MAKERERE  **UNIVERSITY**

RESISTANCE OF COWPEA TO SCAB DISEASE AND DIVERSITY OF *SPHACELOMA* SP. OCCURRING IN UGANDA

BY

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Reg No. 2013/HD02/1879X

A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PLANT BREEDING AND BIOTECHNOLOGY OF MAKERERE UNIVERSITY

FEBRUARY 2017

DECLARATION

The work presented in this thesis is my own research and to my knowledge it has not been presented for the award of degree or diploma in any other University.

Signed……. Date…..……

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This thesis has been submitted for examination with our approval as the university supervisors.

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DEDICATION

This work is dedicated to my wife, Mrs. Stella Afutu and children, Andrew Nii-Kotey Afutu and Aaron Nii-Kotei Afutu for their prayers, love and support during the entire period of my studies.

ACKNOWLEDGEMENT

My sincere gratitude goes to my supervisors, Prof. Patrick R. Rubaihayo and Dr. Moses Biruma for their supervision, mentoring, guidance and encouragement that propelled me to complete my research and write-up on time. Apart from my two supervisors, I acknowledge the other members of my doctoral committee, Prof. P. Tukamuhabwa, Dr. Mildred Ochwo-Ssemakula, Dr. Thomas L. Odong and the Head of Department of Agricultural Production, Dr. Dennis Mpairwe for their time, and guidance in shaping this work. Special thanks to Prof. Ekwamu Adipala and Dr. O-A. Danquah for their encouragement to complete my Ph.D on time. I am grateful for the full doctoral funding received from Carnegie Corporation of New York – *Training the next generation of Scientists* through the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM). Special thanks to the PI for RUFORUM sponsored students in Makerere University, Dr. Agnes Namutebi, for her prompt actions in putting in requisitions for stipends, research and other funds for a smooth stay in Uganda. I am grateful to the Head of Crop Science Department (UCC), the Dean of School of Agriculture (UCC) and the University of Cape Coast, my employer, for granting me study leave. Special thanks to the leader and team of the cowpea scab breeding project at the National Semi-Arid Resources Research Institute (NaSARRI) – Serere, especially, Messrs. John Obuo, Robert Amayo, Robert Omadi, John Ariko Bosco, and Martin Ekurita, and Misses. Teddy Oparok and Tabitha Ruth Atukei for their immense support during field surveys, field evaluation of crops and crossing. I acknowledge the technical advice and supervision of Messrs. Allan M. Ssekamate and Fred Kato, and all the Laboratory Technicians at the International Centre for Tropical Agriculture (CIAT) – Kawanda, especially Messrs. Dennis Bbosa, Yulu Mbembo Simeon and Samuel Kiviiri for their support during the molecular work. I am grateful to Prof. Paul Gibson, Dr. Thomas L. Odong, Dr. Peter Wasswa, Mr. Warren Arinatwe, Naomi Afi Senyo and Bruno Awio for their assistance in data analyses. I am thankful to all my colleagues in the Plant Breeding and Biotechnology programme (Cohort III) for sharing and critiquing to improve the research and write-up. Finally, my sincere gratitude goes to my wife, Mrs. Stella Afutu, Children, Andrew Nii-Kotey Afutu and Aaron Nii-Kotei Afutu, and to my parents, Mr Charles Kotey Afutu and Mrs. Elizabeth Ayeley Arthur-Afutu for their prayers and emotional support. To God be the glory for how far He has brought me.

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ABSTRACT

Cowpea (*Viga unguiculata* L. Walp) is the third most important legume food crop in Uganda with the Eastern and Northern regions accounting for most of the production in the country. However, its mean yield is less than 400 Kg/ha though the crop has a yield potential of 3,000 Kg/ha. Cowpea scab (*Sphaceloma sp*.) is a seed-borne disease and is one of the major constraints of cowpea production in the country, capable of causing yield losses of up to 100%. The disease affects all the above ground parts of the cowpea plant. There is currently a resurgence of the disease in the country leading to significant yield losses in farmers' fields, yet, only one out of the five improved cowpea cultivars recently released in the country is moderately resistant to the disease. The use of resistant cultivars in disease management is the most practical approach, easily adopted and more environmentally friendly. The objectives of this study were to: (i) determine the distribution of scab disease in different cowpea growing agro-ecological zones; (ii) identify sources of resistance and high yield potential; (iii) determine the variability of the Scab fungus (*Sphaceloma sp*) in different cowpea growing agro-ecological zones of Uganda; and (iv) determine the heritability and gene action of the genes controlling scab disease resistance. Field surveys were conducted in 17 major cowpea growing districts in Uganda across three agro-ecological zones in 2013 and 2014, and diseased plant parts were collected from farmers' fields. Amuria and Tororo were hot spots of scab disease in the country with both districts recording severity score of 4 out of a scale of 1-5 and mean disease incidence between 77-92 %. The incidence and severity of scab were significantly ($P < 0.05$) affected by altitude (> 1200 m.a.s.l), cropping systems (intercropped), previous crop grown (legume/cassava) and the cowpea cultivars grown. 100 cowpea lines including five improved cultivars were evaluated at Makerere University Agricultural Research Institute - Kabanyolo (MUARIK) and the National Semi Arid Resources Research Institute (NaSARRI), Serere, between April and July, 2014 in a 10 × 10 lattice design. Ten lines (SECOW3B, NE4, NE20, NE32, NE49, WC5, WC7, WC16, WC62, and WC67B) were moderately resistant to scab at both locations while one line (NE15) was resistant at both locations and high yielding suggesting that these 11 lines could serve as good parents in breeding for resistance to scab disease. Both morphological (based on colony characters on potato dextrose agar, conidia features, radial growth rate and pathogenicity) and molecular (involving inter simple sequence repeat – ISSR markers and sequencing of internal transcribed spacer – ITS region) approaches were employed to determine the variability of scab fungus occurring in Uganda. 495

pure fungal isolates of *Sphaceloma* sp. comprising 419 from infected leaves and 76 from infected pods were obtained following isolation and culture. Morphological characterization resulted in six morphological and three pathogenicity groups with most of the isolates being slow growing (> 14 days to cover 90 mm diameter petri dish). Through the pathogenicity tests, NE31 and NE70 had the widest horizontal resistance followed by ACC12.2W, Alegi, NE 15, NE 23, SECOW5T and WC 35B. Molecular characterization showed similarity coefficients ranging from 0.0248 - 0.684, suggesting a high degree of genetic variability among the isolates, with greater part of the genetic variation occurring within populations (96%; PhiPT = 0.040; $P < 0.001$) than among populations (4%; PhiPR = 0.042; $P < 0.001$). Mantel test indicated that there was no significant correlations between geographic distance and genetic distance among populations suggesting that, for the purpose of breeding for resistance to the cowpea scab fungus occurring in Uganda, there would be no need to develop different cultivars for the different regions or agro-ecological zones. 11 selected parents were crossed using a half diallel mating design and F_2 plants and the parents were evaluated at MUARIK and Serere to study the heritability and gene action controlling scab resistance. Non-additive gene effects were more important for most of the traits (Baker's ratio < 0.5) except for the number of pods per plant and seeds per pod. Alegi, NE15 and NE48 had significant negative GCA effects for scab disease severity while SECOW5T had significant positive GCA effects for high seed weight and grain yield suggesting that these parent could be selected for breeding for resistance to scab and high yield, respectively. Cross between WC35B*WC66 had high SCA effects for scab severity while six crosses had high SCA effects for high yields across the two locations suggesting that these crosses could be selected and advanced in breeding for resistance to scab and high yield, respectively.

PUBLICATIONS

The research from this thesis generated the following papers from the various chapters.

Chapter 3:

Afutu, E., Agoyi, E.E., Amayo, R., Biruma, M., Rubaihayo, P.R. (2017a). Cowpea scab disease (*Sphaceloma* sp.) in Uganda. *Crop Protection*. http://dx.doi.org/10.1016/j.cropro.2016.06.024.

Chapter 4:

Afutu, E., Mohammed, K.E., Odong, T.L., Biruma, M. and Rubaihayo, P.R. (2016a). Evaluation of Ugandan cowpea germplasm for yield and resistance to scab disease. *American Journal of Experimental Agriculture*. 12(2): 1-18. http://dx.doi.org/10.9734/AJEA/2016/25138

Chapter 5:

Afutu, E., Agoyi, E.E., Kato, F., Amayo, R., Biruma, M. and Rubaihayo, P.R. (2016b). Morphological characterization of Ugandan isolates of *Sphaceloma* sp. causing cowpea scab disease. *Journal of Agricultural Science*. Vol. 8, No. 9. http://dx.doi.org/10.5539/jas.v8n9p55

Chapter 6:

Afutu, E., Agoyi, E.E., Odong, T.L., Wasswa, P., Ssekamate, A.M., Biruma, M. and Rubaihayo, P.R. (2017b). Molecular characterization of Ugandan isolates of cowpea scab fungus (*Sphaceloma* sp.). *Plant Pathology*. In press).

Chapter 7:

Afutu, E., Agoyi, E.E., Gibson, P., Biruma, M. and Rubaihayo, P.R. (2017c). Genetics of resistance to cowpea scab disease and yield components of cowpea. *Field crops*. (yet to be submitted).

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

1.1.1 Classification

According to Pasquet (2001), the genus *Vigna* includes about 80 species distributed throughout the tropics. The 80 species are made up of seven domesticated species, out of which five are of Asian origin and two of African origin. Green gram/mung bean (*V. radiata* (L.) Wilczeck), black gram/urd bean (*V. mungo* (L.) Hepper), moth bean (*V. aconitifolia* (Jacq.) Marèchal), adzuki bean (*V. angularis* (Willd.) Ohwi et Ohashi), and rice bean (*V. umbellata* (Thunb.) Ohwi *et* Ohashi) are of Asian origin while Bambara groundnut (*V. subterranea* (L.) Verdc.) and cowpea (*V. unguiculata* (L.) Walp.) are of African origin (Pasquet, 2001). According to Pasquet (1997), the species *V. unguiculata* consists of domesticated forms and wild annual forms. These are *V. unguiculata* ssp. *unguiculata* var. *unguiculata* and ssp. *unguiculata* var. *spontanea* (Schweinf.) respectively, and 10 wild perennial subspecies. This classification is based on morphological (Padulosi, 1993), allozyme (Pasquet, 1999) and cpDNA studies (Vaillan-court and Weeden 1992). The *V. unguiculata* ssp. *unguiculata* var. *spontanea* is the likely progenitor of the domesticated cowpea (Pasquet, 1999).

1.1.2 Growth habit

Growth forms vary and may be erect, trailing, climbing or bushy, usually indeterminate under favourable conditions. Leaves are alternate and trifoliate usually dark green. The first pair of leaves is simple and opposite. Stems are striate, smooth or slightly hairy, sometimes tinged with purple (Aveling, 1999). Cowpea is considered more tolerant to drought than soybean or mung bean because of its tendency to form a deep taproot. It has a competitive niche in sandy soils, does not tolerate excessively wet conditions, and should not be grown on poorly drained soils. It thrives in dry environments and available cultivars produce a crop with as little as 300 mm of rainfall. This makes it the crop of choice for the Sahelian zone and the dry savannahs, though cultivars that flourish in the moist savannahs are available as well (Hall *et al*., 2003). According to Singh *et al.* (2003), cowpea is well-adapted to the semi-arid regions of the tropics where other food legumes do not perform well and this is because it is drought tolerant and a warm weather crop. It has the unique ability to fix atmospheric nitrogen and performs well even in poor soils with more than

85% sand, less than 0.2% organic matter and low levels of phosphorus (Sanginga *et al*., 2000). Also, its ability to tolerate shade makes it compatible as an intercrop with maize, millet, sorghum, sugarcane and cotton as well as with several plantation crops (Singh and Emechebe, 1998). In addition to these attributes, its quick growth and rapid ground cover helps to check soil erosion, and root decay in situ produces nitrogen-rich residues that improve soil fertility and structure (Singh *et al*., 2003). Collectively, these characteristics have made cowpea an important component of subsistence agriculture particularly in the dry savannas of sub-Saharan Africa (Carsky *et al*., 2001).

1.1.3 Uses

Cowpea is the most economically important indigenous African legume crop (Langyntuo, *et al*., 2003). It is an important staple food crop and a major source of plant proteins, vitamins (Rachie, 1985; Kholi, 1990; Asiwe *et al*., 2005), and animal fodder (Tarawali *et al*., 1997) and of considerable importance for human nutrition in the semi-arid and tropical regions of Africa (Gowda *et al*., 2000). It is of key importance to the nutrition and livelihoods of millions of people in less-developed countries of the tropics (Singh *et al*., 2003). Nielsen *et al*. (1997) reported that the young leaves, green pods and green seeds are used as vegetables whereas dry seeds are used in a variety of food preparations. The dry grain is the most important product of this species (Yeung *et al*., 2009).

1.1.4 Production trends

It is grown in more than 60 countries either as a food crop or cash crop (Davis *et al*., 1991) occupying most parts of Asia and Oceania, the Middle East, Southern Europe, Africa, Southern USA, Central and South America (Singh *et al*., 2003). Ba *et al*. (2004) reported that cowpea was cultivated in all tropical areas, as well as under more temperate climates like in California's Central Valley or in the Mediterranean basin. According to Ward *et al*. (2002), it is hard to accurately estimate annual production because cowpea is mostly grown as a subsistence crop or is sold in internal markets. The crop was reported to have been cultivated on at least 12.5 million hectares and with an annual production more than three million tons world-wide (Singh *et al*., 1997). Later, Singh *et al*. (2003) reported that the estimated World wide area under cowpea production was about 14 million ha of which West Africa alone accounted for about 9.3 million ha with annual

production of about 2.9 Mt. Eighty percent of the world production comes from Africa (Singh *et al*., 2003) and Ba *et al*. (2004), also reported that Africa was the main area of production, where the crop was very important for low input agriculture which was a characteristic of most parts of the continent.

1.1.5 Constraints to production

In Uganda, mean yield of cowpea is less than 400 kg ha^{-1} (Collaborative Crops Research Project - CCRP, 2012) and total production is estimated to be at 20,000 t/yr, with Northern and Eastern regions accounting for most of the production in the country (FAO, 1997). The potential grain yield is high under sole cropping (1.5–3.0 t/ha), when insecticide is applied to the crop, however, in the West African sub-region the actual yields obtained are much lower estimated at an average of 250–300 kg/ha (Mortimore *et al*., 1997). According to Ajeigbe and Singh (2006), the low yields recorded are due to a number of insect pests, diseases, parasitic weeds, low soil fertility, drought and lack of inputs. The insect pests cause maximum damage to cowpea from seedling to storage (Ajeigbe and Singh, 2006) and the most damaging of all insect pests are those that occur during flowering and podding stages which include flower thrips (*Megalurothrips sjostedti* Trybom), the legume pod borer (*Maruca vitrata* Fabricius) and a complex of pod and seed suckers, of which *Clavigralla tomentosicollis* Stal, is the dominant species in Africa (Jackai and Adalla, 1997). As a result, remarkable increases in yields, sometimes up to tenfold, have been obtained with application of insecticides (Ajeigbe and Singh, 2006).

According to Isubikalu (1998), some farmers in Uganda resorted to the indiscriminate use of insecticides to reduce pest damage, sometimes applying as many as 8-10 sprays per season. Conversely most Ugandan farmers are resource-poor, and require pest management strategies that are cost-effective and sustainable (Karungi *et al*., 2000a) and therefore, the use of pesticides must be minimised due to their high costs, and harmful effects on human health and the environment (Giliomee, 1997). According to Karungi *et al*. (2000a), application of insecticide once at budding, flowering and podding stages resulted in grain yield of 1,293 kg/ha while weekly application of insecticide throughout the crop's growing season yielded 1,561 kg/ha compared to 268 kg/ha obtained when no insecticide was applied. On the other hand, Nabirye *et al*. (2003) reported that combining cultural practices and spraying once each at budding, flowering, and podding stages was more effective and profitable than spraying cowpea weekly throughout the growing season.

An IPM practice which combined early planting with close spacing cowpea (30 × 20 cm^2) and three insecticide applications once each at budding, flowering and podding stages, yielded 791 kg/ha with a 51% yield gain over the farmers' traditional practices (Nabirye *et al*., 2003). Rusoke and Rubaihayo (1994) reported that there was a yield potential of 3000 kg/ha. Also, cowpea suffers severe grain damage in storage due to the bruchid *Callosobruchus maculatus* (F). The bruchid causes irreversible losses to stored grain, with initial infestation beginning at the field level from where it is carried over to storage (Opolot *et al*., 2006). This results in direct weight loss as well as reduced grain quality making the seed unfit for human consumption or for planting (Singh and Jackai, 1985; Opolot *et al*., 2006).

Different diseases affect different parts of the crop at different stages of growth including bacteria, viruses and fungi (Allen, 1983). Some 40 species of fungi are cowpea pathogens (Allen, 1983). The major and common diseases are anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magnus) Bri. & Car.), scab (*Sphaceloma sp*.), *Sclerotium* stem, root and crown rot, damping off, *Cercospora* leaf spot, *Septoria* leaf spot and *Fusarium* wilt (*Fusarium oxysporum* f. sp. Tracheiphilum (E.F. Sm.) W.C. Snyder & H.N. Hans.), rust (*Uromyces phaseoli* (Pers.) Wint.), white zonate leaf spot (*Dactuliophora tarri* Leakey), zonate leaf spot (*Ascochyta phaseolorum* Sacc.), yellow blister (*Synchytrium dolichi* (Cooke) Gaum), and gray leaf mold (*Cercospora canescens* Ellis & G. Martini) (Dugje *et al*., 2009).

In the plant family Leguminosae, Cowpea (*Vigna unguiculata*), Lima bean (*Phaseolus lunatus*) and Common beans (*Phaseolus vulgaris*) are known to be hosts of the scab disease (Singh and Allen, 1979) and, therefore, though cowpea scab is caused by the fungus *Sphaceloma* sp., the pathogen is said to have other synonymous names such as *Elsinoe phaseoli*, Jenkins; and *Elsinoe vignicola* (Singh and Allen, 1979). *Cladosporium vignae* also causes a scab of cowpea (Singh and Allen, 1979). The conidium of *Sphaceloma* sp are usually regarded as the anamorph of the perfect species, which is *Elsinoe phaseoli* Jenkins (Emechebe, 1980; Mbong *et al*., 2014).

Sphaceloma scab is characterised by development of silvery grey, circular to oval lesions on stems, leaves and their petioles, peduncles and pods. In severe infections, such lesions coalesce, causing distortion and flower bud abortion (Singh and Allen, 1979; Emechebe and Shoyinka, 1985; Iceduna, 1993). Gould (2004) described the symptoms of scab as discrete lesions, sunken or raised which appear on leaves, pods and the stem. Leaves of diseased plants are often cupped and bear

numerous small whitish scab lesions along the veins. Scab has a puckering effect on the leaf lamina. These attacks reduce the photosynthetic surface area of the leaves. According to Singh and Allen (1979), the stem symptoms can be confused with anthracnose, however, scab infections on stem are greyish not tan-brown, and are more often circular. According to Emechebe (1980), infections by scab leads to the formation of chlamydospores and heavily scabbed young pods abort or remain attached to the plant as mummified black masses and flower formation is either completely prevented or flower and pod abortion occurs when there is heavy scab infection of the flowering axis. The longevity of survival is probably mediated by chlamydospores produced on pod and stem tissues. However, the role of the ascospores in the epidemiology of the disease in the tropics is not known (Emechebe, 1980).

Mbong *et al*. (2010a), reported that scab was seed-borne. It attacks all plant parts during all stages of growth (Emechebe, 1980; Emechebe and Shoyinka, 1985). Primary inocula for infection is provided by an infected seed or plant material (Lin and Rios 1985; Emechebe 1985), while the subsequent dispersal of secondary conidial inoculum may be by rain splash, run-off and wind-driven moisture (Emechebe and Shoyinka 1985). Conditions conducive for disease development have been described as moderate temperatures of about 23-28 °C, with 3 or more consecutive days of wet weather resulting in high relative humidity (Emechebe, 1980; Emechebe and Shoyinka, 1985), however, Iceduna (1993) observed more disease during dry conditions in Uganda.

1.2 Problem statement

Scab (*Sphaceloma sp*.) is one of the major and common diseases of cowpea in the semi-arid tropics (Allen, 1983; Dugje *et al*., 2009). Mbong *et al*. (2012) described it as one of the most destructive diseases of cowpea capable of causing yield losses of up to 100%. According to Allen (1983), Scab of cowpea caused by *Sphaceloma sp*. is widespread in Tropical Africa and it is a major disease with the pathogen very damaging in Savannah areas. The disease affects all the above ground parts of cowpea (leaves, stems, peduncles, flower cushions and pods (Emechebe, 1980). Mbong *et al*. (2012) suggested that any factor that positively or negatively affect grain yield of a plant would certainly have an influence on the yield obtained. It has been suggested that there are at least two races of the scab pathogen in West Africa. This follows the observation that the variety

TVx 3236 which was resistant to scab in Nigeria was susceptible in Burkina Faso (Konate and Ouedraogo, 1988).

In Uganda, the cowpea improvement programme was initiated at Makerere University and started with the collection of local and exotic accessions, which were screened for yield potential (Rubaihayo *et al*., 1973), diseases (Edema and Adipala, 1996) and insect pests (Karungi *et al*., 2000a, 2000b). Also, there were earlier studies conducted on the scab disease in an attempt to improve resistance to cowpea scab in the existing locally grown susceptible cowpea varieties such as studies in which 75 cowpea lines were screened against scab of which 10 were identified as resistant (Nakawuka and Adipala, 1997). The 10 resistant lines identified were then used to study the genetics of resistance to scab by (Tumwegamire *et al*., 1998). However, despite the years of intensive research, no improved cultivars with resistance were developed. Also, there is currently no information on the distribution of the disease, morphological and molecular variability of the Scab fungus (*Sphaceloma sp*.) in Uganda. There is the likelihood that the resurgence of the disease could be due to the development of variability in patho-types of the fungus or changing weather patterns. This could also be due to a breakdown in the resistance in the current farmers' varieties. Also, out of the five recently released varieties (SECOW 1T, SECOW 2W, SECOW 3B, SECOW 4W and SECOW 5T), only SECOW 3B has moderate resistance to the scab disease. Although scab caused by *Sphaceloma sp.* is a major disease of cowpea in the tropical and sub-tropical areas, there is little known about the genetics of resistance to the disease (Tumwegamire *et al*., 1998). Cowpea producers in Uganda are mostly small-scale, resource-poor farmers and hence, management strategies that have been proposed such as fungicide spraying coupled with timing of planting (Mbong *et al*., 2010b) are not easily adopted by farmers.

1.3 Justification

Plant breeding efforts at developing resistant varieties to mitigate the effect of scab disease infections on the cowpea crop, its yield, and thus, the livelihoods of cowpea farmers, required an appreciation of the current severity and distribution of the disease across the major cowpea growing districts and agro-ecological zones of the country.

The use of resistant varieties would result in lower infections from scab resulting in boosting production dramatically with a positive impact on the livelihoods of cowpea farmers. More so,

resistant varieties are easily adopted than the adoption of management practices. The development of resistant varieties requires a better understanding of the nature of the pathogen, its variability across the major growing agro-ecological zones. There is therefore, need to isolate and determine the variability of the scab fungus occurring in Uganda to better inform plant breeding decisions. Secondly, in order to develop resistant varieties to mitigate the effects of scab infection, it is necessary to identify new sources of resistance and high yielding potential in Uganda. There is therefore, the need to screen new and a wider range of cowpea lines to identify resistance genes and to further challenge the promising lines with the isolated biotypes from the major growing areas to identify lines that have the highest levels of resistance to the different biotypes of the fungus, thus, wider horizontal resistance to the scab disease. Furthermore, the development of improved cultivars requires a better understanding of the genetics and inheritance of resistance to *Sphaceloma* scab.

1.4 Objectives

1.4.1 Main objective

The main objective of this study was to generate knowledge that will support the breeding of cowpea varieties resistant to the *Sphaceloma* scab disease.

1.4.2 Specific objectives

Specifically, the research sought to;

1. Determine the distribution of scab disease in different cowpea growing agro-ecological zones of Uganda.
2. Identify sources of resistance and high yield potential.
3. Determine the variability of the Scab fungus (*Sphaceloma sp*) in different cowpea growing agro-ecological zones of Uganda.
4. Determine the heritability and gene action of the gene controlling scab disease resistance.

1.4.3 Hypotheses

1. Scab is widely distributed in the different cowpea growing agro-ecological zones of Uganda.

2. Due to the wide genetic diversity in cowpea germplasm in Uganda, there is a potential of identifying resistant and high yielding genotypes.
3. Scab fungus has many biotypes distributed in the different cowpea growing agro-ecological zones of Uganda.
4. Resistance to scab disease is due to highly heritable influence of additive effects of the gene for scab resistance.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sources of resistance to scab disease

According to Iceduna *et al*. (1994), screning cowpea lines for resistance to scab has gained limited success. In screening cowpea for resistance to major diseases in Zambia, Kannayan *et al*. (1987) reported that, out of a total of 210 lines screened for scab disease resistance, none were resistant, but five were moderately resistant to the scab disease (*Elsinoe phaseoli*).

In a study by Iceduna *et al*. (1994), 80 cowpea lines consisting of 30 lines from the International Institute of Tropical Agriculture (IITA), 20 from Uganda, and 30 lines from Kenya and Tanzania were evaluated for resistance to *Sphaceloma* scab at the Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) under field conditions during the second rains of 1991 and the first rains of 1992 in a randomized complete block design (RCBD). They reported that the severity of scab varied with the seasons and on the whole, 25 lines were considered resistant, 20 moderately resistant while 35 lines were rated susceptible based on a modified Horsfall-Barret scal (Campbell and Madden, 1990). Furthermore, there were variations in resistance of cowpea lines to foliar and pod infection as some lines which were resistant to foliar infection succumbed to pod infection or vice-versa, suggesting the possibility that resistance to foliar and pod infection might be under the control of different genes.

Nakawuka and Adipala (1997) carried out field evaluations of 75 cowpea lines comprising 27 lines from IITA, 27 lines from Kenya and Tanzania, and 21 local collections from Uganda to identify sources of resistance to scab disease during the second rains of 1993 and the first rains of 1994 at MUARIK on fields previously planted with maize using an RCBD. Based on a scale of 1-5, where 1 = 0 % infection (no visible symptoms on either foliage or pods); 2 = less than 10 % scab infection (scattered lesions on either foliage or pods); 3 = 10 to 20 % infection (extensive spotting of young stems and branches or on the pods); 4 = 20 to 50 % infection (stem lesions coalescing, covering half the plants or the pods); and 5 = 50 % or greater infection (foliage severely damaged or pods transformed into mummies containing virtually no seeds), 10 lines were rated resistant (score < 2), 30 lines moderately resistant (Score 2-3) and 35 lines were rated susceptible (score >3) based on

foliar infections. On the other hand, 24, 40 and 11 cowpea lines were rated resistant, moderately resistant and susceptible respectively, based on pod infection and concluded that, on the whole, i.e., based on both foliar and pod infections, local lines were less infected than plant introductions.

2.2 Effects of scab infections on yield and yield related traits of cowpea

There is little information on how scab infection relates to yield and yield components of cowpea. Tumwegamire *et al*. (1998) studied the relationship between *Sphaceloma* scab infection and some seed yield components of cowpea in Uganda using principal component and path analyses approaches and reported that severe scab infections both directly and indirectly significantly affected the yield of cowpea. Thus, foliar scab severity exhibited a negative direct effect on yield, and indirectly, by reducing the number of pods per plant and pod length while pod scab severity indirectly reduced cowpea yields through reduced number of pods per plant and pod length suggesting deleterious effects of scab disease directly on yield and indirectly through reduction in the number of pods per plant and pod length, similar to previous reports by Emechebe (1980) and (Iceduna, 1993). Tumwegamire *et al*. (1998) recorded lower but significant ($P < 0.05$) phenotypic coefficients for both foliar ($r = -0.186$) and pod ($r = -0.159$) and attributed the low values to the high genotypic variances for scab resistance among the cowpea lines studied. Also, foliar scab infections showed significant ($P < 0.05$) negative correlations with yield components such as number of seeds per pod ($r = -0.213$) and number of branches ($r = -0.194$).

Other studies relating to the effect of *Sphaceloma* scab on grain yield of cowpea were conducted by Mbong *et al*. (2012) involving three cowpea varieties obtained from the IITA over three cropping seasons and data obtained from the different scab infected plant parts and grain yield were correlated and path coefficient analyses were run to determine the direct, indirect effects and percent contributions of scab to grain yield. They recorded yield reductions ranging from 13.7% to 67.1% depending on the susceptibility of the varieties used. According to Mbong *et al*. (2012), among all the parameters that contributed to scab infection on grain yield of the three varieties of cowpea, pod scab was the major contributor that reduced grain yield of cowpea confirming previous studies by Mbong *et al*. (2010a)

2.3 Morphological characterization

Like other imperfect fungi, the genus *Sphaceloma* which is the imperfect stage of the genus *Elsinoe* are identified according to their conidial or non- sexual states and the number of septa as all imperfect fungi have septa (Barnett and Hunter, 1987). Morphological characterization of *Sphaceloma* and its related genus *Elsinoe* have mainly been based on the colony characters as seen on agar media (Barnett and Hunter, 1987).

The relationship of some *Elsinoe* and *Sphaceloma* species pathogenic on cassava and other Euphorbiaceae collected from farmers' fields in Central and South America was studied by Zeigler and Lozano (1983). Twelve isolates of *Sphaceloma* and *Elsinoe* spp. collected from different hosts including *Euphorbia brasiliensis, Eu. heterophylla* and cassava (*Manihot esculenta*) were cultured on acidified (lactic acid) potato dextrose agar (PDA) in petri dishes while matured ascospores and asci were measured on water mounts of crushed or freeze-microtomed ascomata. Zeigler and Lozano (1983) observed that isolates from all host plants typically formed slow-growing, pulvinate, or raised and deeply fissured, gummy to occasionally mucoid colonies on agar media with tomentose colonies found in all species but more common in isolates of *S. poinsettiae* and the *Sphaceloma* state of *E. brasiliensis*. Furthermore, hair-like aggregations of hyphae and white or pinkish aerial mycelia were commonly formed on the colonies for several transfers after the original isolation from the host and after four to five transfers, however, aerial mycelium production was limited to a few small tufts. Also, Zeigler and Lozano (1983) observed that colony color on PDA was extremely variable even within the same isolates, with all isolates, regardless of original host, ranging from orange to yellow or orange to bright red, rust, and brown. The yellow or orange colonies (from single conidia) were observed to have frequently formed small red sectors which yielded entirely red colonies when the pigmented sectors were transferred for several times. They concluded that cultural characteristics and conidial dimensions on PDA were not sufficiently different to aid in separating the species, and that, placing heavy emphasis on cultural characteristics carried the risk of considering normal variants of one species to be different species. They therefore, proposed a combination of several species, *viz.* a combination of *S. poinsettiae* and *S. krugii* under *S. poinsettiae*; *E. jatrophae, E. brasiliensis* and *E. manihoticola* under *E. brasiliensis.*

Timmer *et al*. (1996) studied the morphological and pathological characters of species of *Elsinoe* and *Sphaceloma* causing scab diseases of citrus obtained from Argentina, Australia and Florida. The study involved a total of 45 isolates obtained from infected leaves and fruits cultured on Whiteside's (1986) selective medium and then transferred to PDA plates and grown for three weeks at 27 ^{o}C in the dark to determine colony morphology and colour. Conidia from the isolates were obtained based on the methods of Whiteside (1978) and Kennedy (1988). Timmer *et al*. (1996) observed that, colonies of all Australian isolates of *S. fawcettii* var. *scabiosa* were blood-coloured at the colony edge and vinaceous in the centre while colonies of *E. fawcettii* from Florida and Argentina varied from pale ochraceous to dark vinaceous in the centre and were generally lighter coloured than Australian isolates. Further, *E. australis* isolates from Argentina were generally found to be highly pigmented with some becoming black near the centre. On the other hand, Timmer *et al*. (1996) observed that the conidia of all isolates produced in culture were hyaline, elliptical to obclavate, non-septate, measuring 4-8 × 2-3 µm in length with no significant differences in means of the lengths of species. They suggested in corroboration with previous reports that the colony colour of *E. australis*, *E. fawcettii* and *S. fawcettii* could vary with the cultural medium (Jenkins, 1931) and colony age, and thus, concluded that colony colour was not very useful for identification purposes, consistent with Zeigler and Lozano (1983).

In an attempt to verify 19 collections of isolates as belonging to the genus *Sphaceloma* prior to molecular and pathogenicity characterizations, Alvarez *et al*. (2003) cultured samples of infected leaves, stem and petioles of cassava on acidified PDA to observe the morphological characteristics. Conidia of the isolates were observed to be small, thin-walled, ellipsoid to (rarely) globose, commonly with one or two guttules, with the conidiophores being phialides, hyaline to slightly pigmented 0-1 septate; while conidiophores from the weedy species were phialides, hyaline to brown 0-2 septate producing hyaline conidia. The morphological characters of scab was described by Singh and Allen (1979) as having a hyaline, scanty and submerged mycelium, with hyaline to pale coloured conidia produced in pycnidia, and ascospores borne on the asci and are hyaline, pale coloured oblong to elliptical and 3 septate.

2.4 Molecular characterization

According to Sudré *et al*. (2010) and Moulin *et al*. (2012), the correct identification and characterization of genotypes or isolates of a pathogen are fundamentally important for crop

genetic improvement programmes. Various types of molecular markers have been used for purposes such as the mapping of specific genes in plants or identification of successfully transformed organisms, selection of superior genotypes in breeding programmes (i.e. marker assisted selections) and the identification of species or isolates of pathogens. The different markers have been found to have strengths or limitations in their application. According to Semagn *et al.* (2006), the presence of various types of molecular markers, and differences in their principles, methodologies, and applications require careful consideration in choosing one or more of such methods. No molecular markers are available yet that fulfill all requirements needed by researchers.

There is little knowledge about the use of molecular marker techniques in characterization of the cowpea scab fungus (*Sphaceloma* sp). On the other hand, molecular marker techniques have been applied in characterization of some closely related genera and species such as the *Sphaceloma manihoticola* in cassava (Alvarez *et al*, 2003), *Elsinoe* ssp. in citrus (Hyun *et al*., 2009) and *E. fawcetti* in citrus (Hou *et al*., 2014).

In characterization of *Sphaceloma manihoticola* (asexual stage of *Elsinoe brasiliensis*), the causal organism of super elongation disease in cassava (*Manihot esculenta* Crantz), the internal transcribed spacer (ITS) region of ribosomal DNA of 19 isolates was amplified by polymerase chain reaction (PCR) with primers ITS4 and ITS5 and the PCR products were digested with the enzymes *Msp*I, *Cfo*I, *Hinf*I, *Hae*III, and *Taq*I (Alvarez *et al*., 2003). Alvarez *et al*. (2003) obtained two different band sizes (600 and 650bp) following ITS amplifications and different band patterns based on the digestion with the enzymes *Msp*I and *Cfo*I and concluded that the isolates were of two groups (600bp and 650bp) indicating that they belong to two species confirming earlier reports that more than one species of *Sphaceloma* infects cassava naturally (Zeigler and Lozano, 1983). Furthermore, Alvarez *et al*. (2003) conducted random amplified polymorphic DNA (RAPD) analysis of the 19 isolates using three RAPD primers (OPA-01, OPA-02 and OPA-03) and based on the bands obtained (20-25 bands) and cluster analysis, the 19 isolates were separated into five groups with a similarity level of 0.6.

According to Hyun *et al*. (2009), two scab diseases have been recognized on citrus: citrus scab, caused by *Elsinoe fawcettii*, and sweet orange scab, caused by *E. australis.* Since the two species could not be reliably distinguished by morphological or cultural characteristics, Hyun *et al*. (2009)

studied the genetic relationships of 76 worldwide collections of *Elsinoe* spp. causing scab disease of citrus using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) assays involving nine RAPD primers. It was observed that, out of the total of 76 isolates, 61 which were identified as *E. fawcettii* based on pathogenicity tests were divided into three subgroups while 15 isolates identified as *E. australis* (based on pathogenicity) were differentiated into two groups. According to Hyun *et al*. (2009), the *E. fawcettii* group was clearly distinguished from the *E. asutralis* group through sequence analysis of the internal transcribed spacer (ITS) region and the translation elongation factor 1 α (TEF) gene. The length of the ITS sequence varied from 653 to 654 bp for *E. fawcettii* and 626 bp for *E. australis* while the length of the TEF sequence was 420 bp for both species and thus, the TEF did not clearly differentiate all the subgroups obtained from the RAPD analysis.

Hou *et al*. (2014) studied the host-specificity and genetic relationships among 46 isolates of *E. fawcettii* collections from China using both internal transcribed spacer (ITS) (ITS 4 and ITS5) sequence data and inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) assays. They reported that based on pathogenicity testing on nine different species, the 46 isolates were divided into 11 pathotypes, however, the ISSR-PCR assays separated the isolates into 10 subgroups which basically corresponded to the pathogenicity test. All the 46 isolates were confirmed to be *E. fawcettii* based on the ITS data.

2.5 Genetics of inheritance of Scab resistance (Gene action)

There is great variability in resistance of cowpea lines to the scab disease (Tumwegamire *et al*., 1998). The use of host resistant varieties is the most recommended in the control of scab (Singh and Allen, 1979; Rusoke and Rubaihayo, 1994; Nakawuka and Adipala, 1997; Mbong *et al*., 2012). Though a lot of research has been done on diseases of cowpea, there has not been much studies done specifically with respect to cowpea scab disease.

Following field evaluations conducted by Nakawuka and Adipala (1997), five cowpea lines of which two were resistant, one intermediate resistant and two susceptible, were selected and crossed in a diallel mating scheme to understand the inheritance of resistance to scab in cowpea. The parents (selfers) and F_2 from the diallel were planted in the field using a randomized complete block design and scab-infested cowpea residues introduced in to the experimental plots to provide

initial source of scab inoculum. They suggested greater role of general combining ability (GCA) effects than specific combining ability (SCA) effects in the inheritance of resistance to scab. Thus, additive genetic variation constituted the major portion of the total genetic variance for resistance to scab in cowpea, indicating a type of resistance referred to as non-race specific (van der Plank, 1963) which was in contrast with earlier observations which suggested monogenic resistance whereby a single recessive gene, rss, governs resistance to scab (*Sphaceloma* sp.) (Abadassi *et al*., 1987). Further, Nakawuka and Adipala (1997) observed significant SCA effects for some crosses and attributed those to dominance and epistatic effects since SCA effects reflect the deviation of a cross from its expected performance (Olunju *et al*., 1990; Olurunju *et al*., 1992).

Tumwegamire *et al*. (1998), studied the genetics of resistance to *Sphaceloma* scab of cowpea involving 10 lines comprising four resistant, five moderately resistant and one susceptible line used in a half-diallel cross and the F_1 and F_2 seeds were grown in the field together with their parents during the second rains of 1996 and first rains of 1997 respectively using a randomized complete block design with scab infected cowpea residues introduced between rows to ensure uniform scab infection. They observed that both GCA and SCA effects were important for resistance to scab infection both on foliage and pods suggesting the importance of both additive and non-additive gene actions for scab disease resistance in contrast to Nakawuka and Adipala (1997) who suggested a greater role of GCA effects than SCA. On the other hand, a high GCA:SCA ratio indicated a dominance of additive genetic variance. Tumwegamire *et al*. (1998) reported high values for both broad sense heritability (93.8%) and narrow sense heritability (79.8%) estimates for resistance to foliar infection obtained by the variance components method. The high heritability estimate for resistance to scab infections was an indication of a high proportionate genetic variance value attributable to the observable phenotypic variance (Falconer, 1989; Tumwegamire *et al*., 1998) and therefore suggested that direct selection based on phenotypic variance for scab resistance would be enough for improvement of resistance against scab infections (Falconer, 1989; Diz and Schank, 1995; Tumwegamire *et al*., 1998). Further, Tumwegamire *et al*. (1998) suggested the high narrow-sense heritability (h^2) estimates indicated a high amount of additive genetic variance value which was attributed to the genetic variance observed (Falconer, 1989). Uguru (1995) and Tumwegamire *et al*. (1998) both concluded that the high h^2 obtained suggested additive nature of inheritance of scab resistance and therefore, recommended phenotypic

recurrent selection for resistance to scab as heritability has a major impact on the choice of the methods of breeding (Fehr, 1987).

Tumwegamire *et al*. (1998) also estimated the phenotypic and genotypic correlation coefficients among nine traits and observed that the number of peduncles exhibited the highest coefficient (r = 0.915) with yield followed by pods per plant, seed weight, number of branches and pod length in that order. They thus concluded that, number of peduncles and the latter yield components were the major yield factors in cowpea. The conclusion of Tumwegamire *et al*. (1998) were consistent with Patil and Bhapkar (1987) and Uguru (1995), and further suggested that a significant ($P < 0.001$) correlation (r = 0.949) between genotypic foliar and pod scab severity was indicative of similar genes or genetic mechanisms being involved in the inheritance of resistance to the two types of scab infection (Wallace and El-Zik, 1989) though Nakawuka (1995) suggested the two traits were inherited differently in some cultivars.

