Estimation of narrow sense heritability using parent offspring regression was also done by regressing F_2 populations on the value of their mid parents. This was obtained by using Vogel *et al.*, (1980) method as shown in the following model:

$$Y_i = \mu + bx_i + E_i \quad (6.6)$$

Where Y_i is the performance of offsprings of ith parent; μ is the mean performance of all parents evaluated, b is the linear regression coefficient, X_i is the performance of the ith parent and E_i the experimental error associated with the measurement of X_i. For effective application of this method, it was assumed that the character of interest is diploid, the population is randomly mated, there is no linkage among loci controlling the trait, parents are non-inbred and that there is no environmental correlation between the performance of parents and offsprings.

Additionally, a chi square test to determine the departure of the observed frequencies from the expected frequencies was based on the critical value of 3.84 for one degree of freedom on the 0.05 probability level

6.3.5 Estimation of number of genes

To verify the possibility of using marker assisted selection for future breeding work, the estimation of the number of genes was carried out following the formulae used by Bjarko and Line (1988):

$$F_2 \text{ generation: } n = \frac{(GR)^2[1.5-2\,h(1-h)]}{8\,(V_{F2} - (V_{PS} + V_{PR} + 2V_{F1})/4)} \quad (6.7)$$

Where n is the estimated number of segregating genes; GR is the genotype range, estimated as phenotypic range of segregating generation or difference between the two parents (P_s - P_R); h being given by $(F_{1m}-P_R)/(P_S-P_R)$; V_{F1} is variance of F1 progenies; V_{F2} is variance of F_2 progenies; P_R is the mean of resistant parent; P_S is the mean of susceptible parent; F_{1m} is the mean of F_1 progenies, VP_R is the variance of resistant parents; and VP_S is the variance of susceptible parents.

6.3.6 Determination of maternal influence

Maternal influence on the inheritance of dry bean anthracnose resistance was determined using the analysis of variance of the reciprocal crosses. As a way of confirmation, a correlation analysis was done for only crosses of K132 x G2333, K20 x G2333, Kanyebwa x G2333, K132 x AB 136, K20 x AB 136 and Kanyebwa x AB 136 and their reciprocals. Using F_2 generations, the general combining abilities of the different crosses and their respective reciprocals were correlated to determine the relationship between them.

6.4 Results

6.4.1 Resistance of parental genotypes

Three parents displayed susceptibility while six lines expressed resistance to *C. lindemuthianum* Race 767 (Table 23). The disease scores ranged from 1 to 5 with parents K132 and Kanyebwa giving the highest susceptibility score and AB136 and G2333, giving the highest degree of resistance.

Table 13: Anthracnose susceptibility/resistance of parental lines

Genotype	No. of plants tested	Anthracnose reaction	Average susceptibility score[1]
K132	20	S	4.8
K20	20	S	3.9
Kanyebwa	20	S	4.4
NAT002	20	R	1.6
NAT003	20	R	1.7
NAT008	20	R	2.3
NAT067	20	R	2.2
AB 136	20	R	1.4
G2333	20	R	1.1

[1]The scores are based on a 1 - 5 scale, where 1 indicates resistance, and 5 indicates susceptible; R= Resistant; S= Susceptible

6.4.2 Anthracnose resistance of F_1 and segregating populations

Results of performance of F_1 progenies showed closer performance to the resistant parents than the susceptible parents (Table 24), indicating the presence of dominant gene action.

Table 14: Mean of anthracnose reaction scores of F_1 progenies arising from nine parental lines (above diagonal) and reciprocals (below diagonal) in a 9 x 9 diallel cross

Parents	K132	K20	Kanyebwa	NAT002	NAT003	NAT008	NAT067	AB136	G2333
K132		4	4	2	2	2	1	1	1
K20	4		4	2	1	2	2	1	1
Kanyebwa	4	4		1	1	2	2	1	1
NAT002	2	3	2		1	1	2	1	1
NAT003	1	2	1	1		1	1	1	1
NAT008	2	2	2	1	2		2	1	1
NAT067	2	2	2	1	2	2		1	1
AB136	1	1	1	1	1	1	1		1
G2333	1	1	1	1	1	1	1	1	

The scores are based on 1 - 5 scale

Further segregation, as depicted in the F_2 progenies, showed increases in susceptible plants. The progenies with the highest degree of resistance were created from crosses between or with G2333, AB136, NAT002, and NAT003, in that order (Table 25). These are indicated by the lower mean values of the infection in the majority of crosses, including their reciprocals.

Table 15: Mean of anthracnose reaction of F2 progenies arising from nine parental lines (above diagonal) and reciprocals (below diagonal) in a 9 x 9 diallel cross

Parents	**K132**	**K20**	**Kany**	**NAT002**	**NAT003**	**NAT008**	**NAT067**	**AB136**	**G2333**
K132		4	4	3	3	4	3	2	1
K20	4		3	2	3	3	4	1	1
Kany	5	4		3	3	3	3	1	2
NAT002	2	2	2		3	2	2	1	1
NAT003	2	2	3	2		2	2	1	1
NAT008	3	3	3	2	2		3	2	2
NAT067	4	3	3	2	1	3		2	1
AB136	1	2	1	1	2	1	2		1
G2333	1	1	1	1	1	1	1	1	

The scores are based on 1 - 5 scale; Kany= Kanyebwa

6.4.3 Genetic analysis observations

Variation among the crosses was highly significant for anthracnose scores (Table 26). Further analysis of variance showed that the array of parental order of dominance Wr+Vr and the difference over the arrays for the Wr-Vr were also significant (Table 26).

