DISTRIBUTION AND CHARACTERIZATION OF SWEETPOTATO *ALTERNARIA* BLIGHT ISOLATES IN UGANDA

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DECLARATION

I Agnes Alajo declare that the work presented in this thesis is from my own research and has not been submitted for the award of a degree in any University.

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To my husband, children and parents.

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ABSTRACT

In Uganda, sweetpotato virus diseases and *Alternaria* are the major diseases affecting sweetpotato production. Most of the improved varieties and landraces grown for home and commercial consumption like NASPOT 1 and New Kawogo are highly susceptible to *Alternaria* blight caused by the fungus *Alternaria.* The population genetic structure of *Alternaria* species in Uganda is currently unknown, consequently, it is difficult to reliably determine whether popular resistant cultivars will remain resistant over a wide geographical area, or not. This information is critical in breeding for resistance to the disease. A study was carried out at Makerere University to characterize sweetpotato *Alternaria* isolates, and to assess their pathogenicity on selected sweetpotato cultivars in Uganda. *Alternaria* isolates (299) were recovered from local popular and improved cultivars from 170 locations in 17 districts of Uganda. Isolates were characterized using morphological characters, pathogenic and molecular techniques; Random amplified polymorphic DNA (RAPDs) and restricted fragment length polymorphism (RFLP). Disease severity was significantly ($P< 0.05$) varied between districts surveyed. Wakiso district had the highest (3.2) severity scores whereas Sironko had the lowest severity scores (1.2). A study of the 299 *Alternaria* isolates revealed substantial variability in growth rate, color, morphology and sporulation. Colony morphologies of isolates were compared on Potato Agar (PDA), Sweetpotato vine decoction medium (SPVDM) and Calcium carbonate ($CaCo_3$). Mean increase in diameter in seven days varied greatly by species type ranging from 0.1 to 3.0 mm. In addition, morphological markers revealed two species of *Alternaria, A. bataticola* and *A. alternata*. Randomly amplified polymorphic DNA (RAPDs) and restricted fragment length polymorphism (RFLP) were able to differentiate the two species into two clusters with many sub groups.

Pathogenicity tests of six isolates were carried out on both detached leaves and potted plants. The cultivars; Ebwanaterak (highly resistant), Magabali (moderately resistant) and NASPOT 1 (highly susceptible) were used as host differentials to determine variation in pathogenicity, aggressiveness and possible host specificity among *Alternaria* isolates. The three cultivars differed significantly ($P < 0.01$) to all *A. bataticola and A. alternata* infection.. The most virulent isolate was NTG 215 which was able to attack the most resistant cultivar, Ebwanaterak. However, *A. alternata* isolates only caused mild symptoms to all the cultivars. This proved occurrence of three different strains of *A. bataticola* and a single strain for *A. alternata. A. alternata* is not a major threat to sweetpotato production since it showed mild symptoms. Existence of 3 different races of *A. bataticola* as revealed by race differentials is a major threat to sweetpotato production. Breeding should either target the most virulent race from Ntungamo or could be location specific for the races.

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CHAPTER ONE

1.0 Introduction

1.1 Origin and Spread of Sweetpotato

Sweetpotato (*Ipomoea batatas* L. Lam) together with the wild *Ipomoea s*pecies originated from Central or South America in the region between Yucatan peninsula of Mexico and Orinoco river in Venezuela, about 8000-6000 BC, where *I. trifida* and *I. triloba* might have crossed to produce the wild ancestor of *I. batatas* (Austin, 1988). Sweetpotato belongs to the *Convolvulaceae* family, genus *Ipomoea*, sub genus *Eriospermum*, section *Eriospermum* and series *batatas* (Purseglove, 1968). It is a self-incompatible species with a ploidy level 2n = 6x = 90 chromosomes (Jones, 1964). It is the only member of the family which is of economic importance. The family *Convulvulceae* comprises of approximately 50 genera (Pursglove, 1968). Sweetpotato was introduced to Uganda from both east and western parts of the African continent (Aldrich, 1963). To date, sweetpotato cultivars are grown in nearly all tropical and sub-tropical parts of the world and in warmer temperate regions such as southern USA (Horton *et al*., 1984), sub-Saharan Africa, West Indies, China and Japan. The secondary centres of diversity are found in Guatemala, Colombia, Equador and Peru (Austin, 1983).

1.2 Importance of Sweetpotato

It is estimated that sweetpotato cultivation in Uganda has extensively increased from 473,000 to 590,000 hectares in the past decade with an annual production of 2.2 million tones per year making it the third largest producer in the world after China and Nigeria (FAO, 2007). The International Potato Centre (CIP) ranked sweetpotato as the seventh most important food crop in the world after wheat, rice, maize, potato, barley and cassava (Mwanga *et al*., 2001b).

Production is concentrated in East Asia, the Caribbean and tropical Africa with the bulk of the crop being in China (Hijmans *et al*., 2001. Despite its rank in world production, sweetpotato potential production of 45 tons/ ha is not yet realized in many production areas.

In Uganda, sweetpotato occupies a national status as the third most important crop after banana and cassava (Mwanga and Wanyera, 1988; Bashaasha *et al*., 1995; Mwanga *et al*., 2001b). It is regarded as a food security crop because of high yields and low input requirements for its production (Aritua and Gibson, 2002; Bashasha *et al*., 1995), and the high rates of production per unit area and time (Woolfe, 1992). The crop is harvested piece meal for supplying fresh daily food for the family throughout the year (Bashasha *et al*., 1995). During dry season and during seasons of scarcity the dried sliced chips form part of the dietary staple mainly. In several African countries, the young leaves are eaten as vegetables (Rees *et al*., 2003; Owori and Hagenimana, 2000). The tuberous roots contain nutrients and minerals in varying proportions as described by Woolfe (1992). The crop provides nutritionally significant quantities of ascorbic acid, riboflavin, iron, calcium and protein. In addition, the orange fleshed sweetpotatoes (OFSP) especially NASPOT 9 and Ejumula are rich in β-carotene a precursor of vitamin A, which effectively fights certain types of cancer and other diseases it is particularly valuable in meeting crucial dietary requirements of pregnant women and children (Mwanga *et al*; 2003; Prakash *et al*., 2001). Traditionally the crop is grown mainly by rural women near their homes to feed their families and to provide them with a source of income, thus the reference to the crop as a woman's crop (Mwanga *et al*., 2001b; Hakiza *et al*., 2000; CIP, 1999).

1.3 Sweetpotato Production Constraints in Uganda

The potential yield of sweetpotato is up to 45 t/ha (PRAPACE, 2003). However, on-farm root yields in Uganda are still as low as 4.1 t/ha far less than the average for Kenya (9.5 t/ha) and Ethiopia (7.7 t/ha) (PRAPACE, 2003). Uganda's average production per hectare is one of the lowest in the entire East and Central Africa region (Osiru, 2008). The major constraints to sweetpotato production have been identified among others as lack of high yielding early maturing varieties with high levels of resistance to disease and shortage of clean, high quality planting materials of superior (Bashasha *et al*., 1995).

Many challenges have been reported that affect sweetpotato production and productivity in Uganda. These challenges include diseases, pests, inadequate farmer access to improved cultivars, poor agronomic practices, and slow rate of technology adoption. Other constraints include poorly adapted cultivars, low yielding indigenous cultivars, poor marketing chain, low soil fertility and shortage of farm inputs (Bua *et al*., 2001; Bashasha *et al*., 1995; Lenne, 1991). Of these, pests and diseases are the most important constraints of sweetpotato production in Uganda. The most economically important viral disease is sweetpotato virus disease (SPVD), caused by *sweetpotato feathery mottle virus* (SPFMV) and *sweetpotato chlorotic stunt virus* (SPCSV) (Mwanga *et al*., 2002a; Aritua *et al*., 2000; Gibson *et al*., 1998). Sweetpotato viruses are of economic importance in all the major sweetpotato growing areas in Uganda (Mukasa *et al*., 2003). Pathogenic bacteria, although not very common, are responsible for important economic losses. They affect vascular tissue as well as storage and fibrous roots, thus causing vine wilting and rots. The most prominent being bacterial stem and root rot (*Erwinia chrysanthemi*), bacterial wilt (*Pseudomonas Ralstenia*), soil rot

(*Streptomyces ipomoea*), leaf and stem scab (*Elsinoe batatas*, *Sphaceloma batatas*), which also attack sweetpotato worldwide (Ames *et al*., 1997).

The main harmful insect pests are the sweetpotato weevils of the genus *Cylas* (*C. puncticollis* and *C. brunneus*) that cause devastating yield losses and quality (Purseglove, 1968). *Cylas formicarius* is mainly distributed over East Africa. It attacks the leaves, stems and roots (Skoglund and Smit, 1994). Other minor insect pests are sweetpotato butterfly (*A. acereta*). Caterpillars of *Acraea acereta* may be considered minor pest but can cause serious losses through feeding on leaves of sweetpotato, occasionally they may become a major pest and defoliate the plants, particularly during dry season in Uganda (Skoglund and Smit, 1994). Several pathogenic fungi cause disease that affect yields of sweetpotato and among these are *Alternaria* species which cause *Alternaria* disease with varying levels of damage (Clark, 1987).

1.4 Problem statement

In East Africa the *Alternaria* blight disease has been ranked as the most important fungal disease (Rees *et al*., 2003). In Uganda, infection by *Alternaria* is becoming very rampant in most districts. Frequent observations have pointed out damages due to *Alternaria* spp in many farmers' fields especially in central and south-western Uganda (Mwanga *et al.*, 2003). The disease affects the shoots destroying the leaf, petiole and stem causing brown lesions that enlarge and become dark grey or black due to the abundance of spores (Clark, 1987, Osiru, 2008).

Yield losses due to *Alternaria* disease range from 2.5-10 t/ha (Turyamureeba *et al*., 1999). In cases where the disease causes plant death yield losses have been speculated to be much

higher (Lopes *et al*., 1994). While the disease is known to be present in epidemic proportions in many parts of Uganda causing severe crop loss depending on the crop stage, susceptibility of cultivar and the prevailing environment conditions. There is no information on prevalence, incidence and severity, variation of the pathogen and disease reaction on the local sweetpotato land races as well as epidemiological studies haven not been carried on sweetpotao. There is general lack of adequate knowledge on the race structure and the pathogenicity of those races in Uganda.

1.5 Justification of the study

In Uganda, there are wide varietal differences in susceptibility to *Alternaria* disease (Mwanga *et al*., 2002). Differences in susceptibility have been an advantage for mitigating breeding programmes for resistance. However, little success has been achieved in breeding for resistance to this disease due to regular resistance breakdown in the resistant genotypes across different areas and growing seasons (Collins *et al*., 1991). This finding may imply that different races display different levels of pathogenicity in some regions. The existence of at least three races of the same genus of *Alternaria* spp in the sweetpotato growing regions in the world namely *A. alternata*, *A. bataticola* and *A. tax sp IV* (Van Bruggen, 1984) is a challenge that needs to be addressed through characterization prior to breeding initiatives. Where there are location specific races of *Alternaria*, location specific breeding is required to alleviate their damage ((Mwanga *et al*., 2002). Accordingly, a study to identify *Alternaria* spp and races is needed because very limited information is available about existing *Alternaria* races, yet it is critical for effective breeding for resistance. Basing on a country map of existing races, their distribution, strategic breeding approaches targeting farmer

preferred high yielding variety would then be adopted. In order to achieve this, adequate information about available *Alternaria* races, their structure and pathogenicity is required.

1.5 Objectives of Study

The main objective of this study was to characterize isolates of the pathogen responsible for leaf petiole and stem blight of sweetpotato, and determine their pathogenicity.

The specific objectives were to:

1. Characterize *Alternaria* spp that cause *Alternaria* blight disease of sweetpotato in Uganda
2. Test the pathogenicity of six *Alternaria* spp isolates on three selected sweetpotato cultivars.

The hypotheses for this study are;

- There is no diversity among *Alternaria* isolates from in Uganda with typical pathogenic effect in sweetpotato.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sweetpotato Production Constraints

The potential production of sweetpotato in Africa as a continent is nearly 7 million tons and nearly all of it is from south of the Sahara (PRAPACE, 2003). Although the inherent yield potential of sweetpotato is up to 80 tons per ha (Woolfe, 1992), the average yield of sweetpotato in Africa is 5 t/ha this is the lowest of any developing region, and less than a third of the average yield in Asia (Scott and Maldonado, 1999). In Africa growth rates in sweetpotato production and in particular, area planted is declining over the last decade (Mwanga**,** *et al.,* 2003**)**. As area continues to expand, the annual average rate of improvement in yields has turned negative in some cases (Uganda: 1.9%), and off setting what would have otherwise been faster rates of growth in production (PRAPACE, 2003). Other constraints to sweetpotato production include weeds, pests, diseases, low yielding varieties, low soil fertility, soil acidity, marketing and post harvest losses and utilization. In addition, farmers' varieties grown have low dry matter (20-30%), too low to be used as a raw material in the processing industry that prefers dry matter above 35% (Mwanga *et al*., 2003). In addition, many sweetpotato varieties have low vitamin A (Mwanga *et al*., 2007c). Fluctuating prices of sweetpotato in the markets are a farmers' main concern, further more the farmers' weak bargaining position with the traders who buy the standing crop in the field and monopolize the marketing system.

There are challenges to increased and sustainable sweetpotato production in Africa and Uganda in particular. Among the major constraints to its production is lack of high yielding,

early maturing varieties that are adapted to a wide range of agro ecologies and tolerant to diseases like SPVD and *Alternaria* (Turyamureeba, 1997). Among the viral diseases, sweetpotato virus disease (SPVD) caused by synergistic interaction of s*weetpotato chlorotic stunt virus disease* (SPCSV) and *sweetpotato feathery mottle virus* (SPFMV) is the most severe and results in causing 56-98% yield losses (Mwanga *et al*., 2002; Mukasa *et al*., 2006, Rukarwa *et al*., 2010).

2.2 Host Range of *Alternaria* spp

Alternaria species are important pathogens of a wide variety of crops and weeds (Abbas *et al*., 1995). This is true for species like *Alternaria solani* that causes serious blight on potato (early blight) and tomato as well as some *Brassicae* species. *Alternaria brassicae* attacks a wide range of cruciferous plants (Parry, 1990). Four of these pathogens (*A. alternata, A. capsici-annui*, *A. solani* and *A. tenuissima*) have also been reported on three other species of *Ipomoea* (Lenne, 1991). There are seven plant diseases caused by *Alternaria* species in which host specific toxins are responsible for their pathogenicity (Rotem, 1994). *Alternaria* species are also capable of infecting numerous plant species (Agrios, 1969), including cotton, tobacco, sunflower, onion, apple, carrot, citrus, pear, pistachio, sunflower, sesame, beans, sinicia and many species from the family Solaneaceae, Brasscaceae and Cucurbitaceae (Rotem, 1994).