2.6 Genetics of inheritance of reproductive traits in cowpea

In heritability and correlation studies involving eight cowpea lines consisting three elite cultivars and five F_5 breeding lines (derived from hybridization of the elite cultivars) laid out in a randomized complete block design, Ajibade and Morakinyo (2000) estimated the broad sense heritability of 10 traits using the variance component method. They reported high heritability estimates for pod length (86%) and 100 seed weight (96%), moderate heritability for the number of seeds per pod (45%), threshing percentage (43%) and seed yield per plant (40%), while number of pods per plant had low heritability (20%). According to Ajibade and Morakinyo (2000), selection for the moderately heritable traits (number of seeds per pod, threshing percentage and seed yield per plant) would have to be done over many generations of inbreeding in order to accumulate the relevant genes and suggested the low heritability estimate for number of pods per plant (20%), was indicative of strong environmental effects.

Omoigui *et al*. (2006) conducted screen house experiments to study the genetic variability and heritability of some reproductive traits involving nine cowpea genotypes selected based on their variability for growth habit, maturity, and seed size. They observed considerable variation among cultivars for duration of reproductive phase, rate of photosynthate partitioning and high genotypic coefficients of variation for days to first flower, 100-seed weight, plant height, and harvest index.

Furthermore, Omoigui *et al*. (2006) reported high broad sense heritability estimate (H^2) for 100-seed weight (98.9%), duration of reproductive phase (94%), days to first flower (84.5%), days to maturity (83.9%), and harvest index (77.3%) and concluded that selection for these traits would be effective for crop improvement as the traits can easily be transferred from parents to offsprings. On the other hand, Omoigui *et al*. (2006) reported low genetic coefficient of variation and heritability for grain yield per plant and suggested that direct selection for grain yield improvement may not be possible, rather, through indirect selection for other secondary traits.

Studies by Nwofia *et al*. (2013) on heritability of yield and yield components of cowpea involving seven cowpea genotypes obtained from IITA evaluated under field conditions in March and September 2011 using a randomized complete block design showed that the genotypic coefficient of variation was lower than the phenotypic coefficient of variation for all the traits measured. They reported broad sense heritability estimates greater than 90% for the number of seeds/pod, pod length, leaf area and plant height, with the number of pods/plant showing higher positive direct effect and correlation on pod yield than other yield traits (0.972 and 0.936, respectively). Nwofia *et al*. (2013) further reported low phenotypic and genotypic variation for the number of branches per plant, 100 seed weight and pod weight and concluded that since the error variances obtained were smaller than the genotypic variances for all the traits, it was an indication that the genotypic component was the major contributor to the total variation for the attributes. Thus, the variations observed were more of genetic than non- genetic contributions. According to Nwofia *et al*. (2013), high variability due to genotypic variances suggested that there was considerable scope for selections among the genotypes as supported by Baye (2002) and Nwofia *et al*. (2006).

Nwosu *et al*. (2013) studied the genetic variability, heritability and genetic advance for six cowpea genotypes under two different agro-ecological environments using an RCBD and reported that phenotypic variances were higher than genotypic variances suggesting that the variations observed among varieties studied were not only due to genotype but also due to environment. They further reported that phenotypic coefficient (PCV) and genotypic coefficient of variation (GCV) were higher at both locations for seed yield per plant, number of seeds per plant, number of seeds per pod, hundred seed weight, dry pod weight, pod length, pods per plant and clusters per plant, relative to other characters studied and they suggested the existence of greater magnitude of variability in these traits indicating possibilities for improvement through selection (Selvam *et al*.,

2000; Vineeta-Kumari *et al*., 2003). Nwosu *et al*. (2013) also reported high broad sense heritability estimates for most of the traits *viz.* pods per plant, seed yield, days to 50% flowering, days to maturity, pod length, pod weight, seeds per pod and 100 seed weight in both environments and suggested the influence of fixable additive gene effects for inheritance of these traits indicating that selection for the traits would lead to fast genetic improvement. Nwosu *et al.* (2013) further reported a high heritability and high genetic advance as percent of mean (GAM) for traits such as pods per plant, peduncle length, pod length, dry pod weight, seeds per pod, number of seeds per plant, 100 seed weight and seed yield per plant, and attributed the observations to the highly additive gene effects, in line with earlier reports by Vineeta-Kumari *et al*. (2003) and Nehru *et al*. (2009). On the other hand, Nwosu *et al*. (2013) recorded high heritability and moderate genetic advance for the number of days to flowering and days to 50% flowering indicating a non-additive gene action which may be epistatic and/or dominance effects. They therefore, suggested mass selection breeding method and family based selection as means of improvement of traits controlled by additive gene action and non-additive gene action, respectively.

CHAPTER THREE

3.0 OCCURRENCE OF COWPEA SCAB DISEASE (*SPHACELOMA* SP.) IN UGANDA

3.1 Abstract

Cowpea (*Viga unguiculata* L. Walp) is the third most important legume food crop in Uganda. It is the main legume food crop in the Eastern and Northern regions of the country, however, its mean yield is less than 400 kg ha^{-1}. Scab (*Sphaceloma sp*.) which is a seed-borne disease is one of the major constraints of cowpea production in the country, capable of causing yield losses of up to 100%. Cowpea scab is the anamorph of *Elsinoe phaseoli* of common bean (bean scab). The disease affects all the above ground parts of the cowpea plant. A study was conducted in the country to determine the incidence, severity and distribution of scab disease in 17 cowpea growing districts across three agro-ecological zones over a two year period. The results indicated that scab disease was widespread in all the districts with mean incidence ranging between 35-70% and mean severity 2-4. Tororo and Amuria districts had the highest incidence and severity, while Bukedea and Arua districts recorded the least disease incidence and severity. Cowpea fields located at altitudes above 1200 m.a.s.l had the highest mean disease incidence (82%) and severity (score = 3.4), while fields located on altitudes lying between 771-990 m.a.s.l registered the least disease incidence (64.7%) and severity (score = 2.7). The type of cultivar grown and cropping system practiced influenced the incidence and severity of the scab disease. The results of this study also showed that scab had high incidence and severity across districts and altitudes in Uganda suggesting the need to develop resistant cultivars. This indicated the need to establish the variability of the pathogen to inform the breeding programme for development of resistant varieties.

Keywords: *Vigna unguiculata*, *Sphaceloma* sp., *Elsinoe*, incidence, severity, distribution.

From this chapter the following paper has been published:

Afutu, E., Agoyi, E.E., Amayo, R., Biruma, M., Rubaihayo, P.R. (2017a). Cowpea scab disease (*Sphaceloma* sp.) in Uganda. *Crop Protection.* http://dx.doi.org/10.1016/j.cropro.2016.06.024.

3.2 Introduction

Cowpea (*Vigna unguiculata* L. Walp) is the most economically important indigenous African legume crop (Langyntuo *et al.*, 2003). It is grown in more than 60 countries either as a food crop or cash crop (Davis *et al.*, 1991) occupying parts of Asia and Oceania, the Middle East, Southern Europe, Africa, Southern USA, Central and South America (Singh *et al.*, 2003). According to Ba *et al*. (2004), Africa is the main area of production, where the crop is very important for low input agriculture which is a characteristic of most parts of the continent.

In Uganda, cowpea is the third most important legume food crop after the common beans (*Phaseolus vulgaris* L.) and groundnuts (*Arachis hypogea* L.), however, it is the main legume food crop in the Eastern and Northern regions (Nabirye *et al*., 2003) where it accounts for most of the production in the country (FAO, 1997). The mean yield of the crop is less than 400 kg ha-1 (CCRP, 2012) with annual production estimated at 20,000 t/yr.

Cowpea farmers face several adverse factors in growing the crop (Asiwe *et al*., 2005) for example, in Nigeria (Singh *et al*., 2003) and Uganda (Rusoke and Rubaihayo, 1994) where diseases are a major production constraint. Insect pests have also been reported as a major production constraint in Uganda (Karungi *et al*., 2000a) and Nigeria (Singh *et al*., 2003). According to Allen (1983), about 40 species of fungi are pathogens of cowpea. Mbong *et al*. (2012), described scab as one of the most destructive diseases of cowpea that was capable of causing yield losses of up to 100% in epidemic infections. Cowpea scab (*Sphaceloma* sp.) is the anamorph of *Elsinoe phaseoli* in common bean (bean scab). Allen (1983) suggested that scab of cowpea is widespread in Tropical Africa and is a major disease in Savannah areas, and is seed-borne. The disease affects all the above ground parts of cowpea (Plate 3.1). Symptoms of leaf infection include the appearance of spots on both leaf surfaces and cupped, small greyish scab lesions along the veins (Iceduna, 1993). The lesions coalesce and cause distortions or rugged appearance under severe infections. Infected stems show oval to elongated silver grey lesions surrounded by red or brown elliptical rings while infected pods show sunken spots with grey centres surrounded by brown borders, malformations, and dark coloured pycnidia formed in the brown spots (Singh and Allen, 1979). Conditions conducive for disease development have been described as moderate temperatures of about 23-28 °C, with three or more consecutive days of wet weather resulting in high relative humidity (Emechebe, 1980).

Though disease assessment is one of the most challenging tasks in working with plant diseases, it is the most important task in the study of plant diseases (Campbell and Neher, 1994). The incidence and severity of fungal diseases, as well as the prevalence rates have been found to be influenced by many factors. These factors have broadly been categorized into three which are, factors relating to the host plant, the pathogen and the environment (Agrios, 2005). The occurrence and the intensity of the disease are dependent on how these three factors interact. However, environmental factors have traditionally been considered to have the most impact on disease development (Keane and Kerr, 1997). According to Cooke and Whipps (1993), infection and disease occurrence on plants due to air-borne fungi are favoured by temperatures ranging between 15-40 ^{o}C. Atmospheric moisture is generally the single most important environmental factor influencing the incidence and severity of fungal diseases on plants (Talley *et al*., 2002).

Practices such as the planting density has also been found to affect the incidence and severity of fungal diseases. Gautam *et al*. (2013) indicated that an increase in biomass can modify the microclimate and affect the risk of infection. On the whole, an increase in plant density is said to increase the duration of leaf surface wetness and regulate temperature, thereby making infection by foliar pathogens more likely (Yáñez-López *et al*., 2012; Gautam *et al*., 2013). The recommended spacing for cowpea has been given as 50 × 20, 75 × 20 and 75 × 30-50 cm for erect, semi-erect and creeping types respectively (Dugje *et al*., 2009). The cultivar of cowpea grown, the source of seeds for planting and other husbandry practices may also influence disease incidence and severity (West *et al*., 2001). Studies on the scab disease in Uganda commenced in 1988 when the disease was reported to be rife in the country in the preceding years (Takan, 1988). Successive studies on the disease were carried out by Iceduna *et al*. (1994), Nakawuka and Adipala (1997) and Tumwegamire *et al*. (1998) but since then, no other studies on the disease was considered until resurgence of the disease in the country in 2010 necessitating further research on the disease by the National Semi-Arid Resources Research Institute (NaSARRI) and to develop resistant varieties to the disease as there is currently no improved cultivar resistant to the disease in the country. There was therefore a need to conduct studies on the occurrence and distribution of the scab disease under different ecological zones in the major cowpea growing areas of Uganda to generate information that will serve as basis for the breeding programme.

3.3 Methodology

Field surveys were conducted in two years (2013 and 2014) within the major cowpea growing areas in Uganda. Fifteen districts were surveyed in 2013 (Figure 3.1a.) while 14 districts were surveyed in 2014 (Figure 3.1b). Twelve districts were common in both years of study while five districts (three for 2013 and two for 2014) had only one year data (two seasons combined).

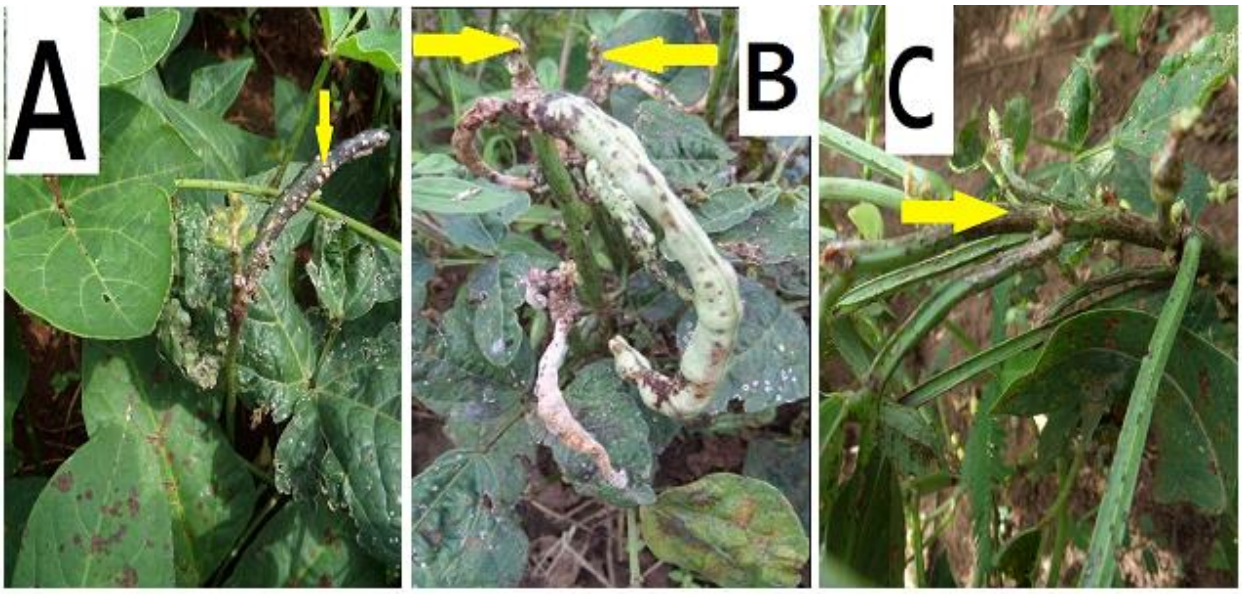

Plate 3.1: Scab disease symptoms on (A) infected leaves and pods, (B) flower cushions and (C) stem of cowpea. Plates show oval to elongated silver grey lesions surrounded by red or brown elliptical rings (1A and 1B) while infected pods show sunken spots with grey centres surrounded by brown borders (1A). Source: Personal photos.

The dropping or adding of new districts in the 2014 study were based on new reports received on high occurrence of the disease on some farmers' fields within some Districts which were not covered in 2013. A total of 17 districts covering three (3) Agro-ecological Zones were surveyed. Districts under the Eastern Agro-ecological Zones (EAEZ) were surveyed in May 2013 and November 2014 while the districts within the North-Eastern Savanna Grasslands (NESG) and North-Western Savannah Grassland (NWSG) were surveyed in November 2013 and December of 2014 reflecting the differences in time of planting across the regions and the need to take observations at mid-podding stage. The average rainfall for the EAEZ, NESG and NWSG are 875 mm – 1125 mm, 1250 mm – 1500 mm and 875 mm – 1250 mm respectively (NEMA, 2009). The number of districts surveyed under the various Agro-Ecological Zones were chosen in consultation with the Cowpea Breeding Programme at the National Semi-Arid Resources Research Institute

(NaSARRI) on the basis of the size of the Agro-Ecological Zones and the areas where the cowpea crop is mostly grown in the districts. A total of 87 Sub-Counties (45 in 2013 and 42 in 2014) were selected across the districts and from each Sub-County, three cowpea farms were selected based on the purposive sampling technique of Gray *et al*., (2007). In all, a total of two hundred and sixty one (261) cowpea farms were surveyed. Data were collected on the incidence and severity of scab disease at three different sampling points on each of the farms. Other data collected were the cropping system employed, previous crop on the field, the variety cultivated, field planting distances and the type of crop/plants surrounding the field. Global Positioning System (GPS) readings of latitude, longitude and altitude were recorded for each location where data were collected.

3.3.1 Data Analysis

Twenty (20) plants were observed for the presence of scab disease symptoms by taking a transact walk across the field and earmarking three sampling points which were 10 m apart along the transact (Ddamulira *et al*., 2014). Disease incidence was expressed as the percentage of infected plants over the 20 plants within the sampling point. Scab disease severity was scored using a scale of 1-5, where 1 = no symptoms, 2 = less than 10% infection, 3 = 10 to 20% infection, 4 = 20 to 50% infection, and 5 = more than 50% infection (Tumwegamire *et al*., 1998). Infected leaves and pods where available, were collected from each sampling point and wrapped in absorbent tissue and further wrapped in aluminium foil and kept on ice for pathogen isolation in the laboratory as shown in Plates 3.2A-2D.

Scab disease incidence and severity maps were developed using GPS survey data points obtained from each sampling location and incidence and severity means generated from data analysis (Ddamulira *et al*., 2014). Correlation between incidence and severity means was done according to Payne *et al*. (2011) and data points were transformed into a point map using Ilwis 3.2 software (Toxopeus, 1997). The maps were exported and visualised in Arc View® GIS3.2 software (Rockware Inc). Disease incidence data was transformed using arcsine transformation of arcsine percentage (Gomez and Gomez, 1984) following a Kurtosis-Skewness test which showed a significant difference from the normal and the transformed data was analysed using Genstat edition 14 (Payne *et al*., 2011). Scab disease incidence and severity means were separated using Fisher's protected Least Significant Difference (LSD) test at $P < 0.05$. Chi-square test for independence or

association of incidence and severity data with altitude was done in Genstat using the maximum likelihood method because it is more accurate (Payne *et al*., 2003). Cluster analysis was performed using R statistical package version 3.1.2 for windows. Clustering was done using Euclidean distances generated and the average method was used to generate hierarchical clusters.

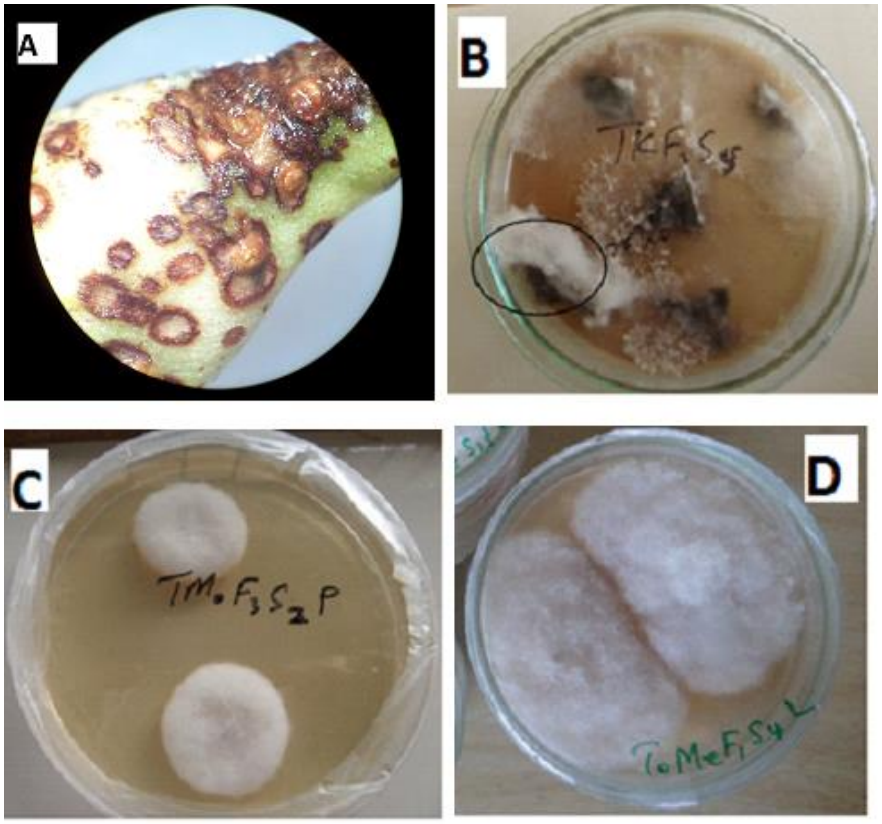

Plate 3.2: (A) Stereoscopic microscope view of scab-infected cowpea pod (magnification = 2X); (B) *Sphaceloma* sp growth on an infected pod (circled in black) on 7-day old PDA culture prior to isolation; (C) Single conidium isolate on PDA; (D) single conidia grown to cover entire petri dish. Note: Labels ending with P (Plate C) and L (Plate D) implies isolation from an infected pod and leaf respectively.

3.4 Results

The results of genotypes and cropping systems practiced in the various districts are presented in Table 3.1. The 17 districts surveyed lie on mean altitudes ranging between 894 and 1221 m.a.s.l. Amuria and Tororo districts predominantly cultivated cowpea genotypes such as Sunshine, WC 29 and WC36 which have been found to be susceptible. The remaining districts were found to have cultivated genotypes which are rated as moderately resistant to the scab disease (Table 3.1).

Table 3.1: Genotypes and cropping systems in districts

District	Altitude (m.a.s.l.)	Genotype	Yield potential (t/ha)[a]	Resistance level[b]	% Inter-cropping	Crops grown
Amuria	1029	Sunshine, WC 29	0.72 and 0.89	Susceptible	56	Beans/ pigeon pea/ groundnuts/ Cassava
Bukedea	1103	SECOW 3B, Alegi	1.45 and 1.42	Moderate	71	Maize/ Cassava/ Groundnut
Kaberamaido	1094	SECOW 2W	1.55	Moderate	60	Sorghum/ Green gram
Kumi	1093	SECOW 2W, SECOW 3B	1.55 and 1.45	Moderate	48	Maize/ Cassava/ Sorghum
Ngora	1073	SECOW 2W, NE 50	1.55 and 1.45	Moderate	75	Maize/ Cassava/ Sorghum
Palisa	1089	SECOW 2W	1.55	Moderate	86	Maize/ Cassava/ Sorghum/ Millet
Serere	1077	SECOW 2W, SECOW 3B	1.55 and 1.45	Moderate	64	Maize/ Cassava/ Sorghum/ Green gram
Soroti	1085	SECOW 2W, SECOW 3B	1.55 and 1.45	Moderate	59	Maize/ Cassava/ Sorghum/ Green gram
Tororo	1221	WC 29, WC 36	0.89 and 1.21	Susceptible	50	Bean/ Maize/ Cassava/ Green gram
Apac	1061	Alegi	1.42	Moderate	48	Bean/ Maize/ Cassava/ Sorghum
Dokolo	1069	WC 10, NE 50	1.61 and 1.45	Moderate	47	Maize/ Sunflower/ Cassava
Kitgum	953	WC 10, NE 50	1.61 and 1.45	Moderate	56	Maize/ Green gram
Lira	1083	Alegi	1.42	Moderate	80	Bean/ Maize/ Cassava/ Millet
Pader	1031	Alegi	1.42	Moderate	69	Bean/ Maize/ Groundnuts/ Sorghum
Arua	926	Alegi, WC 10	1.42 and 1.61	Moderate	88	Maize/ Cassava/ Sorghum/ Pigeon Pea
Nebbi	894	Alegi, NE 48	1.42 and 1.43	Moderate	86	Maize/ Cassava
Yumbe	910	Alegi, NE 50	1.42 and 1.45	Moderate	69	Maize

[a] where two genotypes occur, first yield written corresponds with first genotype and second yield indicated corresponds with second genotype within the districts;
[b] resistance levels are based on results from NaSARRI following field screenings of germplasm.

The genotypes included two improved cultivars for high yield which were SECOW 2W and SECOW 3B, a local cultivar called Alegi, and the remaining genotypes were landraces. The percentage intercropping observed at the districts ranged between 47% and 88% with different crop combinations including cereals, other leguminous crops and root crops.

The results of scab disease incidence are presented in figure 3.1A for 2013 and 3.1B for 2014. There were differences in scab disease incidences among the districts studied. There were also significant difference ($P < 0.05$) across different altitudes (Table 3.3). Mean scab disease incidence in 2013 ranged between 10-52%. The highest was recorded in Tororo (52%), followed by Kumi, Serere and Kaberamaido which recorded between 31-36% with the remaining districts recording incidences less than 30% (Fig. 3.1A).

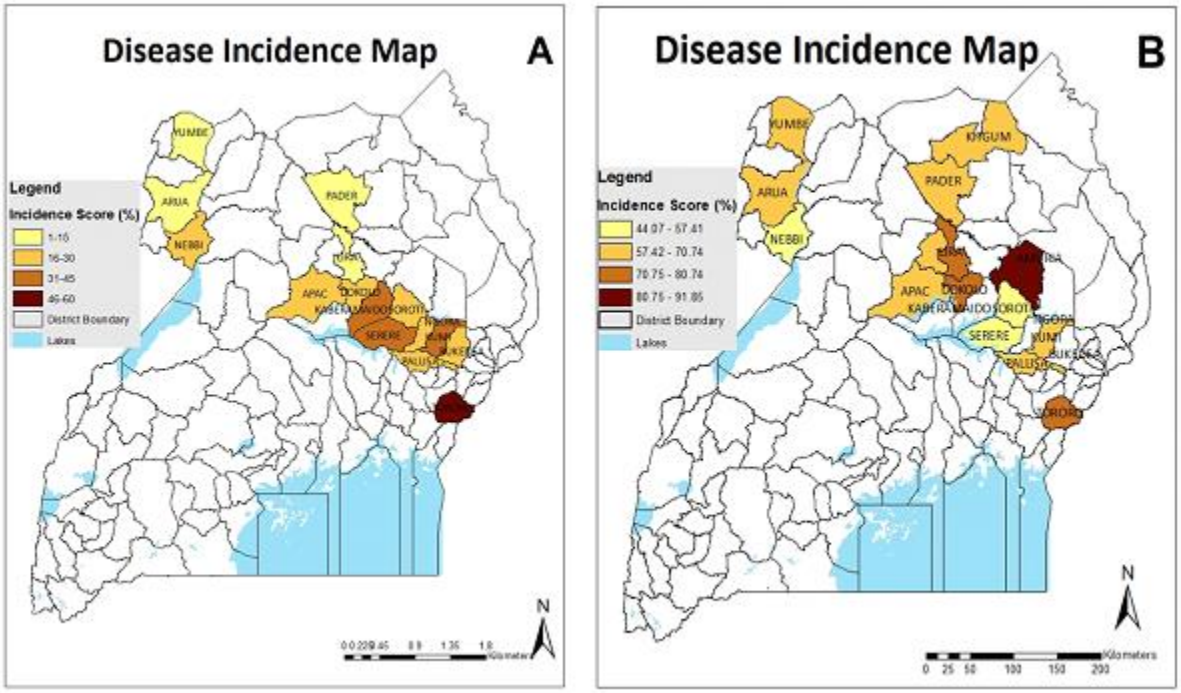

Figure 3.1. A. Incidence of *Sphaceloma* sp in fifteen cowpea growing districts during 2013 cropping season (September-December), B. Incidence of *Sphaceloma* sp in fourteen cowpea growing districts during 2014 cropping season (September-December).

The mean scab disease incidence in 2014 ranged between 44-92% (Fig. 3.1B). Amuria district recorded the highest incidence rate of 92%, followed by Lira, Tororo, Dokolo and Apac districts with mean incidence rates of 80%, 77%, 74% and 71% respectively. Kumi, Arua, Kitgum, Pader, Palisa and Yumbe districts recorded between 64-69%. The remaining three districts, viz., Nebbi, Soroti and Serere recorded incidence rates between 44-57% (Fig. 3.1B).

The results of scab disease severity are presented in Figure 3.2A for 2013 and 3.2B for 2014. Again, the results varied both among the surveyed districts and sub-counties across the two years of the study. The mean scab severity ranged from 2-4 (mild to severe) in both years. In 2013, Tororo district again recorded the highest mean severity score of 4 (severe) while Serere, Soroti, Palisa, Ngora and Bukedea districts recorded moderate severity scores of 3. All the remaining districts recorded mild infections with mean score of 2 (Fig. 3.2A). However, in 2014, Tororo, Amuria and Palisa districts recorded the highest scab disease severity score of 4. All the remaining districts recorded moderate scab severities (mean severity score = 3) except for Arua district which recorded a mean severity score of 2 (mild) (Fig. 3.2B).

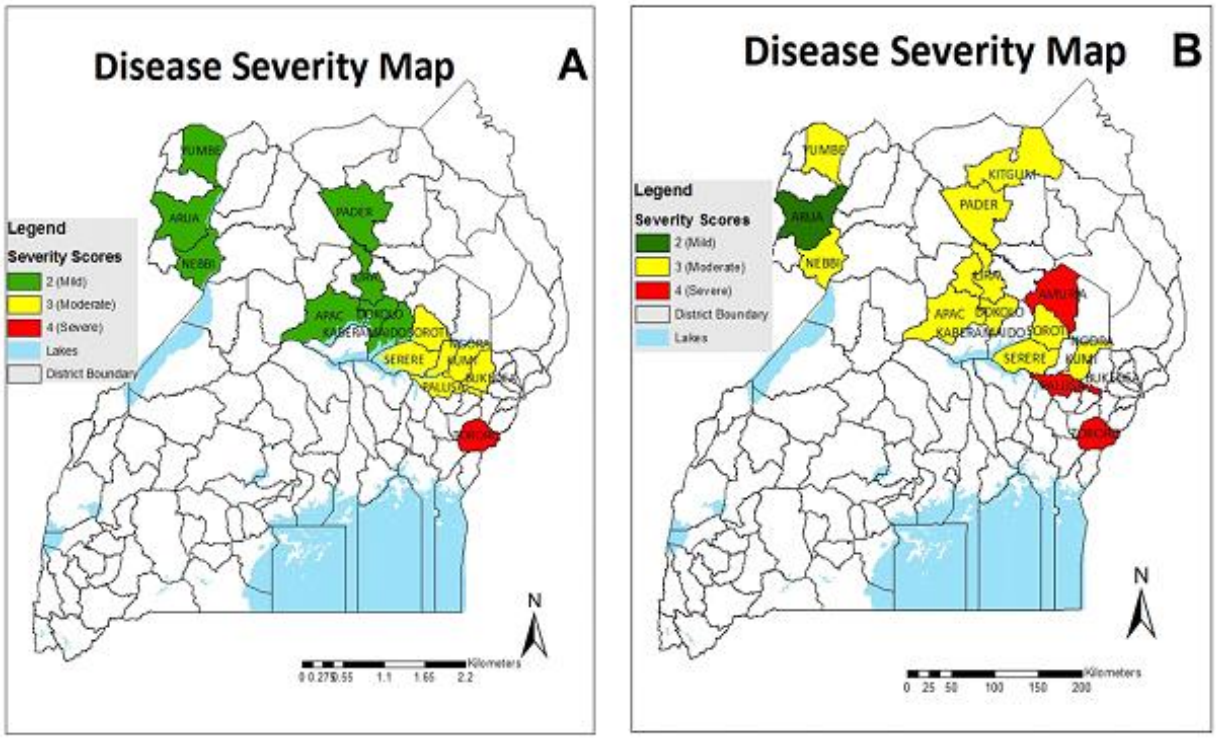

Figure 3.2. A. Severity of *Sphaceloma* sp in fifteen cowpea growing districts during 2013 cropping season (September-December), B. Severity of *Sphaceloma* sp in fifteen cowpea growing districts during 2014 cropping season (September-December).

Results of the effects of cropping system and previous crop history on the occurrence of scab disease are presented in Table 3.2. The results indicated that the mean disease incidence recorded in intercropped fields were significantly higher ($P < 0.05$) than mean incidence recorded in fields where monocropping was practiced. Also, the mean incidence of scab disease on fields where either legumes/cassava was previously cultivated (Table 3.2) was significantly higher ($P < 0.05$)

than incidence recorded on fields where other crops were previously cultivated. However, the severity of scab was not significantly different under the different cropping systems and previous crop cultivated.

Table 3.2: Effect of cropping system and previous crop history on the occurrence of scab disease

Cropping System	Mean disease incidence (%)	Mean disease severity
Sole cropping	65.80a	3.1a
Intercropped	69.00b	3.3a
CV %	22.71	23.58
LSD (0.05)	2.94	0.27
Previous crop		
Other crop	64.14a	3.1a
Legume/Cassava	70.98b	3.2a
CV %	22.36	23.67
LSD (0.05)	4.8	0.27

Figures followed by the same letter indicate lack of significance while those with different letter indicate significant differences.

The results of mean incidence and severity of scab at different altitudes over the 2 years are presented in Table 3.3. The mean incidence recorded at areas which lie above 1200 m.a.s.l. were higher and significantly different ($P < 0.01$) from mean incidence recorded in areas below 1200 m.a.s.l. However, there was no significant difference in mean disease incidence recorded for areas which lie at different altitudes below 1200 m.a.s.l.

The severity of scab also varied significantly ($P < 0.01$) across altitudes (Table 3.3). Thus, a mean scab severity of 3.60 was recorded at altitudes above 1200 m.a.s.l. which was significantly higher ($P < 0.01$) than severity values recorded at altitudes below 1200 m.a.s.l. There was a positive correlation ($r = 0.69$, $P < 0.001$) (Table 3.3) observed between disease incidence and severity across districts indicating that the two disease parameters were conditioned by same environment.

Table 3.3: Mean incidence and severity of scab at three different altitudes in Uganda over 2 years

Altitude (M) a.s.l	Incidence (%)	Severity (1-5)
551-875	23.60a	2.05a
876-1200	37.32a	2.63a
>1200	61.11b	3.60b
$LSD_{(0.01)}$	18.71	0.69
Correlation (r)	0.69	
P	< 0.001	

Figures followed by the same letter indicate lack of significance while those with different letter indicate significant differences.

Results of chi-square test for independence to test the null hypothesis that high scab disease incidence is independent of altitude are presented in Table 3.4. The results gave the test statistic values ($\chi^2 = 23.54$, df = 6, and $P < 0.001$). The highly significant difference in scab incidence with altitude suggested a rejection of the null hypothesis thus indicating that high disease incidence is dependent on altitude. Out of the 15.858 margin contributed by altitude above 1200 m.a.s.l. to the chi-square statistic of 23.54, high incidence contributed 7.783 of the margin.

Table 3.4: Combined chi-square test for both scab disease incidence and severity

	Incidence					Severity					
Altitude (M a.s.l.)	Low	Med-ium	High	Very high	Mar-gin	Low	Mild	Mode-rate	Severe	Very severe	Mar-Gin
551-875	2.675	1.637	2.848	0.008	7.169	1.584	2.248	1.351	1.587	1.387	8.157
876-1200	0.011	0.207	0.236	0.057	0.511	0.021	0.017	0.040	0.015	1.019	1.113
>1200	6.299	0.821	7.783	0.955	15.858	7.562	1.368	0.031	0.276	10.287	19.525
Margin	8.986	2.665	10.867	1.019	23.537	9.168	3.633	1.422	1.879	12.693	28.795
χ^2	23.54					28.8					
Df	6					8					
P	< 0.001					< 0.001					

Also, chi-square test for independence of high scab disease severity from altitude, gave the test statistic values (χ^2 = 28.8, df = 8, and P < 0.001). It thus showed a significant difference in scab severity with altitude indicating that high scab disease severity is dependent on altitude. Out of the 19.525 margin contributed by altitude above 1200 m.a.s.l. to the chi-square statistic of 28.8, 10.287 was contributed by severity level rated very severe.

The results of combined analysis of variance for scab disease incidence and severity at district and sub-county levels are presented in Table 3.5. The results for mean incidence of the disease showed highly significant differences (P < 0.001) among districts, sub-county and between seasons. There was no significant difference among the three agro-ecological zones in scab disease incidence. Also, there were highly significant differences (P < 0.001) in both mean severity scores among districts, sub-county, between seasons and among the three agro-ecological zones.

Table 3.5: Combined Analysis of variance for scab disease incidence and severity at district and sub-county levels.

Source of variation	df	Mean square		Pr > F
		Incidence	Severity	
District	16 (394)	4850.40 (650.60)***	7.12 (0.89)***	0.001
Sub-County	74 (336)	2440.10 (456.50)***	2.75 (0.78)***	0.001
Season	1 (409)	162786.10 (418.50)***	44.68 (1.03)***	0.001
AEZ	2 (408)	457.7 (816.30)	26.47 (1.01)***	0.571

Figures in parenthesis are error values; AEZ = Agro-ecological Zone; *** = significant at P < 0.001.

The results for cluster analysis of the 17 districts are presented in Figure 3.3. The 17 districts were grouped into 3 statistically significant clusters. The clustering of the districts was based on the mean scab disease incidence and severity ratings recorded and the prevalence rate of the disease for the two years combined. Cluster 1 was the least weighted containing two districts followed by clusters 2 and 3 with seven and eight districts respectively. Amuria and Tororo districts were put together in cluster 1. Both districts recorded mean prevalence rates of 100%, mean severity score of 4 and mean incidence between 65 and 70%. The seven districts in cluster 2 recorded mean disease incidence between 35% and 48%, mean severity scores of 2-2.5 and disease prevalence rates ranging from 64% to 76%. The districts in cluster 3 recorded mean incidence ranging from

39% to 52%, mean severity scores between 2.5-3.5 and a mean disease prevalence rate between 85% and 97%.

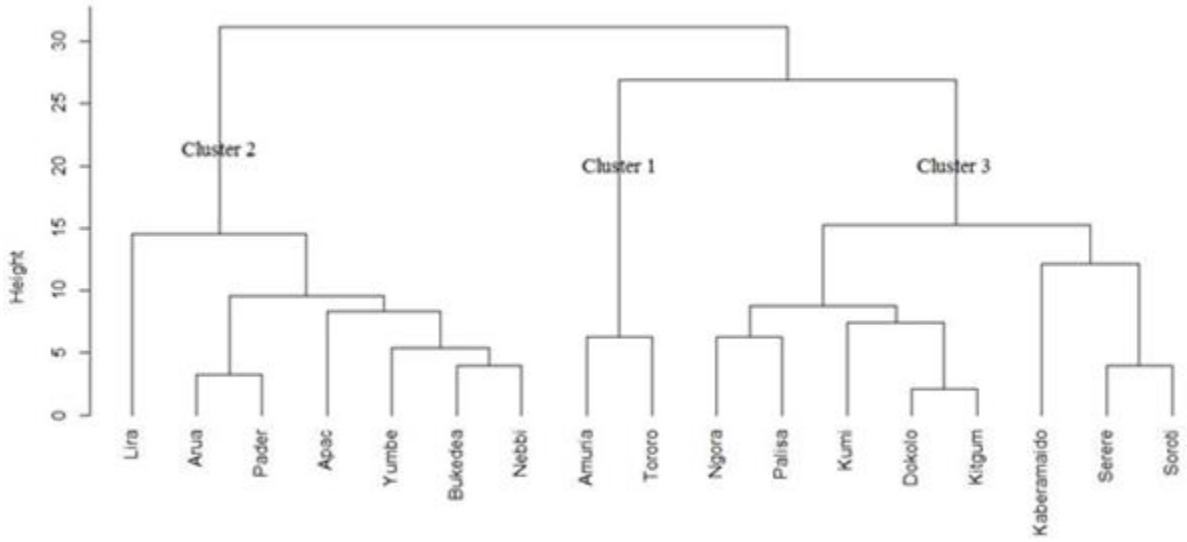

Figure 3.3: Dendogram generated based on mean disease incidences, severity scores and disease prevalence rates across 17 districts over two years period.

3.5 Discussion

The disease was found to occur at varying levels in all districts (Figs. 3.1A-2B), cropping systems (Table 3.2) and altitudes studied (Table 3.3). This clearly indicated the need for the use of scab resistant varieties for cultivation by the farmers. The results show that development of the disease was favoured by the prevailing conditions in all the cowpea growing areas (Table 3.1).

Among other factors that influence the incidence and severity of scab were environmental factors and husbandry practices. More specifically, the results in this study were explained by environmental factors such as temperature and relative humidity. Notwithstanding the differences in altitudes between Amuria and Tororo districts, they both recorded the highest occurrences and severities of the disease. Both districts record temperatures above the 25 ^{o}C threshold for the development of scab disease (Phillips, 1996). Optimum temperatures for development of the disease was reported as ranging between 23-28 ^{o}C (Bressani, 1985). Also, husbandry practices such as the continuous use of a susceptible cultivar overtime and choice of seed source lead to adaptation of pathogens in particular varieties for example, Sunshine and WC 29 (West *et al*., 2001) and locations such as Tororo and Amuria (Sartorato, 2004). It further explains why districts

such as Arua, Yumbe and others recorded relatively lower incidences and severities consistently in both years. These districts were observed to cultivate mostly the local cultivar known as Alegi which has been found to be moderately resistant to the disease (Table 3.1).

Since incidence was found to be correlated with severity (Table 3.3), it was to be expected that areas which recorded high incidence rates would as well record high severity scores. Lawrence (2005) suggested that relative humidity increases as temperature decreases and since relatively higher altitudes are expected to have higher relative humidity due to lower temperatures (Harrison *et al*., 1997), this could in part explain the observation that areas lying above 1200 m.a.s.l. recorded both the highest occurrence and severity of the disease. These results were verified by the chi-square test statistic results which showed highly significant differences ($P < 0.001$) both for occurrence and severity where data recorded at altitudes above 1200 m.a.s.l. for both disease parameters contributed more than half of the test statistic values. High relative humidity implied long periods of leaf surface wetness which was reported to favour the development and sporulation of fungal diseases (Gautam *et al*., 2013).