Table 16: Analysis of variance for crosses, (Wr+Vr) and (Wr-Vr) estimates

	Source	D.F.	S.S.	M.S.
Crosses	Rep	2	1.189	0.594ns
	Genotypes	35	616.416	17.612**
	Error	96	21.504	0.224
	Total	146	641.109	
Wr + Vr	Rep	2	0.584	0.292ns
	Wr + Vr	8	6.592	0.824*
	Error	16	1.560	0.098
	Total	26	8.736	
Wr – Vr	Rep	2	1.024	0.512ns
	Wr-Vr	8	319.912	39.989**
	Error	16	15.024	0.939
	Total	26	350.984	

*, ** Significant at $P \leq 0.05$ and 0.01 respectively; ns = Not significant

The results show that varieties of Kanyebwa and K132 had the lowest variances (Vr) and covariances (Wr) (Table 27). Also varieties G2333, AB 136, NAT002 and NAT003 had relatively low parental and array means for anthracnose resistance as well as negative (Wr–Vr) values but high (Wr+Vr) values.

Table 17: Mean of array variances, covariances and parental lines for anthracnose resistance

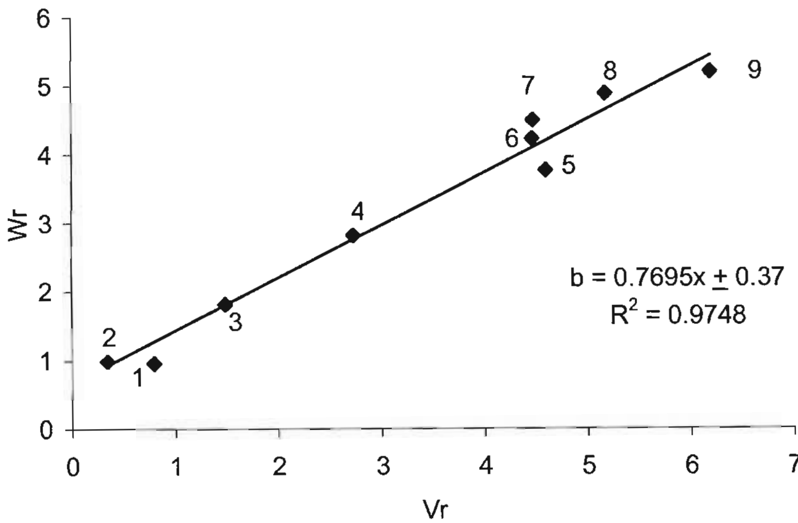
Array	Vr	Wr	Wr+Vr	Wr-Vr	Parent mean	Array mean
G2333	0.39	0.86	1.26	0.47	13.08	12.46
NAAT002	1.49	1.51	3.00	0.15	10.15	11.06
AB136	0.35	0.99	1.35	0.63	13.08	12.30
K20	4.48	4.48	8.95	0.01	8.02	9.37
NAT008	4.60	4.25	8.86	-0.35	6.53	9.00
NAT003	2.73	2.81	5.54	0.08	8.63	9.99
NAT067	4.47	4.21	8.68	-0.26	7.51	9.87
Kanyebwa	5.18	4.86	10.04	-0.34	6.71	8.87
K132	6.44	5.17	11.61	-1.27	6.18	8.59

A joint regression of Wr on Vr over three replications was highly significant (Table 28). The results give a regression coefficient b=0.77 (Figure 11) which was significantly ($P<0.01$) different from unity. The regression line intercept Wr is above the origin, indicating partial dominance.

Table 18: Analysis of variance for joint regression of Wr on Vr

Source	D.F.	S.S.	M.S.
Regression	1	69.757	69.757**
Heterogeneity	26	1.501	0.060
Error	27	71.258	

**Significant at P<0.01



1= AB 136; 2= G2333; 3= NAT002; 4=NAT003; 5=NAT067; 6= NAT008; 7= K20; 8= Kanyebwa; and 9= K132

Figure 11: Linear regression of Wr on Vr

General combining and specific combining ability were highly significant (P<0.01 and 0.001, respectively) for anthracnose resistance scores (Table 29). Data also gives an estimation of narrow sense heritability equivalent to 0.69, with a high $\sigma^2_A:\sigma^2_D$ ratio. The non-significant reciprocal effects implied that the maternal effects were not important in determining resistance to anthracnose. Further analysis showed that the correlation between the GCA values for the crosses and their respective reciprocal GCA values was positive and highly significant (P<0.001) and very close to unity (Table 29). This further confirms the absence of significant differences between reciprocal GCA values.