2.3 History, distribution and occurrence of *Alternaria* blight of sweetpotato

Alternaria blight disease of sweetpotato was first recorded from subsistence food garden in the Nebilyer valley of the Western Highlands province in Papua New Guinea in early 1987

(Lenne, 1991), then it was reported in the southern and western highlands of Papua New Guinea, Brazil, South America and in New Caledonia (Lenne, 1991).

In 1945, Hansford reported the occurrence of a sweetpotato disease in Uganda in districts of Ankole, Kigezi, Toro and Mengo with symptoms commencing as small brown to black spots on the stem surfaces, and later extending until the stem was completely girdled at soil level, or further along the vines. Although reported as *Plenodomus destruens* no evidence is provided to support this, and possibly, this was a case incited by alternaria spp. Later reports indicated the presence of the *Alternaria* leaf petiole and stem blight disease in Kabale district in south–western Uganda (Low, 1996; 2000; Bashasha *et al*., 1995).

In Uganda, surveys conducted have revealed presence of *Alternaria* pathogen in almost all districts (Bua *et al*., 2001; Osiru, 2002). Recently the presence of *Alternaria* leaf and stem blight of sweetpotato was reported in a number of sweetpotato producing countries including Nigeria (Arene and Nwankiti, 1978). In Rwanda Van Bruggen, (1984); Ndamage, (1988) reported the presence of *A. tax sp IV*, in Ethiopia Terefe and Amanuel, (1992) reported the disease to be attacking sweetopotato in medium-altitude regions in Burundi Simbashizweko and Perreaux, (1988) reported alternaria presence in Aiyura Research Station and Gatumbi *et al*., (1990) reported the disease along with 12 other fungi infecting sweetpotato in Kenya. Skoglund, (1994) conducted a survey on sweetpotato non-viral diseases in Kenya and reported *Alternaria* spp to be present in all 13 districts surveyed. It was the only pathogen found in the highland, tea growing areas of Kericho district. Disease incidence and severity were especially high (>50%) in highland areas (Kirinyanga, Muranga, and Kiambu)

Skoglund, (1994). Likewise in Uganda Osiru, (2002) conducted a survey in Kenya which revealed 22 out of 35 districts surveyed as having *Alternaria* However, according to Simbashaizweko and Perreaux, (1988) the disease was serious particularly in infertile, acid soils, and at higher altitudes in Rwanda and Burundi.

2.4 Epidemiology of Sweetpotato *Alternaria* leaf, petiole and stem blight disease

A. alternata, was identified as the causal agent of stem and leaf blight on sweetpotato (Lenne, 1991. Clark and Moyer, 1988) recorded other species namely; *A. solani, A. tenuissima and A. bataticola* as causing stem blight of sweetpotato in other countries. According to Lopes, Embrapa, (1984); *Alternaria* leaf stem and petiole blight is confined to the highlands, he also observed that *A. bataticola is* a causal organism for *Alternaria* blight of sweetpotato that cause blackened lesions on veins observed on leaf undersurface as the disease progresses, the lesions become necrotic usually surrounded by a wide yellow halo causing the whole leaf blade to turn chlorotic/yellow and drop. Bases and middle sections are more affected than the vine terminals. The ground under affected vines is often carpeted with blackened leaf debris. On the petioles and stems, lesions are initially grey, enlarged and become black and sunken. The petioles and stems are girdled and the plant dies.

Alternaria leaf spot is also caused by *Alternaria* spp. (Bruggen, 1984). It has not been considered important, since the damage caused is mostly present in older leaves. *Alternaria* leaf spot is present wherever sweetpotato is grown, but no written information has been found on the geographic distribution references from India and Japan, (Lopes, 1984). It is characterized by presence of brown necrotic lesions on older leaves, with a typical ‘bulls’ eye

appearance of concentric rings, 1 to 5 mm wide with well defined margins, is the most evident symptom of the disease (Ellis, 1971). As the disease develops, light brown lesions are formed with concentric rings. Several lesions can fuse and cover a great area of the spot. When this occurs the leaf drops. Usually spots are surrounded by chlorotic halo (Clark and Moyer, 1988).

The fungus persists or lives as mycelium in infected crop debris in or on the surface of the soil (Van Bruggen, 1984). Many species of *Alternaria* are mostly saprophytic; they cannot infect a living plant tissue but grow on dead or decaying plant tissue and most on senescent or old tissues (Agrios, 1980). *Alternaria* leaf petiole and stem blight disease appears as small, grey to black oval lesions with a lighter centre on stems and petioles (Clark and Moyer, 1988; Van Bruggen, 1984). Infection of stems and petioles occur under favorable weather conditions when spores germinate and enter healthy tissues either directly or through wounds causing enlarged black lesions on stems and petioles, resulting in petiole and stem girdling (Skoglund and Smit, 1994). Brown lesions with typical "target" appearance of concentric rings often occur on leaves, especially older ones (Skoglund and Smit, 1994). Leaves above the affected parts become chlorotic and dry. If the main stem is severely affected when plants are still young, (approximately 2 months after planting) in most cases this may result in death of plant (Lenne, 1991; Van Bruggen, 1984). The plant leaves at close proximity to ground level and middle sections are usually more affected than the terminal vines. The ground under affected vines is often carpeted with blackened leaf debris (Skoglund and Smit, 1994). Stem blight has been reported to manifest in the wet season as stem necrosis and dieback but is especially serious in the drier periods of the cropping season (Lenne, 1991). The amount of

damage on the sweetpotato plant is dependant on the stage of plant growth at infection and environmental conditions (Skoglund and Smit, 1984).

Increased infection by *Alternaria* spp are as a result of stress factors such as drought, inadequate nutrients, infestations by insect pests or other pathogens, wounds inflicted by non-specific agents, and weakening of plant due to production of excessive yield or senescence (Lenne, 1991). Based on other *Alternaria* – host systems, the influence of wounds has been proven to be more important with the weakly pathogenic *Alternaria* spp for example with *A. alternata and A. tenussima* in onions (Rotem, 1994). Similarly, the more virulent *A. solani* is not able to infect unwounded potato tubers, even in the presence of free moisture (Anginyah *et al*., 2001).

2.5 Taxonomy and Characterization of *Alternaria* spp

Simmons, (1995) reviewed the taxonomy of the genus *Alternaria* and found that identification of some *Alternaria* species still offers considerable difficulties, owing to their high variability and polymorphism, which occur even in pure cultures. Morphological characteristics of conidia and conidiophores and sometimes host plant association, provide the major taxonomic criteria for delimitation of fungal species (David, 1991). However, the classification of small spored species, including host-specific toxin producing fungi, has been particularly confused, because of the simple and convergent morphology of conidia and facultative parasitism, resulting in an ambiguous host range (Simmons, 1999). However, according to Pattanamahakul and Strange, (1999), the taxonomy of *Alternaria* on brassicas has been based principally on morphology and sometimes host plant association of each of

the species occurring *(A. brassicicola, A. brassicae and A. raphani)* has a distinct morphology considering the diversity of conidium shapes and sizes among *Alternaria* spp. In general, there have been efforts in developing sub- generic groupings of species based upon similar conidium characteristics. Simmons, 1995) organized the genus into 14 species-groups upon characteristics of conidia and chain formation. In previous work by Simmons, (1995) recovered *Alternaria* isolates from pear and further advanced the species group concept referring to certain groups using a representative species, for instance, the *alternata* group, the *tenuissima* group, and the *infectoria* group. Additional species-groups discussed in other work include the *arborescens*, *brassiciocola*, *porri*, and *racicina* groups (Pryor and Gilbertson, 2000). Although the use of species-group designation does not resolve more definitive species boundaries within *Alternaria*, it has an advantage of morphologically organizing the diverse assemblage of *Alternaria* spp. at sub-generic level. This permits the generalized discussion of morphologically similar species without becoming overly restricted due to nomenclatural uncertainty.

Alternaria alternata has microscopic morphology that is brunched with acropetal chains of multicellular conidia produced sympodially from simple, sometimes branched, shorter elongate conidiophores. According to description by David (1991), conidia of *A. alternata* are sometimes referred to as obclative, obpyriform, ovoid or ellipsoidal often with a short conical or cylindrical beak and have a pale brown, smooth walled or verrucose. It is one of the smaller spored species, bearing dull grey green to pale yellowish brown conidia ranging in size from 10- 43 x 5- 14 µm at maturity. The conidia are formed as short chains borne on conidiophores, rather than single conidia. Like *Alternaria solan*i, the conidia are multi-

septate and muriform. However, there is profuse aerial mycelium that is lighter in color than that of *A. solani*.

2.6 Cultural Studies of Alternaria Leaf Blight

A number of culture media of different types and quality have been recommended for growth and sporulation of different species of *Alternaria*. Van Bruggen (1984) reported maximum growth and sporulation of *Alternaria tax sp. (IV)* on potato dextrose agar (PDA) and malt extract agar (MEA) under 12 hrs light alternating with, 12 hrs darkness. Lopes *et al.* (1996), reported abundant conidia production of the same pathogen in calcium carbonate sporulating medium as opposed to PDA. Anginyah, (2003) obtained maximum growth of *Alternaria* sesame on oatmeal agar (OMA).

2.7 Symptoms and Effects of *Alternaria* Leaf Stem and Petiole Blight on Sweetpotato

Observations made during surveys in Uganda indicate that the extent of stem and petiole infection during favorable climatic conditions is quite extensive (Bua *et al*., 2001; Osiru, 2002). The severe effect of the disease on the vines was seen during growth periods of dry weather when lesions, particularly on the petioles become bleached and cracked leading to development of dieback symptoms. Generally, decline of photosynthetic area due to leaf damage is often the initial effect of *Alternaria* leaf blight. In very severe attacks, vines may be killed within a very short period of symptom development leading to 100% crop failure, while milder attack causes defoliation (Skoglund and Smit, 1984). Premature defoliation may have an adverse effect on growth and development of the plant, and hence its production. If the main stem is affected at a younger stage, the plant dies before it manufactures its food but

attack in later stages of plant development results in no loss in root yield (Lenne, 1991). Disease and lesion size increase as altitude increases (Ndamage, 1988).

The early or initial symptom of the disease in the fields is the appearance of small black oval and circular lesions about 1mm in diameter on the stems and petioles. The lesions become irregular as they enlarge or when they coalesce. Under favorable weather conditions the lesions continue to enlarge and completely girdle the stem and petiole (Skoglund and Smit, 1994). Under stress conditions, severe infections eventually result in the death of the whole terminal shoot or individual leaves. The lesions are initially superficial and become depressed as they increase in size. An individual lesion on the stem may enlarge to 5 cm length. Affected leaves initially show yellowing and eventually the whole leaf blade or lamina dries up. Infection on the lower surface of the leaf can also lead to an even chlorosis of the blade. Occasionally death of leaves on the side of the stem above the lesion is observed. This occurs when a lesion does not completely girdle the stems especially with varieties that have thicker stems (Lopes *et al*., 1994). Shoot or tip dieback is another symptom associated with the disease. It is uncommon in wet weather except on varieties with thinner stems and petioles. Dieback is usually common in dry weather, when the lesions completely girdle stems and petioles and become bleached and cracked (Osiru, 2008).

Alternaria leaf petiole and stem blight can be considered as the most important fungal disease of sweetpotato in East Africa (Anginyah, 2001) and South East Asia (Lopes *et al*., 1994). The amount of damage to the sweetpotato plant is dependent on the stage of growth and prevailing environmental conditions (Skoglund and Smit, 1994). High relative humidity

or free water is necessary for infection and sporulation and the fungus survives in debris and spores are spread through infected planting material, wind, splashing rain and water (Ames *et al*., 1997). It is clear that *Alternaria spp* cause yield loss, unfortunately research has mainly focused on field evaluation but pathogenicity and genetic population variation has not been investigated in Uganda besides, research on relative resistance of sweetpotato cultivars in Uganda has not been done before (Osiru, 2002).

2.7.1 Improved *Alternaria* disease resistance

Sweetpotato is grown primarily as a subsistence crop in most developing countries, it has many diseases that attack it. Owing to the subsistence method of farming chemical control of these diseases is not widely practiced. Frequent planting with pathogen- free stock is also no enduring solution as warm climates lead to a high re-infection rate. Development of cultivars resistant to diseases is a viable option that makes both environmental and economic sense.

Resistance breeding depends on understanding of the range of the different strains of the pathogen that exist in the area of production. Unfortunately there is currently little or no information on strains of *Alternaria spp.* distribution in Uganda. According to Scot *et al*., (1992) the difference between healthy and diseased plants is sometimes due to non-genetic causes resulting in major breeding problem. The presence of healthy escapees in susceptible lines exacerbates the problem (Scot *et al*., 1992), suggesting need for vigorous testing and selection procedure and use of the principal methods of host plant resistance and phytosanitation. This study was conducted to map the geographical distribution of *Alternaria*

pathogen, characterize collected isolates using morphological and molecular markers and generate available information required to design a resistance breeding strategy.

2.7.2 Phytosanitation

Techniques for phytosanitation include rouging, selection of healthy (clean) looking materials to reduce the chances of inoculum (Van Bruggen and Smit, 1984). Phytosanitation as a control strategy for *Alternaria* disease has received limited attention and clear guidelines for the use of its component techniques have not yet been formulated. In practice, phytosanitation involves the use of fungus free planting materials and subsequent rouging of plants that become infected in the field. This however does not work well for fungus since the spores can live in the soil for years and can be washed by floods and splashed on plants. Through use of pathogen free (clean) planting materials, the health of sweetpotato planting materials can be maintained. Cultural methods for control of *Alternaria* blight include, manipulation of planting date, intercropping, use planting of pure varieties with no mixtures, controlling soil erosion (Kapinga *et al*., 2008).

2.8 Genetic variability in plant pathogen population

Evolutionary forces, mutation, genetic drift, reproduction and mating system, gene flow and natural selection result in variability in pathogen population (Okori, 2004). Mutation, a change in the DNA at a particular locus introduces new alleles in a pathogen (Linde, 2002), thus the primary source of new virulent pathotypes in pathogen populations. Genetic drift is a random process that leads to unpredictable changes in pathogen population over a short period of time. Gene flow breaks down the boundaries of isolate population and promote

fixation of different alleles (genes) in local populations by exchanging alleles or individuals (genotypes) among geographically separated populations. Gene flow introduces new selectively adapted alleles in new environments and is responsible for a number of epidemics that have plagued agriculture (McDermont and McDonald, 1993). Natural selection is a directional process that leads to an increase in frequency of selected alleles or genotypes in a population (Zhan *et al*., 2004).

Genetic structure, amount and distribution of genetic variation within and among populations over time are the summed effect of interaction among fore mentioned evolutionary forces (McDonald, 2004). Monitoring of pathogen variation provides information about the structure of pathogen populations that is relevant to breeding programs for development and deployment of host resistance.