A district like Amuria lies at a relatively low altitude but it equally recorded higher incidence and severity than other low lying areas. This means that high relative humidity and lower temperatures might not be the only factors contributing to the high incidence and severity recorded at high altitudes. Other factors such as the cowpea cultivars grown, the source of seeds for planting, and husbandry practices may also influence disease incidence and severity (West *et al*., 2001). For example, it was observed that most sampled fields in Serere, Soroti and Kumi districts were growing SECOW 3B and SECOW 2W varieties (Table 3.1) which are improved cultivars for high yield released by NaSARRI and are moderately resistant to the disease.

The lack of significance in incidence across the different agro-ecologies may be attributable to the fact that there is not much difference in climatic conditions among these three ecological zones. Mugisha (2008) indicated that these zones in general record similar temperatures and relative humidity. The significant differences across districts and sub-counties could be due to the different cowpea varieties grown ranging from local to improved cultivars. Furthermore, different husbandry practices were observed across the locations. For example, moving from one sub-county to another, it was observed that different cropping systems were employed and even where similar cropping systems were carried out, different crop combinations were involved (Table 3.1).

Similar results were reported by Hemannavar (2008) who observed that the occurrence of a disease in a location may greatly be due to provenance effects.

The 17 districts were categorized into 3 statistically significant groups by the cluster analysis (Fig. 3.3). These groups were obtained on the basis of the incidence levels, severity scores and the prevalence of the disease in the districts for two years. Tororo and Amuria districts were categorized as being similar and the two districts were found to be the worst hit by the scab disease during the study. The high occurrence and severity of the disease in Tororo could partly be due to its altitude (mean altitude = 1221 m.a.s.l.) as compared to districts such as Palisa and Kumi which lie between mean altitudes of 1089 and 1093 m.a.s.l. Therefore, Tororo district recorded a relatively high relative humidity (>70%) coupled with high temperatures which were favourable for the development of the disease. According to Yáñez-López *et al*. (2012), this condition causes prolonged periods of leaf surface wetness and therefore favours the development of disease. Secondly, it is partly due to the varieties found to be cultivated in the district (WC 29 and WC 36). Within the whole of the district, no field was found to be cultivated with any improved variety, and this was the same for Amuria district (Sunshine and WC 39). Amuria also recorded high occurrence of the disease because most of the fields in the district were found to be intercropped with other leguminous crops such as common beans which have been reported to be greatly affected by scab (*Elsinoe phaseoli*) (Phillips, 1994). Also, the intercropping of cowpeas with cassava could partly account for the high incidence and severity of the disease in these districts as the scab fungus has also been reported in cassava (*Sphaceloma manihoticola*) and scab fungus isolates obtained from the weed *Euphorbia heterophylla* has been reported to be pathogenic on cassava (Alvarez *et al*., 2003), therefore, suggesting possibilities of cross infections from both common beans and cassava to the cowpea crop.

On the other hand, Apac, Arua, Bukedea, Lira, Nebbi, Pader, and Yumbe districs were found to have clustered together because all these 7 districts recorded incidence rates less than 50% and had mean severity scores between 2-2.5 which was rated as mild infection. The results were partly accounted for by the particular varieties cultivated and the planting densities observed in these districts (Table 3.1). Most of the fields in these districts were observed to have been planted with the local cultivar “Alegi” which has moderate resistance to the disease. Also, the planting distance observed during the study in most of the fields especially for between rows were between 30 and

45 cm which is less than the standard 50-70 cm for the erect and semi-erect varieties (Dugje *et al.*, 2009). Districts such as Serere, Soroti and Bukedea recorded low occurrence of the disease because most of the fields within these districts were found to be cultivating the SECOW 3B and SECOW 2W varieties (Table 3.1) which are moderately resistant to the disease. Although, districts such as Apac, Arua, Lira, Nebbi and Yumbe were found to be predominantly growing unimproved varieties it was observed that the between row distance in most of the fields in these districts ranged between 60-70 cm which is the recommended planting distance (Dugje *et al.*, 2009). Gautam *et al*. (2013) reported that an increase in biomass modifies the microclimate by increasing the duration of leaf surface wetness and regulating temperature, thereby making infection by foliar pathogens more likely.

3.6 Conclusion

The study revealed a wide occurrence of cowpea scab disease in the major cowpea growing areas of Uganda at different altitudes. Altitude, the type of cultivar grown and cropping system practiced influenced the occurrence of the scab disease. Amuria and Tororo districts were found to be hot spots of cowpea scab disease in the country. Future work is needed to establish the variability of the pathogen to better inform breeders working to develop resistant varieties as a management strategy to control the disease.

CHAPTER FOUR

4.0 EVALUATION OF UGANDAN COWPEA GERMPLASM FOR YIELD AND RESISTANCE TO SCAB DISEASE

4.1 Abstract

This study was conducted to identify cowpea (*Vigna unguiculata*) cultivars with high yield potential and resistance to scab disease (*Sphaceloma* sp.) in Uganda. 100 cowpea genotypes were evaluated at two locations (Kabanyolo and Serere) in Uganda between April and July, 2014 using a 10 × 10 alpha lattice design. The cowpea lines differed significantly ($P < 0.05$) in their response to natural disease pressure as determined by disease incidence, apparent infection rate (r) and severity indicated by area under disease progress curve (AUDPC). Analysis of variance showed that there was highly significant differences ($P < 0.001$) in genotypes, locations, AUDPC and other traits and genotype by location (G×L) interaction on AUDPC. The correlation analysis revealed a positive relationship of scab disease incidence with AUDPC (0.8; $P < 0.001$) but a significant ($P < 0.001$) negative relationship with grain yield (-0.8), number of pods per plant (-0.5), number of seeds per pod (-0.5) and 100 seed weight (-0.5). Cluster analysis based on only scab disease indexes produced 4 main clusters while cluster analysis based on disease and yield traits produced 3 main clusters. However, the two different clusters revealed similar grouping patterns in which cowpea lines with similar resistance ratings were shown to form unique clusters. R-mode principal component analysis yielded 4 principal components explaining 62.28% of the variation observed. The study revealed that the use of apparent infection rate "r" alone as an index for rating a genotype for scab reaction was not decisive. One line (NE 15) was found to be resistant to the scab disease at both locations and high yielding and could be used in the cowpea improvement programme to breed for resistance to the scab disease.

Keywords: *Sphaceloma* sp.; severity; AUDPC; apparent infection rate; cluster analysis.

From this chapter the following paper has been published:

Afutu, E., Mohammed, K.E., Odong, T.L., Biruma, M. and Rubaihayo, P.R. (2016a). Evaluation of Ugandan cowpea germplasm for yield and resistance to scab disease. *American Journal of Experimental Agriculture*. 12(2): 1-18. DOI: 10.9734/AJEA/2016/25138

4.2 Introduction

Cowpea (*Vigna unguiculata*) is an important component of subsistence agriculture particularly in the dry savannas of sub-Saharan Africa (SSA) (Carsky *et al*., 2001). This is due to its drought tolerance, quick growth and rapid ground cover to check soil erosion (Singh *et al*., 2003) and unique ability to fix atmospheric nitrogen (Sanginga *et al*., 2000). Its ability to tolerate shade makes it compatible as an intercrop with maize, millet, sorghum, cotton and several plantation crops (Singh and Emechebe, 1998). Though Cowpea is the most economically important indigenous African legume crop (Langyintuo, *et al*., 2003) grown in more than 60 countries either as a food crop or cash crop (Davis *et al*., 1991), its yields are generally low. Mean yield in Uganda is less than 400 kg ha-1 (Omongo *et al*., 1997). The annual production in the country is estimated to be at 20,000 t/yr, with Northern and Eastern regions accounting for most of the production in the country (FAO, 1997). It is the third most important legume food crop in Uganda after the common beans (*Phaseolus vulgaris* L.) and groundnuts (*Arachis hypogea* L.) (Nabirye *et al*., 2003). Rusoke and Rubaihayo (1994) reported that a yield potential of 3000 kg/ha was achievable. According to Ajeigbe and Singh (2006), the low yields recorded were due to a number of constraints including insect pests, diseases, parasitic weeds, low soil fertility, drought and lack of inputs among others.

Scab (*Sphaceloma sp*.) is one of the major and common diseases of cowpea (Dugje *et al*., 2009). It is widespread in Tropical Africa and very damaging in Savannah areas (Allen, 1983), capable of causing yield losses of up to 100% (Mbong *et al*., 2010a). The disease affects all the above ground parts of cowpea (Emechebe, 1980). Cowpea improvement programme in Uganda was initiated in the late 1960s at Makerere University with the collection of local and exotic accessions, which were screened for yield potential (Rubaihayo *et al*., 1973). The promising selections were evaluated under different management practices for control of diseases (Edema and Adipala, 1996) and insect pests (Karungi *et al*., 2000a, 2000b). Nakawuka and Adipala (1997) screened 75 cowpea lines against scab of which 10 were resistant and observed that in general, local lines were less infected than introductions. These were then used to study the genetics of resistance to scab by Tumwegamire *et al*. (1998) using a half-diallel cross set.

There is currently a surge in the occurrence of the scab disease in the country (Afutu *et al*., 2017a) suggesting that over the years, the fungus has developed variability in its patho-types. Only one line (SECOW 3B) out of the five recently released varieties (SECOW 1T, SECOW 5T, SECOW

2W, SECOW 4W and SECOW 3B) is moderately resistant to the disease. Following screening of 70 lines done by the National Semi Arid Resources Research Institute (NaSARRI) in Serere, some promising lines were identified but there is the need to screen a wider collection and at different locations in the country to ensure selection of stable parents both in terms of yield and resistance to scab.

Cowpea producers in Sub-Saharan Africa are mostly small-scale, resource-poor farmers who cannot afford the management strategies that have been proposed such as regular spraying or timing of planting (Mbong *et al*., 2010b). In contrast, resistant varieties are easily adopted resulting in boosting production dramatically with a positive impact on the farmers' livelihoods. Mbong *et al*. (2012) suggested the use of resistant varieties which would help in disease management and improve the yield of the crop as it would result in lower infections. According to Rusoke and Rubaihayo (1994), the use of host resistant varieties is the most practical control measure available to farmers, and it is environmentally friendly. This study was conducted to identify sources of host resistance and yield potential that could be used in the breeding programmes for the purpose of developing improved resistant varieties to scab.

4.3 Methodology

4.3.1 Experimental materials

100 cowpea lines (Appendix 4.1) consisting of 69 landraces, 25 inbred lines at F_7, 1 local and 5 improved cultivars recently released by the National Semi Arid Resources Research Institute (NaSARRI) Serere, Uganda were used in the study.

4.3.2 Experimental Sites

The screening experiments were planted at two locations, Makerere University Agriculture Research Institute - Kabanyolo (MUARIK) (0°28'N and 32°37'E; 1200 m above sea level) in the Central part of Uganda and the National Semi Arid Resources Research Institute (NaSARRI) in Serere (1°39'N and 33°27'E; 1038 m above sea level), Eastern part of Uganda during the first rainy season (April-July) of 2014. The average rainfall and relative humidity recorded during the period in kabanyolo were 162.8 mm and 69-87 % respectively while Serere recorded 136.3 mm and 61-73 % for rainfall and relative humidity respectively for the same period.

4.3.3 Experimental Design

A 10 × 10 alpha lattice design with 3 replications at each site was used to conduct the evaluation. Each replication had 10 blocks with each block having 10 plots. Each genotype was planted on a plot with an area of 3 m × 3 m with a spacing of 1 m between plots and between replications. A spacing of 60 cm between rows and 30 cm within rows was used. The fields were weeded three times and insecticide application was done twice, one just before flowering and the second during pod setting. No fertilizer or fungicide were applied during the entire growing period.

4.3.4 Data Collection and Analysis

Six weeks after planting, five plants were randomly selected in each plot, tagged and severity ratings done visually at seven days intervals (Mbong *et al*., 2010b) for six consecutive weeks. Disease scoring was done using a scale of 1-5, where 1 = no symptoms, 2 = less than 10% infection, 3 = 10 to 20% infection, 4 = 20 to 50% infection, and 5 = more than 50% infection (Nakawuka and Adipala, 1997). Incidence was estimated by counting all the individual plants with scab disease symptoms in each plot and expressing the number as a ratio over the total number of plants in the plot and multiplied by 100 to express it as a percentage.

Data on yield and yield components including number of days to 50% flowering, number of branches per plant, number of peduncles per plant, number of pods per peduncle, number of pods per plant, pod length (cm), seeds per pod, and 100 seed weight (grams) were recorded. Grain yield (tons ha^{-}1) was estimated from yield per plot.

Mean severity scores were estimated using Microsoft Excel and the means obtained were used to calculate Area Under the Disease Progress Curve (AUDPC) for each of the cowpea lines in Microsoft Excel using the following formula of Campbell and Madden (1990):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right) (t_{i+1} - t_i)$$

Where “t” is the time of each reading, “y” is the percent of affected foliage at each reading and “n” is the number of readings. The variable “t” represents days after planting. The AUDPC as a resistance measurement calculated from the estimated percentages of leaf area affected recorded

on weekly basis, was used to measure resistance (Fry, 1978) of the cowpea lines. Percentage incidence data were Arcsine transformed (Ezueh and Amusan, 1988) and used to estimate the Apparent Infection Rate (r) in Microsoft Excel using van der Plank's equation (van der Plank, 1963):

$$r = \frac{1}{t_2 - t_1} \, log_e \left[\frac{x_2 \, (1 - x_1)}{x_1 \, (1 - x_2)}\right]$$

Where: "t_1" = initial time of disease assessment (i.e. days after planting – DAP); "t_2" = final time of disease assessment (DAP); "x_1" and "x_2" represent amounts of disease present at "t_1" and "t_2" respectively.

Plot means for yield, yield components and scab incidence and severity values were subjected to analysis of variance (ANOVA) using R statistical package for Windows v.3.1.2. Correlation among traits and Principal component analysis (PCA) were calculated using IBM SPSS Statistics version 22 (IBM Corporation, 2013). The PCA was performed using varimax rotation method which is generally considered superior to other orthogonal factor rotation methods in achieving a simplified factor structure (Hair *et al*., 2010). Hierarchical cluster analysis was performed using R statistical package for windows v.3.1.2 based on Ward's (1963) method (Odong *et al*., 2011).

4.4 Results and Discussion

4.4.1 Phenotypic variability

The results of analysis of variance for agronomic traits, scab disease incidence, apparent infection rate and AUDPC evaluated at the two locations are presented in Table 4.1. The results showed highly significant differences ($P < 0.001$) among genotypes for AUDPC, yield traits ($P < 0.01$) and grain yield ($P < 0.05$) indicating the presence of sufficient variability in the lines (Noubissie *et al*., 2011) for these traits. This could in part be explained by the highly significant differences ($P < 0.001$) in the AUDPC observed since each of the traits is affected by scab disease (Emechebe, 1980). Similar findings were reported by Sharawy and El-Fiky (2002). The traits, including incidence of the disease showed highly significant differences ($P < 0.001$) between locations suggesting different disease pressures in the locations. There were highly significant ($P < 0.001$) genotype by location (G×L) interaction effects on AUDPC, days to 50% flowering and 100 seed weight suggesting inconsistent performance in the two locations.

Table 4.1: Mean sum of squares for scab disease incidence, AUDPC and agronomic traits of 100 cowpea lines

Sources of variation	Df	Disease Incidence	Apparent infection rate (r)	AUDPC	Days to flowering	No. of branches	Peduncles / plant	Pods/ peduncle	Pods/ plant	Pod length (cm)	Seeds/ pod	100 seed weight (g)	Grain yield (t/ha)
Genotype (G)	99	174	0.0005	989 ***	19.6 ***	2.241 ***	85	0.1221 ***	22.1 ***	43.71	5.3 **	22.0 ***	0.27 *
Location (L)	1	599747 ***	0.0311 ***	333926 ***	1872.7 ***	14.107 ***	9149 ***	1.2150 ***	2488.8 ***	307.81 **	970.3 ***	1513.4 ***	291.50 ***
G×L	99	177	0.0005	1056 ***	17.8 ***	1.309	80	0.0466	6.1	43.77	3.6	6.8 ***	0.25
Error	400	139	0.0005	609	7.5	1.195	77	0.0650	6.3	37.34	3.7	1.7	0.20

Df = degrees of freedom; *** = significant at 0.001; ** = significant at 0.01; * = significant at 0.05

The results also suggested that for the purpose of breeding, different cultivars have to be developed for different locations (Acquaah, 2007).

4.4.2 Interrelationships among disease indexes, yield and yield traits

The results of correlation analysis among the traits studied are presented in Table 4.2. Scab disease incidence was significantly correlated ($P < 0.001$) with AUDPC (0.8) suggesting that the severity of scab disease increased with incidence. This was expected since scab is a polycyclic epidemic disease and thus, as long as there is fresh new leaf tissues to be infected, the severity of polycyclic diseases will increase as the incidence increases (Burdon, 1987). Mbong *et al*. (2010b) also reported that the incidence of scab disease increased with plant age. Scab disease incidence and AUDPC had a significant ($P < 0.001$) negative correlation, -0.793 and -0.591 respectively, with grain yield. This means that as the incidence and severity of the disease increased, the grain yield decreased significantly through the significant negative effects of scab disease on both the morphological and reproductive growth of cowpea plants (Emechebe, 1980). The incidence of scab also showed a significant negative correlation with the number of pods per plant (-0.547; $P < 0.001$), number of seeds per pod (-0.522; $P < 0.001$) and 100 seed weight (-0.505; $P < 0.001$) implying that there was significant reduction in these yield traits as the incidence of scab increased. Grain yield was found to be significantly positively correlated (0.5; $P < 0.001$) with the number of pods per plant, number of seeds per pod and 100 seed weight which were negatively affected by severity of scab disease indicating that yield was directly related to these traits and any factor affecting them would affect the grain yield (Mbong *et al*., 2012).

4.4.3 Disease intensity, resistance and yield potential

Mean values of final scab disease incidence (FI), severity (FS), area under disease progress curve (AUDPC), yield, apparent infection rate (r) and host resistance (HR) of the cowpea lines grown at Kabanyolo and Serere are presented in Table 4.3. In all cases, scab disease incidence, severity, AUDPC and apparent infection rate (r) were more severe under conditions of lower rainfall (136.3 mm in Serere) than of higher rainfall (162.8 mm in Kabanyolo). This suggests that the scab fungus is more virulent under relatively low moisture conditions as was recorded. However, earlier reports suggested that scab was more severe under wet conditions (Allen, 1983; Mbong *et al*., 2010b).

Table 4.2: Correlation of disease incidence, apparent infection rate, AUDPC and agronomic traits

Traits	Disease Incidence	Apparent infection rate	AUDPC	Days to flowering	No. of branches	Peduncles/ plant	Pods/ peduncle	Pods/ plant	Pod length (cm)	Seeds/ pod	100 seed weight (g)	Grain yield (t/ha)
Disease Incidence	-											
Apparent infection rate	0.197***	-										
AUDPC	0.814***	0.146***	-									
Days to flowering	-0.435***	-0.075	-0.290***	-								
No. of branches	-0.106**	-0.037	-0.066*	0.112*	-							
Peduncles/ plant	-0.389***	-0.072	-0.326***	0.087*	0.399***	-						
Pods/ peduncle	-0.155***	-0.039	-0.118**	-0.038	0.133***	0.208***	-					
Pods/ plant	-0.547***	-0.066	-0.408***	0.287***	0.125***	0.327***	0.172***	-				
Pod length (cm)	-0.116**	-0.053	-0.097*	0.113	0.136***	0.050	-0.006	0.042	-			
Seeds/ pod	-0.522***	-0.103*	-0.384***	0.260***	0.062	0.176***	0.059	0.392***	0.154***	-		
100 seed weight (g)	-0.505***	-0.256***	-0.327***	0.326***	0.063	0.156***	0.031	0.321***	0.193***	0.386***	-	
Grain yield (t/ha)	-0.793***	-0.288***	-0.591***	0.361***	0.142***	0.419***	0.165***	0.481***	0.069*	0.466***	0.525***	-

Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

Final scab disease incidence at Kabanyolo ranged between 0 – 43 % while mean final severity ranged between 1.0 – 5.0 and final scab disease incidence at Serere was 100% with mean final severity ranging from 2.1 – 5.0. The range of values for the final scab disease incidence and severity recorded at Kabanyolo indicated that the disease pressure was lower compared to Serere (Table 4.3) suggesting that the environmental conditions in Serere were more favourable to the scab fungus attack and thus overcame the defense system of the plants (Agrios, 2005).

The apparent rate of infection (r) in Kabanyolo was between 0.000 - 0.046 while Serere recorded between 0.000 - 0.059. The smaller range of 'r' recorded in Kabanyolo compared to Serere was suggestive of disease incidence being lower in Kabanyolo and with a more steady progression in infection compared to Serere. Nagesha and Nargund (2005) reported a wide variation in apparent infection rate (r) in sunflower while Mbong *et al*., (2010b) reported similar findings in cowpea with the values varying among genotypes and attributed these observations to the differences in sowing dates and the effect of the scab disease on the genotypes. According to Parry (1990), the more resistant a variety is, the smaller the 'r' value. Since the initial incidence of the disease in Serere was high (mean incidence = 81.26%) and almost all the genotypes were affected by the end of the evaluation with incidence reaching 100% for most of the genotypes, the difference between the final and initial incidence recorded in Serere were of a narrow range. These results did not delineate genotypes as being resistant or susceptible based on the use of the 'r' alone since a lower 'r' value recorded in Serere did not reflect more resistance. This suggests that the use of 'r' alone as an index to measure resistance to disease may not yield useful results. The 'r' estimated was suggested to quantify the rate of disease development and estimate cultivar resistance (van der Plank, 1963), and to evaluate the effectiveness of fungicide application (Fry, 1978).

The values for AUDPC and hence resistance rating showed that the genotypes behaved differently at the two locations with Serere recording a higher range of AUDPC and thus, showing most of the lines to be susceptible to the disease. Lines such as WC 29, WC 36 and WC 58 were found to be susceptible (AUDPC > 105 which is equivalent to a mean severity score > 3.0) at Kabanyolo but were moderately resistant (AUDPC 104 and 105 respectively) to scab at Serere. On the other hand, cowpea lines such as ACC12 × Secow 2W, ACC26 ×ACC2, Alegi, Alegi × ACC2, NE 50, WC 10 and WC 66 rated as resistant (AUDPC 35-70) to scab disease in Kabanyolo were found to be susceptible to scab in Serere (AUDPC > 105 i.e. mean severity score > 3.0).

Table 4.3: Mean values of FI, FS, AUDPC, Yield, r and HR for the 100 cowpea lines grown at Kabanyolo and Serere, Uganda, in 2014.

	Kabanyolo						Serere					
Line	FI[a] (%)	r	FS[b]	AUDPC	HR	Yield (t/ha)	FI[a] (%)	r	FS[b]	AUDPC	HR	Yield (t/ha)
SEC1T × ACC23	11.14 (17.54)	0.038	2.33	49.00	R	1.333	100.00 (85.93)	0.000	4.27	112.93	S	0.773
SEC1T × Alegi	15.43 (19.63)	0.006	3.00	77.00	MR	2.074	100.00 (85.93)	0.030	4.40	112.70	S	1.016
SEC2W × SEC1T	23.52 (29.13)	0.032	4.00	91.00	MR	1.889	100.00 (85.93)	0.011	4.07	115.97	S	0.599
SEC2W × ACC2	0.00 (4.05)	0.000	1.00	35.00	R	2.111	100.00 (85.93)	0.007	4.47	131.83	S	1.129
SEC3B × SEC2W	3.33 (9.00)	0.016	1.67	42.00	R	2.074	100.00 (85.93)	0.036	3.67	102.43	MR	0.722
SEC4W × SEC5T	2.78 (8.46)	0.015	1.33	38.50	R	1.815	100.00 (85.93)	0.003	4.73	138.37	S	0.874
SEC5T × SEC3B	1.69 (7.26)	0.002	1.67	51.33	R	2.148	100.00 (85.93)	0.036	3.20	87.97	MR	0.672
SEC5T × SEC4W	2.22 (7.88)	0.007	1.67	49.00	R	2.370	100.00 (85.93)	0.042	2.67	88.43	MR	0.694
SEC5T × ACC12	2.23 (7.88)	0.000	1.67	57.17	R	2.037	100.00 (85.93)	0.037	3.67	104.30	MR	0.738
ACC12 × SEC2W	0.00 (4.05)	0.000	1.00	35.00	R	2.148	100.00 (85.93)	0.023	3.60	106.63	S	0.571
ACC12 × SEC3B	2.78 (4.86)	0.015	1.67	44.33	R	2.481	100.00 (85.93)	0.043	2.93	91.70	MR	0.725
ACC12 × SEC5T	0.57 (5.56)	0.008	1.33	40.83	R	2.593	100.00 (85.93)	0.018	4.20	125.53	S	0.508
ACC2 × SEC1T	28.00 (28.46)	0.009	3.67	86.33	MR	1.519	100.00 (85.93)	0.023	4.13	127.17	S	0.611
ACC2 × ACC12	0.00 (4.05)	0.000	1.00	35.00	R	1.556	100.00 (85.93)	0.038	3.33	98.47	MR	0.651
ACC23 × SEC2W	8.31 (14.51)	0.013	2.33	59.50	R	1.667	100.00 (85.93)	0.020	3.00	93.10	MR	0.630
ACC23 × SEC3B	5.61 (12.74)	0.017	1.33	45.50	R	1.667	100.00 (85.93)	0.020	3.27	94.03	MR	0.910
ACC23 × SEC4W	5.00 (10.43)	0.002	2.00	54.83	R	1.593	100.00 (85.93)	0.038	2.93	82.60	MR	1.043
ACC23 × ACC12	0.00 (4.05)	0.000	1.00	35.00	R	1.963	100.00 (85.93)	0.019	3.20	96.83	MR	0.680
ACC26 × SEC1T	9.68 (16.55)	0.024	2.33	60.67	R	1.926	100.00 (85.93)	0.012	2.93	89.60	MR	1.011
ACC26 × ACC2	0.00 (4.05)	0.000	1.00	35.00	R	1.741	100.00 (85.93)	0.007	5.00	142.33	S	0.598
Alegi	9.14 (15.94)	0.020	2.33	61.83	R	1.963	100.00 (85.93)	0.009	3.73	116.67	S	0.875
Alegi × SEC3B	14.17 (16.36)	0.007	2.33	71.17	MR	1.815	100.00 (85.93)	0.019	3.73	112.23	S	0.563
Alegi × SEC4W	6.67 (11.68)	0.010	2.00	54.83	R	2.259	100.00 (85.93)	0.040	2.93	80.27	MR	0.513
Alegi × SEC5T	3.90 (9.51)	0.003	1.67	50.17	R	2.444	100.00 (85.93)	0.009	3.73	115.27	S	0.886
Alegi × ACC12	7.33 (14.23)	0.014	2.33	56.00	R	1.704	100.00 (85.93)	0.044	2.93	94.97	MR	1.494
Alegi × ACC2	15.03 (16.86)	0.004	2.33	64.17	R	2.000	100.00 (85.93)	0.011	4.40	123.90	S	1.333
NE13	3.33 (9.00)	0.009	1.67	49.00	R	1.593	100.00 (85.93)	0.012	3.60	107.33	S	0.708
NE15	0.00 (4.05)	0.000	1.00	35.00	R	2.222	100.00 (85.93)	0.052	2.13	70.40	R	1.044
NE18	3.89 (9.51)	0.017	1.67	44.33	R	2.000	100.00 (85.93)	0.043	3.07	97.53	MR	0.985
NE19	32.43 (31.02)	0.008	3.33	94.50	MR	1.111	100.00 (85.93)	0.019	3.67	108.97	S	0.750
NE20	15.86 (20.52)	0.021	3.00	73.50	MR	2.259	100.00 (85.93)	0.020	3.13	98.93	MR	0.777

Table 4.3 continued

	Kabanyolo						Serere					
Line	FI[a] (%)	r	FS[b]	AUDPC	HR	Yield (t/ha)	FI[a] (%)	r	FS[b]	AUDPC	HR	Yield (t/ha)
NE21	1.69 (7.26)	0.013	1.67	44.33	R	2.333	100.00 (85.93)	0.031	3.20	92.87	MR	0.844
NE23	0.00 (4.05)	0.000	1.00	35.00	R	1.815	100.00 (85.93)	0.029	2.80	95.43	MR	0.800
NE30	0.00 (4.05)	0.000	1.00	35.00	R	1.407	100.00 (85.93)	0.015	4.20	119.47	S	0.995
NE31	0.00 (4.05)	0.000	1.00	35.00	R	1.778	100.00 (85.93)	0.024	3.40	96.37	MR	1.144
NE39	4.44 (11.41)	0.027	2.00	47.83	R	1.778	100.00 (85.93)	0.038	3.20	86.10	MR	0.610
NE4	12.88 (18.95)	0.008	3.00	81.67	MR	1.852	100.00 (85.93)	0.037	3.27	96.60	MR	0.686
NE40	12.00 (15.09)	0.025	2.00	45.50	R	1.630	100.00 (85.93)	0.047	3.87	115.97	S	0.789
NE41	1.67 (7.22)	0.012	1.33	38.50	R	1.333	100.00 (85.93)	0.028	4.13	125.30	S	0.818
NE44	14.66 (20.19)	0.028	2.67	57.17	R	1.926	100.00 (85.93)	0.020	3.67	105.00	MR	0.830
NE46	3.45 (9.11)	0.006	2.00	58.33	R	2.444	100.00 (85.93)	0.035	3.00	99.87	MR	0.834
NE48	9.33 (13.46)	0.004	2.33	66.50	R	2.037	100.00 (85.93)	0.034	2.73	84.70	MR	0.818
NE49	19.59 (22.35)	0.008	3.00	89.83	MR	1.852	100.00 (85.93)	0.048	3.00	86.33	MR	1.031
NE5	1.15 (6.52)	0.003	1.33	45.50	R	2.667	100.00 (85.93)	0.015	4.20	122.97	S	0.805
NE50	11.67 (17.99)	0.023	2.67	64.17	R	2.148	100.00 (85.93)	0.013	4.87	140.47	S	0.751
NE51	0.00 (4.05)	0.000	1.00	35.00	R	2.778	100.00 (85.93)	0.046	2.40	73.27	MR	0.893
NE53	3.89 (10.48)	0.016	2.00	52.50	R	1.704	100.00 (85.93)	0.027	3.13	90.30	MR	0.738
NE55	2.22 (7.88)	0.014	1.67	46.67	R	1.852	100.00 (85.93)	0.031	3.73	99.87	MR	1.141
NE6	3.89 (9.50)	0.017	1.67	39.67	R	1.741	100.00 (85.93)	0.000	4.07	116.90	S	0.881
NE70	0.00 (4.05)	0.000	1.00	35.00	R	1.630	100.00 (85.93)	0.030	2.53	81.67	MR	0.863
NE71	20.41 (22.49)	0.022	2.67	74.67	MR	1.185	100.00 (85.93)	0.036	3.73	106.87	S	0.653
SECOW1T	23.10 (28.86)	0.046	4.00	87.50	MR	1.926	100.00 (85.93)	0.014	3.93	118.07	S	1.246
SECOW2W	0.56 (5.53)	0.007	1.33	36.17	R	2.222	100.00 (85.93)	0.055	2.87	86.33	MR	0.878
SECOW3B	18.82 (24.19)	0.023	4.00	85.17	MR	1.963	100.00 (85.93)	0.028	3.07	95.43	MR	0.941
SECOW4W	2.78 (8.46)	0.015	1.33	38.50	R	2.259	100.00 (85.93)	0.040	2.53	76.77	MR	1.000
SECOW5T	5.55 (12.87)	0.023	2.33	53.67	R	2.741	100.00 (85.93)	0.007	3.27	97.53	MR	1.359
WC10	13.61 (17.80)	0.005	3.00	70.00	R	2.370	100.00 (85.93)	0.028	3.47	106.87	S	0.855
WC15	4.67 (11.87)	0.016	2.00	56.00	R	2.000	100.00 (85.93)	0.033	3.67	107.33	S	0.990
WC16	28.82 (32.11)	0.014	4.33	105.00	MR	1.444	100.00 (85.93)	0.025	2.73	82.37	MR	0.735
WC17	0.00 (4.05)	0.000	1.00	35.00	R	1.481	100.00 (85.93)	0.033	3.40	96.60	MR	0.853
WC18	19.84 (19.63)	0.003	2.33	63.00	R	1.926	100.00 (85.93)	0.014	3.93	112.47	S	0.801
WC2	6.67 (11.68)	0.004	2.00	58.33	R	2.444	100.00 (85.93)	0.025	3.27	95.67	MR	0.694

Table 4.3 continued.

	Kabanyolo						Serere					
Line	FI[a] (%)	r	FS[b]	AUDPC	HR	Yield (t/ha)	FI[a] (%)	r	FS[b]	AUDPC	HR	Yield (t/ha)
WC20	8.47 (12.90)	0.006	2.00	57.17	R	2.704	100.00 (85.93)	0.022	3.47	94.27	MR	0.937
WC21	9.60 (14.74)	0.002	2.33	67.67	R	2.000	100.00 (85.93)	0.015	3.20	95.67	MR	1.174
WC26	11.30 (16.81)	0.022	2.33	56.00	R	1.630	100.00 (85.93)	0.025	3.73	118.77	S	0.793
WC27	9.76 (16.62)	0.023	2.67	72.33	MR	2.333	100.00 (85.93)	0.044	3.47	108.03	S	0.874
WC29	42.84 (41.01)	0.039	5.00	116.67	S	1.041	100.00 (85.93)	0.017	3.60	103.60	MR	0.737
WC30	0.00 (4.05)	0.000	1.00	35.00	R	1.667	100.00 (85.93)	0.022	3.40	106.87	S	0.885
WC32	5.00 (10.43)	0.019	1.00	35.00	R	1.778	100.00 (85.93)	0.023	4.27	125.07	S	0.628
WC32A	5.00 (10.43)	0.019	1.67	39.67	R	2.333	100.00 (85.93)	0.047	3.13	94.73	MR	0.744
WC33	2.78 (8.46)	0.015	1.67	44.33	R	1.963	100.00 (85.93)	0.000	4.13	124.37	S	0.761
WC35A	3.09 (8.77)	0.003	1.67	49.00	R	2.111	100.00 (85.93)	0.032	3.73	107.80	S	0.640
WC35B	0.00 (4.05)	0.000	1.00	35.00	R	2.037	100.00 (85.93)	0.020	3.33	105.93	S	0.770
WC35C	23.71 (28.64)	0.008	4.33	105.00	MR	2.037	100.00 (85.93)	0.010	3.87	112.47	S	0.820
WC36	32.18 (34.32)	0.012	4.67	128.33	S	1.630	100.00 (85.93)	0.043	3.27	105.47	MR	0.786
WC37	16.19 (17.52)	0.005	2.33	60.67	R	1.556	100.00 (85.93)	0.016	4.27	116.20	S	0.641
WC41	22.23 (21.05)	0.022	1.67	53.67	R	2.296	100.00 (85.93)	0.010	3.73	106.87	S	0.758
WC42	0.00 (4.05)	0.000	1.00	35.00	R	2.481	100.00 (85.93)	0.039	3.47	103.83	MR	0.779
WC44	13.46 (15.95)	0.015	2.33	64.17	R	1.667	100.00 (85.93)	0.035	3.00	89.83	MR	0.550
WC46	14.44 (20.06)	0.028	2.33	53.67	R	1.963	100.00 (85.93)	0.059	2.73	84.47	MR	0.694
WC48	0.00 (4.05)	0.000	1.33	40.83	R	1.963	100.00 (85.93)	0.010	3.33	110.83	S	0.981
WC48A	14.53 (16.57)	0.004	2.33	64.17	R	1.926	100.00 (85.93)	0.028	4.00	119.23	S	0.669
WC5	14.52 (19.97)	0.022	3.00	72.33	MR	1.778	100.00 (85.93)	0.029	3.73	104.77	MR	0.816
WC52	5.77 (11.02)	0.020	1.67	46.67	R	2.148	100.00 (85.93)	0.041	3.53	101.03	MR	0.820
WC53	7.84 (14.99)	0.034	2.00	43.17	R	1.815	100.00 (85.93)	0.023	2.60	84.00	MR	0.612
WC55	13.34 (21.34)	0.016	3.33	82.83	MR	1.370	100.00 (85.93)	0.000	4.33	127.63	S	0.873
WC58	24.62 (28.26)	0.014	4.33	112.00	S	2.185	100.00 (85.93)	0.039	3.13	94.73	MR	1.106
WC62	15.08 (20.38)	0.023	3.00	73.50	MR	2.148	100.00 (85.93)	0.035	3.00	93.33	MR	0.702
WC63	4.24 (9.81)	0.007	2.00	50.17	R	2.111	100.00 (85.93)	0.024	3.13	99.63	MR	0.867
WC64	0.00 (4.05)	0.000	1.00	35.00	R	2.148	100.00 (85.93)	0.048	3.27	94.03	MR	0.855
WC66	0.00 (4.05)	0.000	1.00	35.00	R	2.074	100.00 (85.93)	0.013	4.60	129.73	S	0.793
WC67	0.00 (4.05)	0.000	1.00	35.00	R	1.963	100.00 (85.93)	0.019	4.00	110.83	S	0.870
WC67B	17.97 (22.41)	0.031	3.33	81.67	MR	1.593	100.00 (85.93)	0.029	3.07	96.60	MR	1.210

Table 4.3 continued

	Kabanyolo						Serere					
Line	FI[a] (%)	r	FS[b]	AUDPC	HR	Yield (t/ha)	FI[a] (%)	r	FS[b]	AUDPC	HR	Yield (t/ha)
WC68	14.05 (22.22)	0.031	3.00	78.17	MR	2.259	100.00 (85.93)	0.059	3.47	107.80	S	0.897
WC69	2.87 (8.56)	0.015	1.67	44.33	R	1.481	100.00 (85.93)	0.012	4.13	128.33	S	0.723
WC7	19.77 (23.33)	0.008	3.33	93.33	MR	1.963	100.00 (85.93)	0.031	3.20	99.17	MR	0.637
WC8	1.82 (7.41)	0.013	1.33	36.17	R	2.148	100.00 (85.93)	0.029	3.27	100.57	S	0.778
LSD (0.05)	18.12	0.032	2.03	44.98		0.921	0.00	0.035	1.10	29.90		0.370
CV (%)	56.50	85.70	49.80	39.10		29.40	0.00	61.80	20.00	17.80		41.70

FI = final incidence, FS = final severity, AUDPC = area under disease progress curve, r = apparent infection rate and HR = host resistance ratings. [a] = incidence at 81 days after planting; figures in parenthesis = arcsine transformed values; [b] = Severity at 81 days after planting; AUDPC (35-70) = severity (1.0-2.0); AUDPC (71-105) = severity (2.1-3.0) and AUDPC (> 105) = severity (> 3.0); SEC = Secow; ACC = Accession; R = resistant; MR = moderately resistant; and S = susceptible.

The variability in responses of these lines to scab where lines which were resistant in one location were found to be susceptible in the other location suggested the existence of different pathotypes of the fungus (Afutu *et al*., 2016a) at the two sites. A significant G×L interaction observed could be explained in part by the different levels of disease pressure in the two locations and the existence of different biotypes of the fungus (Afutu *et al*., 2016a). On the other hand, 11 lines were found to have shown a consistent reaction to the scab disease at both locations. These were Secow 3B, NE 4, NE 20, NE 32, NE 49, WC 5, WC 7, WC 16, WC 62, and WC 67B which were moderately resistant at both locations and NE 15 which was rated as resistant at both locations. The stability in both locations suggested that these 11 lines could serve as good parents for resistance breeding to scab disease.

Mean cowpea yield recorded in Kabanyolo was between 1.0 (WC 29) to 2.8 (NE 51) t/ha while mean yields recorded at Serere were between 0.5 (ACC 12 × Secow 5T) and 1.63 (Alegi × ACC2) t/ha (Table 4.3) but none of the lines was found to be stable in yield in the two locations which was attributable to the significant difference in the disease pressure in the two locations and the significant negative effect of scab disease on the genotypes (Emechebe, 1980).

There was significant negative correlation between both disease incidence and AUDPC with yield (-0.8 and -0.6 respectively) (Table 4.2) suggesting that Serere would have lower yields than Kabanyolo due to the high disease incidence and AUDPC recorded in Serere. According to Ali *et al*. (2012), the loss of active leaf area results in less photosynthetic available region during grain filling stage which eventually results in lower yield.

4.4.4 Principal component analysis (PCA)

Principal component analysis was used to analyze the structure of the interrelationships among the 100 genotypes and to explain these genotypes in terms of their common underlying dimensions. The results of the R-mode principal component analysis (PCA) are presented in Table 4.4. Four principal components were obtained based on components with Eigen values greater than 1 (Ho, 2006), and factor loadings of ±0.3 (Hair *et al.*, 2010) explaining 62.28% of the total variance. The first principal component (PC1) explained 35.34% of the total variance observed and this was mainly due to the high negative factor loadings of disease incidence, severity and AUDPC and

high positive factor loadings of days to 50% flowering, pods per plant, seeds per pod, 100 seed weight and grain yield.