Table 19: Analysis of variance of GCA and SCA in the estimation of narrow sense heritability

Source	D.F.	S.S.	M.S.
General combining ability	8	160.512	20.064***
Specific combining ability	27	56.638	2.098**
Reciprocal effects	36	24.624	0.684ns
Error	71	4.544	0.064
Additive variance (σ^2_A) = 2.93			
Dominance variance (σ^2_D) = 1.28			
Ratio of σ^2_A/σ^2_D = 2.3			
Narrow sense heritability (h^2_n) = 0.69			

, * =Significant difference at P< 0.01 and 0.001 respectively.

Further correlation analysis shows that the GCA of the crosses and their respective reciprocals are close to unity with a correlation coefficient (r) = 0.993 (P<0.001). This further confirms the absence of significant differences between reciprocal GCA values (Table 30).

Table 20: Inbred parent GCA effects for anthracnose resistance scores and the correlation between the GCA and reciprocal GCA values

Parents	GCA effects	GCA effects*
K132	0.46	0.51
K20	0.49	0.42
Kanyebwa	0.34	0.43
NAT002	-0.40	-0.41
NAT003	-0.36	-0.38
NAT008	0.47	0.45
NAT067	0.36	0.50
AB136	-0.64	-0.69
G2333	-0.74	-0.82
r = 0.993; P<0.001; s.e. value =± 0.085		

r = Correlation coefficient; * =Reciprocal GCA values; s.e. =Standard error value

Results indicate significant estimates of GCA and SCA effects (P<0.05). There are four parents with negative significant GCA effects and parents G2333, AB136, NAT002 and NAT003 are envisaged as good combiners. Additionally, negative significant estimates of SCA are indicative of desirable crosses (Table 31).

Table 21: SCA and GCA (diagonal, bold and underlined) effects of parental lines of crosses for resistance to anthracnose

Parents	G2333	AB136	NAT067	NAT008	NAT003	NAT002	Kanyebwa	K20	K132
K132	-0.27	0.16	-0.17	0.53*	-0.20	-0.39*	0.43*	0.23	**0.46***
K20	-0.35	-0.48*	0.72*	0.33	-0.35	-0.49*	0.38*	**0.49***	
Kanyebwa	-0.32	-0.23	0.24	0.16	-0.35	-0.30	**0.34***		
NAT002	0.59*	0.28	-0.12	-0.27	0.68*	**-0.40***			
NAT003	0.49*	0.22	-0.20	-0.29	**-0.36***				
NAT008	-0.37	0.02	-0.11	**0.47***					
NAT067	-0.26	-0.13	**0.36**						
AB136	0.47*	**-0.64***							
G2333	**-0.74***								

*Values are significant at P < 0.05

6.4.4 Parent - offspring regression

The mid-parent on offspring regression analysis was highly significant with a regression coefficient of 0.71 (Table 32). According to Vogel *et al.* (1980) this regression coefficient is an estimate of the measure of heritability in the narrow sense (h^2n). Clearly, a regression value of 0.71 suggests that anthracnose resistance is a highly heritable character.

Table 22: Analysis of variance for mid-parent on F_2 progenies for anthracnose resistance

Source	D.F.	S.S.	M.S.
Regression	1	101.207	101.207**
Residual	7	12.913	1.845
Total	8	121.120	
CV (%) = 5.8			
$R^2 = 0.86$			
$b = 0.71 = h^2_n$			

**Significant at P<0.01

6.4.5 Chi-square goodness of fit for the 3:1 phenotypic ratio of resistance to susceptibility in the F_2 populations

Phenotypic ratios for levels of anthracnose resistance at the F_2 generation are presented in Tables 33 A-C. Chi-square tests on the ratios were obtained for all crosses in which the susceptible lines were used as male parents. Results showed that many of the progenies obtained from the crosses significantly deviated (P>0.05) from the expected 3:1 phenotypic ratio. High deviations were observed for all progenies arising from the AB136 and G2333 parental lines.

Table 23 A-C: Phenotypic ratios of resistant (R): susceptible (S) segregating families of F2 populations when fitted on a 3:1 phenotypic ratio

A: Crosses with Kanyebwa as male parent

F2 population	Reaction	No. of Plants	Observed ratio (R/S)	Expected ratio (R/S)	X^2
AB136 x Kanyebwa	R x S	148	8:1	3:1	8.6*
G2333 x Kanyebwa	R x S	114	10:1	3:1	8.09*
K132 x Kanyebwa	R x S	130	1:3	3:1	34.68*
K20 x Kanyebwa	R x S	141	1:1	3:1	15.14*
NAT002 x Kanyebwa	R x S	139	2:1	3:1	3.37
NAT003 x Kanyebwa	R x S	114	4:1	3:1	1.61
NAT008 x Kanyebwa	R x S	135	2:1	3:1	0.02
NAT067 x Kanyebwa	R x S	156	3:1	3:1	1.17

*Significant at P<0.05; Critical X^2 = 3.84; R= Resistant; S= Susceptible

B: Crosses with K132 as male parent

F2 population	Reaction	No. of plants	Observed ratio (R/S)	Expected ratio (R/S)	X^2
AB136 x K132	R x S	122	11:1	3:1	7.89*
G2333 x K132	R x S	109	21:1	3:1	9.63*
Kanyebwa x K132	R x S	142	1:2	3:1	14.48*
K20 x K132	R x S	149	1:1	3:1	22.13*
NAT002 x K132	R x S	117	8:1	3:1	5.64*
NAT003 x K132	R x S	139	6:1	3:1	4.53*
NAT008 x K132	R x S	136	1:1	3:1	4.61*
NAT067 x K132	R x S	132	3:1	3:1	0.39