2.8.1 Characterization of pathogenic and genetic diversity of plant pathogens

Detection of pathogen variation has traditionally relied upon identification of virulence variation (races) in the pathogen population by inoculating pathogen isolates on host plants and observing the resulting compatible or incompatible disease reaction. Besides time consuming and highly error-prone conventional pathogenicity assays, morphological and biochemical tests have been used as alternative genotyping methods to examine the biodiversity of members within the genus *Alternaria* (Simmons, 1995). Molecular tools have also been used to elucidate principles underlying the evolution, population genetics of pathogens, plant pathogen interaction. They are relatively neutral and differ in their technical

differences, reliability, sensitivity, cost, speed; amount of DNA needed and ease of use (Antonio *et al*., 2004).

With the advancement of molecular techniques, several studies have examined taxonomic relationships among small-spored catenulate *Alternaria* spp using a variety of methods in an attempt to establish consensus with contemporary morphological-based species. Molecular markers have proved to be more reliable than morphological characterization in pathogen characterization. Many molecular markers have been used to simplify genetic diversity assessments. The most commonly employed molecular techniques include amplified fragment length polymorphism, randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and repetitive polymerase chain reaction or rep-PCR (Mueller *et al*., 1996). The genetic differences with these molecular tools reflect variability existing in the whole genome (Mitchell *et al*., 1995). PCR amplification with specific primer sets provides an alternative method for analyzing genetic variation at DNA level within and between populations (Innis *et al*., 1990). Since different molecular techniques differ in their power to resolve genetic differences (Antonio *et al*., 2004), more discriminating techniques such as RAPD-PCR are needed to further characterize genetic diversity of *Alternaria* population. With the advancement of molecular techniques, several studies have examined taxonomic relationships among small-spored catenulate *Alternaria* spp using a variety of methods in an attempt to establish consensus with contemporary morphological-based species. Molecular markers have proved to be more reliable than morphological characterization in pathogen characterization. Many molecular markers have been used to simplify genetic diversity assessments.

2.8.2 Morphological Characterization

Morphological characteristics of conidia and conidiophores provide the major taxonomic criteria for delimitation of fungal species (Hughes, 1953). *Alternaria* species is further complicated by their morphological characters, which is affected by both internal environmental factors. This variability has resulted in descriptions of obscure species never verified by other researchers (Ellis, 1971). Problems result from the similarity of morphological features or characteristics of other species as well as differences in various isolates of same species. Thus in quantifying size dimensions, number of transverse or longitudinal septae, isolates often fit a number of different species. However, the genetic make up, environment and field management practices influence the morphology of the pathogen (Simmons, 1985), limiting the accuracy of morphological pathogen characterization. This creates difficulty in delineating or delimiting isolates as well as identifying new or rare pathogen species that they may be involved. An example of such occurrence could be *A.triticina,* with dimensions similar to *A. alternata* which has only been reported in India (Rotem, 1994).

CHAPTER THREE

3.0 DISTRIBUTION AND SEVERITY OF *ALTERNARIA* BLIGHT OF SWEETPOTATO IN UGANDA

3.1 Introduction

Alternaria species are important pathogens of a wide variety of crops and weeds (Abbas *et al*., 1995). Species like *Alternaria solani* cause serious blight on potato (early blight), tomatoes as well as some *Brassicae* species. *Alternaria brassicae* attacks a wide range of cruciferous plants (Parry, 1990). Four of these species of *Alternaria* genus; *A. alternata*, *A.capsici annui, A. solani* and *A. tenuissima* have also been reported on three other species of *Ipomoea* (Lenne, 1991). *Alternaria* species exhibit considerable morphological plasticity that is dependant upon cultural conditions of substrate, temperature, light, and humidity (Anginyah *et al*., 2001).

In addition, the presence of any *Alternaria* species is influenced by a number of factors such as climatic conditions, host crops and management conditions (Osiru *et al*., 2002). *Alternaria* species may occur as saprophytes or pathogenic on various agricultural crops. However, this occurrence does not provide information on their economic importance nor agricultural impact of the disease in any given area. In the highlands, the disease is known to be very severe and exists throughout all seasons with yield loss on crops. In low lands the disease is seen on senescing plants and appears mainly in dry season cam mistaken to be sun scotch effect. On sweetpotato plants, the disease is frequently observed at various stages of growth, from three months until harvest (Osiru, 2008) despite the fact that there are many species

associated with the disease in sweetpotato plants; research has not addressed the etiology, temporal and spatial distribution of the pathogen diversity in Uganda. Therefore this research was conducted to: i) determine the distribution, incidence and severity of Alternaria blight in the major sweetpotato growing regions in Uganda, gather farmers indigenous knowledge and document information on sweetpotato present in Uganda.

3.2 Materials and Methods

A survey was conducted in 17 major sweetpotato producing districts of Uganda during the cropping seasons of the year 2002. A total of 170 fields in 17 districts were surveyed for *Alternaria* blight disease from January-May 2007. The numbers of fields surveyed were uniform (10). Disease occurrence was recorded in all the 17 districts surveyed.

3.2.1 Selection of districts

A total of 170 farmers in 17 districts (10 farmers from different sub counties were interviewed on the major of sweetpotato production constraints. The surveyed districts were purposively selected on the basis of sweetpotato production (tons) and area (ha) under production statistics obtained from the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF). The districts represented various agro- ecological zones of Uganda consisting of the tall grassland (along Lake Victoria), and western tall grassland zone and the savannah (short grassland). The districts included: Iganga, Kamuli, Busia, Sironko, Soroti, Apac, Masindi, Hoima, Kibale, Luwero, Wakiso, Mpigi, Masaka, Rakai, Mbarara, Ntungamo and Kabale (Fig 1).

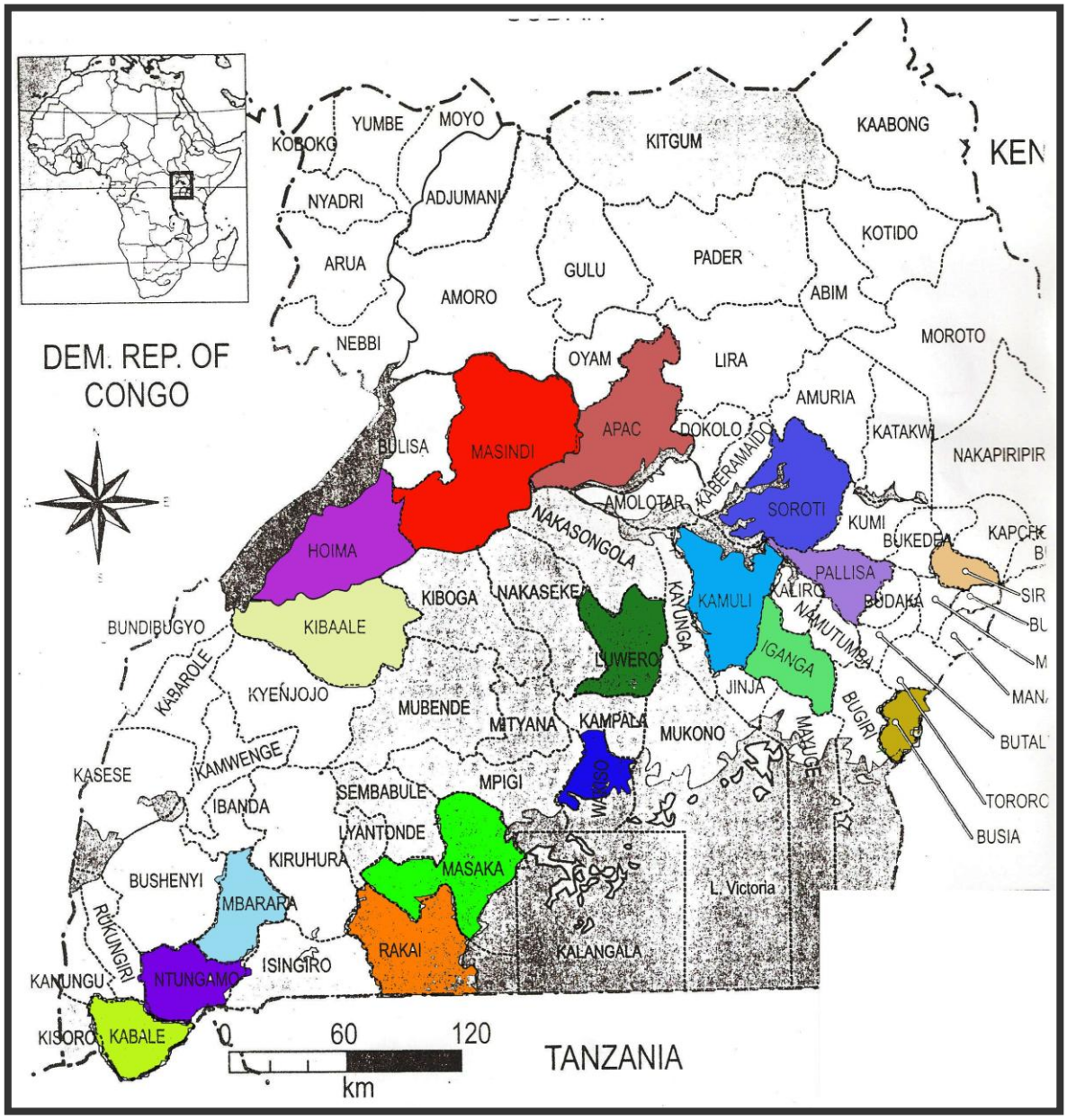

Figure 1: Map of Uganda showing the 17 surveyed districts.

3.2.2 Disease Assessment and interview

A questionnaire and checklist were administered to provide information on farmer perception and indigenous knowledge on *Alternaria* blight disease. The farmers were interviewed to

obtain indigenous knowledge related to the *Alternaria* blight disease. The local name for the disease, age of the crop, variety grown, place of origin history of the field, cropping system, planting system, source of planting material, and *Alternaria* control strategy used. Other areas of interest were susceptibility and resistance of varieties weed challenges, varieties preferred and grown and diseases, pests affecting sweetpotato production and diseases and pests management aspect.

Local collection site information; latitude, longitude and longitude were obtained using a Garmain GPS V2.0. The major areas of concern were variety performance, weed challenges, diseases, pests, proper agronomic practices, availability of planting materials and marketing problems. The unit of sampling in the field was the sweetpotato plot rather than the farmer from whom the sample was collected. Data collection was based on the diseased plants in the field. The presence or absence of *Alternaria* symptoms on sweetpotato plants was recorded. In each, 20 plants selected on field diagonals were assessed for disease severity. Disease severity was based on visual estimation of percentage leaf area affected blighted on 20 randomly selected plants field using the 1-5 scale (CIP, 1995) as follows; guide as follows: 1 = 0 % or No disease; 2 = < 1 or few small lesions; 3 = 1–10 %; 4 = 11–25 %; 5 = > 50 % leaf area affected.

3.2.3 Sample collection

Infected cuttings of each plant with clear *alternaria* lesions (1 to 3) depending on the severity of the disease in the field were collected from the fields. Attempt to spread the sampling points in different parts of the villages to ensure ecological diversity was achieved by driving

6 - 7 km from one field to the next one. We avoided the collection of same species by picking samples from different spots. Samples were stored and cultured in a laboratory at Makekere University Agricultural Research Institute Kabanyolo (MUARIK).

3.3 Data analysis

Social economic data was used to compute frequencies and Disease severity data used for computing means among districts. All the data from the 17 locations were combined and analysed using the SAS program. Entry means were separated by standard by standard error of the Duncan multiple Test at 5 % probability level.

3.4 Results

3.4.1 Assessment of field by researcher

A total of 170 fields in 17 districts were surveyed for *Alternaria* leaf, petiole and stem blight disease. The number of fields surveyed were uniform for all districts, 10 fields were surveyed per district. Farmers reported that they had first seen the *Alternaria* symptoms in their crops starting as long as 3-5 years ago, and as little as one year ago, but most frequently approximately two years ago. *Alternaria* blight disease is rapidly becoming a threat to sustainable sweetpotato production. Survey results demonstrated the prevalence of *Alternaria* blight disease throughout all districts surveyed. Nearly all sweetpotato fields examined displayed diagnostic *Alternaria* symptoms from 3-4 months onwards of growth.

3.4.2 Production constraints

The survey results showed several challenges in sweetpotato production. However, according to farmers' ranking, shortage of planting materials was the most prevalent constraint, and pests least affected production. Farmers also pointed out that there are mainly two diseases (Sweetpotato virus disease (SPVD) and *Alternaria* leaf blight disease) affecting sweetpotato production and productivity (Fig 2).

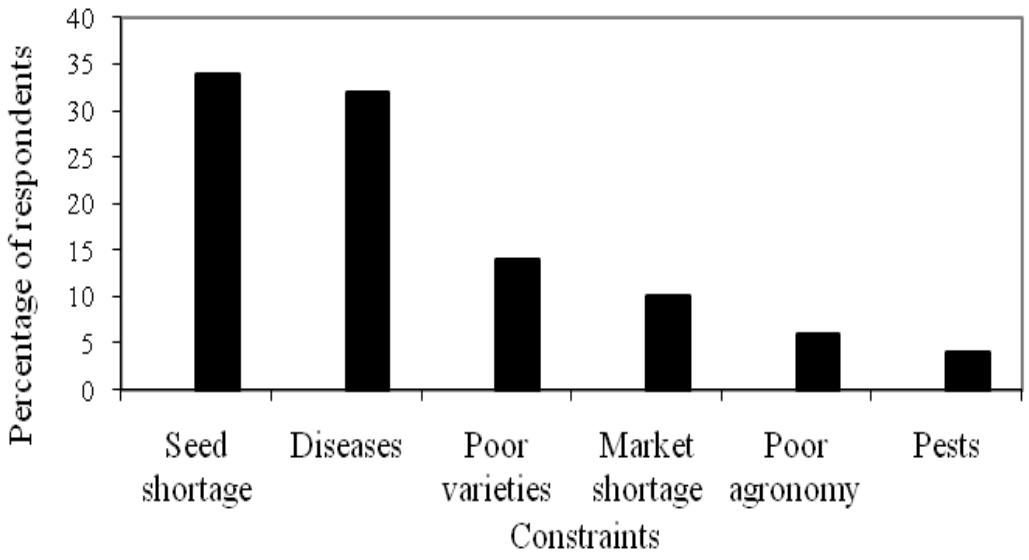

Figure 2: Sweetpotato production Constraints

3.4.3 Source of planting material

Majority of the farmers (81.2 %) reported that they get planting materials from their old sweetpotato fields and sprouts. Other minor sources included neighbors/friends and markets. Many new varieties, which are a result of chance seedlings, were growing in farmers fields. Only 5.9 % of the farmers indicated getting materials from rapid multiplication beds (RMTs). Majority of the farmers interviewed indicated no access to improved cultivars.

3.4.4 Planting method and cropping system

The planting methods deferred from farmer to farmer, 97.1 % of farmers planted varieties as mixtures in the same field and only 2.9 % separated varieties of sweetpotato, these happened to be commercial farmers. The farmers predominantly intercropped sweetpotatoes with other beans (51.2 %) for food security; whereas only 48.8 % of them planted their sweetpotatoes as a pure crop stand.