Table 4.4: Rotated component matrix of four factor model explaining 62.28% of the total variance for traits

Disease and agronomic trait	PC1	PC2	PC3	PC4	Communalities
Disease incidence	**-0.91**	-0.16	0.02	0.12	0.87
Disease severity	**-0.72**	-0.10	0.02	0.10	0.55
Apparent infection rate (r)	-0.10	-0.04	-0.03	**0.93**	0.88
AUDPC	**-0.78**	-0.13	0.06	0.06	0.63
Days to 50% flowering	**0.53**	-0.12	0.37	0.09	0.44
No. of branches	-0.02	**0.74**	0.41	0.05	0.72
Peduncles/ plant	0.32	**0.74**	0.02	0.01	0.65
Pods/ peduncle	0.08	**0.57**	-0.32	-0.13	0.45
Pods/ plant	**0.67**	0.23	-0.04	0.10	0.52
Pod length (cm)	0.05	-0.07	**0.79**	-0.08	0.64
Seeds/ pod	**0.64**	-0.02	0.19	-0.02	0.45
100 seed weight (g)	**0.55**	-0.05	0.32	-0.38	0.56
Grain yield (t/ha)	**0.78**	0.22	0.02	-0.28	0.74
Eigen values	4.59	1.37	1.13	1.01	
Percentage of total variance	35.34	10.50	8.66	7.78	
Cumulative percentage of variance	35.34	45.84	54.50	62.28	

The second principal component (PC2) was primarily positively correlated with the number of branches per plant, peduncles per plant and pods per peduncle. These together explained 10.50% of the total variation observed and was due to the high positive factor loadings of the yield traits. Principal components 3 and 4 were weighted on pod length (cm) and apparent infection rate (r) respectively. These were due to the high positive factor loadings of pod length and apparent infection rate (r) respectively with each explaining 8.66% and 7.78% respectively, of the total variation in the cowpea lines. Chiorato *et al*. (2006) suggested that the greatest loading coefficient in the last component indicated a redundancy of the descriptor (trait) associated to the component and therefore, the apparent infection rate (r) may be described as redundant descriptor in the description and characterization of the lines evaluated.

4.4.5 Cluster analysis (CA)

The results of cluster analysis constructed using Ward's method based on all 13 traits are presented in Figure 4.1. The 100 lines were grouped into three major clusters when the dendogram was cut at a distance of 1.5 (k = 3). Cluster 1 was the heaviest weighted comprising 58 lines which were all moderately resistant to the scab disease. Cluster 2 comprised 9 cowpea lines consisting of 5 susceptible and 4 moderately resistant lines. The third Cluster comprised 33 lines which were made up of 24 resistant lines and 9 moderately resistant lines. Cluster analysis performed based on the 4 disease indexes alone i.e. scab disease incidence, severity, AUDPC and apparent infection rate (r) (Fig. 4.2) produced clusters with similar patterns to the clusters produced based on 13 traits. The dendogram was cut at a distance of 1.5 (k = 4). Cluster 1 consisted 33 lines while cluster 2 was made up of 24 lines, with both clusters consisting lines which were moderately resistant to scab. Cluster 3 was made up of 10 lines comprising 5 susceptible lines and 5 moderately resistant lines while cluster 4 consisted 33 lines comprising 24 resistant and 9 moderately resistant lines. Both figures showed clear patterns of grouping of the lines, where lines with close resistance ratings were clustered together suggesting that the disease indexes had a significant effect on most of the other traits. Thus, even in cluster 3 (Fig. 4.1) and cluster 4 (Fig. 4.2) where there was a mix of resistant and moderately resistant lines, unique and clearly distinct sub clustering of lines based on resistance levels was observed.

The same phenomenon was observed in the pattern of clustering comparing cluster 2 (Fig. 4.1) and cluster 3 (Fig. 4.2) which had a mix of susceptible and moderately resistant lines implying that for the purposes of screening and categorization of cowpea lines based on resistance to scab, cluster analysis based on scab disease indexes alone generated reliable information comparable to information that was generated by disease indexes together with yield and yield parameters involved in the analysis.

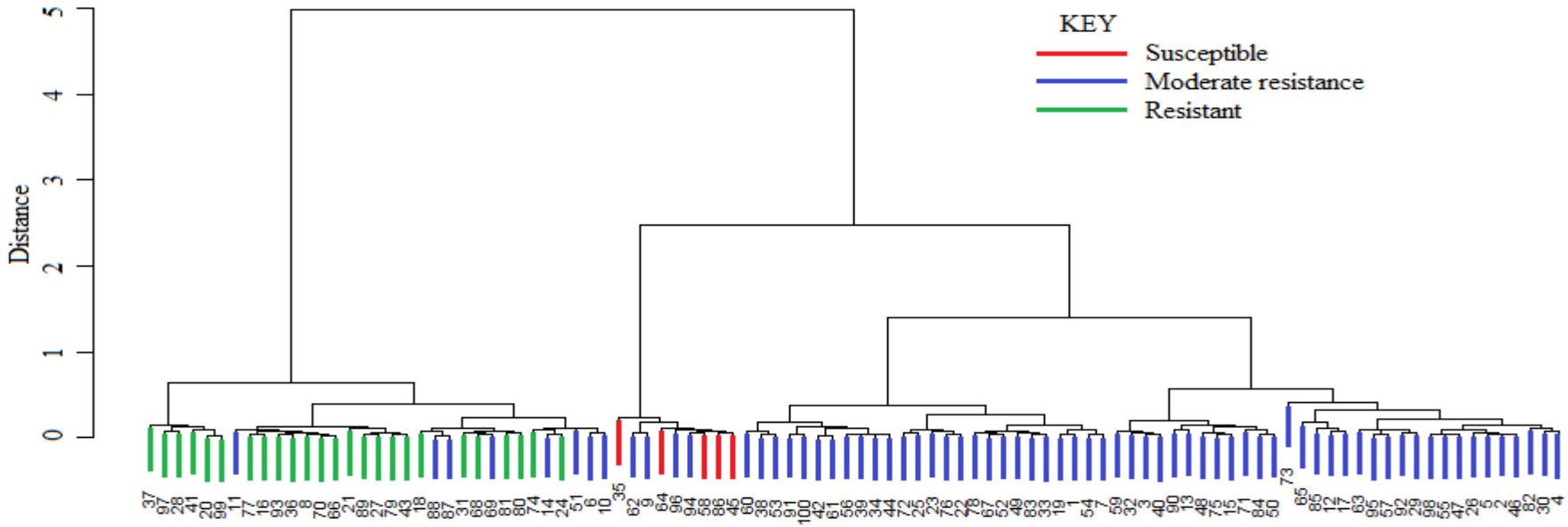

Figure 4.1: Ward's cluster dendogram of the 100 cowpea lines based on 13 traits.

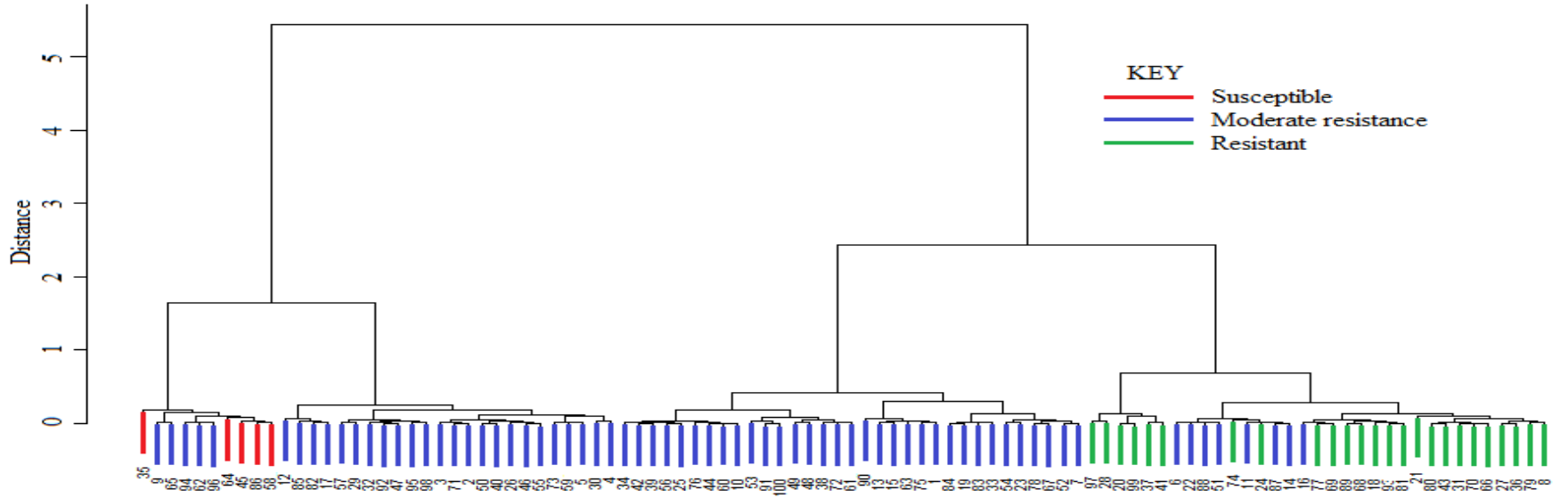

Figure 4.2: Ward's cluster dendogram of the 100 cowpea lines based on 4 disease indexes

4.5 Conclusion

There was variation in the traits among the 100 lines which could be used in selecting parental lines for improving yields and resistance to the cowpea scab disease. One line (NE15) was found to be resistant to the disease at both locations and high yielding and could be used in the cowpea improvement programme to breed for resistance to the scab disease.

CHAPTER FIVE

5.0 MORPHOLOGICAL CHARACTERIZATION, PATHOGENICITY AND GROWTH RATE OF *SPHACELOMA* SP. CAUSING COWPEA SCAB DISEASE IN UGANDA

5.1 Abstract

Scab is an important fungal disease of cowpea, affecting both young and old tissues including stems, leaves and pods of susceptible cowpea genotypes, leading to significant yield losses of up to 100 % under severe infections. Colony characteristics on agar media, symptomatology, phylogenetic affinity of hosts and host range have been used to justify taxonomic distinctions. The correct identification and description of a pathogen is paramount in understanding its control or developing genotypes resistant to it. This study involved the isolation and culture of the scab fungus (*Sphaceloma* sp.) from infected plant parts (leaves and pods) collected from farmers' fields across major cowpea growing districts and agro-ecological zones in Uganda. The fungus was characterized using growth habit on potato dextrose agar (PDA) media, conidia features, variability in radial growth rate (mm/day) among the isolates and pathogenicity and virulence of some isolates on 20 selected cowpea genotypes with different levels of resistance. A total of 495 *Sphaceloma* sp. isolates comprising of 419 from infected leaves and 76 from infected pods were obtained following isolation and culture. There was a wide variation in the isolates based on the amount, nature, colour, depth and rate of mycelia growth, features of conidia and number of septations. Based on the mean incidence, severity, AUDPC and pathogenicity on the 20 genotypes, the isolates were put into three pathogenicity groups. Isolates were mostly slow growing (> 14 days to cover entire 90 mm petri dish). Genotypes NE 31 and NE 70 showed wider level of resistance to the isolates and could therefore be recommended as parental lines in the cowpea breeding programme to develop cultivars with wide horizontal resistance to the scab disease.

Keywords: Pathogenicity, virulence, horizontal resistance, radial growth rate, variability.

From this chapter the following paper has been published:

Afutu, E., Agoyi, E.E., Kato, F., Amayo, R., Biruma, M. and Rubaihayo, P.R. (2016b). Morphological characterization of Ugandan isolates of *Sphaceloma* sp. causing cowpea scab disease. *Journal of Agricultural Science*. Vol. 8, No. 9. http://dx.doi.org/10.5539/jas.v8n9p55.

5.2 Introduction

Cowpea scab, caused by the fungus *Sphaceloma* sp., is one of the major diseases affecting the production of cowpea in Uganda. The disease is capable of causing up to 100% yield loss (Mbong *et al*., 2012). Scab disease is widespread in all the cowpea growing districts of Uganda with mean incidence ranging between 35-70% and mean severity 2-4 out of a severity scale of 1-5, where 1 = no symptoms, and 5 = more than 50% infection, based on which Amuria and Tororo districts were reported to be hot spots of the disease in the country and was found to be more severe at higher altitudes (> 1200 m.a.s.l.) (Afutu *et al*., 2017a).

The genus *Sphaceloma* is made up of more than 50 species, of which most are found in the Tropical and Sub-tropical regions and have been characterized based on the colony characters on agar media, symptomatology, phylogenetic affinity of reported hosts, and to a lesser extent, host range (Zeigler and Lozano, 1983). The fungus is the imperfect state of *Elsinoe* and is considered as lesser fungi because it lacks the perfect stage (sexual reproduction). Though imperfect, it is part of important eukaryotic microorganisms which affects plants and other life forms in diverse ways and thus, the need to determine its identity is important in research, industry, plant pathology and many other disciplines (Barnett and Hunter, 1987). The genus *Sphaceloma* has been described by Barnett and Hunter (1987) as having disc-shaped or cushion-shaped acervuli, waxy; conidiophores simple, closely grouped or compacted, arising from a stroma-like base, sometimes almost appearing as a sporodochium; conidia hyaline, 1-celled, ovoid or oblong; parasitic; imperfect states of *Elsinoe*; similar to *Gloeosporium* and *Colletotrichum*. On the other hand, Ayodele and Kumar (2014) described the genus as having hyaline mycelium, scanty and submerged; hyaline to pale coloured conidia which are produced in pycnidia; have hyaline ascospores borne on the asci; pale coloured oblong to elliptical, and 3 septate. The conidia of isolates of the genus *Sphaceloma* were described as being small, unicellular, and hyaline, formed in more or less acervulus-like structures or, more commonly on continuous fertile layers of densely packed phialidic conidiophores with some species forming a larger, 0-2 septate, pigmented, thick-walled, spindle-shaped spores under certain conditions (Zeigler and Lozano, 1983). The conidia of the genus were later described as small, thin-walled, ellipsoid to (rarely) globose, commonly with one or two guttules and conidiophores being phialides hyaline to slightly pigmented 0-1 septate while conidiophores from

the weedy species were phialides, hyaline brown 0-2 septate producing hyaline conidia (Alvarez *et al*., 2003).

The fungus attacks all the above ground parts of the cowpea plant. Infected leaves show spots on both leaf surfaces, cupped, appearance of small grayish lesions along the veins leading to leaf distortions and ragged appearance under severe infections while infected stems show oval to elongated silver grey lesions surrounded by red or brown elliptical rings with lesions coalescing and forming distortions under severe infections. On the other hand, sunken spots with grey centres surrounded by brown borders appear on infected pods, leading to malformation and formation of dark coloured pycnidia in the brown spots, with heavily scabbed young pods aborting or remaining attached to the plant as mummified black masses (Singh *et al*., 1997).

Screening of local germplasm for sources of resistance to the scab disease in Uganda showed a wide variability in response to the disease where lines which were rated resistant in one location, were found to be either moderately resistant or susceptible in another location (Afutu *et al*., 2016a). The variability in response to the disease, among other factors, suggested a variability in pathotypes of the scab fungus. This phenomenon complicates crop protection programs (Alvarez *et al*., 2003) and the development of resistant cultivars. This study was therefore, conducted to determine the morphological variability, growth rate and pathogenicity of some selected isolates on some local germplasm having varying levels of resistance to the scab disease to identify cowpea lines with broad spectrum of resistance for possible parents in the cowpea breeding programme.

5.3 Methodology

5.3.1 Sampling, isolation, culture and morphological characterization of pathogen

Samples of infected plant materials were collected in a field survey conducted in some of the major cowpea growing areas in the country, for isolation and culture of the scab fungus. Infected leaves and pods were cut into small portions using sterile surgical blades and were disinfested with 75% ethanol for 1 minute and then in 1% sodium hypochlorite for 1 minute, followed by rinsing in sterile water (Hou *et al*., 2014). The intact lesions were plated on commercial preparations of Potato Dextrose Agar (PDA) (Difco, Detroit) amended with Streptomycin Sulphate (1.5 g/L) and rose bengal (0.0025 g/L of agar) for isolation of *Sphaceloma* sp. (Mungo *et al*., 1998). Five lesions

from each cowpea plant part were plated on the media in 9 cm-diameter Petri dishes and incubated at 26 ^{o}C for 5-7 days (Mungo *et al*., 1998).

Following isolation and culture, a total of 495 pure fungal isolates from single conidium cultures were morphologically characterized based on colony characters such as the texture, density, colour, presence of conidial masses and colour of the reverse side of the dish (Talhinhas *et al*., 2002). The isolates were obtained from a total of 14 districts with each having three Sub-Counties and three farms per Sub-County making 126 farms in total. Isolates were named by assigning unique two-letter codes to represent the districts of origin and followed by serial numbers to identify the particular isolates (Appendix 5.1) and a letter "L" or "P" to indicate that the isolate was obtained from an infected leaf or pod respectively.

5.3.2 Pathogenicity of selected isolates

Five isolates were selected for pathogenicity tests based on the Agro-ecological zone and Districts where the crop was mostly cultivated. The number of isolates chosen from a region was based on the size of the region covered during the field survey and the morphological groups to which the isolate belonged. Pathogenicity of the five selected isolates was determined by inoculating seedlings of 20 cowpea genotypes (Table 5.1) selected on the basis of their resistance ratings following field screening under natural infections (Afutu *et al*., 2016b). Seeds of each of the 20 lines were sown in 20 cm diameter by 20 cm high plastic buckets filled with sterilized top soil with one seed per bucket. The plants were grown in the screen house and no supplementary lighting was provided. Temperature ranged from 19^{o}C at night to 27^{o}C during the day. Supplementary irrigation was applied where necessary by directly watering and avoiding water coming into contact with the surface of the leaves. Relative humidity was mainly between 50-80%.

Inoculum was prepared by culturing isolates in half strength (18g/L) Potato Dextrose Broth (PDB) (HiMedia Laboratories Pvt., India) supplemented with Rifampicin antibiotic at 0.03g/L, Streptomycin Sulphate at 1.5g/L and Rose Bengal at 0.0025g/L. Glass wares containing the media and inoculum were put on a magnetic shaker (Stuart Scientific Flask Shaker SF1) set to 100 oscillations per minute (osc/min) for 10 days to prevent mycelia formation within the culture and to promote conidia formation.

Table 5.1: List of 20 selected genotypes used for pathogenicity test and their resistance ratings.

Genotype	Resistance rating[a]	Days to 50% flowering[b] (days)	Yield potential[c] (t/ha)
Accession 12 * Secow 2W	MR	48	1.36
Accession 23	S	47	0.93
Accession 23 * Accession 12	R	50	1.32
Alegi	MR	49	1.42
NE 15	R	49	1.63
NE 23	R	46	1.31
NE 31	R	48	1.46
NE 48	MR	48	1.43
NE 50	MR	49	1.45
NE 70	R	49	1.25
Secow 1T	MR	50	1.59
Secow 5T	MR	49	2.05
Sun shine	S	49	0.72
WC 10	MR	46	1.61
WC 17	R	48	0.87
WC 29	S	51	0.89
WC 35B	R	50	1.40
WC 36	S	49	1.21
WC 66	MR	51	1.43
WC 67	MR	49	1.42

[a] resistance rating by Afutu *et al.* (2016a): R = Resistant, MR = Moderately Resistant, S = Susceptible; [b] average data from two locations in 2014 season A (April-July); [c] average yield obtained from two locations (Afutu e*t al.*, 2016a).

The concentration of inoculum was determined using Neubauer improved bright-light counting chamber (Superior Marienfell – Germany) and the concentration of inoculum was adjusted to 10^6 conidia/ml (Hyun *et al*., 2009). Inoculation of plants were done 4 weeks after sowing by spraying leaves till run-off followed by covering inoculated plants with transparent plastic bags for 18 hours (Mchau *et al*., 1998). In the control treatment, sterile water was sprayed on the plants in place of the conidium suspension. There were four plants per treatment replicated three times and the buckets were arranged in a Completely Randomized Design (CRD).

Assessment of disease was done at 7 and 14 days after inoculation. A plant was considered positive for infection if a clearly distinguishable scab lesion developed, negative where no infections were developed while infections which were spotting but with no clearly identifiable scab lesions were

designated as plus or minus (±) (Hyun *et al*., 2009). Disease severity was measured using a scale of 0-3 where 0 = no visible symptoms; 1 = very small pinprick type; 2 = small dark brown lesions with no chlorosis; and 3 = pale brown lesions surrounded by a chlorotic halo and with some distortion of the lamina (Mchau *et al*., 1998). Where there were any doubts about the identity of the symptoms, re-isolation was made from symptomatic plants inoculated with isolates.

Percentage pathogenic reactions of genotypes to isolates were calculated by expressing the number of isolates that caused clear scab disease symptoms (with the + symbol) over the five isolates (excluding the control treatment) and multiplied by 100 while percentage pathogenicity of isolates were estimated by expressing the number of genotypes with clear scab disease symptoms (with the + symbol) over the 20 genotypes and multiplied by 100. To estimate pathogenicity values for each isolate, pathogenicity codes (0-2) were assigned to the three symbols used in designating the presence or absence of scab disease symptoms (Hyun *et al*., 2009), where, 0 = "-" (no infections developed), 1 = "±" (for infections which were spotting but no clearly identifiable scab lesions), and 2 = "+" (clearly distinguishable scab lesions). Following inoculation with the isolates, scab disease incidence was expressed as the percentage of infected plants and the incidence data was transformed using arcsine transformation of arcsine percentage (Gomez and Gomez, 1984) after a Kurtosis-Skewness test showed a significant deviation from the normal. Mean severity scores were estimated using Microsoft Excel and the means obtained were used to calculate area under the disease progress curve (AUDPC) at 7 and 14 days after inoculation for each cowpea genotype in Microsoft Excel using the formula of Campbell and Maddden (1990):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right) (t_{i+1} - t_i)$$

Where "t" is the time of each reading, "y" is the percent of affected foliage at each reading (severity score) and "n" is the number of readings. The variable "t" represents days after inoculation. The transformed incidence data, mean severity and AUDPC calculated were analysed for variation (ANOVA) using Genstat edition 14 (Payne *et al*., 2011) and the disease incidence, severity and AUDPC means were separated using Fisher's protected Least Significant Difference (LSD) test at $P < 0.05$.

5.3.3 Growth rate

The growth rate of the fungus was measured by culturing the isolates on commercial preparations of Potato Dextrose Agar (PDA) (Difco, Detroit) amended with Streptomycin Sulphate (1.5 g/L) and rose bengal (0.0025 g/L of agar). Radial growth was measured for a total of 42 isolates selected across the 14 districts and from different morphological groups. Three isolates were selected from each district (one from each Sub-County) based on the farm with the highest incidence and severity within a Sub-County (Afutu *et al*., 2017a). Mycelial disks (5 mm) from 7-day-old fungal cultures were transferred to the centre of 90 mm diameter Petri plates and sealed with Parafilm (Miyashira *et al*., 2010; Turkkan and Erper, 2014). Two separate petri dishes were prepared for each isolate representing two replicates and the dishes were incubated under 12 hours of alternating light and darkness at 25 ^{o}C (±1 ^{o}C) for a maximum of 14 days.

Two straight lines were drawn perpendicular to each other on the bottom of the petri dishes ensuring that the crossing point coincided with the centre of the 5 mm fungi disc plugs (Appendix 5.2) and radial growth measurements were recorded following the procedure of Miyashira *et al*. (2010) with slight modifications by measuring growth rates on daily basis instead of weekly. Radial growth measurements were taken on daily basis until the fungus mycelia reached the walls of a dish or for a maximum of 14 days and the daily recordings of growth were expressed as mm/day.

Analysis of variance of fungus growth rate for different time intervals (i.e. 7, 10 and 14 days) after inoculation of media and days to full coverage (DTFC) of petri dish was performed in Genstat edition 14 (Payne *et al*., 2011). For more accuracy, the maximum likelihood method was used (Payne *et al*., 2003) in Genstat analysis for a Chi-square test for independence or association of growth rate data with the Districts and Agro-ecological zones from which isolates were obtained.

5.4 Results and Discussion

5.4.1 Morphological characterization of pathogen

There was a wide variation in the isolates based on the colony characters on PDA. Morphological characterization of the 495 isolates based on the amount, nature, colour, depth and rate of mycelia growth, nature or shape of conidia, number of septations and the colour of the base of petri dishes

when inverted yielded six (6) morphological groupings (A-F) (Table 5.2). Each of these groups had morphological structures similar to those described by Barnett and Hunter (1987) and (Ayodele and Kumar, 2014) but with slight variations in the parameters, hence, the different morphological groupings.

Table 5.2: Description and Morphological Characterization of 495 *Sphaceloma* sp. isolates from Uganda

Group	Isolates[a]	Source of isolates		Total isolates %	Characteristics of group
		Leaf	Pod		
A	70 Isolates: TR175P; NB425P*; AR439L; AR440L; KU88L; LR369L; LR372L; PA132P; PD217L; PD218L; PD219L; PD220L; PD225L; PD226L; PD227L; PD228L; PD233L*; PD234L; YU471L; YU472L; YU481L*; YU482L; YU483L; YU484; YU485L; YU486L; YU487L; YU488L; YU489L; YU490L; YU493L; YU494L; YU497L; YU498L; KT253L; KT254L; KT255L; KT256L; KT257L; KT258L; KT259L*; KT260L; KT261L; KT262L; KT263L; KT264L; KT265L; KT266L; KT267L; KT268L; KT269L; KT270L; KT271L; KT272L; KT273L*; KT274L; KT275L; KT276L; KT277L*; KT278L; KT279L; KT280L; KT281L; KT282L; KT283L; KT284L; KT285L; KT286L; KT287L; KT288L.	67	3	14.1	• Very scanty mycelia • Entirely grey coloured and submerged in the medium • Conidia hyaline with 0-2 septation • Underneath cultures greyish-brown • Slow growing isolates
B	35 Isolates: ST13P*; LR395L; LR396L; AR445L; AR446L*; AR447L; AR448L; AR449L; AR450L; AR451L; AR452L; AR453L; AR454L; AR455L; AR456L; YU469L*; YU470L; YU473L; YU474L; YU475L; YU476L; YU477L; YU478L; YU479L; YU480L; YU491L; YU492L; YU495L; YU496L; YU499L; YU500L; YU501L*; YU502L; YU503L; YU504L.	34	1	7.1	• Scanty pale coloured mycelia • Brownish through pink to black patches of mycelia at the centre • Mycelia submerged in the medium • Hyaline conidia with 1-4 saptation • Underneath cultures peach-orange to pink • Slow growing isolates

Table 5.2: continued

Group	Isolates[a]	Source of isolates		Total isolates %	Characteristics of group
		Leaf	Pod		
C	72 Isolates: ST5L; ST6P; ST9L; ST10L; ST11L; ST12L; ST14L; ST15L; ST16L; ST19L; ST21L; ST22L; ST23L; ST25L*; ST26L; ST27L; ST28L; ST29L; ST30L; ST31L; ST32L; ST33L; ST34L; ST35L; ST36L; PA121L; PA124L; KU76L; KU77L; KU78L; KU79L; KU81P; KU82P; KU84P; KU86P; KU93P; KU94L; KU103L; SE41L; SE42L; SE43L; SE49L; SE50L; AR433L*; AR441L; AR442L; AR443L*; AR444L; LR361L; LR362L; LR363L; LR364L; LR365L*; LR366L; LR367L; LR368; LR371L; LR373L; LR374L; LR375L; LR376L; LR379L; LR380L; LR381L; LR382L; LR383L*; LR384L; LR388L; TR190P; TR173P; TR177P; TR178P.	62	10	14.5	• Hyaline fresh mycelia growth • Old mycelia growths redwood to red-brown with black patches • Submerged scanty mycelia • Conidia hyaline with 1-2 septation • Pink to red-brown underneath cultures • Slow growing Isolates
D	80 Isolates: SE48L; LR393L; LR394L; AR465L*; AR466L; AR467L; AR468L; TR147L; TR158P; TR166L; PA123L; PD229L; PD230L; PD231L; PD232L; PD235L; PD236L; PD239L; PD240L; KU85P; KU101L; KU104L; KU105L*; AM181L; AM182L; AM183L*; AM184L; AM185L; AM186L; AM187L; AM188L; AM189L; AM190L; AM191L; AM192L; AM193P; AM194P; AM195P; AM196P; AM197L; AM198L; AM199L; AM200L; AM201L*; AM202L; AM203L; AM204L; AP289L; AP290L; AP291L; AP292L*; AP294P; AP297L; AP298L; AP299L; AP300L; AP301L; AP302L; AP303L; AP304L; AP305P; AP306P; AP307P; AP308P; AP309L*; AP310L; AP311L; AP312L; AP313L; AP314L; AP315L; AP316L; AP317L*; AP318L; AP319L; AP320L; AP321L; AP322L; AP323L; AP324L.	65	15	16.2	• Grey fresh mycelia growths • Old growths brown coloured • Submerged scanty mycelia • Conidia hyaline with 1-2 septation • Greyish-brown to red-brown underneath cultures • Fast growing isolates
E	36 Isolates: PD241L, PD242L; PD243L; PD244L; PD245L; PD246L; PD247L*; PD248L; PD249L; PD250L;	22	14	7.3	• Abundant hyaline mycelia • Centre Peach coloured

Table 5.2: continued

Group	Isolates[a]	Source of isolates		Total isolates %	Characteristics of group
		Leaf	Pod		
E	PD251L; PD252L; KU87L; KU95P; KU80L; ST24L; TR148P; LR385L; LR386L; LR387L; LR389L*; LR390L; LR391L; LR392L; AM205P*; AM206P; AM207P; AM208P; AM209P; AM210P; AM211P; AM212P; AM213P; AM214P; AM215P; AM216P.				• Fresh hyaline mycelia growths • Mycelia submerged in medium • Conidia hyaline with 1-4 septation • Grey to peach underneath cultures • Fast growing isolates
F	202 Isolates: ST1L; ST2L*; ST3P; ST4L; ST7P; ST8P; KU73L; KU74L*; KU83P; KU89L; KU90L; KU91L*; KU92L; KU95P; KU96L; KU97L; KU98P; KU99P; KU100L; KU102L; KU104P; KU106P; KU107P; KU108L; SE37L; SE38L*; SE39L; SE40L; SE44L; SE45L; SE46L; SE47L; SE51L; SE52L; SE53L; SE54L; SE55L; SE56L; SE57L*; SE58L; SE59L; SE60L; SE61L; SE62L; SE63L; SE64L; SE65L; SE66L*; SE67L; SE68L; SE69L; SE70L; SE71L; SE72L; AR434L; AR435L; AR436L; AR437L; AR438L; AR457L; AR458L; AR459L; AR460L; AR461L; AR462L; AR463L; AR464L; TR145P*; TR146P; TR149L; TR150P; TR151P; TR152P; TR153P; TR154P; TR155L; TR156P; TR157P; TR159P; TR161L; TR162P*; TR163P; TR164L; TR165P; TR167P; TR168P; TR169L; TR170L; TR171L*; TR172L; TR174P; TR179P; TR180L; NB397L; NB398L; NB399L*; NB400L; NB405L; NB406L; NB407L; NB408L; NB413L; NB414L*; NB415L; NB416L; NB417L; NB418L; NB419L; NB420L; NB421L; NB422L; NB423L; NB424L; NB426L; NB427L; NB428L; NB429L; NB430L; NB431L; NB432L; PD221L; PD222L; PD223L; PD224L*; PD237L; PD238L; PA109L; PA110L*; PA111L; PA112L; PA113L; PA114L; PA115L; PA116L; PA117L; PA118L; PA119L; PA120L; PA122L; PA125L; PA126L*;	169	33	40.8	• Abundant and hyaline mycelia • Both old and fresh growths entirely white • Mycelia submerged in medium • Conidia hyaline with 1-4 septation • White underneath cultures • Fast growing isolates

Table 5.2: continued

Group	Isolates[a]	Source of isolates		Total isolates %	Characteristics of group
		Leaf	Pod		
F	PA127L; PA128L; PA129L; PA130L; PA131L; PA133L; PA134L; PA135L; PA136L; PA137L*; PA138L; PA139L; PA140L; PA141L; PA142L; PA143L; PA144L; DK325L; DK326L; DK327L; DK328L; DK329L; DK330L; DK331L; DK332L; DK333L; DK334L*; DK335L; DK336L; DK337L; DK338L; DK339L; DK340L; DK241L; DK342L*; DK343L; DK344L; DK345L; DK346L; DK347L; DK348L; DK349L; DK350L; DK351L*; DK352L; DK353L; DK354L; DK355L; DK356L; DK357P; DK358P; DK359L; DK360L; NB409L; NB410L; NB411L; NB412L; NB401L; NB402L; NB403L; NB404L.				
Total	495 Isolates	419	76	100	-

[a] Isolates with the asterisks sign attached (*) constitute isolates selected from the different groups for radial growth rate measurements.

The amount of mycelia produced by the isolates ranged from very scanty (Group A = 14.1%) through scanty (Groups B, C and D = 37.8 %) to abundant (Groups E and F = 48.1%) while the colour of colonies produced varied widely from white (Plate 5.1F), pale (Plate 5.1A), white and peach (Plate 5.1E), through to pink with red-brown to brownish-black pigmentations (Plates 5.1B, 5.1C and 5.1D) confirming earlier reports that colony colour on PDA was found to be extremely variable even within the same isolates being differently pigmented under the same growth conditions (Zeigler and Lozano, 1983).

The variable colour of the colonies observed in this study, including the dark to black pigmentations and the consistently high pigmentation has also been reported in some of the closely related species such as *Elsinoe fawcettii*, *E. australis* and *Sphaceloma fawcettii*, the causal organisms of scab diseases of citrus (Timmer *et al*., 1996). According to Barnett and Hunter (1987), fungal hyphal cells vary in their size, colour and in their extracellular matrix when present, however, since hyphae among different kinds of fungi are more alike than different, they usually cannot be used as a differentiating character. Zeigler and Lozano (1983) also reported that different *Sphaceloma* sp. proved impossible to distinguish using colony morphology and colour alone.

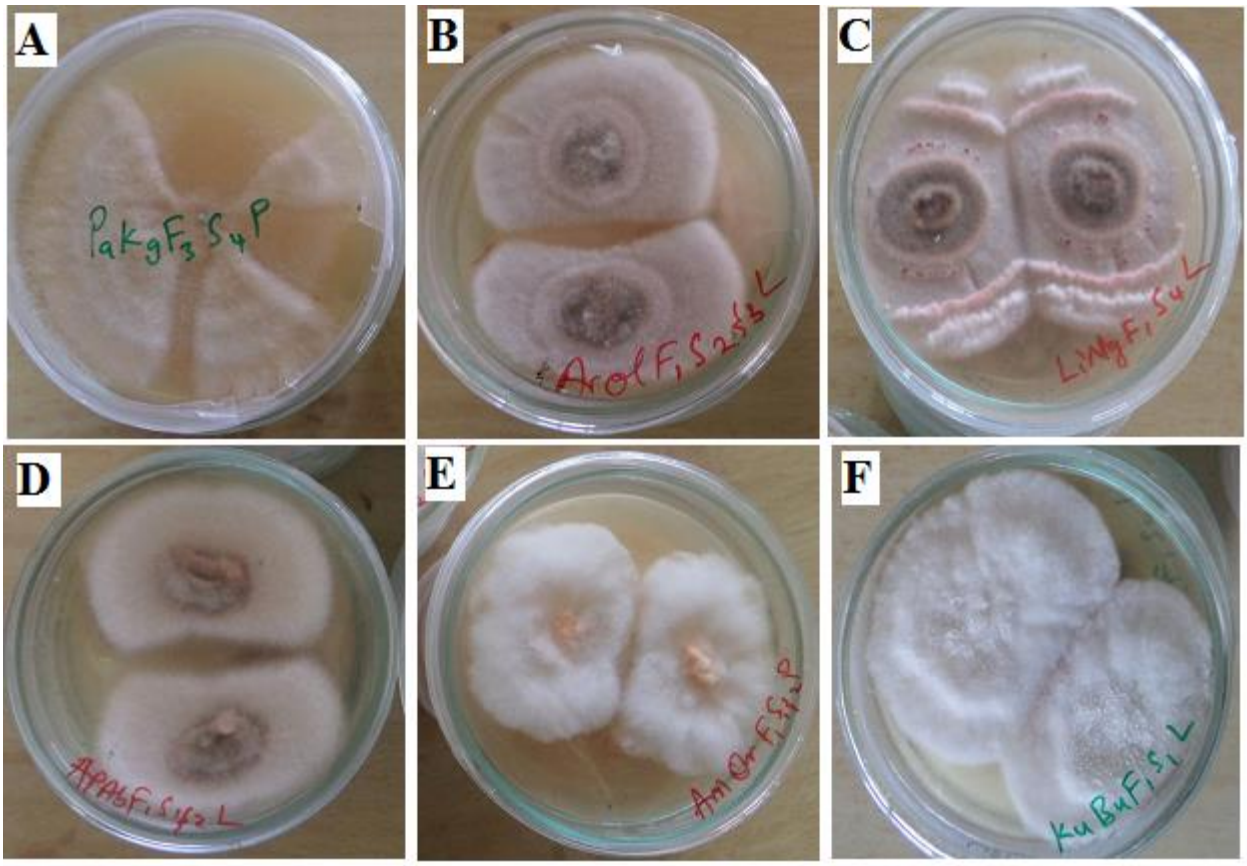

Plate 5.1: Colony characters of the different *Sphaceloma* sp. groups on PDA.

In this study, delineation of isolates into the different morphological groups were further based on the features of conidia belonging to the genus *Sphaceloma* as described by Alvarez *et al*., (2003). Therefore, based on other features apart from those of the fungal mycelia, 70 (14.1 % Group A), 152 (30.7 % Groups C and D) and 273 (55.2 % Groups B, E and F) of the isolates had 0-2, 1-2, and 1-4 septa respectively. The number of septa of species belonging to the genus *Spahceloma* was reported to be 0-2 (Zeigler and Lozano, 1983) and 0-3 (Alvarez *et al*., 2003; Ayodele and Kumar, 2014), however, a greater number of the Ugandan isolates (273 representing 55.2 % belonging to Groups B, E and F) had 1-4 septa, thus suggesting that these isolates may belong to different species within the genus *Sphaceloma*. While some of the isolates (about 37.6 %) had scanty and submerged mycelia as described by Ayodele and Kumar (2014), most of the isolates (Groups E and F = 48.1 %) had abundant mycelia but were also submerged suggesting a variation within the genus.

To ensure more robust grouping of isolates, the colour of the under-side (reverse side) of petri dishes of cultures when inverted as applied in morphological characterization of *Colletotrichum acutatum* isolates causing anthracnose of lupins (Talhinhas *et al*., 2002), was used as an additional parameter to delineate the isolates into groups. The colour of the base of petri dishes when inverted varied from white through grey to peach, and greyish-brown to red-brown (Table 5.2).

5.4.2 Pathogenicity of selected isolates

The results of pathogenicity test of five selected *Sphaceloma* sp. isolates conducted on the 20 cowpea genotypes are presented in Table 5.3. The inoculation procedure proved satisfactory because susceptible genotypes were found to be consistently infected by the isolates though to varying degrees. Characteristic scab disease symptoms were observed on leaves of most infected genotypes by the time of the first observation (7 days after inoculation). No scab disease symptoms were observed on stems even 14 days after inoculation of genotypes. Symptoms produced on leaves included necrotic spots which were only visible on leaf surfaces by the time of first observation but became visible on both leaf surfaces by the second observation. Neither perforations nor leaf distortions were observed as would normally be seen on scab infected plants because the period of 14 days was too short a time for such advanced symptoms to be observed.

Ward's cluster analysis of the 20 genotypes based on the pathogenicity of the isolates, mean incidence, severity and AUDPC, grouped the genotypes into 4 significant clusters (K = 4) (Figure 5.1). Cluster 1 consisted of 5 genotypes (Accession 12 × Secow 2W, NE 50, WC 29, WC 36 and Sun shine) with mean incidence, severity and AUDPC ranging from 51.4-58.3 %, 2.2-3.4, and 13.0-17.7 (Table 5.3) respectively while cluster 2 consisted 7 genotypes (Accession 23, NE 48, Secow 1T, WC 10, WC 17, WC 66 and WC 67) with means of 23.6-44.4 %, 1.5-1.9 and 9.5-11.9 for incidence, severity and AUDPC respectively (Table 5.3). Cluster 3 comprised 6 genotypes (Accession 23 × Accession 12, Alegi, NE 15, NE 23, Secow 5T and WC 35B) with mean incidence, severity and AUDPC ranges of 18.1-31.9 %, 1.4-1.7 and 8.8-10.7 respectively while cluster 4 comprised 2 genotypes (NE 31 and NE 70) with means of 8.3-11.1 %, 0.9-1.2 and 7.4-8.0 for the three disease indexes respectively (Table 5.3). Apart from cluster 4 which consisted of only genotypes rated resistant to the pathogen, the remaining three clusters consisted genotypes with different resistance levels (Fig. 5.1) which implies that these genotypes responded or reacted similarly to infection by the isolates.