*Significant at P<0.05; Critical X^2 = 3.84; R= Resistant; S= Susceptible

C: Crosses with K20 as male parent

F2 population	Reaction	No. of plants	Observed ratio (R/S)	Expected ratio (R/S)	X^2
AB136 x K20	R x S	117	7:1	3:1	6.44*
G2333 x K20	R x S	143	11:1	3:1	11.04*
K132 x K20	R x S	145	1:2	3:1	29.82*
Kanyebwa x K20	R x S	137	1:2	3:1	28.75*
NAT002 x K20	R x S	138	7:1	3:1	6.78*
NAT003 x K20	R x S	124	5:1	3:1	4.98*
NAT008 x K20	R x S	145	3:1	3:1	0.12
NAT067 x K20	R x S	132	3:1	3:1	0.46

*Significant at P<0.05; Critical X^2 = 3.84; R= Resistant; S= Susceptible

Table 34 presents chi-square results obtained from phenotypic ratios of resistant to susceptible segregating families of BC_1F_1 populations. Results revealed that a good fit was obtained for the expected segregation ratio of 1:1 for nearly all the backcrosses apart from the progenies which were obtained from crosses with varieties G2333, AB136 and NAT002. A chi-square test on the ratios obtained from the BC_1F_1 populations for crosses with K132 and K20 did not significantly deviate from the expected 1:1 ratio, suggesting the presence of

a single dominant gene. The backcross populations obtained from cultivar Kanyebwa had a significant deviation which suggested the possibility that more than one independent gene was involved in conditioning resistance (Table 33).

Table 24: Phenotypic ratios of resistant (R) to susceptible (S) segregating families of BC1F1 populations when fitted on a 1:1 phenotypic ratio

Population	Generation	No of plants	Observed ratio (R/S)	Expected ratio (R/S)	X2
K132 x NAT002	BC_1F_1	59	2:1	1:1	0.018
K132 x NAT003	BC_1F_1	60	1:1	1:1	0.01
K132 x NAT008	BC_1F_1	52	1:1	1:1	0.07
K132 x NAT067	BC_1F_1	56	1:1	1:1	0.01
K132 x AB136	BC_1F_1	60	1:1	1:1	0.07
K132 x G2333	BC_1F_1	54	2:1	1:1	0.19
K20 x NAT002	BC_1F_1	60	2:1	1:1	0.08
K20 x NAT003	BC_1F_1	58	2:1	1:1	0.68
K20 x NAT008	BC_1F_1	55	1:1	1:1	0.08
K20 x NAT067	BC_1F_1	59	1:1	1:1	0.13
K20 x AB136	BC_1F_1	60	2:1	1:1	0.56
K20 x G2333	BC_1F_1	58	2:1	1:1	0.92
Kanyebwa x NAT002	BC_1F_1	60	1:1	1:1	0.66
Kanyebwa x NAT003	BC_1F_1	58	1:1	1:1	0.77
Kanyebwa x NAT008	BC_1F_1	60	1:1	1:1	0.09
Kanyebwa x NAT067	BC_1F_1	57	1:1	1:1	0.08
Kanyebwa x AB136	BC_1F_1	54	2:1	1:1	0.53
Kanyebwa x G2333	BC_1F_1	60	3:1	1:1	2.43

Critical X^2 = 3.84; R= Resistant; S= Susceptible

6.4.6 Estimated number of genes conditioning anthracnose resistance to dry bean

One to three genes were involved in controlling anthracnose resistance in the Ugandan market class beans (Table 35) as estimated using the method used by Bjarko and Line (1988). The results suggested that at the F_2 generation, three genes controlled resistance in seven out of 18 crosses (populations), two genes in six populations while one gene controlled anthracnose resistance in five populations (Table 35).

Table 25: Estimated number of genes determining anthracnose resistance in different crosses of dry beans at the F2 generation populations

Population	No. of plants in a cross	Number of genes at F_2
K132 x NAT002	149	2
K132 x NAT003	139	2
K132 x NAT008	151	1
K132 x NAT067	108	1
K132 x AB136	143	2
K132 x G2333	135	3
K20 x NAT002	149	3
K20 x NAT003	124	2
K20 x NAT008	154	1
K20 x NAT067	125	2
K20 x AB136	130	2
K20 x G2333	129	3
Kanyebwa x NAT002	141	2
Kanyebwa x NAT003	117	1
Kanyebwa x NAT008	148	1
Kanyebwa x NAT067	139	1
Kanyebwa x AB136	139	3
Kanyebwa x G2333	136	3
Mean	**136**	**2**

Number of genes were estimated following Bjarko and Line's (1988) method.