3.4.5 Control measures for *Alternaria* blight

Most farmers (78.1 %) did not take any measures of control especially for *Alternaria* blight disease. A small portion of the farmers (17.1 %) were using clean planting materials as a means of controlling *Alternaria* blight and very few practiced rouging method.

3.4.6 Source of information about *Alternaria* blight and its symptoms

The results indicated that the majority of the farmers (80.6 %) did not correlate the wilting symptoms observed to *Alternaria* blight disease. Only 7.6 % of the farmers found about the disease through researchers, 10.6 % got information from friends/neighbors and other farmers in the area. Other sources of information included extension and mass media. These results reveal a lack of proper information flow to the farmers. The results of the major criteria used in the adoption of sweetpotato cultivars show that the farmers (60.6 %) selected the cultivars they grow because of their yield attributes while (24.1 %) simply grew those cultivars which were available in terms of planting materials at the time of planting. Other reasons for selection of a cultivar included disease resistance, drought resistance, high dry matter/ good taste and early maturity.

3.4.7 Disease incidence and severity

The highest incidence of Alternaria disease was observed in Wakiso district (91 %) followed by Mpigi (87 %). Moderate disease incidence of 46.0 %, 50% and 51 %) were recorded in Hoima Masindi and Ntugamo respectively. The lowest disease incidence was noted in the districts of Kibale, 18 %), Mbarara (32 %) and Sironko (33.5 %) respectively. The incidence of *Alternaria* was seen to be low in Kibale and Sironko where some fields were 100 % clean (symptomless) in the same districts. *Alternaria* disease incidence ranged from 18 % -91 %. Disease severity was detected with less than 5 % severity in most fields (Table 1).

Table 1 Mean incidence and severity of sweetpotato fields surveyed for occurrence for *Alternaria* blight disease of sweetpotato in Uganda.

District	Frequency	Mean incidence for Alternaria (%)	Mean severity for *Alternaria*
Wakiso	200	91.0	3.2 a
Mpigi	200	87.0	2.9 b
Apac	200	80.0	2.4 c
Luwero	200	70.5	2.3 d
Busia	200	75.0	2.2 d
Masaka	200	78.5	2.2 d
Rakai	200	61.5	2.2 e
Kamuli	200	62.0	2.2 e
Iganga	200	75.5	2.0 f
Soroti	200	68.5	1.9 f
Kabale	200	71.0	1.8 g
Hoima	200	46.0	1.8 g
Masindi	200	50.0	1.5 h
Mbarara	200	32.5	1.4 i
Ntungao	200	51.0	1.3 i
Kibale	200	18.0	1.3 i
Sironko	200	33.5	1.2 j
Mean			**2.00**
LSD$_{(0.05)}$			**0.17**
CV (%)			**21.90**

Incidence-refers to plants diseased/total plants x100%; Severity-based on a scale of 1-5, where **1** = 0% or No disease; **2** = < 1 or few small lesions; **3** = 1–10 %; **4** = 11–25 % and **5** = > 50 %. Means separated using Duncan's test; followed by the same letter are not significantly different at P <0.05

3.4.8 Distribution of Alternaria blight disease of sweetpotato

In general very low disease severity was recorded was detected during the survey ranging from 1.2-3.2. There were no significant differences in disease occurrence among districts. Great variations were observed in the severity rates of *Alternaria* blight in the cultivars

grown by farmers in different districts. The mean altitudes of fields ranged from 1014 to 2044 m.a.s.l. There was a significant variation (P< 0.05) in severity among surveyed districts. Most cultivars had low to moderate field resistance to sweetpotato virus disease (SPVD) and *Alternaria bataticola* blight. Both diseases can be devastating, causing high storage root yield losses (50 to 90 %) in susceptible clones (Gibson *et al*., 1998; Osiru *et al*., 2009).

Central region had the highest occurrence of the *Alternaria* blight disease followed by South-western region; this was in line with Osiru, (2008) findings on *Alternaria* incidence and severity being high in Mpigi district and low in Kabale. The explanation for this variation is that; the weather conditions were not favorable enough in Kabale the "*Alternaria* hot spot" for disease development at the time of the survey and besides different areas grow different cultivars which have different disease resistance levels, the source of inoculums should be due to movement of planting materials containing inoculum These findings are in line with other findings from other researchers previously reported.

According to most of the farmers (52.4 %) sweetpotato virus disease is the major disease of sweetpotato, followed by *Alternaria* (45 %) and lastly root rot (2.6 %). This was in line with Bua, *et al* (2001) findings on the survey on sweetpotato production constraint. There was a strong positive correlation (0.97, P<0.05) in the *Alternaria* and SPVD severity per district (Fig. 3). A high positive correlation was also observed between SPVD and *Alternaria* in on station trials (Yada, 2009). The strong positive correlation between SPVD and *Alternaria* is expected since plants infected with one disease get weakened and severely succumb to the

other. The districts in the central region have high SPVD and *Alternaria* whereas districts in the eastern Uganda had low SPVD and *Alternaria*. This shows that the environment which favors *Alternaria* also favors the vectors for SPVD thus explaining the relationship. Previous research has shown high SPVD (Mukasa *et al*., 2003; 2006) and high *alternaria* (Osiru *et al*., 2008) in the central regions of Uganda.

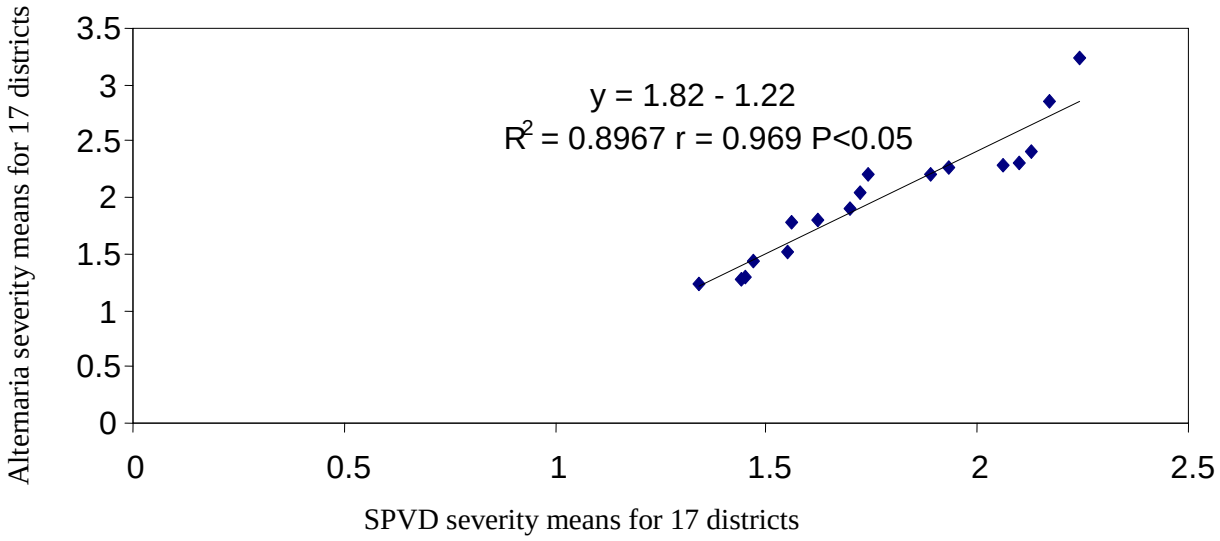

Figure 3: Correlation between *Alternaria* and SPVD severity in the 17 surveyed districts

CHAPTER FOUR

4.0 MORPHOLOGICAL CHARACTERIZATION OF ALTERNARIA BLIGHT ISOLATES IN UGANDA

4.1 Introduction

Morphological characteristics of conidia and conidiophores provide the major taxonomic criteria for delimitation of fungal species (Hughes, 1953; David, 1991). *Alternaria* spp, including *A. alternata*, do exhibit considerable morphological plasticity that is dependent upon cultural conditions. The collected isolates needed to be morphologically characterized in order to identify the causal agent for *Alternaria* disease and to differentiate between species.

Alternaria species is further complicated by their morphological characters, which are affected by both external and internal environmental factors. This variability has resulted in description of obscure species never verified by other researchers (Ellis, 1971). Problems result from the similarity of morphological features or characteristics of other species as well as differences in various isolates of same species. Thus in quantifying size dimensions, number of transverse or longitudinal septae, isolates often fit a number of different species. This creates difficulty in delineating or delimiting isolates as well as identifying new or rare pathogen species that may have evolved. An example of such occurrence could be *A. triticina,* with dimensions similar to *A. alternata* which has only been reported in India (Rotem, 1994).

A total of 299 isolates were assembled at Makerere University Agricultural Research Institute (MUARIK). Although some field evaluation work has been done on *Alternaria* (Osiru *et al*., 2008), the aspect of morphological characterization of the *Alternaria* isolates has not been fully researched on. The characterization of the isolates in this study will facilitate breeding for resistance. The objective of this study was to characterize the *Alternaria* isolates in Uganda using morphological markers in order to improve understanding of the diversity and race differentials to enhance breeding for resistance.

4.2 Materials and Methods

4.2.1 Experimental sites

The laboratory experiments were done in Biotechnology Laboratory, Department of Crop Science at Makerere University and National Crops Resources Research Institute (NaCRRI). Screenhouse experiments were carried out at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) located 19 km north of Kampala at an altitude of 00 28’ N, longitude 32 37’ N and at a mean altitude of 1200 meters above sea level. It receives an annual rainfall of 1300 mm with a mean maximum and minimum temperature of 28.5º C and 13.0º C, respectively. The area has ferrallitic, heavy but well-drained soils.

4.2.2 Isolation and Culturing of Alternaria isolates

4.2.2.1 Collection of isolates and preservation of diseased samples

Isolates of *Alternaria* spp. were obtained from sweetpotato plant tissues collected in 17 districts in Uganda. At most isolates were obtained during dry season of the year. Vines and leaves showing characteristic symptoms of *Alternaria* blight were collected from the 17

districts. From each district at least three samples were collected from every 10 fields in a disctrict and put in paper bags. The infected materials were transported to Makerere University Agricultural Research Institute Kabanyolo (MUARIK).

4.2.2.2 Isolation and culturing of the Alternaria blight fungus

To obtain isolates from sweetpotato plant tissues, 5-10 g of plant material with lesions were placed in 500 ml of sterile H_2O and agitated by hand shaking for 30 minutes. To initiate sporulation, the pieces of plants with lesions were placed in petri dishes lined with moist tissue paper. Excess water was drained using filter paper. Plates were incubated under natural light at room temperature for 7 to 10 days.

4.2.2.3 Incubation of infected tissue

Small pieces of infected leaf petiole and stem/vine tissues were surface sterilized using 3% sodium hypochlorite (NaOCl) for 5 minutes and rinsed in 3 changes of sterile distilled water. Excess water was removed using sterile tissue paper. The samples were placed in moist chambers prepared using tissue paper lined on petri dishes. The surface sterilized infected vines and leaf parts were incubated for 24 hrs to allow sporulation as described by Van Bruggen (1984).

4.2.2.4 Single spore isolation

A total of 40 putative single spore *Alternaria* colonies were randomly picked from the lesions on infected plant parts under binocular microscope, and seeded onto surface of water agar using tip of a sharp sterile inoculating needle. Inoculated plates were incubated on laboratory

bench at room temperature (20-24 ºC). Conidial germination on the plates was checked daily and upon germination, agar blocks bearing single germinated conidia were cut out and aseptically seeded on three media types Calcium Carbonate ($CaCO_3$) and sweetpotato vine decoction media (SPVDM) plates. The plates were incubated for 14 days at room temperature and natural lighting conditions (20-24 ºC and 12-hrs light). Plates of primary media were centrally inoculated with 2-mm diameter plugs taken from the edge of actively growing 4 day old cultures, and then incubated at 25 ºC after 72 hours, the colony diameters were measured and agar blocks transferred to the fresh media. Spore yields were determined with a haemocytometer after 24 hrs of incubation on different media.

4.2.3 Identification of the pathogen

Comparison of cultures/isolates with known cultures of *A. bataticola* and *A.alternata* was done using a modification of the slide culture technique described by Riddle (1950).

4.2.4 Evaluation of morphological characters

For all isolates, morphological characteristics of the colony and sporulation apparatus were determined from single-spored colonies as described earlier. The nature of mycelia growth, nature of conidiophores formation, arrangement of conidia on conidiophores and shape of conidia were noted. The size and shape of conidia (length and width) was determined using ocular and stage micrometer.

4.3 Effect of media type on growth and sporulation of Alternaria blight isolates

4.3.1 Media preparation

The objective of this experiment was to establish the suitable growth conditions for *in vitro* growth and sporulation of *Alternaria* pathogen. Investigation was done on growth rate and conidia formation of the *Alternaria* pathogen. Four types of media were used to determine their effect on growth and sporulation of the pathogen. These were potato dextrose agar (PDA), Calcium carbonate ($CaCO_3$), sweetpotato vine decoction media (SPVDM) and calcium carbonate on technical agar (sporulation media). The four media served the purposes of growing of cultures, cleaning of cultures and sporulation of cultures. Growth media for *Alternaria* species was i) Potato dextrose agar (PDA) made of 39 g PDA and 1000 ml water. ii**)** Ca Co_3 (S- media) made of Calcium carbonate (30 g), agar (20 g) and sucrose (20 g). iii) Sweetpotato vine decoction media (SPVDM) made of Sweetpotato vines (100g), agar (2 g) and water (1000 ml). 100 g of freshly picked vines were cut into inch length and washed with sterile water. The cut pieces of vines were wrapped in a beaker. 39 g of technical agar was prepared in 1000ml of distilled water. The agar and the vine pieces were autoclaved at 121 ^{0}C for 15 min and approximately 20 ml were dispensed into sterile 90 mm plastic petri dishes to form a layer of 2 mm deep.

4.3.2 Inoculation and Incubation

The media was inoculated using a method described by Hyre and Cox (1953). A uniform culture plug of 4 mm in diameter was obtained from a 14-day-old culture plates and placed in the centre of each dish. The plates were incubated at room temperature.

4.3.4 Experimental design

The experiment was arranged in a completely randomized design with 3 replicates. Two factors, media and incubation period were studied in a factorial combination.

4.3.5 Measurement of fungal growth and sporulation

Measurements on radial colony diameter were taken on 2, 4, 6, 8, 10, 12, 14 days after inoculation whereby six plates were sampled each time for each media treatment. To determine conidial concentration of each medium, ten plates were prepared. Two plates were sampled randomly from each media treatment at 8, 10, 12, 14 days after inoculation. 10 ml of sterile distilled water was added to culture plate and using a sterile glass slide, the culture surface was gently scrapped to make a conidial suspension. Conidial concentration was determined using a Neubaure improved heamocytometer.