Table 5.3: Pathogenicity of 5 *Sphaceloma* sp. isolates on 20 selected cowpea genotypes

		Pathogenicity of isolate						Pathothogenic reaction to	Mean		
Genotype	RL[a]	Control	KU78L	TR171L	KT259L	PD232L	AR446L	isolate (%)[b]	Incidence	Severity	AUDPC
Accession 12 × Secow 2W	MR	-	+	+	±	+	±	3 (60.00)	52.8	2.3	13.42
Accession 23	S	-	-	+	+	±	+	3 (60.00)	44.4	1.8	11.86
Accession 23 × Accession 12	R	-	+	±	-	-	-	1 (20.00)	23.6	1.5	9.92
Alegi	MR	-	±	-	+	±	-	1 (20.00)	31.9	1.7	10.69
NE 15	R	-	±	+	±	-	-	1 (20.00)	20.8	1.5	8.75
NE 23	R	-	-	+	±	-	±	1 (20.00)	22.2	1.7	10.69
NE 31	R	-	±	-	-	-	-	0 (0.00)	11.1	1.2	7.97
NE 48	MR	-	-	+	+	-	+	3 (60.00)	41.7	1.8	11.47
NE 50	MR	-	±	+	+	+	±	3 (60.00)	55.6	2.2	14.19
NE 70	R	-	±	-	-	-	-	0 (0.00)	8.3	0.9	7.39
Secow 1T	MR	-	+	+	±	-	+	3 (60.00)	36.1	1.9	11.86
Secow 5T	MR	-	-	±	+	-	-	1 (20.00)	18.1	1.4	9.53
Sun shine	S	-	+	+	±	+	+	4 (80.00)	58.3	3.4	17.69
WC 10	MR	-	-	+	-	+	+	3 (60.00)	33.3	1.8	10.69
WC 17	R	-	±	+	+	-	±	2 (40.00)	37.5	1.8	11.08
WC 29	S	-	+	+	±	+	+	4 (80.00)	52.8	2.3	14.00
WC 35B	R	-	-	±	+	-	-	1 (20.00)	22.2	1.4	9.33
WC 36	S	-	±	+	+	+	±	3 (60.00)	51.4	2.2	13.03
WC 66	MR	-	+	-	+	-	-	2 (40.00)	23.6	1.5	9.53
WC 67	MR	-	±	+	-	+	+	3 (60.00)	36.1	1.8	11.86
Percentage Pathogenicity[c]		-	30	65	45	35	35				
Pathogenicity value[d]		0	20	29	24	16	19				
Pathogenicity group[e]		-	1	3	2	1	1				
$LSD_{(0.05)}$									7.6	0.7	2.7
S.E.									11.6	1.1	1.0
CV (%)									34.0	63.1	36.4

[a] Resistance rating based on Afutu *et al*. (2016a); RL = Resistance level; R = resistant; MR = moderately resistant; S = susceptible;

[b] Pathogenic reaction of genotypes = number of isolates that caused clearly distinguishable scab lesions on genotypes out of the five isolates × 100;

[c] Percent Pathogenicity of isolates = number of genotypes with clearly distinguishable scab lesions caused by each isolate out of the 20 genotypes × 100;

[d] Pathogenicity value = summation of pathogenicity codes 0-2 based on the symptomatic effects of each isolate (Hyun *et al*., 2009);

[e] Pathogenicity groups: Isolates were separated into three groups based on the mean disease incidence, severity, AUDPC and the pathogenicity of isolates on the 20 inoculated cowpea genotypes, using Ward's cluster analysis (Alvarez *et al*., 2003) with 94% level of confidence.

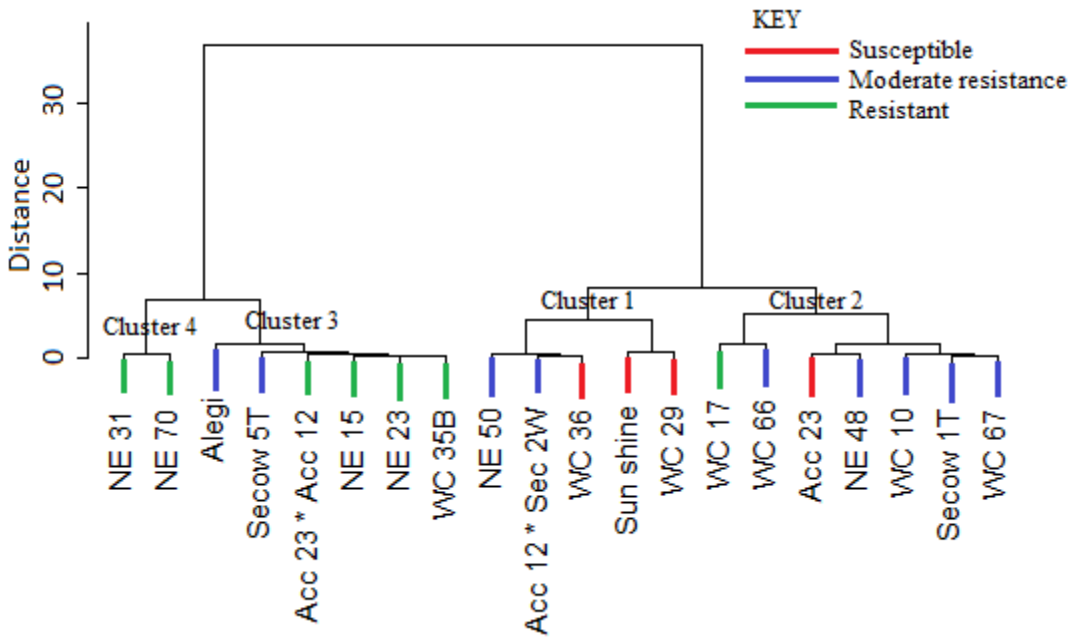

Figure 5.1: Ward's cluster dendogram of the 20 cowpea genotypes based on disease incidence, severity, AUDPC and pathogenicity of isolates.

NE 31 and NE 70 showed a broader spectrum of resistance to infection by all the isolates, thus, confirming their resistance rating reported by Afutu *et al*., (2016a), hence making them good parents for the breeding program to develop cultivars that would have a wide horizontal resistance to the scab disease. According to Acquaah (2007), evolution of new races due to selection pressure against any specific race is absent under horizontal resistance, and provides protection against a wide range of races of a pathogen, hence, is less susceptible to being overcome by a new race making this type of resistance more stable.

There were variable levels of pathogenicity among the isolates (percentage pathogenicity) and different responses of genotypes to isolates (pathogenic reaction to isolates) (Table 5.3). The variable pathogenicity levels as shown in Table 5.3 proved to be significantly different ($P < 0.001$) when the data on pathogenicity were subjected to the analysis of variance procedure (Table 5.4). There were highly significant differences ($P < 0.001$) in virulence of isolates for disease incidence, severity and AUDPC (Table 5.4). The results of the pathogenicity tests carried out in the screen house corroborated the findings from disease evaluations conducted under field pressure conditions at different locations (Afutu *et al.,* 2016a) as cowpea genotypes rated resistant to the disease were found to show very low percentage pathogenic response (0-20 %) to the isolates.

Table 5.4: Combined Analysis of variance for scab disease incidence, severity and AUDPC caused by 5 isolates and a control treatment on 20 selected cowpea genotypes.

Source of Variation	df	Mean square			Pr > F
		Incidence	Severity	AUDPC	
Genotypes	19	4131.9***	4.7***	105.2***	0.001
Isolate	5	22580.9***	10.4***	294.7***	0.001
Genotype × Isolate	95	2594.8***	2.4***	51.9***	0.001
Error	238	134.1	1.3	16.8	

*** = significant at P < 0.001.

Genotype by isolate interaction was also found to be significant (P < 0.001) meaning that the resistance levels of the genotypes to the pathogen had effects on the level of virulence or pathogenicity of the isolates. This was expected because the genotypes selected for the test have been reported to have different levels of resistance when evaluated for resistance to the pathogen under natural conditions in the field and under different environments (Afutu *et al*., 2016a).

Using Ward's cluster analysis (94% confidence level), the isolates were separated into three clusters (Figure 5.2) based on the mean incidence, severity, AUDPC and pathogenicity on the 20 genotypes. Cluster 1 comprised three isolates (KU78L, PD232L and AR446L), all of which were obtained from different agro-ecological zones with mean incidence, severity and AUDPC ranging from 30.0-40.4 %, 1.8-2.0, and 11.1-12.0 (Table 5.5) respectively while clusters 2 and 3 were formed by isolates KT259L and TR171L respectively. The mean incidence, severity and AUDPC for cluster 2 were 45.4 %, 2.0 and 12.1 respectively while cluster 3 had means of 57.1 %, 2.2 and 13.5 for the three traits (Table 5.5).

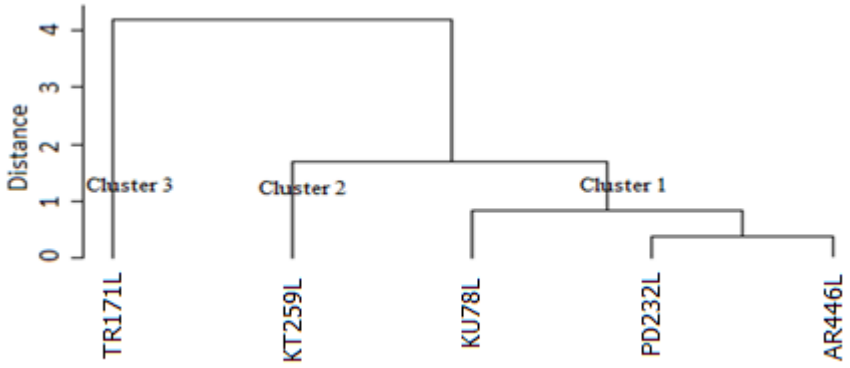

Figure 5.2: Ward's cluster dendogram of five isolates based on disease incidence, severity, AUDPC and pathogenicity of isolates.

Table 5.5: Mean scab disease incidence, severity and AUDPC caused by 5 isolates and a control treatment on 20 cowpea genotypes

	Mean		
Isolate	Incidence	Severity	AUDPC
Control	0.0	1.0	7
KU78L	40.4	1.9	11.8
TR171L	57.1	2.2	13.5
KT259L	45.4	2.0	12.1
PD232L	31.7	1.8	11.1
AR446L	30.0	2.0	12.0
LSD (0.05)	4.2	0.4	1.5
S.E.	11.7	1.1	0.6
CV (%)	34.3	63.2	5.2

5.4.3 Growth rate

The results of radial growth rate (mm/day) for 42 selected isolates are presented in Table 5.6. There were significant variations ($P < 0.001$) among the isolates, districts, morphological groups and agro-ecological zones for radial growth rates at 7, 10, and 14 days after inoculation of media.

Table 5.6: Combined Analysis of variance for radial growth rate of 42 *Sphaceloma* sp. isolates for different time intervals

Source of variation	df	Mean square				Pr > F
		Day 7[a]	Day 10[b]	Day 14[c]	DTFC[d]	
Isolate	41 (41)	331.8 (92.8)***	405.8 (139.7)***	308.0 (81.5)***	8.8 (2.3)***	0.001
District	13 (69)	661.0 (127.8)***	795.6 (174.2)***	581.5 (121.8)***	18.4 (3.2)***	0.001
MG	5 (77)	900.8 (167.6)***	1066.7 (221.2)***	528.1 (173.1)**	18.4 (4.7)**	0.001
AEZ	2 (80)	1875.0 (170.7)***	2450.4 (218.3)***	1586.0 (159.9)***	33.5 (4.9)**	0.001

Figures in parenthesis are error values; MG = Morphological group; AEZ = Agro-ecological Zone; *** = significant at $P < 0.001$.

[a, b] and [c] represents radial growth rates measured at 7, 10 and 14 days respectively after inoculation of media (PDA) with 5 mm diameter disc plugs of isolates.

[d] = days to full coverage of entire surface of the 90 mm petri dishes

The number of days to full coverage (DTFC) was significantly variable among isolates and districts ($P < 0.001$) and also significantly different ($P < 0.01$) among the different morphological

groupings and agro-ecological zones from which the isolates were obtained. The variability in radial growth rate of the isolates were not surprising as this could be accounted for by the fact that the isolates were selected from different morphological groups (indicated by asterisks '*' in Table 5.2). Among the traits used for delineating isolates into the different morphological groups was the amount of mycelia produced by the isolates in culture, ranging from scanty to abundant.

The use of radial growth rate has been shown to be a good measurement approach to differentiate isolates of the same species tested on similar types of media and the approach makes it easier to compare different data obtained within the same experiment or with other experiments to differentiate one isolate from the other or determine which media is best for growth of isolates (Miyashira *et al*., 2010).

There was an association between radial growth rate (mm/day) of *Sphaceloma* sp. isolates and the district and agro-ecological zones from which isolates were obtained. The results of chi-square test of independence to test the hypotheses that radial growth rate of *Sphaceloma* sp. isolates were independent of districts of origin and agro-ecological zones yielded the following test statistic values ($\chi^2 = 67.94$, df = 26, and $P < 0.001$) and ($\chi^2 = 21.08$, df = 4, and $P < 0.001$) for districts and agro-ecological zones respectively (Table 5.7) hence the rejection of the two null hypotheses that growth rate of *Sphaceloma* sp. isolates was independent of districts and agro-ecological zones of origin. Out of the chi-square statistic of 67.94 for districts, isolates from Apac district contributed a margin of 14.869, out of which most (8.680) was due to fast growth rate which means that most of the isolates from the Apac district were fast growing, and covered the entire petri dish by the 8th day. Isolates from two districts, *viz*., Kitgum and Yumbe showed similar growth rates, albeit from different ecological zones, as indicated by their contributions (9.839) each to the chi-square statistic. Also, out of the chi-statistic value of 67.94, slow growth rate contributed most (28.419) indicating that most of the *Sphaceloma* sp. isolates were slow growing (took > 14 days to cover the entire petri dish). On the other hand, out of the test statistic value of 21.08 for the agro-ecological zones, isolates from the Eastern Agro-ecological Zone (EAEZ) contributed the most margin (12.478) out of which a greater part of it was due to moderate growth rate implying that most of the isolates from the EAEZ had moderate growth rate, thus, took between 9-14 days to cover the entire (90 mm) petri dish. On the whole, the observation that most of the *Sphaceloma* sp. isolates were slow (> 14 days) in growth rates (28.419 margin out of $\chi^2 = 67.943$) as shown in

the chi-square test between growth rate and districts of origin, was confirmed by the chi-square test of growth rate being independent of the agro-ecological zones of origin.

Table 5.7: Combined chi-square test of independence for growth rate of *Sphaceloma* sp. isolates with Districts and Agro-ecological zones from which isolates were obtained

District growth rate[a]					Regional growth rate[a]				
District	Slow	Moderate	Fast	Margin	AEZ[b]	Slow	Moderate	Fast	Margin
Amuria	1.016	1.342	0.571	2.929	EAEZ	4.282	4.767	3.429	12.478
Apac	6.143	0.046	8.680	14.869	NESG	3.919	2.697	0.433	7.049
Arua	0.427	0.046	1.077	1.550	NWSG	0.427	0.046	1.077	1.550
Dokolo	1.899	1.661	0.571	4.131					
Kitgum	6.143	3.125	0.571	9.839					
Kumi	0.002	0.046	0.571	0.619					
Lira	0.002	0.046	0.571	0.619					
Nebbi	2.178	5.286	0.571	8.035					
Pader	0.002	0.046	0.571	0.619					
Palisa	1.016	1.342	0.571	2.929					
Serere	2.178	5.286	0.571	8.035					
Soroti	1.016	1.342	0.571	2.929					
Tororo	0.256	0.171	0.571	0.998					
Yumbe	6.143	3.125	0.571	9.839					
Margin	28.419	22.910	16.614	67.943		8.628	7.511	4.938	21.077
χ^2		67.94					21.08		
df		26					4		
P		< 0.001					< 0.001		

[a] Slow, moderate and fast growth rates implies isolates took 1-8, 9-14 and > 14 days respectively to grow to cover entire surface of petri dish (90 mm diameter); b AEZ = Agro-ecological zone, EAEZ = Eastern Agro-ecological zone, NESG = North Eastern Savannah Grassland, NWSG = North Western Savannah Grassland.

Thus, most of the margin (8.628 out of $\chi^2 = 21.08$) was contributed by slow growth rate. This finding that the isolates were mostly slow growing is entirely consistent with earlier reports of studies on the genus *Sphaceloma* and its related genus *Elsinoe* (Zeigler and Lozano, 1983; Timmer *et al*., 1996).

5.5 Conclusion

The study revealed a wide morphological variation in the scab fungus (*Sphaceloma* sp.) occurring in Uganda with the isolates being grouped into six morphological and three pathogenicity groups. Growth rate of the fungus was found to be dependent on the District and Agro-ecological zones of

origin and mostly slow growing. NE 31 and NE 70 cowpea genotypes showed wider resistance to the isolates and could therefore be used to introgress resistance in a breeding programme to develop cultivars with wide horizontal resistance to the scab disease.

CHAPTER SIX

6.0 MOLECULAR CHARACTERIZATION OF COWPEA SCAB FUNGUS (*SPHACELOMA* SP.) OCCURRING IN UGANDA

6.1 Abstract

There is currently no improved cowpea variety resistant to the scab disease (*Sphaceloma* sp.) in Uganda and yet the disease which affects all the above ground parts of the crop has the capacity to cause up to 100% yield loss. An attempt to respond to this challenge requires knowledge about the diversity and population structure of the pathogen in the country. Inter-Simple Sequence Repeat markers were employed to assess the genetic diversity and relationships among 86 isolates from 14 populations of the scab fungus obtained from three different cowpea growing geographical regions of Uganda. The results showed similarity coefficients ranging from 0.0248 to 0.684, indicating the presence of a high degree of genetic variability among the isolates studied. Analysis of molecular variance revealed that there was no variation among the regions and that a greater part of the genetic variation existed within populations (96%) than among populations (4%) and both PhiPT (0.040) and PhiPR (0.042) were significant ($P < 0.001$). Comparison of population pairwise PhiPT values indicated that the greatest differentiation was between Dokolo and Arua populations (PhiPT = 0.564). Both cluster analysis and PCoA did not show clear and distinct patterns of clustering of isolates either based on morphological groups, populations or regions. Mantel test indicated that there was no significant correlations between geographic distance and genetic distance among populations ($R^2 = 0.0192$, $P = 0.044$). Internal transcribed spacer region analysis showed that 22 of the Ugandan isolates were closely related to the *Sphaceloma* sp. and *Elsinoe* sp. isolates in GeneBank.

Keywords: ISSR, variations, *Vigna unguiculata,* molecular variance, demographic factors, seed-borne, internal transcribed spacer region.

From this chapter the following paper has been submitted for publication:

Afutu, E., Agoyi, E.E., Odong, T.L., Wasswa, P., Ssekamate, A.M., Biruma, M. and Rubaihayo, P.R. (2017b). Molecular characterization of Ugandan isolates of cowpea scab fungus (*Sphaceloma* sp.). *Plant Pathology*. (In press).

6.2 Introduction

Cowpea Scab (*Sphaceloma sp*.) is one of the major and common diseases of cowpea in Africa (Dugje *et al*., 2009). In Uganda, the disease was reported to be widespread in all cowpea growing areas with mean incidence ranging between 35-70% and mean severity 2-4 (Afutu *et al.*, 2017a). Mbong *et al*. (2010a, 2012), described it as one of the most destructive diseases of cowpea capable of causing yield losses of up to 100%. According to Allen (1983), Scab of cowpea is widespread in Tropical Africa with the disease very damaging in Savannah areas. The disease affects all the above ground parts of cowpea (leaves, stems, peduncles, flower cushions and pods (Mbong *et al*., 2010a, 2012).

Genetic diversity of pathogens can best be evaluated based on morphological, molecular, biochemical and other characteristics. However, the use of molecular markers in diversity studies is said to have contributed significantly to understanding genetic diversity because they present a higher number of polymorphic loci (Moulin *et al*., 2012). This makes it possible to distinguish between isolates that may have similar morphological traits (Gonçalves *et al*., 2008) or originating from the same location (Moulin *et al*., 2012).

Few molecular marker techniques have been applied in the study of genetic variation in *Sphaceloma* sp. Random Amplified Polymorphic DNA (RAPD) molecular markers have been used in delimiting species among *Elsinoe* isolates (MChau *et al*., 1998) and in the characterization of *Sphaceloma manihoticola* isolates in Brazil (Alvarez *et al*., 2003). Inter-Simple Sequence Repeat (ISSR) markers involving amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction uses microsatellites as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly inter simple sequence repeats of different sizes (Semagn *et al*., 2006). ISSRs exhibit the specificity of microsatellite markers, but need no sequence information for primer synthesis and therefore, enjoy the advantage of random markers (Joshi *et al*., 2000). The technique is simple and usually show high polymorphism (Semagn *et al*., 2006).

Based on the morphological characteristics, six distinct morphological scab fungus groupings were reported to occur in Uganda (Afutu *et al*., 2016b) but there is no information on the molecular variability of the Scab fungus in the country with the likelihood that the resurgence of the disease

in Uganda could be due to the development of variability in patho-types of the fungus. The high occurrence of the disease in recent years, may be evidence of breakdown in the resistance in the current farmers' varieties due to development of virulent patho-types of the fungus. The main objective of this study, was to characterize the *Sphaceloma* sp. isolates occurring in Uganda using ISSR molecular markers and to establish the genetic variability of the populations.

6.3 Materials and Methods

6.3.1 Fungal isolate collection

Monosporic *Sphaceloma* sp. isolates (Appendix 6.1) were used for this study. These were selected from 495 fungal isolates obtained following isolation and culture of infected plant materials collected from farmers' fields across 14 cowpea growing districts and 3 agro-ecological zones in Uganda (Appendix 6.2) (Afutu *et al*., 2016b). A total of 86 isolates were selected based on the morphological groups and the plant part from which isolates were obtained. The isolates selected consisted of 61 and 25 isolated from infected leaves and pods respectively (Appendix 6.1) and were selected across all six morphological groups (Appendix 6.3).

6.3.2 DNA extraction and primer analysis

150 mg of fungal mycelium was scraped from two-week old Potato Dextrose Agar (PDA) cultures of each isolate into a sterilized mortar. DNA extraction protocol outlined by Mahuku (2004) was then followed. The purity of the DNA was estimated from the A_{260}/A_{280} ratio, and the yield was obtained by measuring absorbance at 260 nm using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA). The concentration of DNA of all isolates was adjusted to a standard concentration of 10 ng/µl before being used in the PCR reaction. PCRs were performed using a 200µl PCR tube (Scientific specialties Inc – SSI, USA). The components of the PCR were 0.8 µl of 0.4 mM dNTPs, 3µl of 3 mM $MgCl_2$, 2 µl of 1× PCR buffer, 0.2 µl of 1 U Taq DNA polymerase (Bioneer Inc. Korea), 1 µl of each ISSR primer (0.5 µM) and 1 µl of template DNA (10 ng/µl) and the balance was water to give a total reaction volume of 20 µl. Amplification was performed in a Bioneer MyGenie 96 Thermal block (Bioneer Inc. Korea) programmed for an initial step of DNA denaturation for 5 min at 94^{o}C, followed by 35 cycles of 20 s at 94 ^{o}C, annealing for 40 s at 30-55 ^{o}C depending on the specific primer (Appendix 6.4), and 1 min at 72 ^{o}C., followed by a final extension for 7 min at 72 ^{o}C. A total of thirteen (13) primers consisting of six (6) Random

Amplified Microsatellites (RAMS), five (5) Microsatellites and two (2) ISSRs were used for this study but four did not produce polymorphic bands (Appendix 6.4).

6.3.3 Preparation of samples for sequencing

Following Ward's cluster analysis of ISSR data obtained, 25 isolates were selected for sequencing based on the different morphological groups out of the four different clusters from the 86 isolates. The internal transcribed spacer (ITS) region of fungal ribosomal DNA (rDNA) containing the full length ITS1, the 5.8S rDNA and ITS2 was amplified through PCR with primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White *et al*., 1990) (Operon Technologies, Inc., Alameda, CA). The components of the PCR were 1 µl of 1 mM dNTPs, 5 µl of 3 mM $MgCl_2$, 5 µl of 1× PCR buffer, 0.2 µl of 1 U Taq DNA polymerase (Bioneer Inc. Korea), 2.5 µl of each ITS (ITS 4 and 5) primer (1 µM) and 2 µl of template DNA (10 ng/µl) and the balance was water to give a total reaction volume of 50 µl in 200 µl PCR tubes (Scientific specialties Inc. – SSI, USA). Amplification was performed in a Bioneer MyGenie 96 Thermal block (Bioneer Inc. Korea) programmed for an initial step of DNA denaturation for 5 min at 94 ^{o}C, followed by 35 cycles of 20 s at 94 ^{o}C, annealing for 40 s at 55 ^{o}C, extension for 1 min at 72 ^{o}C. Final extension was for 7 min at 72 ^{o}C, and held overnight at 8 ^{o}C.

6.3.4 Electrophoresis

DNA amplification products from the PCRs were separated by electrophoresis in 1.5% agarose gels in 1× Tris-borate-EDTA (TBE) buffer under a constant 150 volts for 120 min at room temperature. Following staining with ethidium bromide (0.5 µg/mL), fragments were visualized under 500 nm UV light exposure and gel images were captured with Syngene – G:Box documentation system (Syngene, UK). To estimate the size of the amplified DNA fragments, a 100 bp DNA ladder (Bioneer Inc. Korea) was used as molecular weight marker. Profiles for each primer were compared on the basis of the presence or absence of a fragment (Appendix 6.5) presumed to be the same length. Fragments of the same length were scored as identical.

6.3.5 Data analysis

Data for multiloci was transformed to a binary matrix of presence/absence for each individual isolate (Teixeira *et al*., 2014) and used for further analysis. Genetic variation and differential connectivity among populations (PhiPT), regions (PhiRT), and populations within regions (PhiPR) were estimated through analysis of molecular variance (AMOVA) with populations nested within regions using the GenAlEx 6.5 (Peakall and Smouse, 2006; 2012). PhiPT coefficient values $[V_{\mathrm{AP}}/(V_{\mathrm{WP}} + V_{\mathrm{AP}})]$ denote the proportion of the variance among population relative to the total variance and pairwise between populations PhiPT = $(V_{\mathrm{AP}} + V_{\mathrm{AR}})/(V_{\mathrm{WP}} + V_{\mathrm{AP}} + V_{\mathrm{AR}})$. V_{AP} is the variance among populations, V_{WP} is the variance within populations, and V_{AR} is the variance between geographical regions (Keneni *et al*., 2012). The AMOVA procedure in GenAlEx follows the methods of Excoffier *et al*. (1992), which estimates and partitions total molecular variance within and between populations and then tests the significance of partitioned variance components using permutational testing procedures. PhiPT values (analogue of F_{ST}) obtained following 999 permutations were used to determine the genetic differentiation between populations. PhiPT, is a measure that allows intra-individual variation to be suppressed and is, therefore, ideal for comparing codominant and binary data (Teixeira *et al*., 2014).

Latitudinal and longitudinal data for isolates were used to estimate geographic distance. Genetic distance obtained following AMOVA analysis and the geographic distance estimated were used to test for isolation by distance (Mantel, 1967) in GenAlEx. The binary data was used to generate pairwise genetic distance matrix among the 86 isolates using the Euclidean distance method. The distance matrix was used to perform a hierarchical cluster analysis of the 86 isolates based on Ward's (1963) agglomeration method using R statistical package for Windows. Principal co-ordinate analysis was performed based on Nei's distance matrix using NTSYSpc version 2.21L.

Sequences were aligned and edited using ClustalW and evolutionary history was inferred using the Maximum Parsimony method (Hyun *et al*., 2009) and the phylogenetic tree number one out of 1000 most parsimonious trees was selected. Sequence analyses were carried out using the Molecular Evolutionary Genetics Analysis software version 6.0 (MEGA6) (Tamura *et al*., 2013). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar, 2000) with search level 0 in which the initial trees were obtained by the random addition of sequences (1000 replicates). The tree was drawn to scale, with branch lengths calculated using the

average pathway method (Nei and Kumar, 2000) and are in the units of the number of changes over the whole sequence. The analysis involved 29 nucleotide sequences (25 plus 4 reference sequences from GeneBank – [https://blast.ncbi.nlm.nih.gov/Blast.cgi]). All positions containing gaps and missing data were eliminated.

6.4 Results and Discussion

Following various statistical analyses performed on the 311 polymorphic loci obtained from PCR amplifications of the 14 populations with 86 isolates of *Sphaceloma* sp. with the 9 ISSR markers, a wide genetic diversity was observed in the populations of the fungus in Uganda. The results of three-way nested AMOVA analysis based on PhiPT values are presented in Table 6.1.

Table 6.1: Three-Region nested analysis of molecular variance (AMOVA) showing the partitioning of genetic variation within and among populations of *Sphaceloma* sp. based on 311 polymorphic loci

Sources of Variation	df[a]	MS[b]	Variation	% of total variation	Phi Statistic	Value	*P*-value[d]
Among regions	2	59.524	0.000	0	PhiRT	-0.002	0.666
Among populations	11	60.237	2.077	4	PhiPR	0.042	0.001
Within populations	72	47.943	47.943	96	PhiPT[c]	0.040	0.001
Total	85		50.019	100			

[a] Degrees of freedom; [b] Mean Squared deviations; [c] Analogue of F_{ST} fixation index; [d] P-Value is based on 999 permutations

The partitioning of genetic variation following AMOVA showed that there was no variation among the regions but indicated that most (96%) of the molecular variation in *Sphaceloma* sp. occurred within populations while diversity among populations contributed only 4% of the observed genetic diversity. The results of the AMOVA indicated that genetic differentiation was not equally distributed but was significant ($P = 0.001$) both within and among populations and non-significant ($P = 0.666$) among the regions. The lack of genetic structure detected among regions was suggestive of a form of interaction occurring among the regions. Overall Phi values for diversity among populations (PhiPR = 0.042) and within populations (PhiPT = 0.040) were both significant ($p = 0.001$), indicating that the observed differences among and within populations were significant (Breinholt *et al.*, 2009) suggesting significant differentiation and low gene flow across all populations (Hartl and Clark, 1997; Jackson, 2003). Random permutations of the data for AMOVA (Figure 6.1) indicated a non-random partitioning of the genetic variation suggesting that some

external factors other than those of the isolates themselves are driving or influencing the patterns of variation or interactions among the populations.

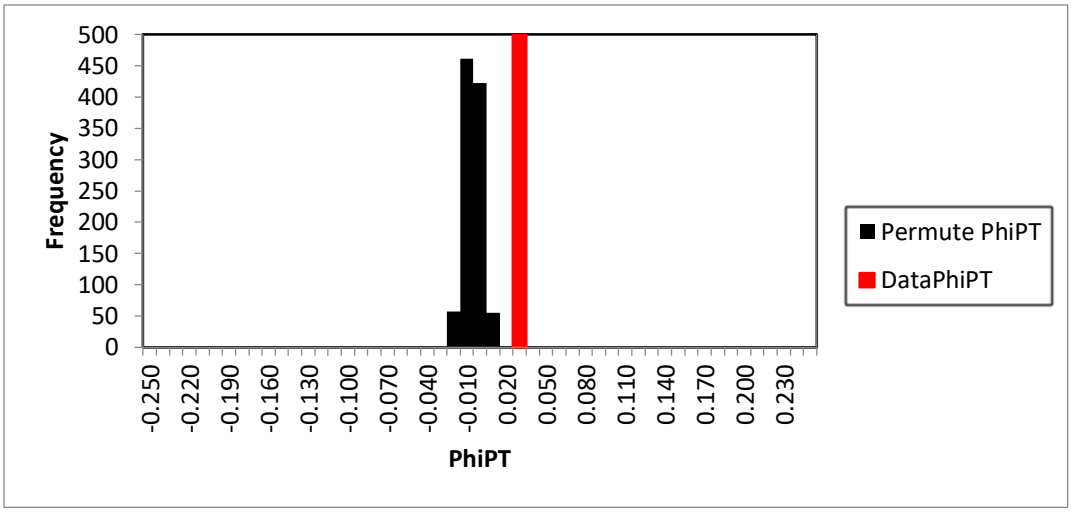

Figure 6.1: Frequency distribution for three-way AMOVA of ISSR band variation for 14 populations of *Sphaceloma* sp

The results of mean genetic distance between individuals in each population (above diagonal) and comparison of population pairwise PhiPT with probability values based on 999 permutations (below diagonal) are presented in Table 6.2. The results showed varying levels of differentiation among all populations. The pairwise PhiPT values showed that no two populations were found to be similar. Thus, all pairwise comparisons were different from one another. The highest differentiation was between Dokolo and Arua populations (PhiPT = 0.564) and the lowest was between Amuria and Palisa and Kumi and Apac populations (PhiPT = 0.002).

Another relatively low value of 0.008 was found between populations of Tororo and Yumbe which are the most separated apart in terms of distance and isolation. Other relatively low PhiPT values were found between some adjacent populations; Amuria and Soroti (0.006), and Kumi and Soroti (0.008). On the other hand, the greatest mean population binary genetic distance (110.357) occurred between individuals in Lira and Soroti populations while the smallest mean genetic distance (63.400) occurred between Pader and Tororo Populations which are geographically far apart (Appendix 6.2).

Table 6.2: Mean genetic distance between individuals in each population (above diagonal). Population pairwise PhiPT values with probability values based on 999 permutations (lower diagonal).

Population	Amuria	Kumi	Serere	Tororo	Soroti	Palisa	Apac	Dokolo	Kitgum	Lira	Pader	Yumbe	Arua	Nebbi
Amuria	-	100.154	104.167	97.640	108.800	103.689	100.933	100.300	98.400	104.857	103.700	101.500	102.125	101.133
Kumi	0.017	-	99.474	92.123	103.365	99.179	98.103	97.654	94.692	105.231	96.141	99.846	100.356	92.744
Serere	0.008	0.235	-	95.467	106.208	102.574	99.611	95.667	98.667	106.262	99.556	103.583	102.542	97.389
Tororo	0.003	0.028	0.027	-	99.350	92.756	92.000	91.100	90.400	101.257	63.400	95.750	95.050	88.200
Soroti	0.006	0.141	0.278	0.027	-	106.639	104.333	104.375	101.250	110.357	102.542	103.375	105.469	102.000
Palisa	0.002	0.008	0.020	0.049	0.008	-	98.370	96.056	99.889	107.651	100.574	105.556	100.986	96.630
Apac	0.088	0.002	0.032	0.003	0.059	0.010	-	96.500	95.667	102.000	97.833	100.833	96.708	92.556
Dokolo	0.140	0.290	0.116	0.154	0.400	0.336	0.216	-	91.500	102.357	95.667	98.750	91.750	98.833
Kitgum	0.102	0.066	0.099	0.027	0.092	0.034	0.201	0.339	-	100.571	90.333	84.750	96.375	91.667
Lira	0.075	0.016	0.189	0.007	0.138	0.004	0.036	0.512	0.151	-	103.119	104.357	104.839	101.095
Pader	0.003	0.457	0.281	0.019	0.458	0.017	0.011	0.386	0.158	0.285	-	97.167	99.958	93.833
Yumbe	0.098	0.029	0.062	0.008	0.246	0.012	0.028	0.265	0.456	0.157	0.230	-	99.813	99.333
Arua	0.027	0.055	0.185	0.033	0.252	0.037	0.046	0.564	0.120	0.385	0.215	0.184	-	96.125
Nebbi	0.119	0.452	0.423	0.258	0.400	0.185	0.186	0.250	0.229	0.480	0.394	0.215	0.485	-

The relatively low PhiPT values observed between adjacent populations were expected because exchange of genetic material is usually expected to occur between adjacent locations than locations which are far separated in distance and isolation because the disease is seed-borne (Mbong *et al*., 2010a). However, the low PhiPT values between populations which are very distant from each other, such as observed between Tororo and Yumbe, were surprising.

This was suggestive of a relatively recent exchange of genetic material between the two populations and therefore the low differentiation (Jackson, 2003). The highest differentiation observed between Dokolo and Arua populations (PhiPT = 0.564), was indicative of a high degree of isolation between those two populations (Rotkopf *et al*., 2010) which was not surprising as Dokolo is far from Arua.

The results of dendogram constructed from the ISSR analysis of the *Sphaceloma* sp. isolates separated the 86 isolates into four clusters at a distance of 16 (Figure 6.2). The results showed that two isolates, AP313L and AP294P, occurred at a relatively shorter distance within cluster 1. Also, isolates AR457L and AR438L were shown to be very closely related to isolate DK358P which is from a different region. Overall, cluster 4 was the least weighted with eight isolates followed by clusters 3 and 1 with nine (9) and thirty-two (32) isolates respectively, with cluster 2 being the heaviest weighted comprising 37 isolates. These results showed no clear patterns of clusters formed either based on belonging to the same morphological group, populations or regions. They revealed a lot of overlapping between populations and regions.

The results of Principal Coordinate Analysis (PCoA) based on pairwise genetic differences are shown in Figure 6.3. Three grouping patterns were formed by the isolates, however, these grouping patterns generated by the principal coordinate analysis confirmed the results of the cluster analysis as there were no clear patterns of grouping of individuals based on either populations or regions. They also revealed a large amount of overlap among populations or sample sites. The three principal coordinates accounted for only 20.56% of the total genetic variation observed. The results of both cluster and principal coordinate analyses did not show clear patterns of genetic variation among the populations. The PCoA did not show significant spatial genetic structure among adjacent populations.

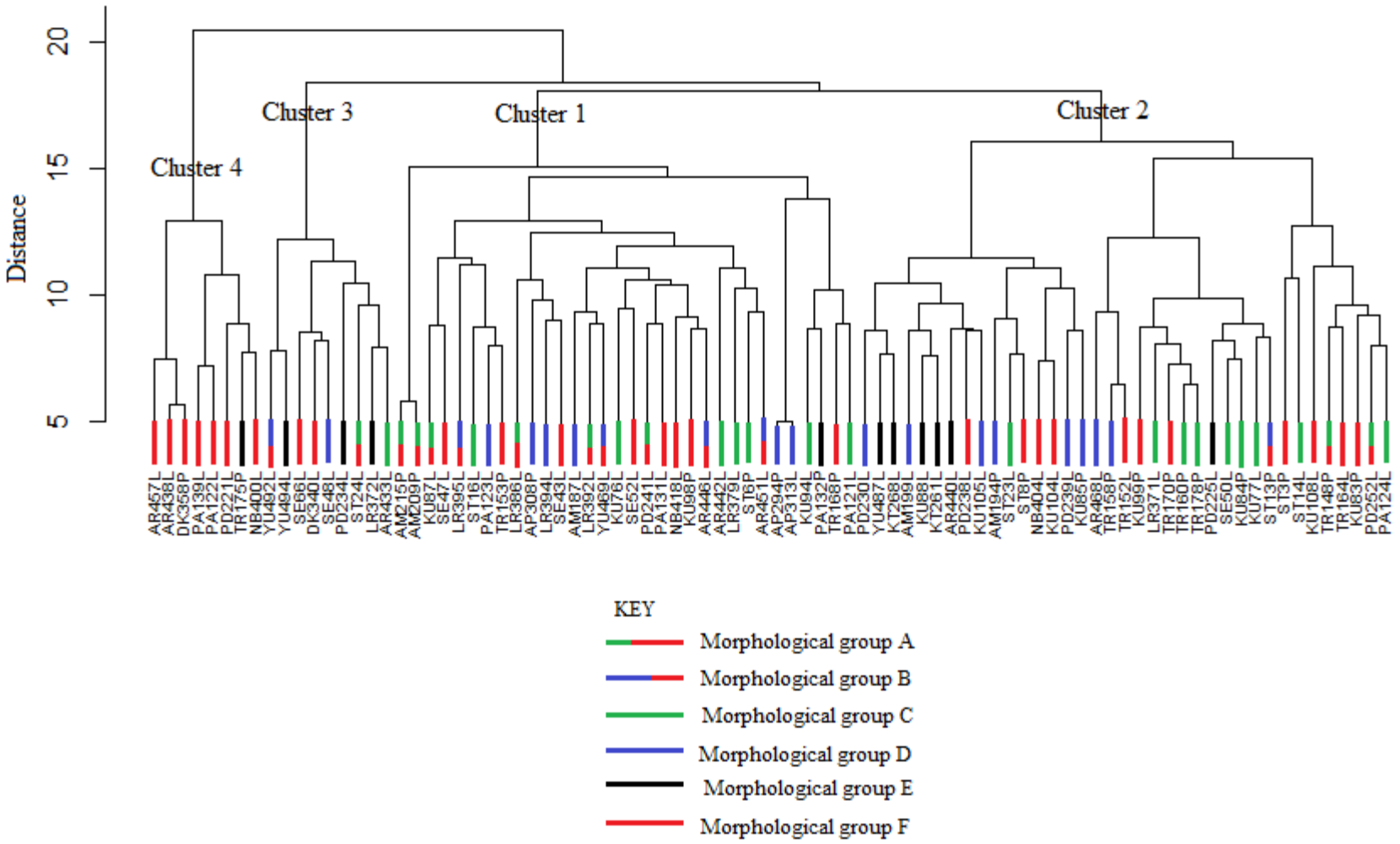

Figure 6.2: Ward's cluster dendogram of 86 *Sphaceloma* sp. isolates based on 9 ISSR markers.