6.5 Discussion and conclusion

This study was undertaken to investigate the nature of resistance in anthracnose resistant inbred lines obtained from the screened Ugandan germplasm. In addition, the study was meant to establish the mechanism of inheritance of anthracnose resistance to the Ugandan market class cultivars from crosses made with these resistant lines as the first step in planning an efficient breeding programme.

The significant values of parental order of dominance (Wr+Vr) and significant differences over the arrays (Wr-Vr) as depicted in Table 27 are indicative of the inadequacy of the additive-dominance model, and reflected the importance of epistasis interactions in controlling anthracnose resistance in this set of bean lines. Christie and Shattuck (1992), Dabholkar (1992) and Kearsey and Pooni, (1996) reported that significant differences arising from Wr+Vr and Wr-Vr values are evidence of epistatic gene action. Results indicated that parental lines G2333, AB136, NAT002 and NAT003 possessed the highest anthracnose resistance. These cultivars were characterised by low variances (Vr) and covariances (Wr) suggesting predominance of dominant alleles (Kearsey and Pooni, 1996). On the other hand, parental

lines Kanyebwa, K132 and K20 had relatively high parental and array means for anthracnose resistance as well as negative (Wr-Vr) values but high (Wr+Vr) values portraying susceptibility to anthracnose in these parents (Fig 11), suggesting predominance of recessive genes for the anthracnose resistance at every locus. Generally, the array means (7 out of 9) were higher than the parental means over the 9 arrays (Table 27), suggesting that the genes for enhancement of anthracnose resistance were dominant over genes for susceptibility (Christie *et al.*, 1988). This is also true for results presented in Tables 23 and 24. In the same way, the results on joint regression of Wr on Vr were significantly different from unity, which provided further evidence for epistasis (Jana, 1975). However, the Hayman's (1954) diallel analysis method assumes a situation of no epistasis. Presence of epitasis suggests that the additive-dominance model would not be adequate to explain variation for anthracnose resistance. This would then imply that the dominance level would have been biased by epistatic action.

When use was made of the mid-parent offspring regression analysis, a regression coefficient (b) of 0.71 was obtained. This is a direct estimate of the heritability in the narrow sense (Dabholkar, 1992; Griffiths *et al.*, 1997). This estimate of h^2_n also portrayed the anthracnose resistance as a highly heritable trait. Also using the variance ratio from the Griffing's method a heritability value of 0.69 was obtained, indicating further that anthracnose resistance is highly heritable. Considering the provisions for heritability standard errors, these two heritability estimates were similar, suggesting that the values obtained represent true estimates for heritability of the resistance trait. On the whole, these heritability estimate values depict the dry bean anthracnose resistance trait as highly heritable, which means that selection for dry bean anthracnose resistance in this case can be readily achieved during the conventional breeding process.

The significant mean squares due to the general combining ability and specific combining ability suggest the importance of both additive and non-additive gene action, respectively. However, the high σ^2_A/σ^2_D ratio indicated that the additive gene action may have been responsible for some of the contributions towards the inheritance of anthracnose resistance in some of progenies. This ratio is also indicative of epistasis gene action (Griffing, 1956; Goffman and Becker, 2001). It is reported that effective selection can be done using F_2 progenies of such a population (Christie *et al.*, 1988).

The parents with highest positive GCA effects were K132, Kanyebwa and K20. A high GCA indicates that there is an increase in susceptibility among their progenies (Christie and Shauttuck, 1992). Therefore, it would be a waste of effort to make crosses between these three cultivars. This would be expected as two of these cultivars (K132 and Kanyebwa) are not known to posses any anthracnose resistance genes. The other cultivar, K20 had initially been bred for resistance to anthracnose (Leakey, 1970). On the other hand, the parental lines with high negative GCA effects were G2333, AB136, NAT002 and NAT003, indicating that these parents were most suited for transferring resistance to anthracnose in their progenies.

Considering the SCA effects, crosses G2333 x AB136, G2333 x NAT002, G2333 x NAT003, and AB136 x NAT002 had the lowest SCA in general and using the susceptible parents, crosses G2333 x Kanyebwa, AB136 x Kanyebwa, G2333 x K132 and G2333 x K20 gave the lowest SCA value, indicating that they included several plants which are resistant to anthracnose, an indication that the parents involved in these crosses are good combiners for anthracnose resistance. On the other hand, crosses NAT003 x Kanyebwa, NAT008 x K20, NAT002 x Kanyebwa, K20 x K132, NAT008 x K132, Kanyebwa x K20, NAT067 x K20, NAT067 x K132 and NAT067 x NAT003 had large SCA effects, suggesting predominance of susceptible plants in their progenies. The above crosses showed that the desirable SCA effects were due to poor x good GCA parents for the anthracnose disease resistance, indicating that desirable transgressive segregates may be released from these crosses in subsequent generations.