4.3.6 Statistical analysis

Averages of colony diameter and number of conidia/ ml for each treatment combination were for subsequent data analysis. Analysis of variance and separation of means to determine differences in growth rates on media types was carried out at 5 % probability levels of F-test. Significant effects were identified using Duncan's multiple range test and least significant difference test at 5 % probability level.

4.4 Results and Discussion

4.4.1 Morphological characterization

A total of 299 *Alternaria* isolates collected from the 17 districts were studied. Based on characteristics of single spored colonies, all sweetpotato *Alternaria* isolates could be grouped into two colony types (Table 2). Group 1 consisted of colonies that were lettuce green to olive green and usually had a prominent (2-5 mm) white margin (Plate 1a). Colony texture was felty to woolly. This group produced a red maroon pigment but 30 % of the isolates produced whitish crystals in the agar medium underneath the mycelial mat. Isolates typically produced colonies that were brown, ellipsoidal, 20-60 x 7.5–1.5 µm (over 70 mm) in diameter after 7 to 10 days. These characteristics were most similar to those of the *A. alternata* descriptors (Plate 1b). Group 2 isolates produced colonies that were typically dark olive gray to iron gray to castor gray color. The colony margin of these isolates was often wavy or torn. Colony texture was generally felty to woolly. Colony growth was generally much reduced, < 50 mm after 7 to 10 days. The mycelia appeared to be clear. These characteristics were most similar to those of the *A. bataticola* descriptors.

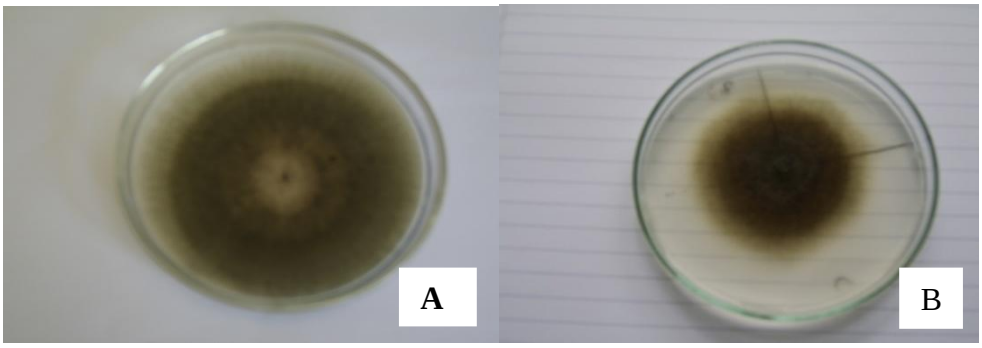

Figure 4: *A. bataticola* on PDA (**A**) and *A.alternata* on PDA (**B**)

Based upon sporulation habit of single-spored colonies, all sweetpotato isolates were grouped into two sporulation types. Group A isolates were characterized by the formation of conidial

chains (bead like chains) 6 to 14 conidia in length and the development of numerous secondary, and occasionally tertiary, chains 2 to 8 conidia in length. Short beaked, with 2 to 6 transverse septa, and 0-4 longitudinal septa and were catenulate at the apex of the conidiophores (Plate A and B). Chain branching occurred in a sympodial manner through the elongation of secondary conidiophores from distal terminal conidial cells and subsequent conidium formation, conidia were typical ovate in shape and the development of apical extensions was minimal. These characteristics were most similar to those of the *A. alternata* representative culture. Based on these results, these isolates were placed in the *A. alternata* species- group.

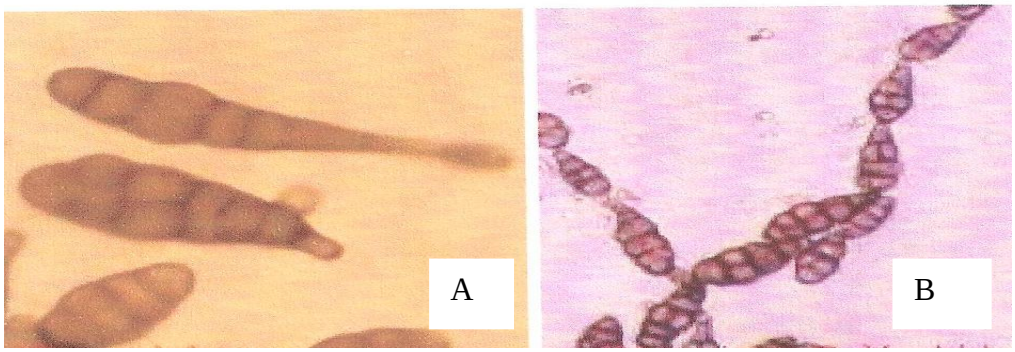

Figure 5: *A. bataticola* conidia with long armed conidia (**A**) and *A. alternata* conidia chained conidia (**B**)

A total of 299 isolates were successfully characterized morphologically and 97.7 % were found to be *A. bataticola* and only 2.3 % were belonged to *A. alternata* species. From the findings *A. bataticola* is most widespread; it occurs in all districts surveyed, whereas *A. alternata* occurs in only 8 districts (Table 2).

Table 2: Sweetpotato *Alternaria spp* isolates collected from different districts of Uganda during 2007

District	*A. bataticola*	*A. alternata*	Total
Iganga	25	0	25
Busia	26	1	27
Sironko	10	0	10
Soroti	41	1	42
Apac	10	0	10
Masindi	9	1	10
Hoima	22	1	23
Masaka	26	1	27
Rakai	21	1	22
Mbarara	15	1	16
Ntungamo	15	0	15
Kabale	21	1	22
Mpigi	18	0	18
Luwero	10	0	10
Wakiso	13	0	13
Kamuli	10	0	10
Total	**292**	**7**	**299**

4.5 Effect of media on isolate sporulation

The study also tested the influence of medium on sporulation of *A. alternata* and *A. bataticola* on three different media. The different media showed variable responses to isolate sporulation. Calcium carbonate media (CaCO3) showed the most conidial (4.0×10^6 /ml) compared to PDA with lowest spore (0.8×10^6/ml). SPVDM and CaCO3 were used as

sporulation media since the spore yields were high at a temperature of 22ºC - 25ºC. (Plate A). Potato dextrose agar (PDA) was used as a growth media since it contains growth promoting nutrients suitable for faster growth of cultures.

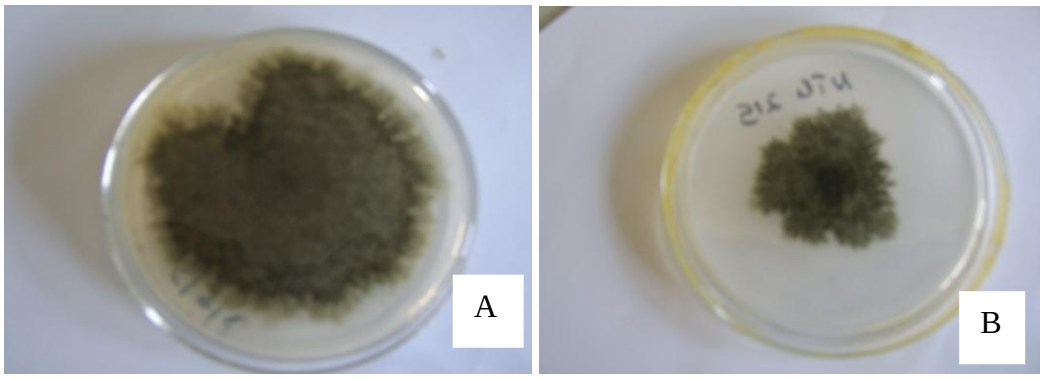

Figure 6: Sporulation of *A. bataticola* on $CaCO_3$ **(A)** and *A.alternata* on $CaCO_3$ **(B)**

4.6 Incubation conditions

Alternaria isolate cultures grew slowly on Potato Dextrose Agar as opposed to host based decoction media. Potato Dextrose Agar also exhibited the least growth in the entire incubation period. There was markedly more growth on Sweetpotato Vine Decoction Medium (SPVDM) and Calcium carbonate sporulation medium ($CaCO_3$). By the 7^{th} day, both SPVDM and $CaCo_3$ had grown to full plates. By that time the growth on plates had reached $\geq$ 2.5 cm (Table 3). The horizontal mycelial growth was faster in $CaCO_3$ and in SPVDM whereas vertical growth on PDA was greater. Analysis of variance revealed that incubation period, media interaction was highly significant. The highest growth occurred in $CaCO_3$ (4.32 cm) and (2.55 cm) on isolates APC 101 and MSD 111 which were *A. bataticola* and *A. alternata* after 7 days of incubation while after the same period SPVDM and PDA recorded 3.82 cm, 1.82 cm and 3.60 cm, 1.34 cm respectively. Out of the 299 isolates 25 produced a diffusible pale orange pigment and abundant crystals in the agar medium underneath the mycelial mat.

Table 3: Influence of primary medium on growth and sporulation of 2 *Alternaria* species

Medium	Isolate	Diameter	Spores /plate
$CaCO_3$	APC 101 (*A.b*)	4.33 cm	4.0×10^6 a
SPVDM	APC 101 (*A.b*)	3.82 cm	2.8×10^6 b
PDA	APC101. (A.b)	3.60 cm	2.6×10^6 b
$CaCO_3$	MSD 111 (A.a)	2.55 cm	1.7×10^6 c
SPVDM	MSD 111 (A.a)	1.82 cm	1.4×10^6c
PDA	MSD 111 (A.a)	1.34 cm	0.8×10^6d

Data followed by same letter do not differ significantly, P = 0.05 according to Duncan's multiple range test.

Cultures of *alternaria* grew and sporulated on the culture media tested. However, appreciable variations under these cultures were observed with optimal peaks noted. Host decoction media, sweetpotato vine decoction media and calcium carbonate supported faster growth and abundant sporulation than potato dextrose agar (PDA).

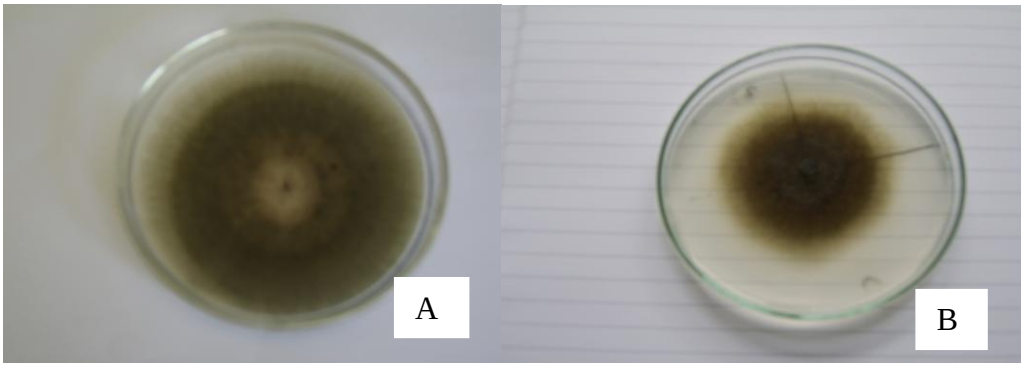

Figure 7: Mycellia growth of *A.bataticola* on CaCO3 **(A)** and *A. Alternata* **(B)**

David, (1999) reported less growth and poor sporulation of *Alternaria* bataticola on PDA, moderate growth on sweetpotato tuber agar and abundantly on tap water agar with wheat straw. Ojiambo (1997) reported less growth and sporulation of *Alternaria sesame* on PDA and MEA as opposed to host decoction media and oatmeal agar. Ann (1993) noted faster growth and abundant sporulation of *Alternaria tax sp. (IV)* on PDA and MEA. The faster growth of the pathogen on host based media can be attributed to lower nutrient availability especially carbon, thus promoting efficient utilization of nutrients by the fungus to finish its growth cycle. On the other hand Calcium carbonate ($CaCO_3$) supports abundant sporulation which can be attributed to the pH level present in the media. Acidic media conditions in the media favoured abundant sporulation of the pathogen while alkaline conditions enhance vegetative growth (Anginyah *et al*. 2001). There was an increase in conidia production with prolonged incubation period, reaching a peak after 13 days, and then starting to decline, with germination of some conidia to give vegetative growth. This could be attributed to media alteration by the fungus during growth.

In conclusion, the results showed that conidia formation may be influenced by media conditions, temperature and time of incubation as reported by Anginyah *et al*. (2001). The implication to these natural conditions is that, most growth and sporulation occurred with specific media conditions ($CaCO_3$) and SPVDM), optimum temperatures (22 ºC-25 ºC). Since the growth conditions are not easily regulated, separation of Alternaria species was achieved by using molecular markers; restriction fragment length polymorphism (RFLP) and random amplified polymorphism DNAs (RAPDs).

CHAPTER FIVE

5.0 MOLECULAR CHARACTERIZATION OF UGANDAN *ALTERNARIA* BLIGHT ISOLATES ON SWEETPOTATO USING RAPDs AND RFLP MARKERS

5.1 Introduction

Alternaria leaf vine and stem blight of sweetpotato caused by *Alternaria* species occurs on sweetpotato hosts throughout the sweetpotato growing areas of Uganda. This has resulted in considerable damage to the sweetpotato crop. This study suggests the existence of two species referred to as *A. alternata* and *A. bataticola* morphotypes. However, these morphological differences may not be consistent due to environmental plasticity. Some isolates exhibiting high morphological similarity may not necessarily be similar genotypically. This could lead to erroneous characterization of the isolates. This necessitates the use of molecular techniques that can accurately distinguish isolates at genetic level. Restriction fragment length polymorphism (RFLP) and Random Amplified Polymorphic DNAs (RAPDs) markers were used to study the relatedness of these two species. Relationship among isolates was determined using cluster analysis. The objective of this study was therefore to characterize diversity among Ugandan *Alternaria* sweetpotato isolates using RFLP and RAPD markers.

5.2 Materials and Methods

5.2.1 Raising of Fungal Cultures for DNA Extraction

A total of 50 isolates putatively classified as A. *alternata* and *A. bataticola* species were used

in this work. The isolate cultures were maintained on potato dextrose agar (PDA). Fungal cultures were raised by using actively growing fungal plugs from mother cultures with a sterile scalpel and then placed on fresh PDA media (39 g of PDA/ 1 litre of distilled water). Some cultures were raised in sweetpotato decoction vine media. The cultures were then placed on laboratory benches and left to grow for about 7-10 days or until there was enough mycelia to harvest for DNA extraction.

5.2.2 Isolation of DNA

Fungal mycelia (about 2 g) from actively growing cultures were carefully peeled off from the PDA surface with a sterile scapel. The mycelia were then placed on a clean pre-chilled mortar, frozen with 10 – 15 ml of liquid nitrogen and crashed with a pestle into fine powder. The powder was then poured into a 1.5 ml eppendorf tube maintained at -196 ^{o}C in liquid nitrogen- bath. This procedure was repeated for all isolates. The powder was suspended by adding 1 ml of hot (65 ^{o}C) CTAB extraction buffer [2 % w/v Hexa decyltrimethyl-ammoniumbromide (CTAB), 8.2 % w/v NaCl (1.4M), 0.2 % v/v Mecaptoethanol (98 %), 1.2 % w/v EDTA (20 mM), 1 % w/v Tris-Base (pH 8, 100 mM) and 1 % w/v Polyvinylpyrodilone (PVP) 40) to each tube, mixed gently by inversion followed by incubation in a water bath (65 ^{o}C) for two hours.