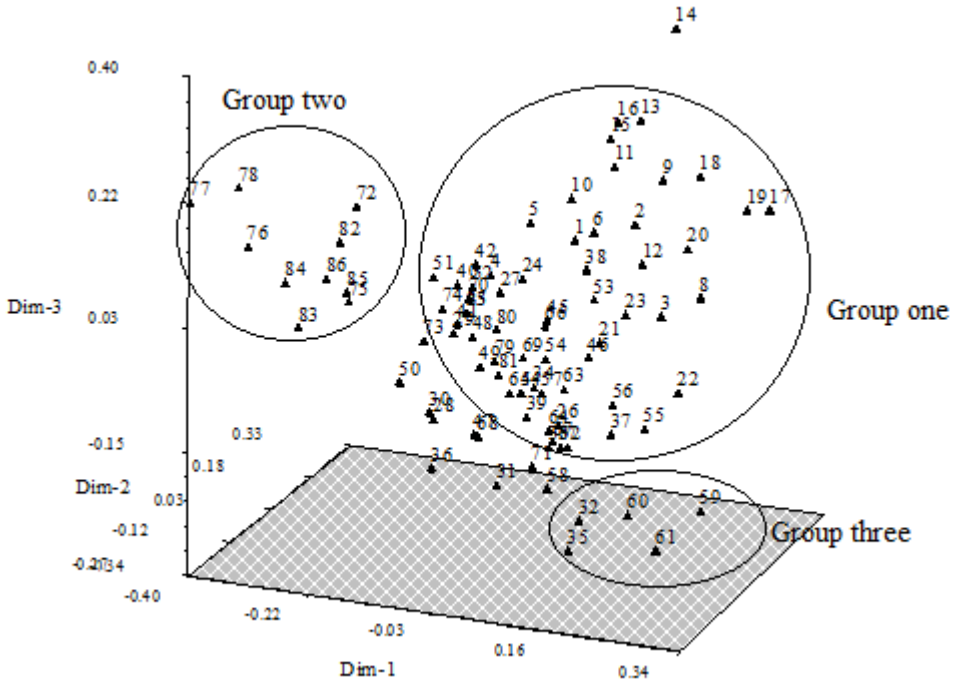

Figure 6.3: Principal coordinate analysis (PCoA) of genetic differences among 14 populations (86 individuals). See Appendix 6.1 for definitions (labels) of individuals. The three axes combined accounted for 20.56% of the total variation.

Thus, results from the two analyses did not suggest relationships among adjacent populations but showed fragmented patterns of genetic variation among the populations of *Sphaceloma* sp. through the groupings of populations and with a lot of overlapping of populations without clear discrete groupings being identified. This explains why the first three axes combined explained only 20.56% of the total variation observed. These findings were corroborated by the Mantel test based on geographic distance matrix and Nei's (1978) genetic distance matrix among the populations (Figure 6.4). The test revealed a weak and non-significant correlation between geographic and genetic distances ($R^2 = 0.0192$, $P = 0.044$) among the *Sphaceloma* sp. populations included in this study suggesting that these populations did not conform to an isolation by distance model to explain the patterns of differentiation (Jackson, 2003).

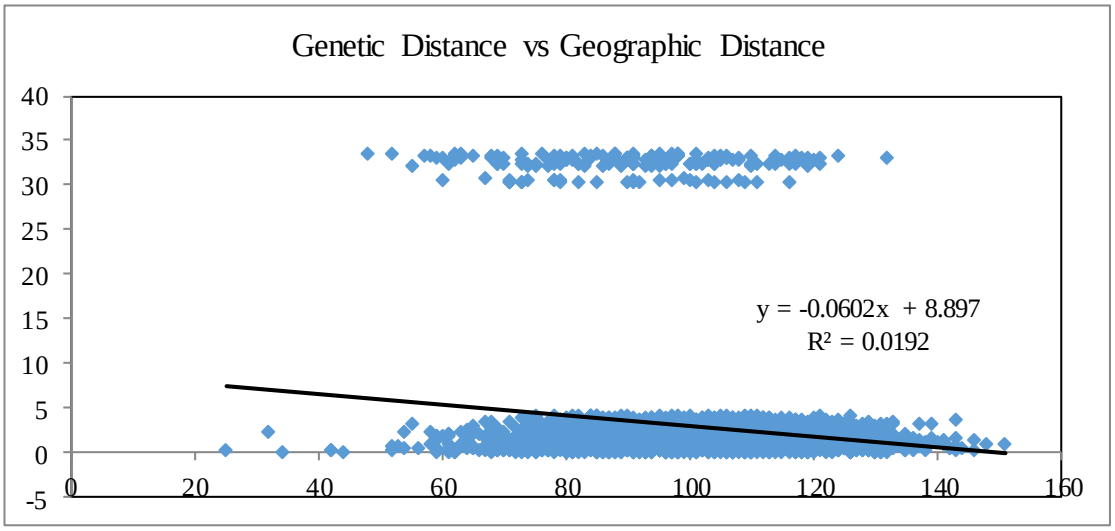

Figure 6.4: Mantel test of genetic distance matrix vs. geographic distance matrix for 14 populations of *Sphaceloma* sp. (Prand >= data = 0.044).

Therefore, isolation of populations caused by geographic distance can be excluded (Marosi *et al*., 2013). The weak correlation and lack of significance between genetic distance and geographic distance was likely to be due to the high within population genetic diversity observed (Teixeira *et al*., 2014). This was supported by the PhiPT value for among regions which showed a weak genetic differentiation between populations in each of the studied regions (*P* value = 0.666) (Table 6.2). In theory, genetic diversity should decline in recently derived populations (Lande, 1999; Jackson, 2003). Using neutral genetic markers, expected levels of genetic differentiation between populations was expected to decline linearly (Morgan *et al*., 2001, Jackson, 2003). The seed-borne nature of the pathogen might have contributed largely to the high level of genetic diversity within populations.

No discrete single cluster was found devoid of isolates from other morphological groups, populations or regions, suggesting an evidence of ancestral relatedness (Breinholt *et al*., 2009) as demonstrated in the PCoA and Cluster analysis with multiple individuals clustering more closely to other regions than their own. The results of the ISSR analyses of the 86 *Sphaceloma* sp. isolates demonstrated variability at the molecular level, therefore, provided genetic evidence of variability among the isolates. These findings are significant as fungal populations with high genetic variability are more able to develop resistance to fungicides and rapidly overcome host resistance than populations with low genetic variability (Sanchez, 1998; Alvarez *et al*., 2003).

The ITS sequences of all 25 selected isolates shared high similarity (82-92 %) with sequences of *Sphaceloma* sp., *S. manihoticola*, *S. fawcettii* and *Elsinoe fawcettii* isolates in GeneBank. The results of evolutionary history inferred using the maximum parsimony method for a total of 352 positions in the final dataset are presented in Figure 6.5.

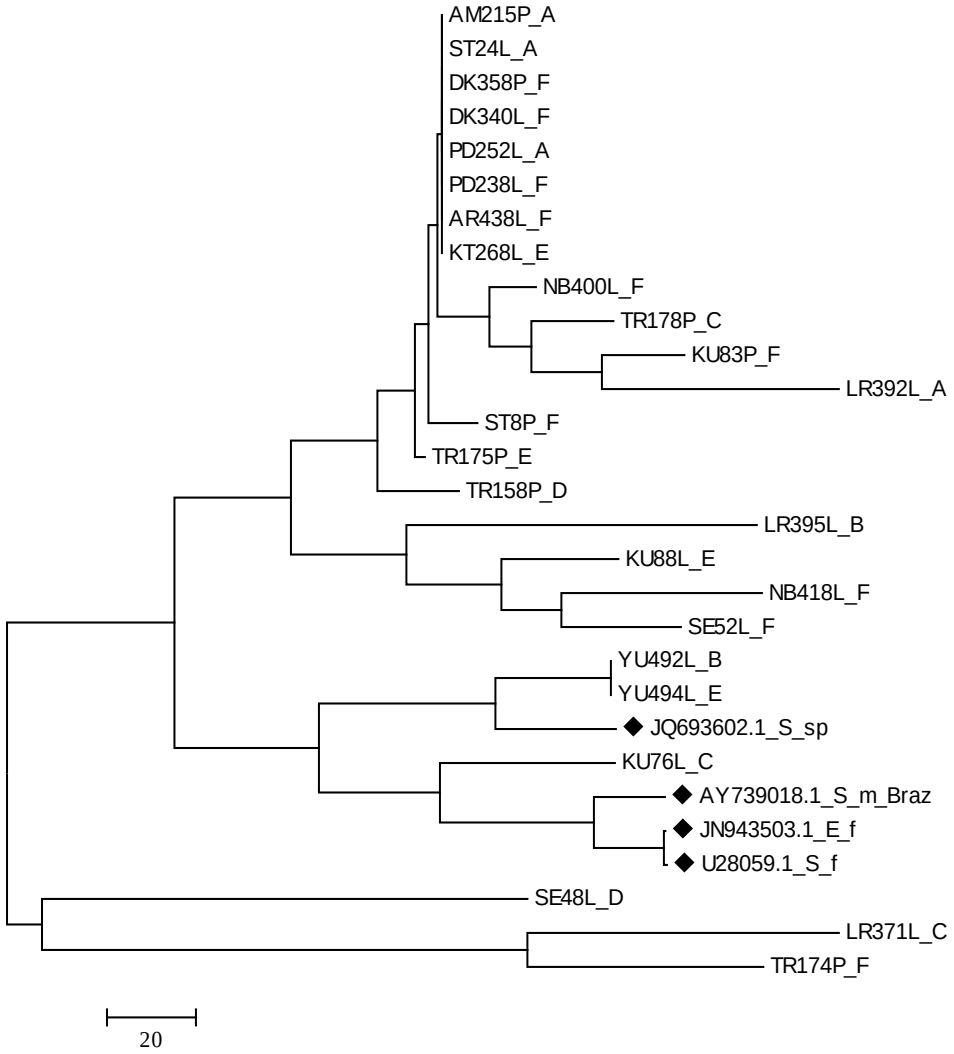

Figure 6.5: Phylogenetic tree based on maximum parsimony analysis of sequences of internal transcribed spacer region (ITS) for *Sphaceloma* sp. isolates causing cowpea scab disease in Uganda. Black shaded diamond shapes represent 4 reference sequences of *Sphaceloma* sp., *S. manihoticola*, *S. fawcettii* and *Elsinoe fawcettii* isolates downloaded from GeneBank.

The consistency index was 0.624215 (0.591618), the retention index was 0.675947 (0.675947), and the composite index was 0.421937 (0.399903) for all sites and parsimony-informative sites (in parentheses). On the whole, no clear distinct groups nor sub-groups (sub-clusters) were formed based on the morphological groups from which isolates were selected indicating that though

isolates were separated into different morphological groupings, they in fact were closely related (Figure 6.5) than predicted by the morphological characters reported by Afutu *et al*. (2016b). Zeigler and Lozano (1983) reported that within-species variability in cultural characteristics for *Sphaceloma* and *Elsinoe* isolates may exceed between-species differences, and concluded that cultural characteristics are unstable, thus, placing heavy emphasis on cultural characteristics carries the risk of considering normal variants of one species to be different species and thereby making molecular characterization a more robust approach to characterization.

Analysis of the ITS region showed that isolates AM215P, ST24L, DK358P, DK340L, PD252L, PD238L, AR438L and KT268L were 100% similar and closely related to isolates NB400L, TR178P, KU83P, and LR392L albeit from different Districts and Agro-ecological zones thus explaining the observed lack of significant difference (i.e. no genetic variation) among the regions (agro-ecological zones) from the AMOVA results (Table 6.1). This finding implied that there was no need to develop different varieties for the different regions to manage the scab disease in Uganda. Except for isolates SE48L, TR174P and LR371L which were clearly distinct, all remaining 22 *Sphaceloma* sp. isolates from Uganda clustered with the four reference isolates *viz. Sphaceloma*. sp. (JQ693602.1 from China), *S. manihoticola* (AY739018.1 from Brazil), *S. fawcettii* (U28059.1 from Australia) and *Elsinoe fawcettii* (JN943503.1 from Brazil) from the GeneBank (Figure 6.5) indicating that, except for the three distinct isolates, the remaining 22 Ugandan isolates were closely related to those reported and deposited in the GeneBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

6.5 Conclusion

There was high genetic differentiation among the *Sphaceloma* sp. populations which were independent of spatial or demographic factors. Genetic diversity within populations was higher than among populations indicative of an active interaction and exchange of seeds across populations and regions. The Ugandan isolates were highly similar to the *Sphaceloma* sp. (from Australia, Brazil and China) and *Elsinoe* sp. (from Brazil) deposited in GeneBank.

CHAPTER SEVEN

7.0 GENETICS OF RESISTANCE TO COWPEA SCAB DISEASE AND YIELD COMPONENTS OF COWPEA

7.1 Abstract

Eleven cowpea lines (four resistant, five moderately resistant and two susceptible) were selected as parents and crossed using half diallel mating design to generate information on the genetics of control of scab disease resistance, yield and yield related traits in cowpea (*Vigna unguiculata*). 55 F_2 progenies and their parents (11) were evaluated at two locations (Kabanyolo and Serere) between April and July, 2016 using an 11 × 6 lattice design. Data were analysed using Griffing's method 4 model 1. Combined analysis of variance for the two locations revealed highly significant differences ($P < 0.001$ and $P < 0.01$) among the genotypes for all 12 traits studied but showed no significant genotype by location (G × L) interaction effect on any of the traits. Coefficient of genetic determination in the narrow and broad sense, analogous to narrow and broad sense heritability respectively, were generally low ranging from 1.23 % - 27.12% (h^2) and 7.4 % -71.91 % (H^2). The low values of Baker's ratio (BR) (< 0.5) for most of the traits suggested that non-additive gene effects were more important except for number of pods per plant which indicated additive gene effects were more important (BR = 0.80) and number of seeds per pod (BR = 0.5) which indicated both additive and non-additive gene effects were important. Alegi, NE15 and NE48 were identified as parental lines with negative general combining ability (GCA) effects for improvement of scab disease resistance for both locations while SECOW5T was identified as the only parent with high positive GCA for improvement of both 100 seed weight (g) and grain yield (K/ha) of cowpea across the two locations. Cross between WC35B*WC66 had high SCA effects for scab disease resistance. Six crosses were identified as the best hybrids for the improvement of cowpea yields across the locations.

Keywords: Coefficient of genetic determination; narrow sense; broad sense; diallel; phenotypic variability; genetic variability; combining ability.

From this chapter the following paper has been prepared to be submitted for publication:

(Afutu, E., Agoyi, E.E., Gibson, P., Biruma, M. and Rubaihayo, P.R. (2016). Genetics of resistance to cowpea scab disease and yield components of cowpea. *Field crops*. (Yet to be submitted)

7.2 Introduction

Scab disease, caused by the fungus *Sphaceloma* sp. is one of the most devastating diseases of cowpea capable of causing 100% yield loss under severe infections (Mbong *et al.*, 2012). Only one of the recently released varieties of the crop is moderately resistant to the disease and yet the disease is widespread in the country (Afutu *et al.*, 2017a). Breeding efforts to develop varieties with resistance to the disease require an understanding of the genes that confers resistance to the disease in terms of its heritability and gene action. Earlier studies on the subject were carried out by screening 80 cowpea lines for sources of resistance (Iceduna *et al.*, 1994), followed by Nakawuka and Adipala (1997) and Tumwegamire *et al*. (1998) who studied the inheritance and genetics of resistance to the scab disease. Recent studies to identify new sources of resistance showed that there were cowpea landraces in Uganda with wide horizontal resistance that could be used as parents in a breeding programme (Afutu *et al*., 2016a; 2016b) to develop resistant varieties for farmers. There is therefore, a need to understand the heritability and gene action within the identified populations.

Economically important traits have complex inheritance and are environmentally influenced, thus, making selection for such traits more difficult (Smalley *et al*., 2004). Heritability is based on the hypothesis that individuals more closely related are more likely to resemble one another than those less closely related (Falconer and MacKay, 1996) and can also be estimated in the broad sense (H^2) or narrow sense (h^2), but it is the narrow-sense heritability which depends on the additive portion of genetic variance that is used to predict how quickly selection can change the average phenotype of the population (Falconer, 1960). Heritability estimates help the breeder in determining the resource allocation necessary to effectively select for the trait of interest and to achieve maximum genetic gain with use of minimum time and resources (Smalley *et al*., 2004).

Nakawuka and Adipala (1997) suggested that general combining ability (GCA) effects were more important than specific combining ability (SCA) effects in the inheritance to scab disease resistance, however, Tumwegamire *et al*., (1998) suggested both GCA and SCA effects were important for both foliar and pod resistance to scab infections as high estimates were obtained for both broad-sense and narrow-sense heritability for resistance to foliar (H^2 = 93.8 %; h^2 = 79.8 %) and pod (H^2 = 97.0 %; h^2 = 84.5 %) scab infections, thus, indicating both additive and non-additive gene actions were important to scab resistance. Broad sense heritability for traits such as pod length

(H^2 = 76.0 %) (Roquib and Patnaik, 1990), the number of seeds per pod (H^2 = 75.3 %) and seed size measured as 100-seed weight (H^2 = 94.4 %) (Siddique and Gupta, 1991) have been reported. Additive gene effects were reported to be more important than non-additive effects for pod length inheritance (Ogunbodede and Fatunla, 1985), however, additive, dominance and epistatic gene effects were observed to be of equal importance in conditioning inheritance for the number of seeds per pod (Drabo *et al.*, 1985). Any estimate of heritability is only relevant to a particular population in a particular set of environments as different populations have different gene frequencies. This study was, therefore, conducted to determine the relative importance of GCA and SCA, and hence additive and non-additive effects in the inheritance of resistance to scab infections, and heritability of yield and yield related traits in the new breeding populations.

7.3 Material and Methods

7.3.1 Experimental materials

Following field evaluations of 100 cowpea lines carried out at the Makerere University Agricultural Research Institute – Kabanyolo (MUARIK) during the first raining season (April-July) of 2014 to identify sources of resistance (Afutu *et al*., 2016a), 20 cowpea lines with varying levels of resistance were selected and later challenged in the screen house with five isolates of the scab fungus (*Sphaceloma* sp.) obtained from different districts and agroecological zones of Uganda to identify lines with wider horizontal resistance to the scab disease (Afutu *et al*., 2016b). Subsequently, eleven cowpea lines (Table 7.1) comprising 4 resistant, 5 moderately resistant and 2 susceptible lines were selected based on their resistance levels and yield potential and used as parental lines.

7.3.2 Crossing and advancing of F_1s to F_2

Crosses were made among the eleven selected parents in the screen house according to Griffing's (1956) diallel method 4 model 1 mating design. Flowers to be used as females were emasculated in the evening using sterile scapel blade regularly sterilized using alcohol (95 %) and hand pollinated in the morning hours of the next day (Tumwegamire *et al*., 1998). To ensure that enough successful crosses were obtained, flowers at all plant growth stages were used in crossing and tagged accordingly. In order for inferences from analysis to be limited to the parents themselves

and the resultant crosses, the parents were considered as a fixed sample (Nakawuka and Adipala, 1997).

Table 7.1: List of selected parents and their resistance ratings

Genotype	Cultivar type	Resistance rating[a]	Yield potential (t/ha)	Maturity
Acc 12*Sec 2W	Inbred line	Resistant	1.36	Early
NE 15	Landrace	Resistant	1.63	Medium
WC 35B	Landrace	Resistant	1.40	Medium
WC 66	Landrace	Resistant	1.43	Medium
Alegi	Local	Moderate	1.42	Early
NE 48	Landrace	Moderate	1.43	Medium
NE 50	Landrace	Moderate	1.45	Early
Secow 5T	Improved	Moderate	2.05	Early
WC 10	Landrace	Moderate	1.61	Medium
WC 36	Landrace	Susceptible	1.21	Medium
Sun shine	Introduction	Susceptible	0.72	Medium

[a] Resistance rating according to Afutu *et al*., (2016a); Acc = Accession; Sec = Secow

At maturity, the F1 seeds from successful crosses were harvested separately and were grown in the field at MUARIK together with the parents during the second raining season (September-December) of 2015 to advance F_1s to F_2. The F_1 seeds and the parents were planted in two rows 1.5 m long and spaced 60 cm between rows and 30 cm within rows and the field was laid out in a randomized complete block design (RCBD) with three replications. A field previously cultivated with Sorghum (*Sorghum bicolor*) was selected in order to reduce the incidence of diseases and insect pests of cowpea. The experiment was weeded three times and sprayed with Roket ® 44 EC (Profenofos 40% + Cypermethrin 4%) insecticide at the rate of 30 ml to 15 L of water to control insect pests three times, one at four weeks after emergence, second at flower initiation stage and the third during pod setting. No fertilizer or fungicide were applied during the entire growing period.

7.3.3 Evaluation of F_2 plants and parents for combining ability studies

The F_2 plants and their parents were evaluated in two locations, MUARIK (0°28'N and 32°37'E; 1200 m above sea level) in the Central part of Uganda and the National Semi Arid Resources Research Institute (NaSARRI) in Serere (1°39'N and 33°27'E; 1038 m above sea level), Eastern

part of Uganda during the first rainy season (April-July) of 2016. The experiments were laid out in an 11× 6 alpha lattice design with 3 replications at each site. Each replication had 11 blocks with each block having 6 plots. Each genotype was planted on a plot with an area of 1.5 m × 1.2 m with a spacing of 1 m between plots and between replications. A spacing of 60 cm between rows and 30 cm within rows was used. The experiment was planted on fields previously cultivated with cowpea to create high disease pressure conditions in the field. Weed and insect control were as described for advancing of F_1s to F_2s.

7.3.4 Data collection and analysis

Five plants were randomly selected from each plot and tagged at six weeks after planting and scab disease incidence and severity data were collected at seven days intervals (Mbong *et al*., 2010a) for six consecutive weeks. Scab disease severity scores were determined using a scale of 1-5, where 1 = no symptoms, 2 = less than 10% infection, 3 = 10 to 20% infection, 4 = 20 to 50% infection, and 5 = more than 50% infection (Nakawuka and Adipala, 1997). Scab disease incidence was estimated by counting all individual plants with scab disease symptoms in each plot and expressed as a ratio over the total number of plants in each plot.

Data were collected for grain yield and yield related traits such as days to 50% flowering, number of branches per plant, number of peduncles per plant, number of pods per peduncle, number of pods per plant, pod length (cm), seeds per pod, and 100 seed weight (g). Yield (Kg ha^{-1}) was estimated from yield per plot. The plot means for yield and yield related traits, scab disease incidence and severity values were estimated using Microsoft Excel and the means were subjected to the analysis of variance (ANOVA) procedure using Genstat edition 14 (Payne *et al*., 2011). Mean severity scores were used to estimate the area under disease progress curve (AUDPC) for both parental lines and F_2 plants as according to Campbell and Madden 1990);

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right) (t_{i+1} - t_i)$$

Where "t" is the time of each reading, "y" is the percent of affected foliage at each reading and "n" is the number of readings. The variable "t" represents days after planting. Percent incidence data were Arcsine transformed (Ezueh and Amusan, 1988) before using for further analysis.

Means of scab disease incidence, severity, yield and yield related traits were analysed using restricted maximum likelihood (REML) procedure in GenStat® edition 14 (Payne *et al*., 2011) to determine the general and specific combining ability (GCA and SCA) effects using Griffing's method 4 model 1 (Griffing, 1956) and the t-test was calculated and used to measure the significance of the GCA and SCA effects (Tumwegamire *et al*., 1998) using Microsoft Excel for Windows. The parents were considered as fixed and therefore, the model fitted was the fiexed effects model of Singh and Chaudhary (2004):

$$Y_{ij} = \mu + GCA_i + GCA_j + SCA_{ij} + e_{ij}$$

Where: Y_{ij} = the observed mean; μ = overall population mean; GCA_i = effect of the i^{th} parent; GCA_j = effect of the j^{th} parent; SCA_{ij} = interaction effect of the i^{th} parent and j^{th} parent; and e_{ij} = experimental error.

The GCA effects for the parents, SCA effects for the crosses and their standard errors were calculated according to Kearsey and Pooni (1996). Coefficient of genetic determination in the narrow (CGD-NS) and broad sense (CGD-BS), analogues of the narrow sense (h^2)and broad sense heritability (H^2) respectively, were estimated using the variance component method as according to Ruming (2004) as follows:

$$CGD - NS \approx h^2 = \frac{(2 * \delta^2 GCA)}{(2 * \delta^2 GCA + \delta^2 SCA + \delta^2 e)}$$

$$CGD - BS \approx H^2 = \frac{(2 * \delta^2 GCA + \delta^2 SCA)}{(2 * \delta^2 GCA + \delta^2 SCA + \delta^2 e)}$$

Where; h^2 = narrow sense heritability; H^2 = broad sense heritability; $\delta^2 GCA$ = variance of general combining ability; $\delta^2 SCA$ = variance of specific combining ability of parents; $\delta^2 e$ = error variance.

The relative contribution of GCA and SCA were estimated using Baker's ratio (BR) (Baker, 1978), computed as:

$$BR = \frac{(2 * \delta^2 \mathrm{GCA})}{(2 * \delta^2 \mathrm{GCA} + \delta^2 \mathrm{SCA})}$$

Where; δ^2GCA and δ^2SCA are the variance components of GCA and SCA, respectively. Phenotypic correlation coefficients (rp) for scab disease incidence, severity, AUDPC, yield and yield related traits were determined using data obtained from the 55 crosses alone (without parents) from the two locations using IBM SPSS Statistics version 22 (IBM Corporation, 2013).

7.4 Results and Discussion

7.4.1 Phenotypic variability

The results of analysis of variance for scab disease incidence, severity, yield and yield related traits for Kabanyolo and Serere are shown in Table 7.2. Location-wise, in Kabanyolo, there were significant differences among the genotypes for scab disease incidence ($P < 0.01$), severity ($P < 0.001$), the number of pods per plant ($P < 0.05$), 100 seed weight ($P < 0.001$) and grain yield ($P < 0.05$) indicating significant diversity for these traits among lines evaluated. On the other hand, scab disease incidence was the only disease parameter that showed significant difference ($P < 0.05$) among the genotypes in Serere, attributable to the extreme drought conditions experienced at the location during the evaluation, thereby suppressing the growth and spread of the fungus compared to Kabanyolo (Table 7.3). The scab fungus has been reported to be more severe under wet conditions (Allen, 1983; Mbong *et al*., 2010b), thus, explaining this observation. The genotypes also showed highly significant differences ($P < 0.001$) in grain yield (Kg/ha) and the days to flowering, significant differences ($P < 0.01$) for yield related traits such as the number of pods per plant and pod length (cm), while the number of pods per peduncle, number of branches and peduncles were shown to be significant at $P < 0.05$. These results implied a significant wide diversity (Noubissie *et al*., 2011) in the yield and yield related traits among the 66 lines evaluated from which potentially high yielding and early maturing lines could be selected in a breeding programme. According to Acquaah (2007), response to selection and thus rapid genetic gain in breeding for a trait is enhanced when there is a wide diversity in the particular trait among the germplasm from which selections are to be made as the two (genetic gain and amount of variation within the population) are directly proportional.

Table 7.2: Analysis of variance for scab disease incidence, severity, yield and yield related traits at different locations

Location	Source	Incidence	Severity[a]	AUDPC	Days to flowering	No. of Pods/ peduncle	No. of branches	No. of Pods/ plant	No. of seeds/ pod	Pod length[a]	No. of peduncles	Seed weight (g)	Yield (Kg/ha)
Kabanyolo	Rep	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
		5188.1***	1.37***	1286.97***	142.24***	0.11	5.11	72.5	51.25*	600.3	80.9	3.49	1331975*
	Block	(28)	-	(29)	(29)	(28)	(28)	(29)	(28)	-	(29)	(29)	(29)
		190*		115.74*	19.96*	0.14	1.96	416.4	14.87		171.4	2.62*	643142**
	Genotype	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)
		356.04**	0.30***	106.09	19.37	0.11	1.33	450.36*	14.07	250.7	132.75	9.37***	554819.98*
	Residual	(102)	(130)	(101)	(101)	(102)	(102)	(101)	(102)	(130)	(101)	(100)	(101)
		197.4	0.1296	82.86	14.29	0.12	1.75	294.6	12.44	246.1	126.3	1.64	317065
	LEE	(100)	-	(103)	(106)	(102)	(104)	(106)	(106)	-	(106)	(104)	(106)
		195.62		88.4	15.25	0.12	1.80	314.94	12.90		134.10	1.80	357948.38
	SED	11.42	0.29	7.67	3.19	0.29	1.09	14.49	2.93	12.81	9.46	1.1	488.5
Serere	Rep	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
		1717.1***	0222*	325.75***	10.24**	1.47***	9.88***	38.67	47.29***	7.26	37.14	1.23	544658***
	Block	(29)		(29)	(30)	(28)	(30)	(30)	(28)	(29)	(30)	(28)	(30)
		343.90*	-	60.29*	4.18***	0.17	1.35***	87.5***	6.82	4.40	38.57***	1.06	73700***
	Genotype	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)
		345.63*	0.07	53.43	3.88***	0.26*	0.95*	63.09**	6.61	6.47**	21.00*	14.07***	42928.90**
	Residual	(101)	(130)	(101)	(100)	(102)	(100)	(100)	(102)	(101)	(100)	(101)	(100)
		215.6	0.07	42.28	1.53	0.17	0.51	28.89	6.02	3.22	11.78	0.91	20116
	LEE	(106)		(105)	(106)	(102)	(105)	(107)	(103)	(103)	(107)	(101)	(106)
		235.13	-	45.28	1.789	0.17	0.59	34.10	6.18	3.42	14.00	0.95	24117.36
	SED	12.52	0.21	5.49	1.09	0.33	0.63	4.77	2.03	1.51	3.06	0.79	126.8

[a] Empty spaces (i.e. no values) for block mean squares and Lattice effective error (LEE) implies blocking was not significant for the traits and therefore were analyzed using RCBD. Figures in brackets represent degrees of freedom; Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

Table 7.3: Mean values of scab disease indexes and Yield of parents and crosses grown at Kabanyolo and Serere in April-July, 2016

		Kabanyolo				Serere			
Genotype	Pedigree	Final Incidence (%)	Final Severity	AUDPC	Yield (Kg/ha)	Final Incidence (%)	Final Severity	AUDPC	Yield (Kg/ha)
SECOW5T	Parent	76.67 (65.00)	2.47	66.27	1429.64	59.67 (54.70)	2.00	42.00	687.04
SECOW5T*Sunshine	Cross	62.67 (52.90)	2.40	59.03	2155.57	53.67 (47.51)	2.00	44.10	731.49
SECOW5T*WC10	Cross	61.33 (55.76)	2.40	55.30	2029.65	90.67 (74.35)	2.00	51.57	638.89
SECOW5T*WC36	Cross	66.67 (55.30)	2.27	57.40	1077.79	80.00 (69.06)	2.00	49.35	435.19
ACC122W	Parent	40.00 (38.41)	2.20	59.03	1837.05	93.33 (78.55)	2.00	50.87	474.08
ACC122W*SECOW5T	Cross	70.00 (60.76)	2.33	49.70	2505.58	83.67 (69.15)	2.00	50.63	566.67
ACC122W*ALEGI	Cross	53.33 (47.53)	2.13	54.60	1025.93	68.33 (56.84)	2.00	49.00	490.75
ACC122W*NE15	Cross	86.67 (74.31)	2.40	60.43	1148.16	95.33 (80.10)	2.00	52.73	490.75
ACC122W*NE48	Cross	60.00 (51.24)	2.20	60.67	1775.94	66.67 (56.12)	2.00	52.03	512.97
ACC122W*NE50	Cross	63.33 (54.22)	2.33	60.43	1450.01	62.67 (53.59)	2.00	49.23	464.82
ACC122W*Sunshine	Cross	53.33 (47.21)	2.07	50.40	1687.05	45.33 (42.60)	2.33	53.90	672.23
ACC122W*WC10	Cross	66.67 (58.84)	2.33	62.53	1814.83	84.33 (69.87)	2.00	51.57	531.49
ACC122W*WC35B	Cross	73.33 (63.08)	2.60	62.53	1735.20	44.67 (42.18)	2.00	49.82	414.82
ACC122W*WC36	Cross	83.33 (68.94)	2.80	72.10	2044.46	74.33 (64.44)	2.00	49.23	427.78
ACC122W*WC66	Cross	96.67 (81.30)	2.80	66.73	1477.79	86.67 (72.82)	2.00	49.00	640.75
ALEGI	Parent	58.00 (51.13)	2.13	59.50	1172.23	64.00 (53.61)	2.00	47.95	435.19
ALEGI*SECOW5T	Cross	70.00 (60.85)	2.60	60.90	1951.87	79.00 (66.34)	2.00	50.40	527.78
ALEGI*NE48	Cross	93.35 (78.55)	2.27	59.03	1475.94	51.33 (46.04)	2.00	52.03	468.52
ALEGI*NE50	Cross	56.67 (49.13)	2.20	53.20	2925.95	95.33 (80.10)	2.00	52.50	392.60
ALEGI*Sunshine	Cross	86.67 (74.31)	2.87	69.07	1088.90	62.67 (52.79)	2.00	47.72	459.26
ALEGI*WC10	Cross	63.33 (56.91)	2.13	56.70	1114.82	79.67 (67.43)	2.00	50.63	546.30
ALEGI*WC36	Cross	57.00 (49.64)	2.33	57.87	2166.68	83.33 (72.38)	2.00	52.85	948.16
NE15	Parent	63.33 (56.91)	2.27	56.70	1485.20	96.67 (81.30)	2.00	50.17	559.26
NE15*SECOW5T	Cross	64.33 (57.45)	2.00	54.83	2316.69	93.33 (78.55)	2.00	53.67	929.64
NE15*ALEGI	Cross	90.33 (76.53)	2.33	61.83	1092.60	93.33 (78.55)	2.00	52.15	729.64
NE15*NE48	Cross	54.33 (47.81)	2.13	57.40	1672.24	71.33 (61.62)	2.00	45.97	429.63
NE15*NE50	Cross	53.33 (47.21)	2.13	57.40	1764.83	83.67 (69.15)	2.00	49.47	679.64
NE15*Sunshine	Cross	41.00 (40.05)	2.07	55.53	1062.97	71.00 (58.54)	2.00	51.10	490.75

Table 7.3: continued.

		Kabanyolo				Serere			
Genotype	Pedigree	Final Incidence (%)	Final Severity	AUDPC	Yield (Kg/ha)	Final Incidence (%)	Final Severity	AUDPC	Yield (Kg/ha)
NE15*WC10	Cross	90.00 (72.04)	2.73	67.90	1242.60	70.00 (60.76)	2.00	47.72	596.30
NE15*WC35B	Cross	76.33 (65.51)	2.40	61.60	2311.13	78.67 (66.15)	2.00	47.95	618.52
NE15*WC36	Cross	75.67 (62.18)	2.60	64.87	1231.49	80.00 (66.93)	2.00	47.02	775.93
NE15*WC66	Cross	50.00 (45.61)	2.27	56.47	1664.83	72.67 (59.13)	2.00	47.95	635.19
NE48	Parent	65.00 (54.23)	2.13	57.40	1785.20	75.00 (61.09)	2.00	48.07	412.97
NE48*SECOW5T	Cross	86.67 (71.69)	3.00	64.63	1655.57	75.00 (63.85)	2.33	56.93	637.04
NE48*NE50	Cross	46.67 (43.36)	2.00	56.00	2051.87	66.67 (58.86)	2.00	43.63	450.00
NE48*Sunshine	Cross	83.33 (72.38)	2.27	61.60	1346.31	80.00 (63.79)	2.00	45.03	666.67
NE48*WC10	Cross	70.00 (60.85)	2.53	66.97	1798.16	96.00 (80.68)	2.00	55.07	614.82
NE48*WC36	Cross	66.67 (55.30)	2.07	56.70	2409.28	52.33 (46.65)	2.00	46.90	687.04
NE50	Parent	86.67 (74.31)	2.73	66.50	966.67	72.00 (59.17)	2.00	48.07	494.45
NE50*SECOW5T	Cross	85.33 (73.53)	2.53	66.03	1761.13	70.00 (57.28)	2.00	49.93	622.23
NE50*Sunshine	Cross	75.33 (61.92)	2.47	63.23	1479.64	67.00 (55.29)	2.00	49.47	464.82
NE50*WC10	Cross	70.00 (60.85)	2.47	65.10	1251.86	63.00 (53.14)	2.00	50.17	405.56
NE50*WC36	Cross	56.00 (48.35)	2.87	67.90	1016.67	80.00 (70.46)	2.00	48.77	401.86
Sunshine	Parent	86.00 (71.07)	2.20	52.27	1270.38	80.00 (66.93)	2.00	53.20	468.52
WC10	Parent	48.33 (44.27)	2.00	56.00	1533.35	58.00 (50.30)	2.00	52.97	396.30
WC10*Sunshine	Cross	80.00 (63.79)	2.53	57.63	1996.31	70.00 (57.28)	2.00	49.00	477.78
WC10*WC36	Cross	56.67 (49.22)	2.20	58.57	2466.69	60.00 (54.91)	2.00	48.53	594.45
WC35B	Parent	76.67 (65.83)	2.87	66.50	1677.79	(71.67 (61.93)	2.33	54.37	761.12
WC35B*SECOW5T	Cross	90.00 (73.92)	2.87	66.50	1920.39	45.67 (42.68)	2.00	47.83	472.23
WC35B*ALEGI	Cross	70.00 (57.31)	2.40	58.80	2064.83	75.33 (64.63)	2.00	50.63	803.71
WC35B*NE48	Cross	65.33 (54.92)	2.20	50.17	1640.75	88.67 (72.66)	2.00	50.63	555.56
WC35B*NE50	Cross	90.33 (76.53)	3.27	77.93	1075.93	64.67 (54.63)	3.00	70.70	414.82
WC35B*Sunshine	Cross	90.00 (76.32)	3.13	73.03	2042.61	48.33 (44.45)	2.00	47.83	705.56
WC35B*WC10	Cross	83.33 (72.38)	2.60	64.63	1238.90	73.33 (62.77)	2.00	51.33	475.93
WC35B*WC36	Cross	76.67 (61.56)	2.40	61.37	2240.76	60.00 (54.91)	2.33	60.67	551.86
WC35B*WC66	Cross	50.00 (45.28)	2.07	56.00	1803.72	68.67 (59.98)	2.00	49.00	375.93
WC36	Parent	60.00 (51.45)	2.27	60.20	2592.61	56.33 (49.69)	2.00	47.13	640.75

Table 7.3: continued.

		Kabanyolo				Serere			
Genotype	Pedigree	Final Incidence (%)	Final Severity	AUDPC	Yield (Kg/ha)	Final Incidence (%)	Final Severity	AUDPC	Yield (Kg/ha)
WC36*Sunshine	Cross	70.00 (60.76)	2.33	59.27	1085.19	80.00 (66.71)	2.00	54.60	546.30
WC66	Parent	60.00 (54.99)	2.27	53.90	1851.87	71.00 (61.32)	2.00	48.30	640.75
WC66*SECOW5T	Cross	66.67 (55.39)	2.27	57.63	1925.94	77.67 (65.75)	2.00	47.37	683.34
WC66*ALEGI	Cross	60.00 (52.28)	2.13	54.83	1935.20	69.00 (60.23)	2.00	53.32	546.30
WC66*NE48	Cross	81.33 (65.06)	2.27	61.13	1364.83	63.67 (53.68)	2.00	47.83	727.78
WC66*NE50	Cross	66.37 (55.39)	2.33	59.50	1287.05	45.67 (42.86)	2.00	44.80	509.26
WC66*Sunshine	Cross	64.33 (54.12)	2.60	63.93	1588.90	88.00 (72.45)	2.00	50.17	729.64
WC66*WC10	Cross	96.67 (81.30)	3.53	80.03	1887.05	46.67 (43.36)	2.33	61.37	551.86
WC66*WC36	Cross	50.00 (45.02)	2.20	57.87	1983.35	71.33 (58.69)	2.00	48.07	661.12
LSD(0.05)		22.60	0.58	15.32	1011.50	25.31	0.42	11.00	294.10
CV (%)		23.50	14.90	15.60	37.30	25.60	12.80	13.50	32.00

Table 7.4: Combined analysis of variance for scab disease incidence, severity, yield and yield related traits across two locations

Source	Degrees of freedom	Incidence	Severity	AUDPC	Days to flowering	No. of Pods/ peduncle	No. of bran-ches	No. of Pods/ plant	No. of seeds/ pod	Pod length	No. of pedun-cles	Seed weight (g)	Yield (Kg/ha)
Location (L)	1	192.8	9.12	7084.26	1040.06	1.23	185.56 *	106857.04 ***	990.39 *	1456.68	24239.44 ***	157.41 **	81589856 **
REP	4	6905.2 ***	1.59 ***	1612.72 ***	152.48 ***	1.58 ***	14.99 ***	111.17	98.54 ***	607.56 ***	118.04	4.73 **	1876633 ***
Genotype (G)	65	215.2 *	0.17 **	67.60 ***	8.86 ***	0.15 ***	0.75 **	191.12 ***	7.92 ***	85.74 **	54.05 ***	13.35 ***	228772 ***
G × L	65	124.8	0.04	19.46	3.33	0.05	0.38	77.26	2.91	42.96	24.5	0.9879	82136
Pooled error	204 -260	215.95	0.1	66.63	8.52	0.14	1.19	173.86	9.54	3.42	73.77	1.378219	191032.9
SED		3.46	0.07	1.92	0.69	0.09	0.26	3.11	0.73	0.44	2.02	0.28	103.02
CV (%)		24.32	14.14	14.70	5.65	17.82	25.13	39.20	22.27	70.51	44.16	8.35	38.85

Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively.