The results in Table 23, showed parental lines with varying anthracnose susceptibility levels which on crossing in a diallel design gave F_1 progenies whose reaction to anthracnose was almost similar to that of the resistant parents. This was suggestive of partial dominance for resistance. Further analysis of the crosses at F_2 showed various phenotypic ratios for different levels of anthracnose resistance. The chi-square results (Table 33 A-C) showed significant deviation from the expected 3:1 phenotypic ratio, especially for progenies arising from the crosses with the parents NAT002, NAT003, AB136 and G2333. This was similar to previous observations made in other studies where AB136 and G2333 have been used as sources of anthracnose resistance genes (Young *et al.*, 1998; Alzate-Martin *et al.*, 1999; Poletine *et al.*, 2000). These deviations are suggestive of dominant epistasis and presence of one to three genes being responsible for the observed resistance. At F_2, close to half of all families had a ratio greater than the expected three resistant to one susceptible phenotypic

ratio, which is also suggestive of dominant epistasis. A good number of families showed ratios significantly differing from the expected ratio and this gives an indication that more than one gene was involved. These ratios represent progenies which are still at the segregating stage and as such, for effective selection, there is a need for further assessment in progressive generations.

There is also a possibility that the genes at the same locus are interacting, resulting in resistant alleles being dominant over alleles for susceptibility, plus the non allelic interaction. According to Wyman (1991) and Johnson and Gepts (2002), in self pollinated plants like dry beans, epistasis is more important than dominance which lasts for a short time with progressive selfing, but non-allelic interaction can generate different phenotypes, some of which represent real genetic advances over their parents. It has even been recommended to identify loci with favourable epistatic combinations in the improvement of economically important traits in crops (Hallauer and Miranda, 1988). According to Kearsey (1993), non-allelic interactions may pose limitations when predicting response to selection, by preventing identification of the most promising crosses at an early stage in the breeding programme. Kearsey and Pooni (1996) reported that the effect of epistasis is more profound on means as opposed to variances and epistatic effects of generation means and are more amenable to analysis. Where additive epistasis is important, recurrent selection and reciprocal recurrent selection have been recommended as efficient techniques for selecting favourable epistatic gene combinations (Allard, 1960; Moreno-Gonzalez and Cubero, 1993).

The lack of significant differences in the reciprocal effects in Table 29 and Table 30 indicated the absence of maternal influence in the inheritance of dry bean anthracnose resistance. This implies that there will not be any variation related to genetic gain among segregating populations whether a susceptible or resistant parental line is used as a female during the hybridization process of introgressing resistant genes into susceptible cultivars.

It can thus be concluded that the diallel analysis techniques detected epistatic gene action for anthracnose resistance among the crosses used. Both GCA and SCA effects were significant, with GCA predominant over SCA for anthracnose resistance. Also it was shown that anthracnose resistance was highly heritable. It was further established that resistance to *C. lindemuthianum* Pathotype 767 in these dry beans was being conditioned by one to three dominant alleles. Reciprocal effects were not significant, indicating that maternal effects did

not influence resistance to anthracnose. This implies that simple pedigree breeding procedures such as backcrossing could be used to improve resistance to anthracnose of the market class dry bean cultivars in Uganda. In addition, the limited number of dominant genes could suggest the possibility of using marker assisted selection in a pedigree breeding programme. Lastly, local parental lines NAT002 and NAT003 can be used as a good source of anthracnose resistance genes.

References

Allard, R.W. 1960. Principles of Plant Breeding. John Wiley and sons, Inc. New York.

Allen, D.J., Ampofo, J.K.O. and Wortman C.S., 1996. Pests, diseases, and nutritional disorders of the common bean in Africa: A field guide. Cali Colombia. International Centre for Tropical Agriculture; Wageningen, The Netherlands: technical centre for Agricultural and Rural Cooperation, 132p.

Alzate-Martin, A.L., Menarim, H., Assis de Carvallo, G., Jose de Paula Junior, T., Goncálves de Barros, E. and Moreira, M.A. 1999. Improved selection with newly identified RAPD markers linked to resistance gene to four pathotypes of *Colletotrichum lindemuthianum* in common bean. *Phytopathology*, 89:4:281-285

Anju, P., Sharman, P., Sharman, O., Chahota, R. and Bilal, A. 2006. Evaluation of resistance sources and inheritance of resistance in kidney bean to Indian virulences of *Colletotrichum lindemuthianum*: Evaluation of resistance in bean to anthracnose. *Euphytica*, 149: 97-103.

Bjarko, M.E. and Line, R.F. 1988. Heritability and number of genes controlling leaf rust resistance. *Phytopathology*, 78:457-461.

Champion, M.R., Brunet, D., Maudit, M.L., and Ilami, R. 1973. Methode de controle de la resistance desvarietes de haricots a l'antracnose (*Colletotrichum lindemuthianum* (Sacc & Magn.) Briosi et Cav . *C. R. Hebd. Seances Academic Agriculture de France*, 59:951-958.

Christie, B.R. and Shattuck, V.I. 1992. The diallel cross: design, analysis and use for plant breeders. *Plant Breeding Reviews*, 9:9-36.

Christie, B.R., Shattuck, V.I. and Dick, J.A. 1988. The diallel cross: Its analysis and interpretation. Department of Horticulture. University of Guelp, Ontario, Canada.

Cockerham, C.C. 1986. Modifications in estimating the number of genes for a quantitative character. *Genetics,* 114:659-664.

Dabholkar, R.R. 1992. Elements of Biometrical Genetics. Ashok Kumar Mittal Concept Publishing Company, New Delhi, India.