The tubes were then cooled down to room temperature, and 0.5 ml of chloroform isoamylalcohol (24:1) was added, and the tubes shaken (100 times) by inversion. This was followed by centrifuging at 13,000 rpm for 15 minutes. The aqueous top layer (about 0.9 ml) was then pipetted to new tubes. Ice- cold isopropanol (0.6 ml) was then added, mixed the

tubes gently by inversion and incubated them at - 20 ^{o}C overnight in order to precipitate DNA. This was followed by spinning the DNA samples at 12,000rpm for 10 minutes. Isopropanol was then decanted carefully leaving a DNA pellet at the bottom of the tubes. The pellet was then washed with 1 ml of 70 % ethanol by mixing well by inversion, and then spinned at 12,000rpm for 10 minutes. The supernatant was ten poured off carefully by inverting the tubes slowly to save the DNA pellet, which was then dried in air (for about 2 hours). Fully dried DNA pellet was dissolved in 0.15 ml of TE [0.122 %w/v Tris-Base (pH8, 10 mM), 0.02 % w/v EDTA (l mM], left to dissolve and then stored at -20 ^{o}C.

For checking the DNA quality, approximately 3 µl of genomic DNA samples were mixed with 2.0 µl of loading dye and electrophoresed for 1 hour at 70 volts in 1 % TBE agarose gel stained with 10 µl of 10 mg / ml ethidium bromide. The DNA samples were run on 1 % agarose gel alongside 4.0 µl of standard molecular weight lambda DNA. The gel images were visualized under 100 % UV light and then photographed in the biotechnology laboratory at Makerere University.

5.2.3 Polymerase chain reaction (PCR)

Fifty *Alternaria* isolates were analyzed by PCR using the primers ITS 1 and ITS4. These primers amplify a fragment of DNA of 550 bp. Primer pairs (ITS1) 5'- TCC GTA GGT GAA CCT GCG-3' and (ITS4) 5'- TCC TCC GCT TAT TGA TAT GC- 3' were used for PCR amplification of rDNA containing the internal transcribed spacer ITS1 0.8 µl, and ITS2 0.8 µl regions (Jasalavich *et al*., 1995). Each DNA sample (50 ng) and the two primers (10 pmol) were added to a PCR bead) which contained 0.4 units of Taq DNA Polymerase, 2.5 µl

of $MgCl_2$ and 5 µl of each dNTP when brought to a final volume of 2.5 µl. The PCR reaction was performed for 35 cycles, with initial 2 min at 94^{o}C for denaturation and a final 7 min in a Progene Thermal cycler (Techne (Cambridge) Ltd, UK) at 72 ^{o}C for extension. Each cycle consisted of 30 s at 94 ^{o}C, followed by 30 s at 58 ^{o}C for annealing and 2 min at 72 ^{o}C for extension. Successful amplification was checked by electrophoresis in 1.2 % agarose gel.

5.2.4 Restriction fragment length polymorphism (RFLP)

Two restriction enzymes Hinf1, Hae*111* (Promega Corporation, Madison, WI) were used to digest PCR-amplified nuclear internal transcribed spacer. PCR amplification using total DNA was carried out in 25-µl reaction mixture containing 5 µl of DNA, 0.8 mM of each primer 0.5 µl dNTP, 3 µl $MgCl_2$, and 0.2 unit of AmpliTaq DNA polymerase in 5x AmpliTaq PCR buffer and 9.7 µl. PCR was carried out in a thermal cycler (Model 480) programmed for the following parameters: 94 ^{o}C for 2 min, 94 ^{o}C for 1.30 sec, 33 ^{o}C for 1.30 sec, 72 ^{o}C for 2 min, and 72 ^{o}C for 10 min for 35 cycles.

5.2.5 Random amplified polymorphic DNA (RAPD)

Genomic DNA from each isolate was used in the RAPD PCR analysis with the 2 operon-primers OPA-2 and OPA-4. Amplification was done in Progene Thermal cycler (Techne (Cambridge) Ltd, UK). The OPA-2 primer, (5'–TGC CGA GCTG-3') and OPA-4 primer ('5-AAT CGGG CTG–3') were used in this study. RAPD PCR was routinely performed in reaction mixture containing 2 mM $MgCl_2$, 1x PCR buffer (10x), 0.5 µl each dNTPs, 0.2 unit of Taq polymerase. 5.0 µl genomic DNA variable amount of PCR water to make up to a 25 µl. PCR reaction was performed as described above.

The amplified PCR products were resolved in 1.0 % agarose gels in 10x TBE buffer and 90 ml of distilled water and Visualized by UV illumination after staining in ethidium bromide. Restriction digestions were carried out using a 2 µl of PCR product in 10 µl reaction mixtures for 2 hrs according to enzyme manufacturer's recommendations. Digestion products were resolved in 1.8 % agarose gels in TBE buffer and visualized by UV illumination after staining in ethidium bromide.

5.2.5 Statistical data analysis

Samples that showed the clearest bands were scored. Both RFLP and RAPDs marker types were evaluated for presence or absence of hybridized fragments at specific positions. The PCR amplification products were scored as presence (1) or absence (0) of polymorphic DNA fragments in binary characters. RFLP and RAPD respective dendograms were obtained using the coefficient of Simple Matching. Cluster analysis was conducted using the unweighted pair group method with arithmetic mean algorithm (UPGMA) in the software (NTSYS-pc) version 2.2 (Rohlf, 1993) to generate a dendrogram.

5.3 Results and Discussion

DNA was successfully extracted from the fungal isolates using CTAB method (Connolly *et al*., 1994) and yielded the PCR products. Preliminary fingerprinting of the 50 representative isolates using internal transcriber spacer (ITS1) (5'TCC GTA GGT GAA CCT GCG 3') and (ITS 4) (5' TCC TCC GCT TAT TGA TAT GC 3') confirmed that these isolates to be of *Alternaria* species (Fig 8). All isolates were identical to a fungus previously identified as

Alternaria species by other studies. Analysis of the ITS regions revealed 100 % identity among the fifty isolates in the A. a*lternata* and A. *bataticola* species – group.

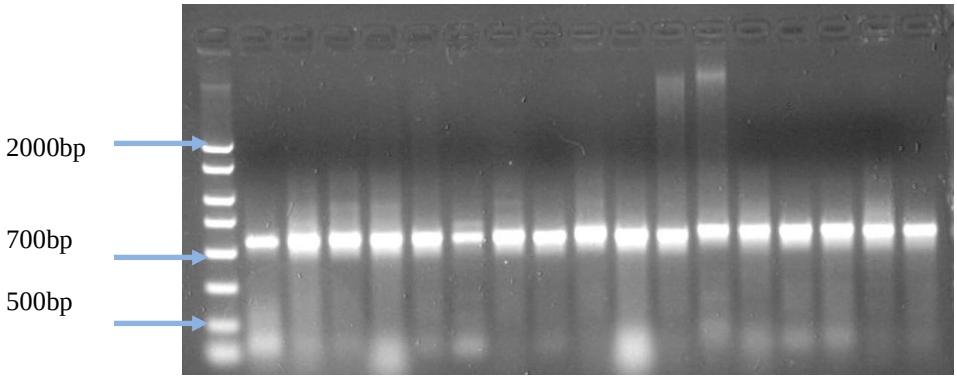

Figure 8: Agarose gels showing restriction banding pattern for ITS-PCR patterns for Alternaria isolates Lanes M: 100 bp ladder; 1: IGA22; 2: BSA23; 3:BSA27; 4:KML286; 5: MSK141;6:MBR196;7:MBR194;8:APC19;NTG215;10:MBR190;11:HMA137;12:SRT97;13:MSD111;14:MSD116; 15:MSD117;16:MPG254; 17:MPG259

5.4 Polymorphisms of Restriction patterns in Alternaria species

Target fragments amplified at 750bp. Restriction enzymes employed produced resolvable fragments *Hae111* gave 2 fragments with fragment size of 400 bp and 350 bp respectively (Fig 9). The second fragment gave 3 banding patterns with fragment sizes of 350 bp, 250 bp and 150 bp. Restriction enzyme Hinf1 also gave 2 banding patterns, the first one had 2 fragments with size of 500 bp and 250 bp. The other banding pattern had fragment size of 350 bp, 250 bp and 100 bp giving a total of 3 bands. In total both Hae111 and Hinf 1 gave a total of 7 fragments. From observation made, the isolates did not vary by species in the grouping because isolate 3 was *A.alternata*, 4 was *A. bataticola*, 5 was *A.alternata* and 7 was *A. bataticola* had similar patterns, suggesting that there are strains within the species, which could have been a result of mutation or recombinations. It would therefore be good to sequence these isolates to further understand their differences.

5.5 Phylogenetic analysis of Alternaria species using RFLPs

A cluster analysis of the RFLP individually revealed a low mean genetic difference of 0.62 in the *Alternaria* isolates. Two distinct clusters were evident: with isolates of the *A. alternata* group, and those for *A. bataticola* clustering regardless of the geographic origin. Therefore RFLP did not group isolates according to geographical origin neither did it group them according to morphological characters because groups contained isolates from more than one morphological group being clustered together (Fig 10). This implies that morphological structures tend to overlap, and thus making identification difficult.

However, this showed that though these isolates are morphologically different, they do share some allele in common that makes them cluster in one group. Group one rooted many clusters of both species. Similarly, cluster 2 had only a few clusters consisted of isolates of both species. This clustering is explained to be a result of movement of inoculums with planting material or some of the isolates sharing the same alleles.

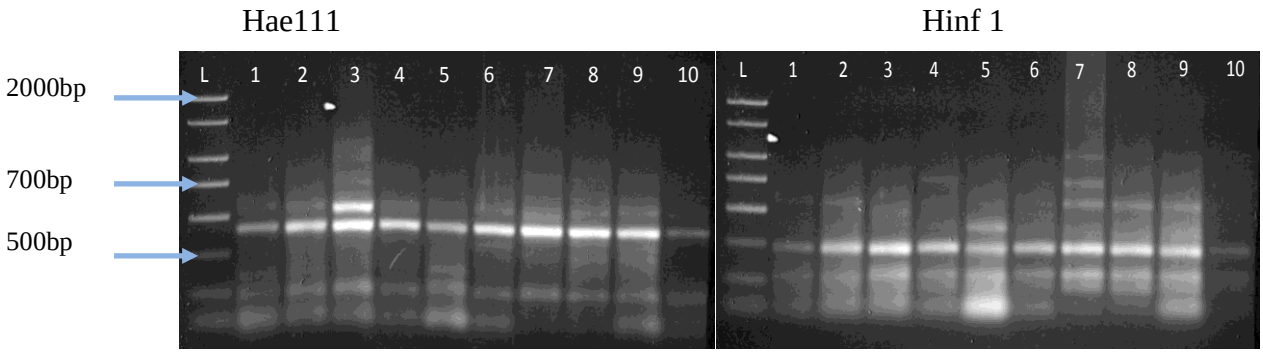

Figure 9: Agarose gels showing restriction banding pattern for RFLP *Alternaria* isolates on ITS rDNA with Hinf 1 and Hae111LanesM: 100 bp ladder; 1: IGA22; 2: BSA23; 3:BSA27; 4:KML286; 5: MSK141; 6:MBR196; 7:MBR194; 8: APC101; 9;NTG215; 10:MBR190; 41:MSK 142; 42:APC 103; 44: KBL230

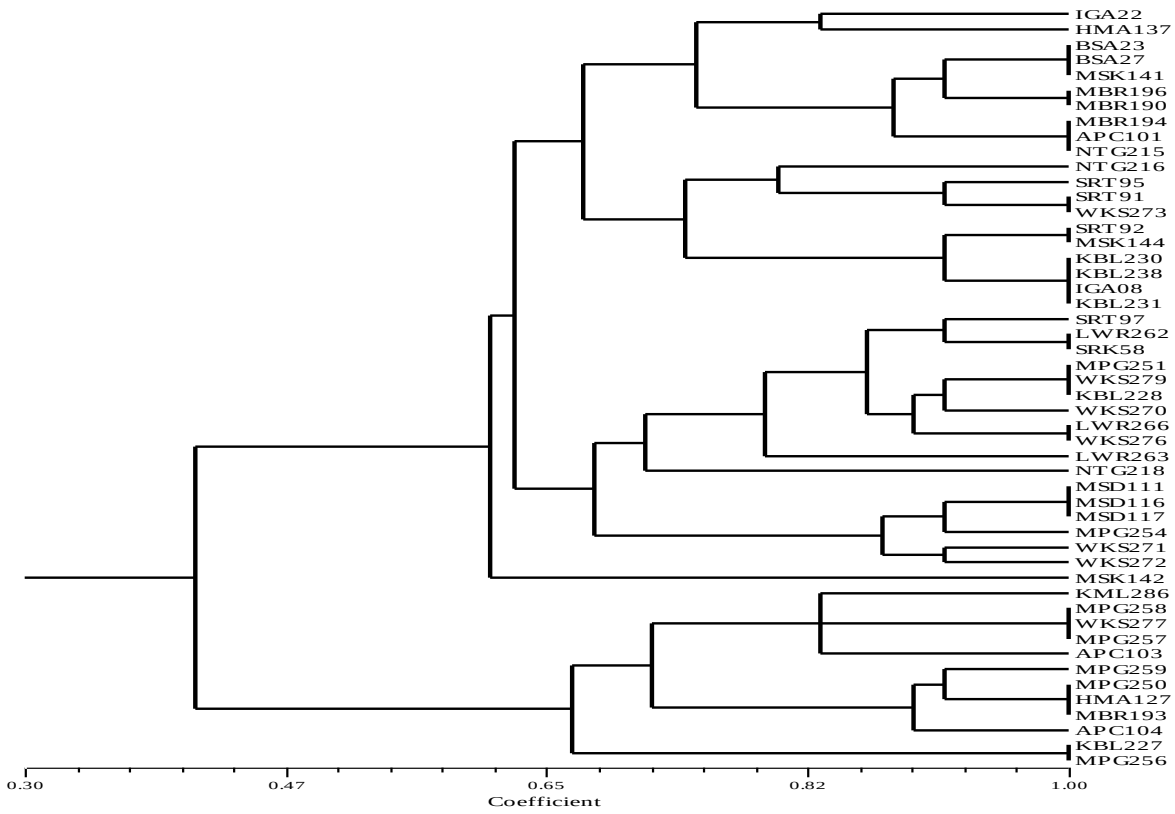

Figure 10: RFLP dendrogram showing genetic relationships among 50 Ugandan *Alternaria* isolates of sweetpotato.