Results of analysis of variance for scab disease incidence, severity, yield and yield related traits for the two locations combined are presented in Table 7.4. The results showed significant differences among the genotypes for all the 12 traits studied. Traits such as the AUDPC, days to flowering, the number of pods per peduncle and per plant, number of peduncles, 100 seed weight and grain yield were shown to be highly significant ($P < 0.001$), scab disease severity, number of branches and pod length were significantly different ($P < 0.01$) suggesting that differences exist among the cowpea genotypes across the locations, indicating high variability. Disease incidence across the locations was also shown to be significantly different ($P < 0.05$). On the other hand, the data showed that there was no significant genotype by location (G × L) interaction effects among the genotypes for any of the 12 traits studied suggesting that the effects of the locations on the genotypes for the traits were negligible. This observation suggested that the genotypes (parents and crosses) performed consistently across the two locations indicating that there would be no need to develop different cultivars for the two locations (Acquaah, 2007).

7.4.2 Genetic variability, heritability and gene action

The results of analysis of variance for combining ability of the 11 parents and resulting crosses for scab disease severity, yield and yield related traits evaluated at the different locations are presented in Table 7.5. At MUARIK, general combining ability of the selected parents showed significant differences for only scab disease severity ($P < 0.05$) and 100 seed weight ($P < 0.001$) while the specific combining ability of the resultant crosses showed highly significant differences ($P < 0.001$) in disease severity and 100 seed weight, and significant differences ($P < 0.05$) for the number of pods per plant and grain yield (Kg/ha), indicating significant diversity among these traits. The results indicated a wide variation in these traits both among the parents and the resultant crosses, thus, suggesting a high potential for selections among both the parents and crosses for improvement in these traits, especially 100 seed weight and grain yield. The coefficient of genetic determination – narrow sense (h^2) were generally low ranging from 0.00 % (number of branches) to 25.12 % (100 seed weight) while the coefficient of genetic determination – broad sense (H^2) ranged between 0.00 % (number of branches) and 81.83 % (100 seed weight).

Table 7.5: Analysis of variance for combining ability studies with variance components and coefficient of genetic determination for scab disease severity, yield and yield related traits at different locations

Location	Source	Severity	Days to flowering	Pods/ peduncle	No. of branches	Pods/ plant	Seeds/ Pod	Pod length	No. of peduncles	Seed weight (g)	Grain yield (Kg/ha)
Kabanyolo	GCA	0.098*	5.796	0.047	0.513	189.8	5.546	88.8	48.51	4.329***	146138
	SCA	0.107***	6.261	0.036	0.496	158.9*	5.142	101.9	51.88	2.47***	195720*
	Residual	0.043	5.085	0.040	0.598	104.980	4.301	82.033	44.700	0.600	119316.130
	σ GCA	0.0061	0.079	0.001	0.000	9.424	0.138	0.752	0.423	0.414	2980.21
	σSCA	0.0638	1.176	0	0.000	53.92	0.841	19.867	7.181	1.87	76403.88
	Baker's ratio	0.16	0.12	1.00	0.00	0.25	0.25	0.07	0.11	0.31	0.07
	CGDNS (%)	10.20	2.5	3.65	0.00	10.6	5.1	1.5	1.6	25.12	3
	CGDBS (%)	63.7	20.78	3.65	0.00	40.93	20.62	20.67	15.23	81.83	40.84
Serere	GCA	0.018	0.836	0.153**	0.201	29.86**	2.65	4.469***	9.995*	8.16***	17257*
	SCA	0.027	1.265***	0.070	0.262	18.86*	2.280	1.755*	6.329	3.887***	14653**
	Residual	0.023	0.596	0.056	0.197	11.370	2.060	1.142	4.667	0.315	8039.120
	σ GCA	0.000	0.027	0.011	0.001	2.055	0.066	0.37	0.592	0.872	1024.21
	σ SCA	0.004	0.669	0.015	0.064	7.493	0.221	0.613	1.662	3.572	6613.88
	Baker's ratio	0.00	0.07	0.59	0.03	0.35	0.37	0.55	0.42	0.33	0.24
	CGDNS (%)	0.00	4.04	23.45	0.69	17.89	5.45	29.64	15.76	30.96	12.26
	CGDBS (%)	14.20	54.77	39.52	25.12	50.51	14.6	54.24	37.89	94.4	51.87

Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

Values of Baker's ratio estimated for all the traits in MUARIK were less than 0.5 (BR < 0.5) suggesting that the traits were under non-additive gene effects implying that the heritability of the traits from the parents were not highly predictable, thus explaining the very low values obtained for the narrow sense heritability.

Evaluation at location two (Serere) showed that the general combining ability of the selected parents were highly significantly different (P < 0.001) for pod length (cm) and 100 seed weight (g) while GCA for traits such as number of pods per peduncle and per plant were significant at P < 0.01 with GCA for the number of peduncles and grain yield being significant at P < 0.05. On the other hand, the specific combining ability of the crosses showed highly significant difference (P < 0.001) for days to flowering and 100 seed weight (g), significant difference (P < 0.01) for grain yield (Kg/ha) while SCA for number of pods per plant and pod length were significant at P < 0.05. These results suggested the performance of parents (GCA) and progenies (SCA) were more variable at Serere than in Kabanyolo suggesting a wider significant diversity among the genotypes which could be attributed to the harsh conditions experienced at Serere. Also, values for CGD narrow sense (h^2) were generally low ranging from 0.00 for scab severity to 30.96 % for 100 seed weight while CGD broad sense (H^2) was between 14.20 % (scab severity) and 94.40 % (100 seed weight. Apart from the number of pods per peduncle and pod length (cm) which had Baker's ratio greater than 0.5, all the remaining traits had BR values < 0.5 suggesting that additive gene action was more important for traits such as number of pods per plant and pod length indicating progress in breeding for these traits could be made through selection of parents with high GCA for the traits (Olweny, 2016).

The results of analysis of variance for combing ability for the two locations combined are presented in Table 7.6. The results showed highly significant differences (P < 0.001) between the two locations for all the traits studied except for the number of pods per peduncle which showed significance at P < 0.01, indicating significant variation between the two locations and thus the environments within which the lines were evaluated. GCA for the combined analysis showed significant differences for only three of the parameters, *viz.* 100 seed weight (P < 0.001), number of pods per peduncle (P < 0.01) and scab severity (P < 0.05). On the other hand, SCA showed significant difference for only two parameters, 100 seed weight (P < 0.001) and scab severity (P < 0.01).

Table 7.6: Combined analysis of variance for general and specific combining ability across two locations for scab disease severity, yield and yield related traits

Source	Df	Severity	Days to flowering	Pods/ peduncle	No. of branches	Pods/ Plant	Seeds/ Pod	Pod length	No. of peduncles	Seed weight (g)	Grain yield (Kg/ha)
Location (L)	1	4.134***	442.33***	0.416**	74.243***	43981.29***	410.225***	682.66***	10033.12***	73.48***	34977717***
GCA	10	0.087*	4.057	0.171**	0.417	152.348	5.628	47.41	39.72	11.69***	87754.1
SCA	44	0.089**	4.033	0.051	0.399	91.806	4.124	51.08	29.09	5.18***	126509.8
GCA*L	10	0.030	2.576	0.029	0.301	67.264	2.568	42.31***	18.79	0.8	75640.8
SCA*L	44	0.045	3.493	0.055	0.359	85.999*	3.297	53.39***	29.12	1.18***	83862.93
LEE	204-260	0.033	2.841	0.048	0.397	57.954	3.180	1.14	24.59	0.46	63677.62
σGCA		0.003	0.082	0.008	0.006	4.727	0.170	0.284	1.163	0.605	672.961
σSCA		0.022	0.270	0.000	0.020	2.903	0.414	0.000	0.000	2.000	21323.432
σGCA*L		0.000	0.000	0.000	0.000	1.034	0.000	4.574	0.000	0.037	1329.242
σSCA*L		0.011	0.652	0.007	0.000	28.046	0.117	52.252	4.531	0.719	20185.309
Baker's ratio		0.23	0.40	1.00	0.40	0.80	0.50	1.00	1.00	0.40	0.10
CGDNS (%)		8.80	4.20	22.16	3.00	9.8	8.40	0.90	7.40	27.12	1.23
CGDBS (%)		39.00	11.06	22.16	7.60	12.30	18.61	0.90	7.40	71.91	20.76

Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

These observations were particularly important as the main thrust of this study was towards identifying promising lines to be selected for improvement in yield and resistance to scab disease. GCA by location interaction (GCA×L) effects were only significant ($P < 0.001$) for pod length while SCA by location interaction (SCA×L) effects were highly significant ($P < 0.001$) for both pod length and 100 seed weight, and, the number of pods per plant showed a significant SCA×L interaction effects at $P < 0.05$ suggesting that the performance of the parents (GCA) and crosses (SCA) were inconsistent in the two environments. This further suggested that for selection of these traits, specific crosses (progenies) will have to be selected for the different environments to derive the benefits in the hybrids (progenies). The combined analysis also showed very low CGD narrow sense (h^2) (1.23 % - 27.12 %) and broad sense (H^2) between 7.4 % (number of peduncles) and 71.91 % (100 seed weight) suggesting that most of these traits studied were not highly predictable. This was confirmed by values of BR estimated for the combined locations which suggested that all the traits had values < 0.5 except for number of seeds per pod (0.50) and number of pods per plant (0.80). The results further suggested that for a trait such as the number of seeds per pod, both additive and non-additive gene action are important while for a trait such as the number of pods per plant, additive gene action was more important than non-additive effects implying that heritability of the trait from the parents is very predictable.

7.4.3 Combining ability effects

Results of the general combining ability effects of the 11 selected parents for scab disease severity, yield and yield related traits in Kabanyolo are shown in Table 7.7. The results showed that none of the parents had significant GCA effects for the number of pods per peduncle and branches. SECOW5T variety showed significant positive GCA effects for days to flowering (DTF) ($P < 0.05$), seeds per pod (SPP) ($P < 0.05$), grain yield ($P < 0.01$) and 100 seed weight ($P < 0.001$) suggesting that the cultivar performed far better in the crosses than predicted for these specific traits and therefore could be selected as a good parent for breeding for high number of seeds per pod, high seed weight and grain yield. This was not surprising as SECOW5T is an improved cultivar released for cultivation in the country. On the other hand, ACC12.2W, Alegi, NE15 and Sunshine showed significant negative GCA effects for 100 seed weight suggesting that these three genotypes would not be good as parents for breeding to increase seed weight, but cultivar WC35B could be selected as it had a significant positive GCA effect for the trait.

Table 7.7: General combining ability (GCA) effects for scab disease severity, yield and yield related traits in Kabanyolo

Parent	Severity	Days to flowering	pods/ peduncle	No. of branches	Pods/ plant	Seeds/ pod	Pod length	No. of peduncles	Seed weight	Grain yield (Kg)
SECOW5T	0.04	1.56*	0.07	0.14	1.00	1.54*	-0.97	2.16	1.47***	261**
ACC12.2W	-0.03	-0.77	-0.03	-0.31	2.59	-0.61	-2.68	1.64	-0.53*	0
ALEGI	-0.10	-0.49	0.11	0.00	4.52	-0.23	2.95	0.94	-0.71**	-58
NE15	-0.14*	1.04	0.07	0.34	7.70*	-0.36	-3.04	4.26*	-0.70**	-125
NE48	-0.15*	-0.20	0.07	0.35	2.79	0.01	-2.11	0.94	0.51*	-23
NE50	0.03	-0.86	-0.03	0.16	0.19	-0.83	3.02	-1.13	0.27	-92
Sunshine	0.05	-0.65	0.03	-0.23	-1.98	-0.07	-1.89	-0.12	-0.60**	-167
WC10	0.13*	0.74	-0.04	-0.11	0.72	-0.88	3.91	-0.92	-0.23	-28
WC35B	0.18**	-0.47	-0.08	-0.06	-5.55	0.41	5.44*	-3.58	0.75**	172
WC36	-0.03	-0.18	-0.10	0.08	-3.87	-0.22	-2.40	-0.91	-0.26	86
WC66	0.02	0.31	-0.07	-0.35	-8.08**	1.24*	-2.19	-3.29	0.03	-26
SE_{GCA}	0.07	0.72	0.06	0.25	3.26	0.66	2.88	2.12	0.25	109.78

Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

Among the parents which had negative GCA effects for scab disease severity, *viz*., ACC12.2W, Alegi, and WC36, only NE15 and NE48 had significant negative effects suggesting that these two parents were good combiners in reducing the severity of scab disease. These results implied that additive gene effects were important for this trait and significant breeding progress could be achieved through effective selection of good parents. Surprisingly, parents such as WC10 (moderate resistant) and WC35B (resistant line) had significant positive GCA effects on scab severity suggesting that they were not good combiners for reducing scab disease severity.

The results of specific combining ability (SCA) effects for the crosses evaluated in MUARIK are presented in Table 7.8. A total of 35 crosses showed negative SCA effects for scab disease severity out of which two (ACC12.2W*SS and WC35B*WC66) showed significant negative effects suggesting that these crosses performed better than predicted and implying that the use of these crosses could result in a significant reduction in the severity of scab disease. On the other hand, eight crosses showed significant positive SCA effects for scab severity, an indication that these crosses were poor performers and could not be selected for resistance to the disease.

Table 7.8: Specific combining ability (SCA) effects for scab disease severity, yield and yield related traits in Kabanyolo

Cross	Severity	Days to flowering	Pods/ peduncle	No. of branches	Pods/ plant	Seeds/ Pod	Pod length	No of peduncles	Seed weight (g)	Grain yield (Kg/ha)
5T*SS	-0.11	-0.11	0.16	-0.74	1.66	-1.66	3.43	-0.64	0.82	291.88
5T*WC10	-0.20	6.67***	-0.10	-0.14	-9.89	-0.06	-2.02	-7.58	0.19	153.50
5T*WC36	-0.18	-2.08	-0.03	0.00	-1.79	-1.49	2.63	-0.52	1.59*	-943.19**
ACC12.2W*5T	-0.10	-1.96	0.23	-0.98	0.20	1.08	3.03	-6.07	0.30	651.40*
ACC12.2W*ALEGI	-0.16	1.41	-0.13	-0.50	-5.14	1.81	-2.77	1.08	-0.84	-460.63
ACC12.2W*NE15	0.15	-2.96	-0.10	-0.23	10.69	0.50	2.97	3.44	-0.23	-329.62
ACC12.2W*NE48	-0.04	-0.34	-0.11	0.42	6.15	-0.02	2.97	10.31	0.64	110.18
ACC12.2W*NE50	-0.10	-0.15	0.00	0.63	-9.36	-1.06	-4.23	-5.56	0.67	58.65
ACC12.2W*SS	-0.38*	-0.52	-0.06	0.12	-4.52	-0.52	2.87	-5.83	-0.40	215.05
ACC12.2W*WC10	-0.19	1.49	-0.01	-0.01	-1.01	-0.14	-4.10	-1.19	0.13	134.07
ACC12.2W*WC35B	0.02	1.87	0.04	0.22	-11.03	1.34	-2.27	-5.40	1.34*	-264.63
ACC12.2W*WC36	0.43*	0.63	0.08	0.12	8.81	-1.36	0.36	10.57	-0.40	182.75
ACC12.2W*WC66	0.38*	0.51	0.06	0.23	5.20	-1.62	1.17	-1.35	-1.20	-297.23
ALEGI*5T	0.23	-1.93	-0.23	-0.98	-16.06	-0.23	-2.49	-10.67	0.75	-17.91
ALEGI*NE48	0.09	0.93	0.43*	0.83	8.24	0.44	-3.11	0.56	-2.36***	-347.68
ALEGI*NE50	-0.16	0.81	-0.16	1.01	17.37*	0.86	42.07***	10.73	-0.65	1274.12***
ALEGI*SS	0.49**	-1.04	-0.22	-1.26*	-18.62*	0.40	-2.68	-8.22	2.73***	-284.91
ALEGI*WC10	-0.32	-1.94	0.22	0.61	7.23	-1.69	-9.54	6.53	0.59	-480.10
ALEGI*WC36	0.03	-1.00	0.24	0.05	10.97	-0.36	-4.26	4.54	0.44	379.13
NE15*5T	-0.33	0.44	0.12	0.74	26.60**	-1.37	1.90	22.25***	0.82	514.47
NE15*ALEGI	0.14	2.50	0.09	0.15	-10.04	0.01	-4.24	-6.36	1.72	-490.08
NE15*NE48	-0.01	4.19*	0.13	-0.16	18.54*	0.80	0.15	2.97	-3.14***	82.94
NE15*NE50	-0.19	-1.14	0.21	0.01	15.56	2.28	-3.17	2.72	-0.65	259.22
NE15*SS	-0.28	0.72	0.15	-0.27	-6.98	-0.77	1.49	-2.43	0.60	-196.09
NE15*WC10	0.31	-2.61	-0.43*	-0.01	-11.83	0.20	-4.22	-2.54	-1.15	-236.82
NE15*WC35B	-0.08	-1.41	-0.07	0.22	-3.56	-0.44	-3.98	0.38	0.21	554.42
NE15*WC36	0.33	3.35	-0.03	0.70	-13.30	0.26	4.13	-7.47	-0.85	-357.81
NE15*WC66	-0.04	-3.08	-0.07	-1.12	-25.67**	-1.46	4.95	-12.96*	2.68***	199.36
NE48*5T	0.68***	1.78	-0.23	-0.34	0.34	-2.74	2.19	-3.37	-0.44	-363.89

Table 7.8: continued.

Cross	Severity	Days to flowering	Pods/ peduncle	No. of branches	Pods/ plant	Seeds/ Pod	Pod length	No of peduncles	Seed weight (g)	Grain yield (Kg/ha)
NE48*NE50	-0.31	0.03	-0.11	-0.70	-12.27	1.25	-1.04	-3.79	3.71***	469.17
NE48*SS	-0.06	0.62	-0.19	0.78	0.42	1.33	3.08	4.78	0.54	-284.01
NE48*WC10	0.12	-1.99	0.20	-0.34	-3.32	-0.66	-2.90	-2.30	0.39	41.83
NE48*WC36	-0.18	-3.62	-0.02	-1.25*	-11.90	2.60	5.21	-5.58	3.27***	644.21*
NE50*5T	0.03	-1.87	-0.10	0.24	-6.07	-3.11	-4.02	-5.55	-2.23***	-206.52
NE50*SS	-0.05	1.45	0.27	0.28	11.11	0.80	-4.29	6.78	-1.72**	-192.99
NE50*WC10	-0.13	-2.86	0.01	0.50	10.96	-0.81	-12.15	5.35	-0.57	-404.25
NE50*WC36	0.43*	2.79	0.09	-1.04	-13.12	-0.69	-5.39	-8.17	-1.05	-624.38*
WC10*SS	-0.08	-4.21*	-0.07	-0.16	2.42	1.81	-3.37	-0.75	-1.07	430.12
WC10*WC36	-0.33	0.33	0.10	0.89	12.18	1.01	-4.50	6.91	-1.16	737.45*
WC35B*5T	0.22	2.10	0.26	0.47	2.93	-1.90	-5.25	2.06	-1.00	-119.88
WC35B*ALEGI	-0.11	-1.52	-0.12	0.26	8.57	0.69	-9.00	4.47	-0.78	320.61
WC35B*NE48	-0.26	-1.25	-0.06	0.58	-7.68	-1.90	-7.91	-2.81	-1.39*	-122.41
WC35B*NE50	0.63***	-1.79	-0.27	-0.28	-16.19	1.85	-6.18	-2.05	2.36***	-477.95
WC35B*SS	0.47*	1.68	-0.05	-0.17	-3.18	-0.07	-2.98	-3.02	0.24	350.72
WC35B*WC10	-0.14	4.81**	0.05	-0.36	5.06	0.08	42.96***	2.48	-0.32	-441.96
WC35B*WC36	-0.19	-1.41	0.12	-0.56	20.54*	0.59	-1.71	2.79	-0.81	209.65
WC35B*WC66	-0.56**	-3.08	0.10	-0.39	4.54	-0.23	-3.69	1.10	0.15	-8.56
WC36*SS	-0.12	-0.91	-0.31	0.67	-9.67	1.10	2.29	-3.81	-0.83	-417.83
WC66*5T	-0.22	-3.05	-0.07	1.74**	2.09	11.49***	0.60	10.10	-0.81	40.13
WC66*ALEGI	-0.22	1.78	-0.12	-0.16	-2.52	-1.93	-3.99	-2.67	-1.60*	107.45
WC66*NE48	-0.03	-0.35	-0.05	0.18	1.48	-1.11	1.35	-0.77	-1.23	-230.32
WC66*NE50	-0.14	2.74	0.05	-0.66	2.02	-1.36	-1.60	-0.45	0.15	-155.07
WC66*SS	0.10	2.32	0.32	0.76	27.37**	-2.40	0.15	13.15*	-0.91	88.06
WC66*WC10	0.95***	0.30	0.03	-0.98	-11.79	0.27	-0.16	-6.90	2.97***	66.17
WC66*WC36	-0.22	1.92	-0.25	0.41	-2.72	-1.65	1.22	0.75	-0.20	190.02
SE_{SCA}	0.19	2.02	0.18	0.69	9.16	1.85	8.10	5.98	0.69	308.95

5T = SECOW 5T; SS= Sunshine; Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

Further, eight crosses had significant positive SCA effects (P < 0.001 to P < 0.05) for 100 seed weight suggesting that these crosses could be selected for the improvement of seed weight. Crosses ACC12.2W*5T, ALEGI*NE50, NE48*WC36 and WC10*WC36 had significant positive SCA effects for grain yield (Kg/ha) which implied that the hybrid effects of these crosses could be harnessed to achieve high grain yields. Among the 55 crosses evaluated in MUARIK, only the cross between WC10*Sunshine showed significant negative SCA effects for days to flowering signifying that this cross could be selected for the purpose of reducing the number of days to flowering in the location.

Results of general combining ability (GCA) effects for scab disease severity, yield and yield related traits evaluated at Serere are shown in Table 7.9.

Table 7.9: General combining ability (GCA) effects for scab disease severity, yield and yield related traits in Serere

Parent	Severity	Days to flowering	pods/ peduncle	No. of branches	Pods/ plant	Seeds/ pod	Pod length	No. of peduncles	Seed weight	Grain yield
SECOW5T	-0.01	-0.04	0.09	0.13	0.08	-0.03	0.59	-0.37	2.13***	36.01
ACC12.2W	-0.01	-0.44	0.05	0.04	-1.51	0.28	0.05	-0.40	-0.29	-56.09*
ALEGI	-0.05	-0.39	0.27***	0.08	2.43*	-1.00*	-1.07**	1.5*	-0.94***	7.61
NE15	-0.05	0.47*	0.05	0.24	3.16**	0.24	-0.26	1.45*	-0.85***	34.31
NE48	-0.01	-0.25	0.09	-0.06	0.85	0.53	0.25	-0.16	0.68***	12.41
NE50	0.06	0.41	0.01	0.02	-2.09*	-0.26	-0.53	-0.99	-0.13	-91.89**
SS	-0.01	0.11	-0.02	0.05	-1.05	-0.18	0.08	-0.21	-0.19	16.71
WC10	-0.01	0.14	-0.17*	0.03	-0.25	-0.84*	-0.24	-0.63	-0.79***	-6.99
WC35B	0.10*	-0.07	-0.17*	-0.33*	-2.60*	0.74	1.50***	-1.85**	1.16***	-34.39
WC36	-0.01	-0.18	-0.09	-0.18	1.35	0.10	-0.77*	1.16	-0.23	41.11
WC66	-0.01	0.25	-0.10	-0.02	-0.33	0.41	0.41	0.50	-0.55**	41.21
SE_{GCA}	0.05	0.25	0.07	0.14	1.07	0.46	0.34	0.69	0.18	28.50

Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

Of the 11 parents, nine were shown to have negative GCA effects for scab disease severity suggesting that these nine parents were good combiners for reducing scab severity in Serere and could therefore be selected for breeding for resistance to the disease. For 100 seed weight, four of the parents were shown to have positive GCA effects out of which three (SECOW5T, NE48 and WC35B) were highly significant (P < 0.001) positive effects indicating that these parents were good combiners and could therefore be selected for the purpose of breeding for increased seed weight. For grain yield (Kg/ha), two parents, ACC12.2W (P < 0.05) and NE50 (P < 0.01) had

significant negative GCA effects suggesting that they were generally bad combiners for improvement of yield in Serere.

The results of specific combining ability (SCA) effects of the 55 crosses evaluated in Serere for scab disease severity, yield and yield related traits are presented in Table 7.10. Out of the 55 crosses, 35 were shown to have negative SCA effects for scab disease severity implying that these crosses could be selected among possible progenies for improvement of resistance to scab disease. 16 out of the 55 crosses showed significant ($P < 0.001$ to $P < 0.05$) positive SCA effects for 100 seed weight suggesting that there was high potential for achieving significant gains in selecting among these crosses in Serere for improvement of seed weight. On the other hand, five crosses (ACC12.2W*SS, Alegi*WC36, NE15*SECOW5T, WC35B*Alegi and WC66*NE48) had significant ($P < 0.001$ to $P < 0.05$) positive SCA effects for grain yield indicating high chances of improving the grain yield of cowpea when these crosses are selected for the location (Olweny, 2016). The results also indicted possibilities of selecting progenies when the target is to reduce maturity period in the location as four of the progenies (ACC12.2W*NE50, NE48*5T, WC10*WC36 and WC35B*SS) showed significant negative SCA effects for days to flowering.

Table 7.10: Specific combining ability (SCA) effects for scab disease severity, yield and yield related traits in Serere

Cross	Severity	Days to flowering	Pods/ peduncle	No. of branches	Pods/ Plant	Seeds/ Pod	Pod length	No. of peduncles	Seed weight (g)	Grain yield (Kg/ha)
5T*SS	-0.02	0.64	-0.25	-0.63	-2.45	0.89	-1.51	0.27	2.60***	28.28
5T*WC10	-0.02	1.79**	-0.11	0.01	-3.07	0.46	1.81	-0.30	1.22*	18.46
5T*WC36	-0.02	0.36	-0.18	-0.48	-5.27	-1.77	0.64	-2.76	0.30	-217.38**
ACC12.2W*5T	-0.02	0.45	0.00	0.68	0.47	0.52	-0.27	-0.28	-1.59**	8.07
ACC12.2W*ALEGI	0.02	0.04	-0.18	-0.26	-4.21	1.67	0.99	-4.25*	0.81	-36.22
ACC12.2W*NE15	0.02	-2.81	-0.30	-0.41	-4.57	-0.13	1.52	-2.53	-0.02	-126.14
ACC12.2W*NE48	-0.03	-0.12	0.34	-0.10	-0.45	2.10	1.01	0.80	1.11*	31.92
ACC12.2W*NE50	-0.10	-1.33*	0.43*	0.44	1.07	-1.71	-1.68	0.47	-1.05*	88.97
ACC12.2W*SS	0.31*	0.03	-0.22	-0.22	5.6*	-1.82	-0.74	3.44	1.40**	179.51*
ACC12.2W*WC10	-0.01	0.00	-0.06	0.06	4.42	-0.05	0.14	2.15	0.57	10.87
ACC12.2W*WC35B	-0.14	-0.12	0.27	-0.02	-1.00	0.05	0.61	-0.52	1.01*	-84.87
ACC12.2W*WC36	-0.02	2.17**	-0.14	-0.46	-3.47	0.28	-0.96	-2.75	-0.58	-124.92
ACC12.2W*WC66	-0.02	1.69*	-0.14	0.29	2.07	-0.91	-0.61	3.47	-1.66***	52.83
ALEGI*5T	0.02	-0.89	-0.22	-0.25	-0.03	0.43	0.26	1.41	-0.96*	-103.08
ALEGI*NE48	0.01	-0.83	-0.22	0.00	-3.20	-0.33	0.62	-0.14	-1.23*	-203.49*
ALEGI*NE50	-0.06	0.91	-0.48*	-0.55	-2.50	0.19	-0.86	-0.27	0.58	-29.02
ALEGI*SS	0.01	-0.66	0.24	-0.21	-1.90	-2.05	-0.97	-1.28	-0.74	-169.39*
ALEGI*WC10	0.02	1.20	0.38	0.62	-0.10	-0.16	0.49	-1.76	0.67	-17.15
ALEGI*WC36	0.01	-0.20	0.30	0.30	8.66**	-0.03	-0.81	3.99*	0.56	322.46***
NE15*5T	0.01	-0.38	0.02	0.50	6.81*	0.22	0.05	0.59	-0.42	287.00***
NE15*ALEGI	0.05	1.08	0.49*	0.25	1.07	0.14	-0.05	-0.19	2.03***	93.48
NE15*NE48	0.02	0.29	-0.33	-0.13	-7.34*	-0.14	-1.86*	-2.57	-2.25***	-153.12*
NE15*NE50	-0.06	0.97	-0.26	1.29**	1.02	-0.59	0.12	0.00	-0.84	77.74
NE15*SS	0.02	2.21***	0.12	-0.15	-6.40*	-0.17	-0.51	-1.91	-1.41**	-114.01
NE15*WC10	0.01	-1.00	-0.07	-1.01*	1.27	-1.46	-1.67	1.67	0.48	-51.09
NE15*WC35B	-0.10	0.70	-0.07	-0.44	7.08*	1.69	1.37	-0.77	-0.74	8.73
NE15*WC36	0.02	-0.22	-0.14	0.31	3.91	1.78	1.37	6.99***	0.96*	71.43
NE15*WC66	0.02	-0.84	0.52*	-0.22	-2.85	-1.32	-0.35	-1.28	2.21***	-94.03
NE48*5T	0.31*	-1.79*	0.64**	-0.11	1.60	-1.19	-0.76	1.16	-0.27	-25.89

Table 7.10: continued.

Cross	Severity	Days to flowering	Pods/ peduncle	No. of branches	Pods/ Plant	Seeds/ Pod	Pod length	No. of peduncles	Seed weight (g)	Grain yield (Kg/ha)
NE48*NE50	-0.09	-0.09	0.05	-0.08	-0.32	-0.52	1.81*	-0.09	2.71***	40.96
NE48*SS	-0.02	0.56	-0.26	0.53	3.22	2.53*	2.09*	-2.60	1.01*	-19.75
NE48*WC10	-0.02	1.10	-0.11	0.24	6.00*	-1.10	-1.69	0.41	-1.69***	19.75
NE48*WC36	-0.02	-0.22	-0.18	-0.28	-6.74*	2.01	2.10*	-1.97	5.44***	119.02
NE50*5T	-0.10	0.15	0.04	0.17	3.02	-1.72	-1.58	0.62	-1.42**	74.82
NE50*SS	-0.10	-0.48	0.15	-1.02*	-0.27	0.51	0.39	1.46	-0.15	9.85
NE50*WC10	-0.10	-0.98	-0.03	-0.43	-2.07	-0.26	-0.44	0.03	-0.16	-31.12
NE50*WC36	-0.10	-0.39	0.23	0.53	0.70	0.84	-0.45	-0.52	-1.09*	-129.00
WC10*SS	-0.02	0.78	0.01	0.34	2.28	-1.33	0.21	2.60	-1.79***	-0.54
WC10*WC36	-0.02	-1.80**	0.08	-0.18	-3.53	0.34	-0.47	-1.76	-0.75	47.80
WC35B*5T	-0.13	-0.49	-0.11	0.08	-3.91	2.07	2.46**	-1.43	1.61**	-89.85
WC35B*ALEGI	-0.09	-0.91	0.05	0.30	6.38*	-2.00	-0.06	5.49**	-0.40	194.40**
WC35B*NE48	-0.13	1.42*	-0.11	-0.31	5.39	-0.67	-1.70	3.18	-4.30***	-22.43
WC35B*NE50	0.79***	0.94	-0.03	-0.88*	-2.35	0.28	0.89	-2.20	2.51***	-37.06
WC35B*SS	-0.13	-2.01**	0.01	1.10**	-5.10	0.54	-0.15	-3.09	2.54***	82.85
WC35B*WC10	-0.13	-0.04	0.16	0.46	-0.13	1.37	-1.03	1.30	-2.77***	56.22
WC35B*WC36	0.20	0.00	-0.25	0.30	-2.98	-2.49*	-0.91	-1.49	-1.76***	-14.86
WC35B*WC66	-0.13	0.51	0.08	-0.58	-3.40	-0.84	-1.47	-0.47	2.31***	-93.14
WC36*SS	-0.02	-0.03	0.27	0.12	3.32	0.82	0.19	-0.60	-1.73***	-72.19
WC66*5T	-0.02	0.17	0.16	0.03	2.84	0.09	-1.11	0.72	-1.06*	19.58
WC66*ALEGI	0.02	0.26	-0.36	-0.19	-4.17	2.15	0.40	-2.99	-1.32**	-51.97
WC66*NE48	-0.02	-0.32	0.16	0.24	1.84	-2.68*	-1.60	1.81	-0.52	213.02**
WC66*NE50	-0.10	0.28	-0.11	0.54	1.70	3.00*	1.81*	0.51	-1.09*	-66.14
WC66*SS	-0.02	-1.05	-0.07	0.14	1.63	0.09	0.99	1.71	-1.74***	75.39
WC66*WC10	0.31*	-1.03	-0.25	-0.10	-5.08	2.20	2.64**	-4.36*	4.23***	-53.20
WC66*WC36	-0.03	0.33	0.01	-0.16	5.41	-1.78	-0.70	0.88	-1.35**	-2.35
SE_{SCA}	0.14	0.69	0.21	0.40	3.02	1.28	0.96	1.93	0.50	80.20

5T = SECOW 5T; SS= Sunshine; Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

The results of general combining ability (GCA) effects for scab disease severity, yield and yield related traits for the two locations combined (Table 7.11) indicated that out of the 11 parents, five had negative GCA effects for scab disease severity of which three (Alegi, NE15 and NE48) were significant ($P < 0.05$) negative effects, suggesting that these three parents were good combiners and could be selected and used as parents in breeding for resistance to scab for across the two locations. For 100 seed weight, the combined analysis showed that four of the parents had positive GCA effects, out of which three (SECOW5T, NE48 and WC35B) had highly significant ($P < 0.001$) positive GCA effects suggesting that genes with additive effects were important and therefore, high chances of improving the seed weight of cowpea when these parents are selected. Of the 11 parents, four had positive GCA effects for grain yield (Kg/ha) of which only one (SECOW5T) had a significant ($P < 0.01$) positive GCA effect on yield. The desirable GCA effect associated with SECOW5T for high yield indicated that it combines well for increased grain yield and that genes with additive effects were more important for the trait. For pod length, four (Alegi, NE50, WC10 and WC35B) out of the 11 parents showed highly significant ($P < 0.001$) positive GCA effects suggesting that they were good combiners and could be good parents for increasing pod length of cowpea which could lead to increased yields as pod length has been found to be highly correlated with grain yield in cowpea (Afutu *et al.*, 2016a).

Table 7.11: General combining ability (GCA) effects for scab disease severity, yield and yield related traits for two locations combined

Parent	Severity	Days to flowering	pods/ peduncle	No. of branches	Pods/ plant	Seeds/ pod	Pod length	No. of peduncles	Seed weight	Grain yield
SECOW5T	0.01	0.76*	0.079	0.13	0.53	0.76*	-0.14	0.90	1.80***	148.46**
ACC12.2W	-0.02	-0.61	0.009	-0.14	0.54	-0.16	-1.28***	0.62	-0.41**	-28.04
ALEGI	-0.07*	-0.44	0.19***	0.04	3.47*	-0.61	0.85***	1.22	-0.83***	-25.14
NE15	-0.09*	0.75*	0.057	0.29*	5.43**	-0.06	-1.64***	2.86**	-0.77***	-45.44
NE48	-0.08*	-0.22	0.080	0.14	1.81	0.27	-0.93***	0.40	0.59***	-5.34
NE50	0.05	-0.23	-0.006	0.09	-0.96	-0.55	1.23***	-1.06	0.07	-91.84
SS	0.02	-0.27	0.004	-0.09	-1.52	-0.13	-0.98***	-0.16	-0.39**	-75.04
WC10	0.06	0.44	-0.11*	-0.04	0.23	-0.86*	1.83***	-0.77	-0.51***	-17.44
WC35B	0.14***	-0.27	-0.12*	-0.19	-4.08*	0.58	3.40***	-2.71**	0.95***	68.86
WC36	-0.02	-0.18	-0.10*	-0.05	-1.27	-0.06	-1.52***	0.13	-0.25	63.46
WC66	0.01	0.28	-0.083	-0.18	-4.21**	0.83*	-0.83***	-1.39	-0.26	7.46
SE_{GCA}	0.04	0.38	0.049	0.14	1.71	0.40	0.24	1.11	0.15	56.71

Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

The results of specific combining ability effects for scab disease severity, yield and yield related traits for the two locations combined are presented in Table 7.12. Only one cross, WC35B*WC66, had significant ($P < 0.01$) negative effect for scab severity suggesting that the use of this hybrid (cross) could result in improved resistance to scab disease for the two locations. This was not surprising as the cross was from two parents both rated resistant to scab (Afutu *et al*., 2016a) suggesting that additive effects were important for scab disease resistance under the environments evaluated (Olweny, 2016). Six crosses, *viz*., Alegi*NE50 ($P < 0.001$), ACC12.2W*SECOW5T, Alegi*WC36, NE15*SECOW5T, NE48*WC36 and WC10*WC36 ($P < 0.05$) showed significant positive SCA effects for seed weight and grain yield suggesting high potential of selecting from among these lines for increased seed (100 seed weight) and grain yield across the two locations. Similarly, the combined analysis of the two locations showed that crosses such as ACC12.2W*NE15 and NE15*WC66 could be selected towards breeding for early maturity as these two hybrids had significant negative SCA effects for days to flowering across the two locations.

7.4.4 Interrelationships among disease indexes, yield and yield related traits for Crosses (hybrids)

The results of correlation analysis for scab disease parameters, grain yield and yield related traits of the 55 crosses for the two locations combined are presented in Table 7.13. The results indicated that scab disease incidence had highly significant ($P < 0.001$) positive correlations with disease severity (0.39) and AUDPC (0.41) suggesting that the scores of the two disease indexes increased with disease incidence. Similar results were reported by Afutu *et al*. (2016a) in which scab disease incidence was shown to be significantly positively correlated with AUDPC (0.80, $P < 0.001$). Burdon (1987) suggested that as long as there was fresh new leaf tissues to be infected, the severity of polycyclic diseases such as scab, would increase as the incidence increases. Further, the incidence of scab disease was reported to increase with plant age (Mbong *et al.*, 2010b). On the other hand, as expected, scab disease incidence showed significant ($P < 0.05$) negative correlations with 100 seed weight (g) (-0.10) and grain yield (Kg/ha) (-0.11) as disease severity was also shown to be significantly ($P < 0.001$) negatively correlated with 100 seed weight (-0.24) and grain yield (-0.30) indicating that as the incidence and severity of scab increased, the 100 seed weight (g) and grain yields (Kg/ha) decreased significantly.