David S., Kasozi S., and Wortmann C. 1997. An investigation of alternative bean seed marketing channels in Uganda. Network on Bean Research in Africa, Occasional Publications Series, No.19. CIAT, Kampala, Uganda.

Drijfhout, E. and Davis, J.H. 1989. Selection of new set of homogeneously reacting bean (*Phaseolus vulgaris*) differentials to differentiate races of *Colletotrichum lindemuthianum*. *Plant Pathology*, 38:391-396.

Dudley, J.W., and Moll, R.H. 1969. Interpretation and use of estimates of heritability and genetic variance in plant breeding. *Crop Science*, 9:257-262.

Falconer, D.S. 1960. Introduction to Quantitative Genetics. Ronald Press Co., New York.

Fernandez, G.C.J. and Miller, J.C. 1985. Estimation of heritability by parent-offspring regression. *Theoretical Applied Genetics*, 70:650-654.

Goffman, F.D. and Becker, H.C. 2001. Diallel analysis for tocopherol contents in seeds of rapeseed. *Crop Science*, 41:1072-1079.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences*, 9:463-493.

Griffiths, A.J.F., Miller, J.H., Suzuki, T.D., Lewontin, R.C. and Gelbart, M. 1997. An Introduction to Genetic Analysis. W.H. Freeman and Company. New York.

Hallauer, A.R. and Miranda, J.B. 1988. Quantitative Genetics in Maize Breeding. Iowa State University Press, USA.

Hayman, B.I. 1954. Theory and analysis of diallel crosses. *Genetics*, 39:789 - 808.

Jana, S. 1975. Genetic analysis by means of diallel graph. *Heredity*, 35:1-19

Johnson W.C. and Gepts, P. 2002. The role of epistasis in controlling seed yield and other agronomic traits in Andean x Mesoamerican cross of common bean (*Phaseolus vulgaris* L.). *Euphytica*, 125:69-79.

Kearsey, M.J. 1993. Biometrical genetics in breeding. In: *Plant breeding, Principles and Prospects.* Chapman and Hall. London

Kearsey, M.J. and Pooni, S. 1996. The Genetic Analysis of Quantitative Traits. Chapman and Hall. London.

Lawes Agricultural Trust. 2005. GenStat Release 8.1. PC/Windows XP. Rothamsted Experimental Station.

Leakey, C.L.A 1970. The improvement of bens (*Phaseolus vulgaris*) in East Africa. In: Leakey, C.L.A. (ed.). *Crop improvement in East Africa.* Pp. 98-128. The Commonwealth Agricultural Bureaux, London.

Mather, K. and Jinks, J. 1982. In: Biometrical Genetics. Chapman and Hall, London.

Menezes, J.R. and Dianese, J.C. 1988. Race characterization of Brazilian isolates of *Colletotrichum lindemuthianum* and detection of resistance of anthracnose in *Phaseolus vulgaris. Journal of Phytopathology*, 78:650-655.

Melo, L.C., dos Santos, J.B. and Ramalho, M.A.P. 1997. Choice of parents to obtain common bean (*Phaseolus vulgaris*) cultivars tolerant to low temperatures at the adult stage. *Brazilian Journal of Genetics*, 20:283-292.

Moreno-Gonzalez, M. and Cubero, F. 1993. Selection methods. In: Plant Breeding, Principles and Prospects. Chapman and Hall. London.

Opio, F., Mugagga., Mawejje, D. and Nkalubo, S. 2006. Progress report on bean anthracnose research in Uganda. *MUARIK bulletin*, (in press).

Pastor-Corrales, M. and Tu, J.C. 1989. Anthracnose. In: Schwartz, H.F. and Pastor-Corrales, M.A. (eds.). *Bean Production Problems in the Tropics.* Pp.77-93. Centro Intemacional de Agricultura Tropical, (CIAT). Cali. Colombia.

Pastor-Corrales, M.A., Erazo, O.A., Estrada, E.I., and Singh, S.P. 1994. Inheritance of anthracnose resistance in common bean accession G2333. *Plant Disease*, 78:959-962.

Peloso, M. J., Cardoso, A., Vieira, C., Saraiva, L.S. and Zimmerman, M.J.O. 1989. Genetic system for the reaction of *Phaseolus vulgaris* to the BA-2 (alpha) race of *Colletotrichum lindemuthianum. Review of Brazilian Genetics*, 12:313-318.

Poletine J.P., Gonçalves-Vidigal M.C., Filho P.S.V., Scapim, C.A., Silvério, L. and Thomazella, C. 2000. Inheritance of Resistance to Races 69 and 453 of *Colletotrichum lindemuthianum* in the Common Bean. *Brazilian Archives of Biology and Technology*, 43:479-485.

Singh, S.P., Pastor-Corrales, M., Molina, A. and Otoya, M. 1992. Breeding common bean for resistance to anthracnose. In: *La Antracnosis del Frijol Común, Phaseolus vulgaris, en América Latina*. Pp. 198-211. Doc. de trabajo.

Ugen M.A., Wien H.C. and Wortmann S.C. 2002. Dry bean competitiveness with annual weeds as affected by soil nutrient availability. *Weed Science,* 50:530-535.