5.6 Phylogenetic analysis of Alternaria species using RAPDs

All the primers used in analysis revealed similar RAPD fingerprints for most Alternaria isolates, regardless of geographic origin. Bands were consistently hared by most isolates. The tested isolates that did not amplify with some of the primers were not used in RAPD data analysis. Based on fingerprints obtained using two RAPD primers, *Alternaria* isolates investigated are not genetically homogeneous. Fingerprints of representative isolates obtained with primers OPA-2 and OPA-4.

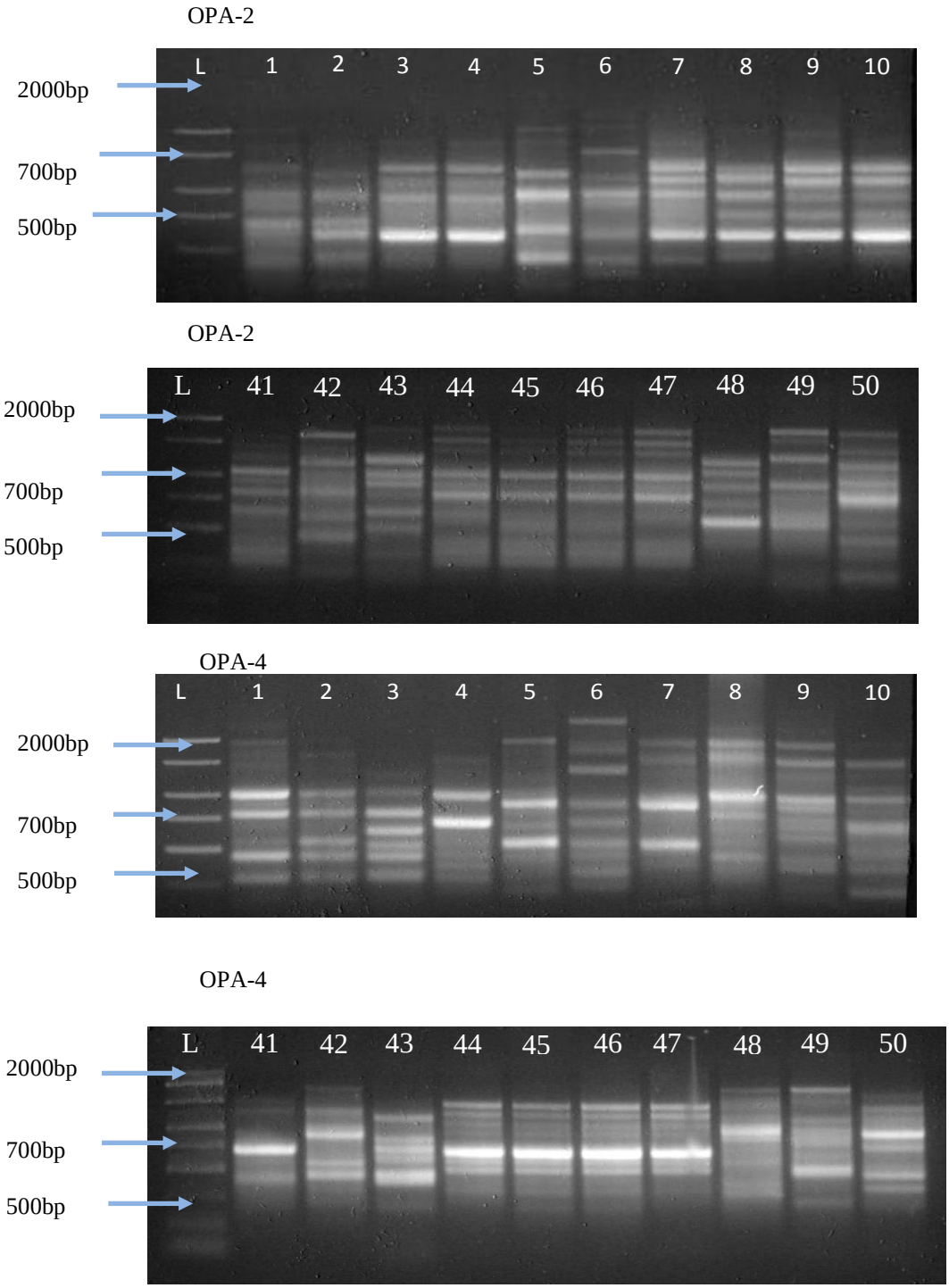

Figure 11: Agarose gels showing restriction banding pattern for RAPD *Alternaria* isolates basing on ITS rDNA with OPA-2 and OPA-4 Lanes M: 100 bp ladder; 1: IGA22; 2: BSA23; 3:BSA27; 4:KML286; 5: MSK141; 6:MBR196; 7:MBR194; 8: APC101; 9:NTG215; 10:MBR190; 41:MSK 142; 42:APC 103; 44: KBL230; 45:IGA08; 46:KBL236; 47:KBL238; 48:MPG250; 49:MBR193; 50:HMA127

Based on binary band- share data generated by a matrix of 50 isolates , Jacquards' coefficient of relationships were computed and used to generate a pair wise matrix of genetic distances and a dendrogram depicting relationships were observed in the diagram (Fig 12). A RAPD based dendrogram with correlation coefficient of 0.5 was generated using NTSYS-pc computer program. A UPGMA cluster analysis based on RAPDs banding pattern of the two primers ranked *Alternaria* spp isolates into three major groups with ten sub-groups. Each sub-group was composed of isolates from different regions suggesting some specialization within the species. Most isolates clustered individually while others clustered as a group. Several sub clusters of isolates were observed in one group in the dendrogram (Fig 12). There was also a high degree of pattern similarity among isolates of the *bataticola* and *alternata* species. A considerable RAPD fragment pattern variation among isolates existed among isolate BSA 27 and isolate SRT 96 that produced a red pigment. These results suggest that there could be isolates from *Alternaria* spp that could be undescribed species of *Alternaria*.

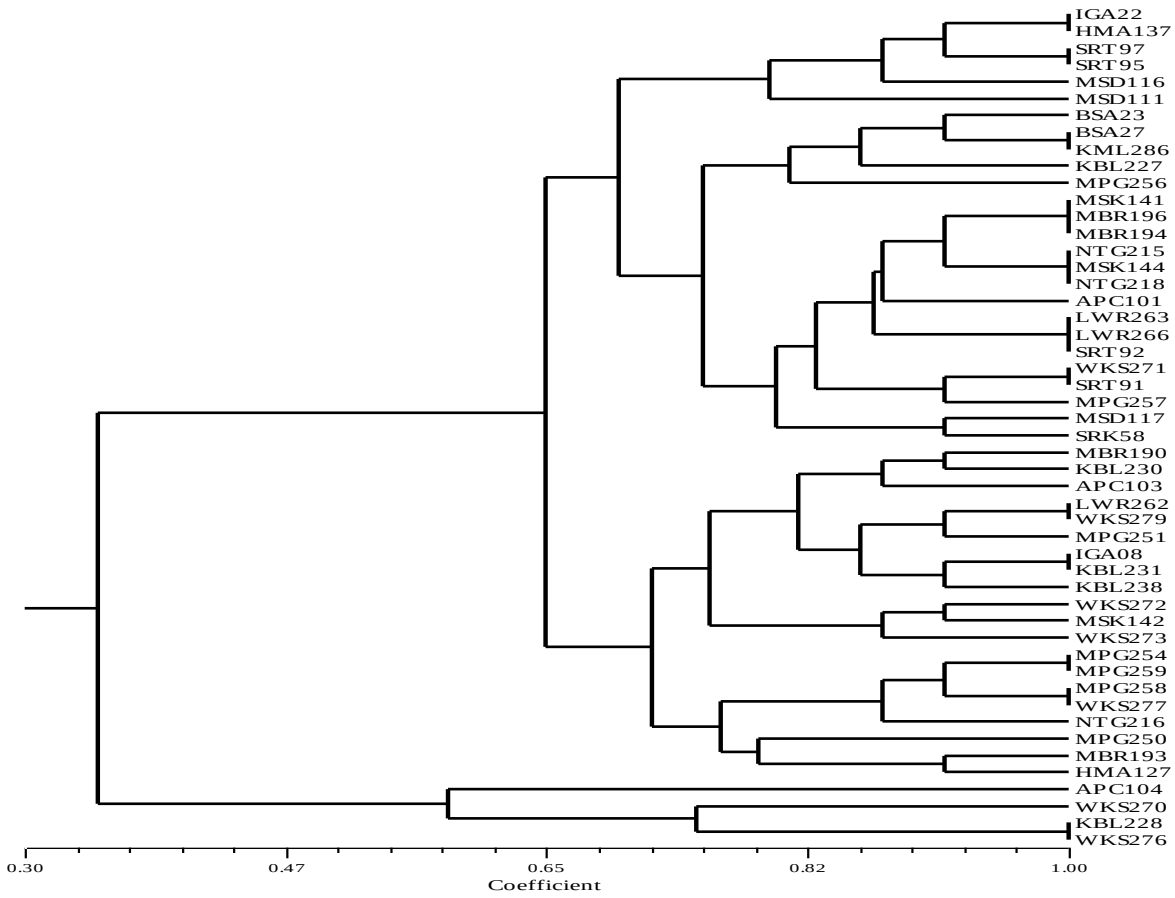

Figure 12: RAPD dendrogram showing genetic relatedness among 50 Ugandan *Alternaria* isolates of sweetpotato

CHAPTER SIX

6.0 PATHOGENICITY OF *ALTERNARIA* BLIGHT ISOLATES ON THREE SELECTED CULTIVARS OF SWEETPOTATO

6.1 Introduction

A fungus *A. alternata* was identified as the causal agent of *Alternaria* stem and leaf blight of sweetpotato (Clark and Moyer, 1988; Lenne, 1991). Other species namely; *A. solani*, *A. Tenuissima* and *A. bataticola* have also been recorded as causing stem blight of sweetpotato in other countries (Lenne, 1991). Pathogenicity tests were conducted to provide proof that the *Alternaria* isolates collected and isolated from infected materials from the 17 districts in Uganda were pathogenic to the selected sweetpotato cultivars.

6.2 Materials and Methods

To study the variability in pathogenicity and virulence of *Alternaria* spp. causing *Alternaria* blight of sweetpotato in Uganda, plants originating from micropropagation were transplanted in well drained sterilized soils contained in 30-cm diameter plastic pots. Plants were used for inoculation at 8 week old stage. three sweetpotato cultivars were inoculated with three *Alternaria* isolates of *A. alternata* and three isolates of *A. bataticola*. Three of the cultivars used; cultivar NASPOT 1 (susceptible), cultivar Magabali (intermediate) and cultivar Ebwanaterak (resistant) represented a range of susceptibility/resistance to *Alternaria* isolates collected.

6.2.1 Inoculum preparation

Conidial suspensions were prepared from 14-day old monosporic cultures grown on

sweetpotato vine decoction media (Angiyah *et al*., 2001). The surface was scraped gently with a Pasteur pipette to remove conidia. Conidial suspensions obtained were filtered through gauze mesh (40 µm) to remove pieces of mycelium. Conidial concentration was determined using a hemocytometer. Suspensions were then diluted with distilled water to give a final concentration and standardized at 6.0 x 10^6 per conidia/ml. The conidial suspensions used in different experiments were prepared from the same stock cultures in order to reduce intra-isolate variation.

6.2.2 Inoculation and incubation of test plants

Conidial germination was carried out by placing droplets of the conidial suspension onto a microscope slide and incubated for 24 hours. Ten plants of each variety were inoculated with each isolate. Experiments were performed twice, once under screenhouse conditions and once under controlled environment (laboratory) conditions. The foliage of plants was inoculated with a hand-pump sprayer held directly at 20 cm above the plant and the conidial suspension was applied until run off. Control plants were similarly treated with sterile distilled water. Plants were covered with polythene sheet to maintain high relative humidity and avoid cross-contamination between isolates. Incubation temperatures were 25 ^{0}C and 29 ^{0}C under laboratory and screenhouse conditions respectively.

Detached leaves from eight-week old plants of Ebwanateraka, Magabari and Naspot 1 were inoculated with six *Alternaria* isolates using a syringe. For each isolate tested, three fully expanded leaves were placed on plastic containers lined with moist sponge and tissue paper to avoid leaf desiccation. Tests were conducted wounded leaves. For the wounded leaf

assays, each leaf was slightly wounded on the mid rib with a razorblade prior to inoculation. To inoculate leaves 20 µl of a conidial suspension was sprayed on the leaf surface using a hand sprayer on each leaf or wound. Control plant leaves were sprayed with sterile distilled water. The plastic containers were covered with lids to maintain high humidity and were incubated at 25 ^{o}C for 7 days. The main visible symptom observed at the beginning of the disease development was foliar necrosis only a small amount of the petiole necrosis was observed. When the disease was more advanced enlarged lesions were observed. We used a numerical scale from 1 to 4, in which disease severity was on a 4- point rating system: 1 = no lesions, 2 = < 1 mm in diameter, 3 = lesions 1-5 mm in diameter, and 4 lesions > 5 mm in diameter. The experiment was a factorial experiment in RCBD replicated 8 times.

Disease symptoms on potted plants in the screen house were scored after 5 to 7 days of incubation. Lesions were scored for size using the same scale as on detached leaves. To minimize the effect of environment variation, all inoculations were performed in a limited time from June to September 2008. Severity scores were recorded weekly for four weeks for screenhouse potted plants. All data was subjected to analysis of variance at 5 % probability levels of the F-test. Significant differences in treatment effects were identified using Duncan's multiple range test and least significant differences test at 5 % probability level.

6.2.4 Re- isolation and culturing

Inoculated plants were examined daily for symptom development and the colour, shape and size of lesions produced on the leaves and vines were noted. Seven days after inoculation, leaves and vines showing symptoms were detached and re-isolation of causal agent

performed to fulfill Koch's postulates.

6.3 Results

6.3.1 Pathogenicity tests

All inoculations were successful with a high percentage of conidial germination (70-95%) obtained with each inoculation. Results of the analyses of variance on disease severity from studying the effects of isolate, cultivar, (isolate x cultivar) interaction and experimental effects are presented in (Table 4). No significant experimental effects were found, isolate and cultivar were significant ($P < 0.05$) as observed in (Fig 13). Cultivar NASPOT 1 was very susceptible to all the isolates, while cultivar Magabari varied from susceptible to intermediate according to the isolate type used and cultivar Ebwanaterak was either intermediate or resistant. Isolate type had an effect on the virulence; *A. bataticola* (KML 4, MPG 259 and NTG 215) isolates differed in virulence from the *A. alternata* isolates ($P < 0.05$), Isolates NTG 215 and KML289 that were aggressive in the growth cultures were able to cause disease in a short time compared to *A. alternata* that caused only mild symptoms. The isolates of the same species did not differ in virulence. The same experiment was repeated on detached leaves. Results from the two separate experiments could not be combined due to lack of variance within certain treatments. However, the trends exhibited in both experiments were nearly identical. Figure 13 and 14 show the effects of different isolate spp behavior on the cultivars tested. Isolates belonging to the same group, developed in a similar pattern as shown in the graph and they had the same pattern of pathogenicity. However between the two groups (*A. alternata*; BSA 27, HMA 137 MSK 141 and *A. bataticola* (MPG 259, KML 4, NTG 215) the trends are not the same, the *A. alternata* showed weak virulence compared

to *A. bataticola* group which were highly virulent.