Table 7.12: Specific combining ability (SCA) effects for scab disease severity, yield and yield related traits for two locations combined

Genotype	Severity	Days to flowering	Pods/ peduncle	No. of branches	Pods/ plant	Seeds/ Pod	Pod length	No. of peduncles	Seed weight (g)	Grain yield (Kg/ha)
5T*SS	-0.07	0.26	-0.69	-0.40	-0.04	-0.39	0.96	-0.19	1.71***	160.08
5T*WC10	-0.11	4.23***	-0.07	-6.48	-0.11	0.20	-0.10	-3.94	0.70	85.98
5T*WC36	-0.10	-0.86	-0.24	-3.53	-0.10	-1.63	1.64*	-1.64	0.94*	-580.29***
ACC12.2W*5T	-0.06	-0.75	-0.15	0.34	0.11	0.80	1.38*	-3.17	-0.65	329.74*
ACC12.2W*ALEGI	-0.07	0.73	-0.38	-4.68	-0.15	1.74	-0.89	-1.59	-0.01	-248.43
ACC12.2W*NE15	0.08	-2.88**	-0.32	3.06	-0.20	0.18	2.25***	0.46	-0.13	-227.88
ACC12.2W*NE48	-0.03	-0.23	0.16	2.85	0.12	1.04	1.99**	5.56	0.87*	71.05
ACC12.2W*NE50	-0.10	-0.74	0.53	-4.15	0.21	-1.39	-2.95***	-2.55	-0.19	73.81
ACC12.2W*SS	-0.03	-0.24	-0.05	0.58	-0.14	-1.17	1.07	-1.20	0.50	197.28
ACC12.2W*WC10	-0.10	0.74	0.02	1.71	-0.03	-0.09	-1.98**	0.48	0.35	72.46
ACC12.2W*WC35B	-0.06	0.87	0.10	-6.01	0.15	0.69	-0.83	-2.96	1.17**	-174.75
ACC12.2W*WC36	0.20	1.40	-0.17	2.67	-0.03	-0.54	-0.30	3.91	-0.49	28.91
ACC12.2W*WC66	0.18	1.10	0.26	3.64	-0.04	-1.26	0.28	1.06	-1.43***	-122.20
ALEGI*5T	0.12	-1.41	-0.61	-8.05	-0.22	0.10	-1.12	-4.63	-0.10	-60.50
ALEGI*NE48	0.05	0.05	0.42	2.52	0.10	0.06	-1.25	0.21	-1.80***	-275.59
ALEGI*NE50	-0.11	0.86	0.23	7.43	-0.32*	0.52	20.61***	5.23	-0.04	622.55***
ALEGI*SS	0.25*	-0.85	-0.74*	-10.26*	0.01	-0.83	-1.83**	-4.75	1.00*	-227.15
ALEGI*WC10	-0.15	-0.37	0.61	3.56	0.30*	-0.93	-4.52***	2.39	0.63	-248.63
ALEGI*WC36	0.02	-0.60	0.17	9.81*	0.27*	-0.19	-2.53***	4.26	0.50	350.79*
NE15*5T	-0.16	0.03	0.62	16.70***	0.07	-0.58	0.98	11.42***	0.20	400.74*
NE15*ALEGI	0.10	1.79	0.20	-4.48	0.29*	0.07	-2.14***	-3.28	1.88***	-198.30
NE15*NE48	0.00	2.24*	-0.15	5.60	-0.10	0.33	-0.86	0.20	-2.69***	-35.09
NE15*NE50	-0.13	-0.08	0.65	8.29	-0.02	0.84	-1.53*	1.36	-0.75	168.48
NE15*SS	-0.13	1.46	-0.21	-6.69	0.13	-0.47	0.49	-2.17	-0.41	-155.05
NE15*WC10	0.16	-1.81	-0.51	-5.28	-0.25	-0.63	-2.95***	-0.43	-0.34	-143.95
NE15*WC35B	-0.09	-0.36	-0.11	1.76	-0.07	0.63	-1.30*	-0.20	-0.27	281.58
NE15*WC36	0.17	1.56	0.50	-4.69	-0.08	1.02	2.75***	-0.24	0.06	-143.19
NE15*WC66	-0.01	-1.96*	-0.67	-14.26**	0.22	-1.39	2.30***	-7.12*	2.44***	52.66
NE48*5T	0.50***	0.00	-0.22	0.97	0.21	-1.97	0.71	-1.11	-0.36	-194.89

Table 7.12: continued

Genotype	Severity	Days to flowering	Pods/ peduncle	No. of branches	Pods/ plant	Seeds/ Pod	Pod length	No. of peduncles	Seed weight (g)	Grain yield (Kg/ha)
NE48*NE50	-0.20	-0.03	-0.39	-6.30	-0.03	0.36	0.38	-1.94	3.21***	255.06
NE48*SS	-0.04	0.59	0.66	1.82	-0.22	1.93	2.59***	1.09	0.78	-151.88
NE48*WC10	0.05	-0.44	-0.05	1.34	0.04	-0.88	-2.29***	-0.95	-0.65	30.79
NE48*WC36	-0.10	-1.92	-0.76*	-9.32*	-0.10	2.30*	3.66***	-3.78	4.35***	381.61*
NE50*5T	-0.03	-0.86	0.21	-1.53	-0.03	-2.42	-2.80***	-2.47	-1.83	-65.85
NE50*SS	-0.07	0.49	-0.37	5.42	0.21	0.66	-1.95**	4.12	-0.94*	-91.57
NE50*WC10	-0.11	-1.92	0.04	4.45	-0.01	-0.54	-6.29***	2.69	-0.37	-217.65
NE50*WC36	0.17	1.20	-0.26	-6.21	0.16	0.07	-2.92***	-4.35	-1.07**	-376.69*
WC10*SS	-0.05	-1.72	0.09	2.35	-0.03	0.24	-1.58*	0.93	-1.43***	214.79
WC10*WC36	-0.18	-0.74	0.35	4.33	0.09	0.68	-2.48***	2.57	-0.96*	392.63*
WC35B*5T	0.04	0.80	0.27	-0.49	0.07	0.08	-1.39	0.31	0.31	-104.86
WC35B*ALEGI	-0.10	-1.21	0.28	7.48	-0.04	-0.66	-4.53*	4.98	-0.59	257.51
WC35B*NE48	-0.20	0.09	0.14	-1.14	-0.08	-1.29	-4.81***	0.19	-2.84***	-72.42
WC35B*NE50	0.71***	-0.42	-0.58	-9.27*	-0.15	1.06	-2.65***	-2.12	2.43***	-257.50
WC35B*SS	0.17	-0.16	0.47	-4.14	-0.02	0.24	-1.56*	-3.05	1.39***	216.78
WC35B*WC10	-0.13	2.38*	0.05	2.47	0.11	0.72	20.96***	1.89	-1.54***	-192.87
WC35B*WC36	0.01	-0.70	-0.13	8.78*	-0.07	-0.95	-1.31*	0.65	-1.28**	97.39
WC35B*WC66	-0.35**	-1.28	-0.49	0.57	0.09	-0.53	-2.58***	0.31	1.23**	-50.85
WC36*SS	-0.07	-0.47	0.40	-3.18	-0.02	0.96	1.24	-2.21	-1.28**	-245.01
WC66*5T	-0.12	-1.44	0.88*	2.47	0.04	5.79***	-0.26	5.41	-0.93*	29.85
WC66*ALEGI	-0.10	1.02	-0.17	-3.35	-0.24	0.11	-1.79**	-2.83	-1.46***	27.74
WC66*NE48	-0.03	-0.33	0.21	1.66	0.05	-1.89	-0.12	0.52	-0.88*	-8.65
WC66*NE50	-0.12	1.51	-0.06	1.86	-0.03	0.82	0.10	0.03	-0.47	-110.60
WC66*SS	0.04	0.63	0.45	14.50**	0.13	-1.16	0.57	7.43*	-1.33**	81.73
WC66*WC10	0.63***	-0.36	-0.54	-8.44	-0.11	1.24	1.24	-5.63	3.60***	6.49
WC66*WC36	-0.12	1.13	0.13	1.35	-0.12	-1.72	0.26	0.82	-0.78	93.84
SE_{SCA}	0.12	1.07	0.40	4.81	0.14	1.13	0.68	3.14	0.43	159.60

5T = SECOW 5T; SS= Sunshine; Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively

Table 7.13: Correlation of disease parameters, yield and yield related traits of crosses (without parents) for two locations combined

Trait	Disease incidence	Severity	AUDPC	Days to flowering	Pods/ peduncle	No. of branches	Pods/ plant	Seeds/ pod	Pod length	No. of peduncles	Seed weight (g)	Yield (kg/ha)
Disease incidence	-											
Severity	0.390***	-										
AUDPC	0.409***	0.881***	-									
Days to flowering	0.187***	0.402***	0.417***	-								
Pods/ peduncle	0.007	-0.103*	-0.094*	-0.046	-							
No. of branches	-0.008	0.170***	0.221***	0.250***	-0.022	-						
Pods/ plant	-0.020	-0.272***	0.331***	0.402***	0.051	0.709***	-					
Seeds/ pod	-0.081	-0.202***	0.223***	0.291***	-0.099*	0.301***	0.325***	-				
Pod length	0.047	0.152**	0.133**	0.185***	-0.060	0.059	0.172**	0.132**	-			
No. of peduncles	-0.038	0.214***	0.266***	0.318***	-0.036	0.758***	0.919***	0.349***	0.159**	-		
Seed weight (g)	-0.087*	-0.241***	-0.185***	0.142**	-0.099*	0.130**	0.172**	0.257***	0.133**	0.174**	-	
Yield (kg/ha)	-0.105*	-0.250***	-0.263***	-0.308***	-0.048	0.488***	0.697***	0.442***	0.206***	0.651***	0.299***	-

Values with *, ** and *** implies significant at $P = .05$, $P < .01$ and $P < .001$ respectively. (N = 55 genotypes evaluated at two locations)

Scab has significant negative effects on leaf area and reproductive parts of cowpea plants and this explains the significant negative correlations of scab severity with other traits such as the number of pods per peduncle (-0.10, $P < 0.05$), number of pods per plant (-0.30, $P < 0.001$) and number of seeds per pod (-0.20, $P < 0.001$). Yield related traits such as the number of branches (0.50), number of pods per plant (0.70), number of seeds per pod (0.44), pod length (0.21), number of peduncles (0.70) and 100 seed weight (0.30) were all shown to have highly significant ($P < 0.001$) positive correlations with grain yield (Kg/ha) implying that grain yield (Kg/ha) of theses hybrids (crosses) was directly related to these traits and therefore, suggests that any factor that affects them would affect the grain yield of cowpea (Mbong *et al*., 2012).

7.5 Conclusion

The study identified Alegi, NE15 and NE48 as parental lines with negative GCA effects for improvement of scab disease resistance for both locations while SECOW5T was identified as the only parent with high positive GCA for improvement of both 100 seed weight (g) and grain yield (K/ha) of cowpea across the two locations. Cross WC35B*WC66 had the best SCA effects for reduction of scab disease severity. The study confirmed earlier reports that additive gene effects were important for scab disease resistance. Six crosses, *viz.* Alegi*NE50, Alegi*WC36, ACC12.2W*SECOW5T, NE15*SECOW5T, NE48*WC36 and WC10*WC36 were identified as the best hybrids (crosses) for the improvement of cowpea yields across the locations.

CHAPTER EIGHT

8.0 GENERAL DISCUSSION, CONCLUSSIONS AND RECOMMENDATIONS

8.1 General discussion

There is currently no improved cowpea cultivar which is resistant to the cowpea scab disease in Uganda, yet, the disease which attacks all the above ground parts of the crop has capacity to cause up to 100 % yield loss. Five improved cowpea varieties (SECOWS 1T, 2W, 3B, 4W and 5T) were recently released by the National Semi-Arid Resources Research Institute (NaSARRI, Serere) for cultivation in Uganda but only one (SECOW 3B) is moderately resistant to the scab disease. Meanwhile, there is currently a resurgence of the disease in the country leading to significant yield losses in farmers' fields. Disease management approaches suggested such as timing of planting, regular spraying of fungicides, and so on, have not been effective as cowpea production is mostly by small-scale and resource poor farmers who cannot afford these management practices suggested. Thus, due to the cost of buying and applying chemicals and the inability of farmers to plan and effectively follow recommendations concerning the timing of planting in order to escape the peak of disease infection, disease management approaches suggested have not been effective. The use of resistant cultivars in disease management is the most practical approach, easily adopted and more environmentally friendly. To develop resistant cowpea cultivars to manage the disease in the country, breeders require a better understanding of the occurrence and distribution of the disease in the country; screening to identify new sources of resistant genes; understanding the variability of the causal pathogen (*Sphaceloma* sp.) and a better understanding of the gene action conditioning resistance to scab, yield and yield related traits in the crop plant. These were therefore, the main thrusts of the studies conducted.

In the first chapter of results (Chapter 3), the study which was centered on determining the occurrence and distribution of scab disease in Uganda, led to surveying some of the major cowpea growing districts in the country across three agro-ecological zones and collection of diseased materials for isolation of the pathogen for use in the subsequent studies. Results of field surveys across a total of 17 cowpea growing districts over a period of two years revealed that Amuria (incidence = 92 %) and Tororo (incidence = 77 %) were hot spots of scab disease in the country with both districts recording severity score of 4 out of a scale of 1-5. Altitude (> 1200 m.a.s.l),

cropping system (intercropped), previous crop grown (legume/cassava) and the cultivar grown significantly affected the incidence and severity of scab (Afutu *et al*., 2017a).

Close to a decade ago, NaSARRI, Serere, a National Agricultural Research Organization in charge of dry land crops such as sorghum, sesame, groundnuts and cowpea, embarked on a nation-wide germplasm collection of cowpea landraces and local varieties under cultivation by farmers in Uganda. The exercise resulted in the assembling of a large collection of cowpea germplasm with a wide diversity which has still not been tapped. In earlier studies to identify sources of resistance, it was observed that local lines in Uganda were less infected by scab than plant introductions (Tumwegamire *et al*., 1998). Therefore, the results of the second study (Chapter 4), that is, identification of sources of resistance to scab disease and high yield potential were obtained following field evaluations of one hundred (100) cowpea lines, all from among the local collections and improved cultivars. Following field evaluations at two locations (Makerere University Agricultural Research Institute - Kabanyolo and NaSARRI - Serere), analysis of variance, coefficients of variations and mean separations showed a variation among the 100 lines for scab disease severity, yield and yield related traits. A highly significant ($P < 0.001$) genotype by location (G×L) interaction effects on AUDPC, days to 50% flowering and 100 seed weight was observed suggesting inconsistent performance in the two locations. This suggested that for the purpose of breeding, different cultivars have to be developed for the different locations (Acquaah, 2007). Cluster analysis based on only scab disease indexes (incidence, severity, Apparent infection rate and AUDPC) grouped the 100 lines into four main clusters while cluster analysis based on disease and yield traits (13 traits) produced 3 main clusters but similar grouping patterns in which cowpea lines with similar resistance ratings were shown to form unique clusters. 11 lines were found to have shown a consistent reaction to the scab disease at both locations. These were Secow 3B, NE 4, NE 20, NE 32, NE 49, WC 5, WC 7, WC 16, WC 62, and WC 67B which were moderately resistant at both locations and NE 15 which was rated as resistant at both locations. The stability in both locations suggested that these 11 lines could serve as good parents for resistance breeding to scab disease (Afutu *et al*., 2016a).

The correct characterization of genotypes or isolates of a pathogen are fundamentally important for crop genetic improvement programmes (Sudre *et al*., 2010; Moulin *et al*., 2012). Therefore, the results of the third study (Chapter 5) were obtained in a quest to identify and characterize the

causal pathogen of the scab disease occurring in Uganda. Thus, following the field surveys conducted in the major cowpea growing districts of the country (Study one; Chapter three) (Afutu *et al*., 2017a), the infected plant materials collected from farmers' fields were plated on potato dextrose agar for isolation and characterization of the pathogen. A total of 495 *Sphaceloma* sp. pure fungal isolates consisting of 419 from infected leaves and 76 from infected pods were obtained following isolation and culture. This chapter (five) focused on characterization of the pure fungal cultures using three approaches, *viz*. (1) variability in morphological features such as colour of colony, coulour of the under-side of culture when petri dishes are inverted, amount of mycelia produced, depth of mycelial growth in media, features of conidia and number of septations; (2) variability in radial growth rate (mm/day) among the isolates; and (3) variation in pathogenicity and virulence of some selected isolates on 20 promising resistant and high yielding cowpea lines identified from the second study (Chapter four) (Afutu *et al*., 2016a). Based on the first approach, the results showed a wide variation in the isolates and six morphological groups were identified. From the second approach, the selected isolates were put into three pathogenicity groups based on the mean incidence, severity, AUDPC and pathogenicity on the 20 promising cowpea genotypes. The third approach confirmed one of the characteristic features of the genus of the cowpea scab fungus (*Sphaceloma*) and its related genus *Elsinoe* as slow growing (Zeigler and Lozano, 1983; Timmer *et al*., 1996) as most of the Ugandan isolates were slow growing (> 14 days to cover the entire surface of a 90 mm diameter petri dish). Among the 20 cowpea genotypes challenged, NE 31 and NE 70 had the widest horizontal resistance followed by ACC12.2W, Alegi, NE 15, NE 23, SECOW5T and WC 35B (Afutu *et al*., 2016b).

An attempt to respond to the challenge of developing resistant cowpea cultivars requires more knowledge about the diversity and population structure of the pathogen in the country. Some limitations of morphological characterization of fungal pathogens have been reported, some of which stemmed from the fact that hyphae among different kinds of fungi are more alike than different and therefore, usually cannot be used as a differentiating character (Barnett and Hunter, 1987). Also, different *Sphaceloma* sp. proved impossible to distinguish using colony morphology and colour alone (Zeigler and Lozano, 1983). Therefore, this study (Chapter six) went a step further to characterize the scab fungus isolates using molecular marker techniques in order to contribute significantly to understanding the genetic diversity within the Ugandan isolates. Molecular marker techniques make it possible to distinguish between isolates that may have similar morphological

traits (Gonçalves *et al*., 2008) or originating from the same location (Moulin *et al*., 2012). Inter-Simple Sequence Repeat markers were employed to assess the genetic diversity and relationships among 86 isolates from 14 populations of the scab fungus obtained from three different cowpea growing geographical regions of Uganda and cutting across the six morphological groups identified from the previous study (Chapter five) (Afutu *et al*., 2016b). The results of this chapter (Chapter six) indicated from the analysis of molecular variance that there was no variation among the regions and that a greater part of the genetic variation existed within populations (96%; PhiPT = 0.040; $P < 0.001$) than among populations (4%; PhiPR = 0.042; $P < 0.001$). The results further showed similarity coefficients ranging from 0.0248 - 0.684, suggesting a high degree of genetic variability among the isolates. Both cluster analysis and principal coordinate analysis did not show clear and distinct patterns of clustering of isolates either based on morphological groups, populations or regions and this observation was confirmed by a Mantel test which indicated that there was no significant correlations between geographic distance and genetic distance among populations ($R2 = 0.0192$, $P = 0.044$). This therefore suggested that, for the purpose of breeding for resistance to the cowpea scab fungus occurring in Uganda, there will be no need to develop different cultivars for the different regions or agro-ecological zones. Internal transcribed spacer region analysis confirmed the isolates as *Sphaceloma* sp. (Afutu *et al*., 2017b; in press).

Following field evaluations (screening) of 100 cowpea genotypes at the two locations (Kabanyolo and Serere) to identify sources of resistance and high yielding potential (Chapter four) (Afutu *et al*., 2016a), and the subsequent challenging of 20 genotypes (selected from the 100 evaluated) with selected isolates (Chapter five) (Afutu *et al*., 2016b), 11 cowpea genotypes were finally selected as potential parents. The 11 parents were crossed using a diallel mating design and were used to study the heritability, gene action and combining ability effects for scab disease resistance, yield and yield related traits and these formed the core of the last chapter of results (Chapter seven). The 11 parents yielded 55 progenies which were advanced to F2 and these progenies were evaluated at two locations. A combined analysis of data from the two locations indicated that there was no significant genotype by environment ($G \times E$) interaction for any of the traits which suggested that the lines performed consistently at both locations. Values of Baker's ratio for most of the traits suggested that non-additive gene effects were more important except for the number of pods per plant and seeds per pod. Three parents, Alegi, NE15 and NE48, had significant negative GCA effects for scab disease severity and these parents could therefore be selected for breeding for

resistance to scab while SECOW5T had significant positive GCA effects for high seed weight and grain yield. The results suggested these parents transmitted genes for resistance and high yield respectively. The cross between WC35B*WC66 had high SCA effects for scab severity while six crosses had high SCA effects for high yields across the two locations.

8.2 General conclusions

Cowpea scab disease occurred widely across the major cowpea growing areas of Uganda and Altitude (> 1200 m.a.s.l), cropping system (intercropped), previous crop grown (legume/cassava) and the cultivar grown significantly affected the incidence and severity of scab. Amuria and Tororo districts were hotspots of scab disease in the country. Cultivars SECOW3B, NE4, NE20, NE32, NE49, WC5, WC7, WC16, WC62 and WC67B were moderately resistant at Kabanyolo and Serere and NE15 was resistant at both locations. The Ugandan isolates of *Sphaceloma* sp. were put into six distinct morphological groups, three pathogenicity groups and were mostly slow growing. NE31, NE70, ACC12.2W, Alegi, NE15, NE23, SECOW5T and WC35B had wide horizontal resistance to scab isolates. There was lack of genetic differentiation among the regions indicating an active exchange of genetic material among the regions. Non-additive gene effects was more important for most of the traits except for number of pods per plant and seeds per pod. The study identified three parents (Alegi, NE15 and NE48) which .had significant negative GCA effects for improvement of resistance to scab disease while SECOW5T had significant positive GCA effects for improvement of high seed weight (g) and grain yield (Kg/ha). The study also identified a cross between WC35B*WC66 to have high SCA effects for resistance to scab while six crosses namely, Alegi*NE50, Alegi*WC36, ACC12.2W*SECOW5T, NE15*SECOW5T, NE48*WC36 and WC10*WC36 were identified as the best hybrids (crosses) for the improvement of cowpea yields.

8.3 Recommendations and future prospectives

Genotypes NE31, NE70, ACC12.2W, Alegi, NE15, NE23 and WC35B which showed wide horizontal resistance are recommended as parental lines in the cowpea breeding programme to develop cultivars with wide horizontal resistance to the scab disease. The cross between WC35B*WC66 with the best SCA effects for resistance to scab; and the six crosses namely, Alegi*NE50, Alegi*WC36, ACC12.2W*SECOW5T, NE15*SECOW5T, NE48*WC36 and WC10*WC36 as the hybrids (crosses) with the best SCA effects for grain yield are recommended

for further evaluation of the F_3 and later generations across locations in target environments for possible selection of resistant lines.

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LIST OF APPENDICES

Appendix 4.1: Description of Uganda collection of 100 cowpea lines screened for yield and resistance to scab disease.

No.	Name	Cultivar type	Maturity	Disease reaction[a]	No.	Name	Cultivar type	Maturity	Disease reaction[a]
1	WC 62	Landrace	Early	Moderate	51	WC52	Landrace	Late	Unknown
2	NE4	Landrace	Early	Unknown	52	NE41	Landrace	Late	Unknown
3	NE49	Landrace	Early	Moderate	53	NE6	Landrace	Late	Moderate
4	WC68	Landrace	Early	Unknown	54	NE46	Landrace	Late	Unknown
5	WC48A	Landrace	Early	Moderate	55	WC5	Landrace	Late	Moderate
6	NE55	Landrace	Early	Moderate	56	WC2	Landrace	Late	Unknown
7	WC15	Landrace	Early	Moderate	57	NE20	Landrace	Late	Susceptible
8	NE31	Landrace	Early	Unknown	58	WC55	Landrace	Late	Unknown
9	NE50	Landrace	Early	Moderate	59	NE71	Landrace	Late	Resistant
10	WC63	Landrace	Early	Resistant	60	NE36	Landrace	Late	Moderate
11	NE53	Landrace	Early	Unknown	61	WC20	Landrace	Late	Unknown
12	WC16	Landrace	Early	Moderate	62	NE19	Landrace	Late	Resistant
13	NE44	Landrace	Medium	Moderate	63	WC33	Landrace	Late	Unknown
14	WC30	Landrace	Medium	Unknown	64	WC29	Landrace	Late	Susceptible
15	WC21	Landrace	Medium	Unknown	65	WC58	Landrace	Late	Moderate
16	NE18	Landrace	Medium	Unknown	66	NE21	Landrace	Late	Unknown
17	WC7	Landrace	Medium	Unknown	67	NE40	Landrace	Late	Moderate
18	WC42	Landrace	Medium	Resistant	68	WC32A	Landrace	Late	Unknown
19	NE5	Landrace	Medium	Unknown	69	WC46	Landrace	Late	Unknown
20	NE70	Landrace	Medium	Unknown	70	ACC23 × SECOW4W	Inbred line	Early	Susceptible
21	WC53	Landrace	Medium	Unknown	71	ACC26 × ACC2	Inbred line	Early	Susceptible
22	WC67	Landrace	Medium	Resistant	72	ACC23 × SECOW2W	Inbred line	Early	Moderate
23	WC35A	Landrace	Medium	Unknown	73	ALEGI × SECOW3B	Inbred line	Early	Resistant
24	WC35B	Landrace	Medium	Unknown	74	ACC23 × SECOW3B	Inbred line	Early	Moderate
25	WC44	Landrace	Medium	Moderate	75	ALEGI × SECOW5T	Inbred line	Early	Susceptible
26	WC26	Landrace	Medium	Moderate	76	ALEGI × ACC12	Inbred line	Early	Moderate
27	NE23	Landrace	Medium	Unknown	77	SECOW5T × SECOW3B	Inbred line	Early	Moderate
28	WC64	Landrace	Medium	Unknown	78	SECOW5T × ACC12	Inbred line	Early	Moderate
29	WC18	Landrace	Medium	Susceptible	79	ACC23 × ACC12	Inbred line	Early	Moderate
30	WC27	Landrace	Medium	Susceptible	80	ALEGI × SECOW4W	Inbred line	Early	Moderate
31	WC8	Landrace	Medium	Susceptible	81	ACC12 × SECOW3B	Inbred line	Early	Susceptible
32	WC37	Landrace	Medium	Moderate	82	SECOW1T × ALEGI	Inbred line	Early	Susceptible
33	WC66	Landrace	Medium	Moderate	83	ACC12 × SECOW5T	Inbred line	Early	Susceptible
34	NE30	Landrace	Medium	Resistant	84	SECOW2W × ACC2	Inbred line	Early	Resistant
35	WC36	Landrace	Medium	Susceptible	85	ALEGI × ACC2	Inbred line	Early	Susceptible
36	WC17	Landrace	Medium	Unknown	86	ACC2 × SECOW1T	Inbred line	Early	Susceptible
37	NE15	Landrace	Medium	Resistant	87	ACC12 × SECOW2W	Inbred line	Early	Resistant
38	NE13	Landrace	Medium	Unknown	88	SECOW3B × SECOW2W	Inbred line	Early	Moderate

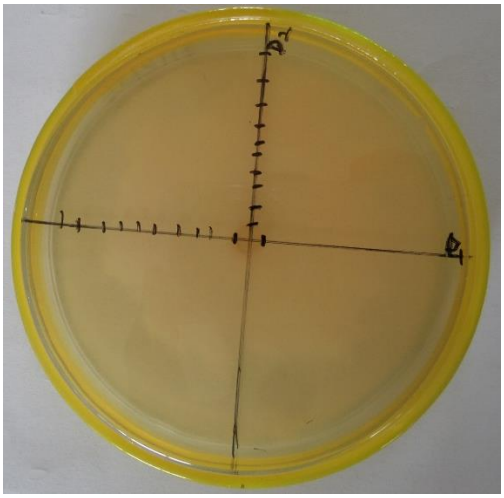
Appendix 4.1: continued

No.	Name	Cultivar type	Maturity	Disease reaction[a]	No.	Name	Cultivar type	Maturity	Disease reaction[a]
39	WC48	Landrace	Medium	Moderate	89	ACC2 × ACC12	Inbred line	Early	Resistant
40	WC69	Landrace	Medium	Susceptible	90	SECOW1T × ACC23	Inbred line	Early	Moderate
41	NE51	Landrace	Medium	Unknown	91	ACC26 × SECOW1T	Inbred line	Early	Moderate
42	NE32	Landrace	Medium	Resistant	92	SECOW4W × SECOW5T	Inbred line	Early	Moderate
43	NE39	Landrace	Medium	Moderate	93	SECOW5T × SECOW4W	Inbred line	Early	Moderate
44	NE48	Landrace	Medium	Moderate	94	SECOW2W × SECOW1T	Inbred line	Early	Moderate
45	WC35C	Landrace	Medium	Susceptible	95	ALEGI	Local	Early	Moderate
46	WC10	Landrace	Medium	Moderate	96	SECOW1T	Improved	Early	Susceptible
47	WC67B	Landrace	Medium	Susceptible	97	SECOW2W	Improved	Early	Moderate
48	WC41	Landrace	Late	Unknown	98	SECOW3B	Improved	Early	Moderate
49	WC32	Landrace	Late	Resistant	99	SECOW4W	Improved	Early	Susceptible
50	NE37	Landrace	Late	Unknown	100	SECOW5T	Improved	Early	Susceptible

[a] = disease reaction as described by NaSARRI; ACC = Accession; NE = Northern and Eastern; WC = Western and Central; Inbred lines at F_7 generation.

Appendix 5.1: Names of Districts and Sub-counties from which isolates (infected plant parts) were obtained.

Agro-Ecological Zone	District	Sub-County	Given Code	Isolates
Eastern Agro-Ecological Zone	Soroti	Soroti	ST	1-12
	Soroti	Gweri	ST	13-24
	Soroti	Arapai	ST	25-36
	Serere	Kyere	SE	37-48
	Serere	Atira	SE	49-60
	Serere	Kateta	SE	61-72
	Kumi	Kumi	KU	73-84
	Kumi	Atutu	KU	85-96
	Kumi	Bukedea	KU	97-108
	Palisa	Kameki	PA	109-120
	Palisa	kamuge	PA	121-132
	Palisa	Opwateta	PA	133-144
	Tororo	Kisoko	TR	145-156
	Tororo	Mollo	TR	157-168
	Tororo	Mella	TR	169-180
	Amuria	Kuju	AM	181-192
	Amuria	Asamuk	AM	193-204
	Amuria	Orungo	AM	205-216
North Eastern Savannah Grassland	Pader	Pajule	PD	217-228
	Pader	Puranga	PD	229-240
	Pader	Kilak	PD	241-252
	Kitgum	Kitgum	KT	253-264
	Kitgum	Matidi	KT	265-276
	Kitgum	Amida	KT	277-288
	Apac	Akalo	AP	289-300
	Apac	Aduku	AP	301-312
	Apac	Abomola	AP	313-324
	Dokolo	Dokolo	DK	325-336
	Dokolo	Agwata	DK	337-348
	Dokolo	Adok	DK	349-360
	Lira	Lira	LR	361-372
	Lira	Agweng	LR	373-384
	Lira	Ngetta	LR	385-396
North Western Savannah Grassland	Nebbi	Nebbi	NB	397-408
	Nebbi	Pakwach	NB	409-420
	Nebbi	Kucwiny	NB	421-432
	Arua	Arua	AR	433-444
	Arua	Oluko	AR	445-456
	Arua	Manibe	AR	457-468
	Yumbe	Yumbe	YU	469-480
	Yumbe	Odravu	YU	481-492
	Yumbe	Kululu	YU	493-504



Appendix 5.2: Radial growth rate measurement for scab fungus isolates on PDA.

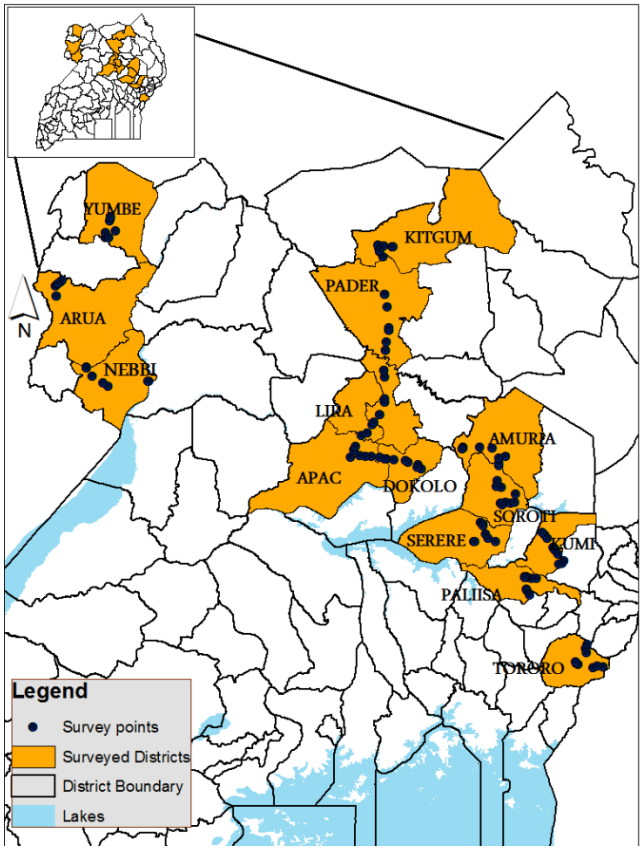
Appendix 6.1: List of isolates, Morphological groups, districts and GPS coordinates of sampled locations of isolates used in this study.

No	Isolate[a]	District	Region	Latitude	Longitude	MG[b]	No	Isolate[a]	District	Region	Latitude	Longitude	MG[b]
1	ST3P	Soroti	EAEZ	1.714	33.676	F	44	TR178P	Tororo	EAEZ	0.7097	34.242	C
2	ST6P	Soroti	EAEZ	1.7092	33.646	C	45	AM187L	Amuria	EAEZ	2.0537	33.518	D
3	ST8P	Soroti	EAEZ	1.7092	33.646	F	46	AM194P	Amuria	EAEZ	1.9422	33.634	D
4	ST13P	Soroti	EAEZ	1.7122	33.708	B	47	AM199L	Amuria	EAEZ	1.998	33.678	D
5	ST14L	Soroti	EAEZ	1.7122	33.708	C	48	AM209P	Amuria	EAEZ	2.0508	33.415	A
6	ST16L	Soroti	EAEZ	1.7122	33.708	C	49	AM215P	Amuria	EAEZ	2.0445	33.407	A
7	ST23L	Soroti	EAEZ	1.7672	33.135	C	50	PD221L	Pader	NESG	2.9915	32.931	F
8	ST24L	Soroti	EAEZ	1.7672	33.135	A	51	PD225L	Pader	NESG	2.9912	32.931	E
9	SE43L	Serere	EAEZ	1.4952	33.568	F	52	PD230L	Pader	NESG	2.6482	32.937	D
10	SE47L	Serere	EAEZ	1.4762	33.612	F	53	PD234L	Pader	NESG	2.6484	32.937	E
11	SE48L	Serere	EAEZ	1.4762	33.612	D	54	PD238L	Pader	NESG	2.6978	32.944	F
12	SE50L	Serere	EAEZ	1.5952	33.522	C	55	PD239L	Pader	NESG	2.6978	32.944	D
13	SE52L	Serere	EAEZ	1.5952	33.522	F	56	PD241L	Pader	NESG	2.7698	32.959	A
14	SE66L	Serere	EAEZ	1.4776	33.483	F	57	PD252L	Pader	NESG	2.7853	32.96	A
15	KU76L	Kumi	EAEZ	1.4959	33.931	C	58	KT261L	Kitgum	NESG	3.2941	32.89	E
16	KU77L	Kumi	EAEZ	1.5146	33.915	C	59	KT268L	Kitgum	NESG	3.2899	32.927	E
17	KU83P	Kumi	EAEZ	1.5311	33.9	F	60	AP294P	Apac	NESG	2.1405	32.827	D
18	KU84P	Kumi	EAEZ	1.5311	33.9	C	61	AP308P	Apac	NESG	2.0398	32.743	D
19	KU85P	Kumi	EAEZ	1.4419	33.967	D	62	AP313L	Apac	NESG	1.9959	32.85	D
20	KU87L	Kumi	EAEZ	1.4419	33.967	A	63	DK340L	Dokolo	NESG	1.9627	33.074	F
21	KU88L	Kumi	EAEZ	1.4419	33.967	E	64	DK358P	Dokolo	NESG	1.9912	32.9	F
22	KU94L	Kumi	EAEZ	1.3999	34.001	C	65	LR371L	Lira	NESG	2.2529	32.903	C
23	KU98P	Kumi	EAEZ	1.3554	34.035	F	66	LR372L	Lira	NESG	2.2529	32.903	E
24	KU100P	Kumi	EAEZ	1.3554	34.035	F	67	LR379L	Lira	NESG	2.5237	32.93	C
25	KU104P	Kumi	EAEZ	1.3435	34.026	F	68	LR386L	Lira	NESG	2.3297	32.932	A
26	KU105L	Kumi	EAEZ	1.3392	34.005	D	69	LR392L	Lira	NESG	2.3299	32.932	A
27	KU108L	Kumi	EAEZ	1.3392	34.005	F	70	LR394L	Lira	NESG	2.3499	32.933	D
28	PA121L	Palisa	EAEZ	1.181	33.805	C	71	LR395L	Lira	NESG	2.3499	32.933	B
29	PA122L	Palisa	EAEZ	1.181	33.805	F	72	NB400L	Nebbi	NWSG	2.5466	31.1	F

Appendix 6.1: continued

No	Isolate[a]	District	Region	Latitude	Longitude	MG[b]	No	Isolate[a]	District	Region	Latitude	Longitude	MG[b]
30	PA123L	Palisa	EAEZ	1.181	33.805	D	73	NB404L	Nebbi	NWSG	2.5408	31.096	F
31	PA124L	Palisa	EAEZ	1.181	33.805	C	74	NB418L	Nebbi	NWSG	2.4599	31.478	F
32	PA131L	Palisa	EAEZ	1.1492	33.823	F	75	AR433L	Arua	NWSG	3.0413	30.91	C
33	PA132P	Palisa	EAEZ	1.1492	33.823	E	76	AR438L	Arua	NWSG	3.0517	30.919	F
34	PA139L	Palisa	EAEZ	1.2513	33.837	F	77	AR440L	Arua	NWSG	3.0517	30.919	E
35	TR148P	Tororo	EAEZ	0.7211	34.12	A	78	AR442L	Arua	NWSG	3.0572	30.923	C
36	TR152L	Tororo	EAEZ	0.7261	34.11	F	79	AR446L	Arua	NWSG	2.9842	30.916	B
37	TR153P	Tororo	EAEZ	0.7365	34.109	F	80	AR451L	Arua	NWSG	2.9827	30.915	B
38	TR158P	Tororo	EAEZ	0.8448	34.176	D	81	AR457L	Arua	NWSG	3.0687	30.938	F
39	TR160P	Tororo	EAEZ	0.8448	34.176	C	82	AR468L	Arua	NWSG	3.079	30.951	D
40	TR164L	Tororo	EAEZ	0.8189	34.169	F	83	YU469L	Yumbe	NWSG	3.273	31.114	B
31	TR168P	Tororo	EAEZ	0.7917	34.174	F	84	YU487L	Yumbe	NWSG	3.3429	31.217	E
42	TR174P	Tororo	EAEZ	0.7081	34.275	F	85	YU492L	Yumbe	NWSG	3.3407	31.218	B
43	TR175P	Tororo	EAEZ	0.7081	34.275	E	86	YU494L	Yumbe	NWSG	3.3406	31.238	E

[a] L implies isolate was obtained from infected cowpea leaf = 25; P implies isolate was obtained from infected pod = 61;
[b] MG = Morphological group to which isolates belong (Afutu *et al*., 2016b);
[c] EAEZ = Eastern Agro-ecological Zone; NESG = North-Eastern Savanna Grasslands; NWSG = North-Western Savannah Grasslands.



Appendix 6.2: Map of Uganda showing sampling points and districts from which isolates were obtained.

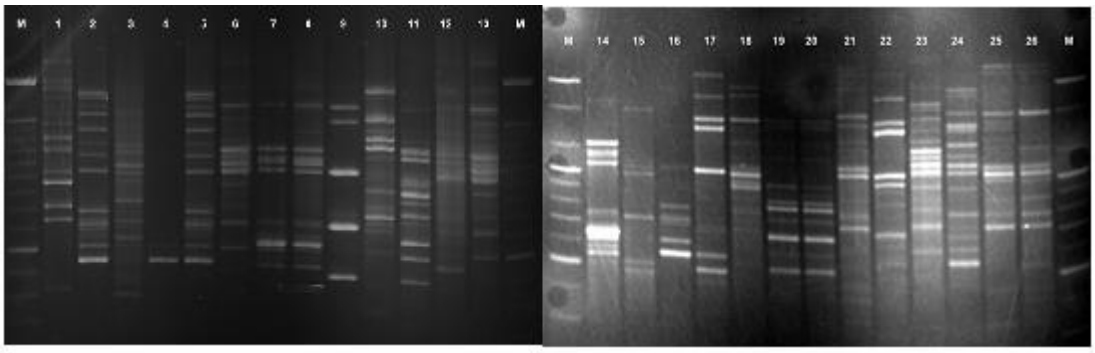
Appendix 6.3: Proportion of isolates selected from the different morphological groups

Morphological group	Total number of isolates within group	Number of isolates selected	Selection intensity (%)
A	36	9	25
B	35	6	17
C	72	17	24
D	80	15	19
E	70	11	16
F	202	28	14
Total	495	86	-

Appendix 6.4: Primers used in this study, their sequence, motifs, length and annealing temperature.

Primer[a]	Nucleotide Sequence (5'-3')[b]	Motif	Length	AT (°C)[c]	Authority
RAMS1	CACACAACAACAACAACA	ACA	18	45	Hantula *et al*., (1996)
RAMS 2	GACCACCACCACCACCA	CAC	17	55	Hantula *et al*., (1996)
RAMS 3	ATCCGACGACGACGACGA	CGA	18	50	Hantula *et al*., (1996)
RAMS 4	AGGGTGTGTGTGTG	GT	14	35	Hantula *et al*., (1996)
RAMS 5	AACACACACACA	AC	12	35	Hantula *et al*., (1996)
RAMS 6	ACCAGAGAGAGAG	AG	13	35	Hantula *et al*., (1996)
*TRI 3	TCCTCCTCCTCCTCC	TCC	15	38	Bahkali *et al*., (2012)
*TRI 4	ACGACGACGACGACG	ACG	15	38	Bahkali *et al*., (2012)
TRI 6	GTAGTAGTAGTAGTA	GTA	15	30	Bahkali *et al*., (2012)
*TET 3	TAGGTAGGTAGGTAGG	TAGG	16	35	Bahkali *et al*., (2012)
*TET 4	TTTCTTTCTTTCTTTC	TTTC	16	35	Bahkali *et al*., (2012)
UBC 809	AGAGAGAGAGAGAGAGG	AG	17	35	Abadio *et al*., (2012)
UBC 836	AGAGAGAGAGAGAGAGYA	AG	18	35	Abadio *et al*., (2012)

[a] Primers with asterisks (*) implies failed to amplify or produce polymorphic bands; [b] Y = (C, T); [c] AT = Annealing Temperature.



Appendix 6.5: DNA fingerprinting profiles amplified from first 26 *Sphaceloma* sp. fungal isolates (lanes 1 - 26) using the UDG 809 primer. M = 100-bp DNA ladder.