Vogel, K.P., Haskins, F.A. and Gorz, H.J. 1980. Parent progeny regression in indiangrass: inflation of heritability estimates by environment covariances. *Crop Science,* 20:580-582.

Wortman, C.S. and Allen, D.J. 1994. African bean production environments: their definition, characteristics and constraints. *Network on Bean Research in Africa.* Occasional paper series No. 11, Dar Salaam, Tanzania.

Wyman E.N 1991. Estimation of heritability and prediction of selection response in plant populations. *Critical Reviews in Plant Sciences*, 10:235-322.

Young, R. and Kelly, J.D. 1996. Characterization of the genetic resistance to Colletotrichum lindemuthianum in common bean differential cultivars. *Plant Disease*, 80:650-654.

Young, R.A, Melotto, M., Nodari, R.O. and Kelly, J.D. 1998. Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar, 'G2333'. *Theoretical and Applied Genetics*, 96:87-94.

OVERVIEW

OVERVIEW OF RESEARCH FINDINGS AND THEIR IMPLICATIONS FOR DRY BEAN ANTHRACNOSE MANAGEMENT IN UGANDA

The common bean (*Phaseolus vulgaris* L.) is an important crop grown and consumed widely in Uganda. It provides a cheap source of human dietary protein in both rural and urban areas. In addition, the crop is also an important source of income for the smallholder farmers in the rural areas, particularly the women. Despite its importance, the production of dry beans continues to decline due to various constraints. Anthracnose disease as a dry bean production constraint in Uganda was the main focus of this study.

After in-depth studies of the dry bean growing communities, germplasm screening, anthracnose pathotype-host relationships, yield loss assessments and resistance inheritance studies of anthracnose, a number of significant findings have been made. These are outlined below;

1. Farmers are aware of the damage caused by anthracnose to their dry bean crop but are not knowledgeable about the disease itself. In addition, they mostly utilise the home saved seed to grow the next season's dry bean crop as they lack access to certified seed sources.

2. A range of anthracnose pathotypes were found to be present in the different bean growing regions in Uganda and one pathotype (Race 767), was conspicuously the most virulent and dominant.

3. The findings from the germplasm screening studies showed that there were some local and exotic dry bean varieties which were resistant to the predominant anthracnose pathotypes in Uganda.

4. In the yield loss assessment study, it was established that the use of susceptible cultivars in the presence of anthracnose pathogen may result in a yield loss of over 70%. This loss is minimised if a resistant cultivar is used or a fungicide is used to

protect the crop against the effects of the pathogen. Unfortunately the available resistant cultivars are not as marketable as the susceptible cultivars.

5. The studies of inheritance of resistance established that anthracnose resistance was being conditioned by a few partially dominant genes with an additive gene action. Additionally, the resistance was found to be highly heritable.

These findings have brought to light important implications with regard to future approaches in the management of dry bean anthracnose in Uganda. First and foremost, the fact that farmers are less knowledgeable about anthracnose implies that it may not be easy to do away with anthracnose as the infection and re-infection processes are heightened by the farmers' use of home saved seed. In this case the management of anthracnose will involve the need to educate farmers about the disease and the agronomic control practices they could use to keep the fungus at bay. Consequently, this will enable farmers to control or reduce the transmission of the fungus from one season to the next or from one farmer's field to another. The lack of adequate certified seed sources implies that there could be potential for seed marketing in this region.

Secondly, the variability in the anthracnose pathotypes implies that breeding for vertical resistance to this pathogen may not be the best option as the resistance may not be durable. But then, the use of the gene pyramiding breeding technique could help improve the durability of vertical resistance. On the other hand, one could consider the use of horizontal resistance which is able to withstand variability in pathogen pathotypes. However, horizontal resistance is more difficult to manipulate because it requires population breeding strategies and statistical techniques to determine which progenies have improved resistance and it is more time consuming. Then again, the fact that one pathotype was the most virulent and dominant, suggests that breeding for resistance to this particular pathotype could result in varieties that are resistant to all the other races. In this case there is a need to weigh the pros and cons of both vertical and horizontal resistance before conclusions are made on the type of resistance to use.

In another finding, resistant lines were identified from the screened germplasm collection implying that these varieties could be used as sources of anthracnose resistance genes for the breeding programme. Although the exotic varieties may have better resistance, it would

be advisable to utilise resistance from the local varieties because of having a similar genetic background with the susceptible cultivars. In addition, the screening of landraces in search of resistance genes should be considered an important part of a disease resistance breeding programme as they possess unique qualities desired by farmers. As for the significant differences between the yield loss of susceptible and resistant cultivars, this suggests that the use of resistant cultivars would be the most economic option in controlling and managing dry bean anthracnose disease.

Lastly, the findings that anthracnose resistance in the Uganda market class beans is conditioned by highly heritable, partially dominant additive genes, implies that resistant progenies can be selected using simple pedigree breeding methods such as backcrossing. Because of the limited number of genes responsible for the observed anthracnose resistance, the use of marker assisted selection can be employed to speed up the breeding process. The breeding process can be done together with farmers through either the participatory varietal selection (PVS) or participatory plant breeding (PPB) processes.