Table 4. Combined ANOVA of Area Under Disease Progress Curve (AUDPC) for all varieties used in the screen house

Source of variation	df	SS	MS	Vr	Fpr
Isolate	5	2564.753	512.951**	74.15	< .001
Variety	2	4475.590	2237.795**	323.5	< .001
Isolate x Variety	10	157. 626	15. 763ns	2.28	0.012
Residual	1355	9373. 284	6. 918		
Total	1379	16923. 638			

** Significant at (P<0.05), ns =non significant

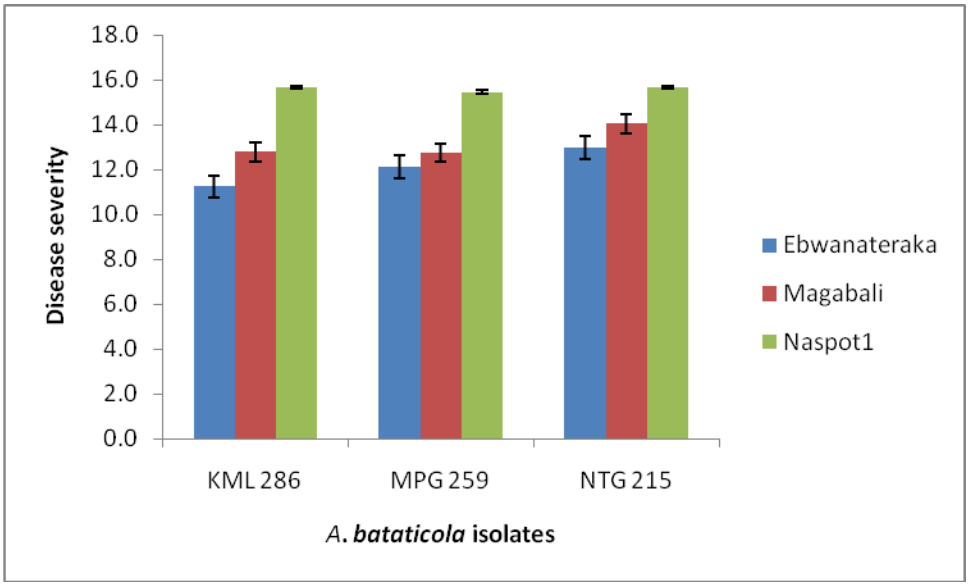

Figure 13: Severity of *A. bataticola* on three sweetpotato varieties

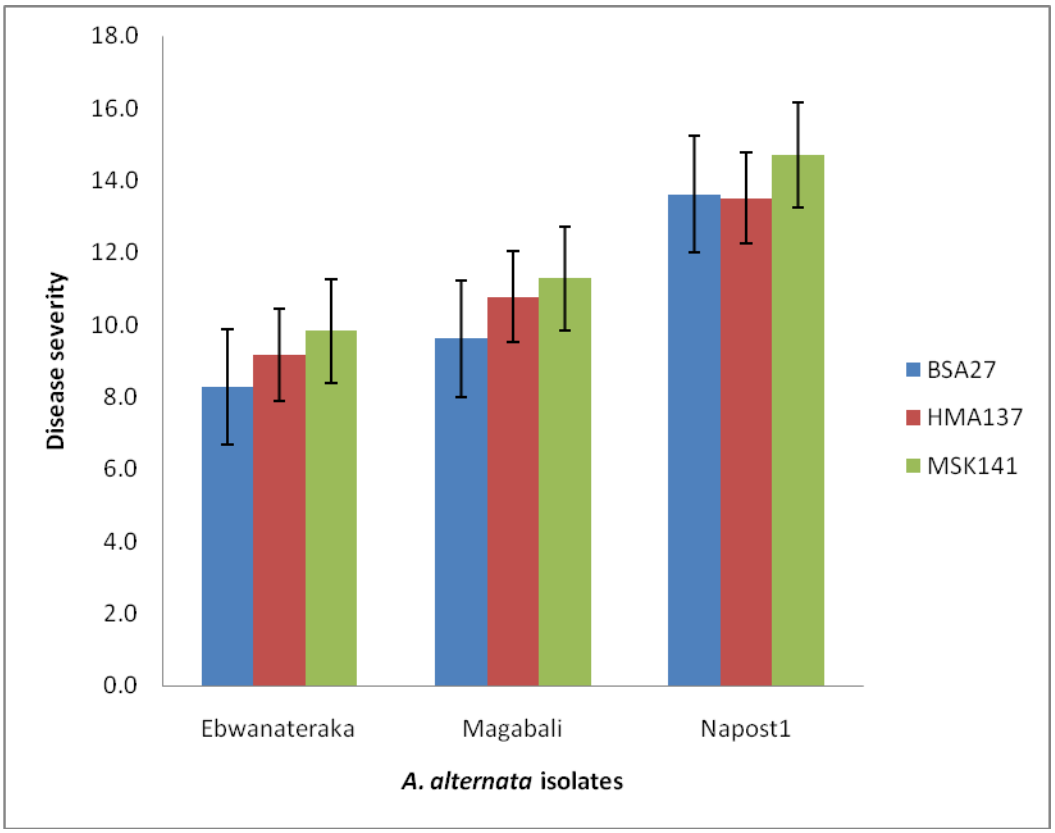

Figure 14: Severity of *A. alternata* isolates on three sweetpotato varieties

Pathogenicity tests conducted on both detached leaves and potted plants revealed that the six isolates from the two groups are pathogenic to sweetpotato. Pattern of pathogenicity of six *Alternaria* isolates were observed as having significant difference ($P<0.01$).Likewise cultivars were significantly different in response ($P<0.01$). Supporting these findings, previous reports by (Osiru, 2008) show that different cultivars respond differently to *Alternaria* infection. Naspot1 was found to be highly susceptible to all the isolates. Aggressive isolates are also known to have a lower latent period and high sporulation index thus enhancing fitness of survival (Anginyah *et al*., 2001). All isolates expressed virulence to all cultivars. The most virulent isolates were those of *A. bataticola* type collected from Kamuli and Ntungamo. The *A. alternata* types seem to have had higher latent period because

it was slow in sporulation and disease development thus causing mild symptoms.

Pathogen isolation from infected tissues yielded colonies of typical *Alternaria* lesions. However, Naspot 1 variety was the most susceptible host plant, it showed disease symptoms 5 days after inoculation for all isolates, Magabari and Ebwanaterak showed symptoms 10 days after inoculation for isolate 5 and 6 and for isolate 1, 2, 3, 4 the symptoms appeared 2 weeks after inoculation, whereas control plants remained healthy during the entire period of evaluation. The lesions enlarged, became black and sunken and finally girdled the petioles and at the abaxial point causing the leaf to droop. The resistant response levels exhibited by Magabari and Ebwanaterak is in agreement with previous field observations (Mwanga 2003; Turyamureeba, 1997; Yada, 2009), observed differences in cultivar reaction to *Alternaria* blight in improved cultivars and landraces.

Table 5. Area Under Disease Progress Curve (AUDPC) analysis of variance (ANOVA) means on three Cultivar response to inoculation with six *Alternaria* isolates

Isolate	Species	Origin	Cultivar disease response				Isolate/cultivar
			Ebwanaterak	Magabari	Naspot1	Mean	Interaction
BSA27	*A.alternata*	Busia	8.268	9.606	13.612	10.495	1
HMA137	*A.alternata*	Hoima	9.162	10.774	13.549	11.152	1
MSK141	*A.alternata*	Masaka	9.818	11.281	14.666	11.933	1
MPG259	*A. bataticola*	Mpigi	11.231	12.231	15.656	13.226	2
KML286	*A. bataticola*	Kamuli	12.158	12.766	15.480	13.468	2
NTG215	*A. bataticola*	Ntungamo	13.016	14.059	16.216	14.430	2

Average of 8 replications; means followed by same number do not differ significantly at p=0.05

Pathogenicity test conducted with selected *Alternaria* isolates from sweetpotato on wounded leaves resulted in significant lesion development for all the isolates (Fig 15 A and B). However, there was a significant difference among cultivars in the number and size of lesions on stems, petioles and leaves. The size of lesions on the vines, leaves and petioles differed significantly ($P< 0.05$) from one variety to another. Lesion expansion was faster on vine, petioles and leaves of Naspot 1 variety. Most petioles of NASPOT 1 cultivar became girdled five days after inoculation, five days earlier than the other cultivars and most plants in the buckets had lost their leaves.

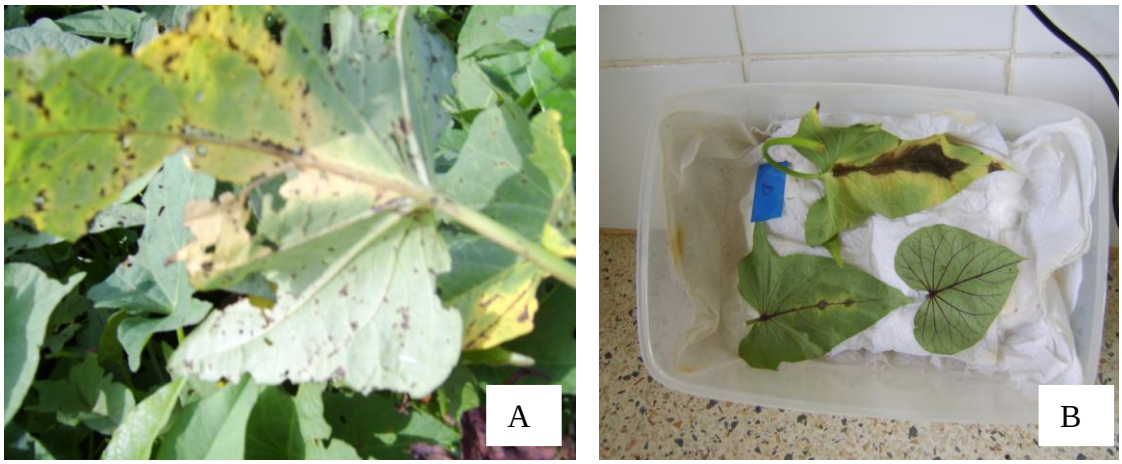

Figure 15: Symptoms of *Alternaria* infection after inoculation on potted plants **(A)** and on detached leaf assays of three cultivars **(B)**

Virulence was evident on all the three sweetpotato cultivars tested. Different reactions were observed on stems, leaves and petioles. The susceptible cultivar NASPOT 1 (Fig 15 A had grey large lesions on vine nodes, petiole and abaxial, on the main vine/stem and small lesions on the leaves Ebwanaterak and Magabari only had pale lesions on the petioles, and the lesions on leaves were surrounded by a yellow halo (Fig 15 B)

6.4 Discussion

This study has shown that sweetpotato cultivars differ in susceptibility levels. As such, control of alternaria leaf blight on sweetpotato is most critical between levels of resistance. As some researchers have investigated the relative susceptibility of different germplasm of sweetpotato to Alternaria leaf blight (Osiru, 2008) no information was available on the relative susceptibility of different sweetpotato germplasm found in Uganda. In this study none of the cultivars tested appeared resistant to the pathogen, while some cultivars showed high degree of susceptibility (NASPOT 1) the other two (Magabali and Ebwanateraka) were only mildly susceptible to Alternaria. No significant differences were observed among the isolates one week after inoculation of the same infected plants. However, the most prevalent *alternaria* species was *A. bataticola,* and it was also proved to be the most aggressive in terms of virulence compared to *A. alternata*. A possible explanation might be that Alternaria innoculum was introduced into Ugandan production fields along with planting material from different geographic origins that straight away goes to infect any growing sweetpotato crop or it falls in the soil were it will stay until it gets conducive conditions and a host. The significantly lower frequency of *A. alternata* in the sweetpotato production fields further indicates that *A. alternata* isolates have lower field fitness than *A. bataticola.* The susceptible cultivars are seen to have shorter latent period compared to the mild resistant ones. (Osiru, 2008)

CHAPTER SEVEN

7.0 GENERAL CONCLUSION AND RECOMMENDATIONS

The survey showed that *Alternaria* blight is widespread in all sweetpotato growing areas of Uganda and high proportions of farmers' fields are harbouring the pathogen. The most prevalent *Alternaria* species is *A. bataticola*, and it is also the most aggressive in terms of virulence compared to *A. alternata.* All the commonly grown and preferred cultivars by farmer's fields are susceptible to *Alternaria* blight pathogen. Results showed that most farmers did not take any measures of control especially for *Alternaria* blight disease. With time, the diseases can escalate as an epidemic. There is need to device measures to control the disease at farmer level. The results indicate that the majority of the farmers did not correlate the wilting symptoms observed to *Alternaria* blight disease only.

The survey results showed that most farmers did not take any measures of control for *Alternaria* blight disease there is a possibility of the disease escalating causing an epidemic. There is therefore need to device measures to control the disease at farmer level since majority of the farmers did not correlate the wilting symptoms observed to *Alternaria* blight disease only. *Alternaria* blight is widespread in all sweetpotato growing areas of Uganda with high proportions of farmers' fields harbouring the pathogen. The most prevalent *Alternaria* species is *A. bataticola*, and it is also the most aggressive in terms of virulence compared to *A. alternata.* All the commonly grown and preferred cultivars by farmer's fields are susceptible to *Alternaria* blight pathogen. This trend indicates need for more research to design ways of control measures for the pathogen

This study revealed that morphological characters were useful in differentiating between species (*Alternata and A. bataticola*) e.g. spore size, spore shapes, growth rates color of mycelia; but not accurate in separating strains within a species. There was no regional no clustering of some isolates of the *Alternaria* strains in Uganda as revealed by the regional clustering patterns observed. Both RAPD and RFLP dendograms had two main groups with many sub clusters, suggesting that the morphological characterization should be used hand in hand with the molecular work to come up with disease control strategies

The ability to distinguish the *Alternaria* isolates by means of molecular analysis will facilitate regulation of movement of infected areas free from these species or pathotypes. The RAPDs and RFLP techniques are sensitive, reliable, and much quicker than pathogenicity studies, thereby enabling more rapid decisions regarding disease control. Moreover without a complete knowledge of the genetic variation and distribution of the *Alternaria* spp. involved in *Alternaria* blight disease of sweetpotato in Uganda, development of a disease management or breeding programme would not be possible.

Since one of the sweetpotato breeding objectives is addressing disease resistance and future work should aim at;

(1) Studying the genetic inheritance of resistance to each group of isolates using genetic design. Identification of a genetic control of resistance would allow us to optimize the selection for *Alternaria* resistance.
(2) Carrying out a study to see if there is a synergistic interaction between the isolate species so as to design appropriate disease management options.

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