

Research Application Summary

## **Cowpea resistance to scab fungus in Uganda: Can resistance be achieved using landraces as source of genes?**

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### **Abstract**

Cowpea (*Vigna unguiculata* L. Walp), the third most important legume food crop in Uganda is faced with a lot of challenges, paramount among them being scab disease. Mean yield is less than 400 kg/ha though the crop has a yield potential of 3,000 kg/ha. The cowpea scab disease caused by the fungus *Sphaceloma* sp., is seed-borne and is capable of causing yield losses of up to 100% as 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 none of the five improved cowpea cultivars recently released in the country is resistant to the disease. The use of resistant cultivars in disease management is the most practical approach, easily adopted and more environmentally friendly. A better understanding of the distribution of the disease in the country, identification of sources of resistance; knowing how variable the pathogen is, and, understanding the gene action conditioning resistance to the disease, yield and yield related traits in the crop plant are fundamental to developing resistant cowpea cultivars to manage the disease. This paper reviews information on these important facets to explore the possibility of developing cowpea cultivars with resistance to the scab fungus in Uganda. The literature suggests that breeding for resistance to the cowpea scab disease and high yield can be achieved using Ugandan landraces as source of genes.

**Key words:** Breeding, local germplasm, resistance, *Sphaceloma* sp., Uganda, *Vigna unguiculata*

### **Résumé**

Le Niébé (*Vigna unguiculata* L. Walp), la troisième plus importante culture alimentaire des légumineuses en Ouganda est confronté à de nombreux défis, le plus primordiale parmi eux

étant la maladie de la gale. Le rendement moyen est inférieur à 400 kg / ha si la culture a un potentiel de rendement de 3000 kg / ha. La maladie de dolique tavelure causée par le champignon *Sphaceloma sp.* est transmise par les semences et est capable de provoquer des pertes de rendement allant jusqu'à 100%, la maladie affectant toutes les parties de la plante au-dessus du sol. Il existe actuellement une résurgence de la maladie dans le pays conduisant à des pertes de rendement importantes dans les champs des agriculteurs, mais aucun des cinq cultivars de niébé améliorés récemment publiés dans le pays est résistant à la maladie. L'utilisation de cultivars résistants à la gestion de la maladie est l'approche la plus pratique, facilement adoptée et plus respectueuse de l'environnement. Une meilleure compréhension de la distribution de la maladie dans le pays, l'identification des sources de résistance; savoir comment la variable de l'agent pathogène est, et la compréhension de la climatisation résistance à l'action des gènes de la maladie, le rendement et les caractéristiques de rendement liées à la plante cultivée sont fondamentales pour le développement de cultivars résistantes de niébé à gérer la maladie. Ce document passe en revue les informations sur ces aspects importants à explorer la possibilité de développer des cultivars de niébé avec une résistance au champignon de la tavelure en Ouganda. La littérature suggère que la sélection pour la résistance à la maladie de la gale dolique et un rendement élevé peuvent être obtenu en utilisant des variétés locales ougandaises comme source de gènes.

Mots clés: l'élevage, le germoplasme, matériel génétique local, la résistance, *Sphaceloma sp.*, l'Ouganda, *Vigna unguiculata*

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## Introduction

Cowpea (*Vigna unguiculata* L. Walp), the third most important legume food crop in Uganda is faced with a lot of challenges, paramount among them being scab disease. 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 (Emechebe, 1980; Emechebe and Shoyinka, 1985) has capacity to cause up to 100 % yield loss (Mbong *et al.*, 2012). The disease is seed-borne (Mbong *et al.*, 2010) and 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 three 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. 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 none of them was resistant to the scab disease. Yet, 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 and regular spraying with fungicides (Mbong *et al.*, 2010a), have not been effective as cowpea production is mostly done by small-scale and resource poor farmers who cannot afford these management practices.

The use of resistant cultivars in disease management (Mbong *et al.*, 2012) is the most practical approach, easily adopted and more environmentally friendly (Rusoke and Rubaihayo, 1994). To develop resistant cowpea cultivars to manage the disease, breeders require a better understanding of the occurrence and distribution of the disease in the country or region; 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. This paper reviews available literature on the possibility of breeding for resistance to the scab fungus in Uganda using landraces as the source of resistance genes. It reviews the occurrence and distribution of the disease in the country, considers efforts at identifying sources of resistance, examines the diversity of the causal pathogen in the country, and, reviews the current knowledge in terms of the genetics of inheritance and combining ability of the gene sources (landraces). Finally, it discusses the implications for breeding for resistance to the scab fungus in Uganda and other cowpea growing areas.

### **State of knowledge on cowpea scab**

**Influence of cropping system and the environment.** Literature on scab disease occurrence and distribution in Uganda confirms the resurgence of the disease in the country as mean disease incidence has been shown to have increased from a range of 10-52% in 2013 to 44-92% in 2014 while mean disease severity for both years were reported to be 2-4 (mild-severe) (Afutu *et al.*, 2016a). The resurgence could be attributed to two main factors, human causes and changing climate (nature). First, literature shows that human practices such as the choice of farming system (mixed or sole cropping), the type of cultivar grown, and, the previous crop history of the land selected for cowpea cultivation, have significant effect on the incidence and severity of scab disease (Afutu *et al.*, 2016a). According to Afutu *et al.*, 2016a, both scab disease incidence and severity were higher in fields where cassava and or other legumes such as common beans were previously grown or were intercropped with cowpea than in fields where other crops were used as intercrops. Further, disease levels were low in fields where mono cropping was practiced. These observations are not surprising as cassava and other leguminous crops such as the common beans, are also hosts to the scab fungus *Sphaceloma manihoticola* (Alvarez *et al.*, 2003) and *Elsinoe phaseoli* – perfect state of *Sphaceloma* (Phillips, 1996). For instance, Alvarez *et al.* (2003) showed that there was a cross infection of cassava by the scab fungus isolated from the weed *Euphorbia heterophylla*, thus explaining why scab incidence and severity were higher in cowpea fields previously or currently intercropped with cassava and or other leguminous crops such as beans. The choice of varieties cultivated was also observed by Afutu *et al.* (2016a) to have contributed to the resurgence of scab in the country as the disease was found to be severe in fields where susceptible cultivars were grown compared to fields in which moderately resistant cultivars were grown. This could be explained by the fact that scab is seed-borne (Mbong *et al.*, 2010b) and the infected seed or plant material provides the primary inocula for infection (Emechebe, 1985; Lin and Rios, 1985), while the subsequent dispersal of secondary conidial inoculum is by rain splash, run-off and wind-driven moisture (Emechebe and Shoyinka, 1985). Hence, the more an infected seed or a

susceptible cultivar is grown on a particular field, the more likelihood that more infections will occur.

The resurgence of the disease could also be attributable to the changing climate (nature) leading to the development of more virulent biotypes of the scab fungus or suppressing the resistance of existing cultivars grown by farmers. Afutu *et al.* (2016a) pointed that the occurrence of the disease, both in terms of its incidence and severity were significantly ( $P < 0.05$ ) higher in fields located at altitudes above 1200 m.a.s.l. than in fields located at lower altitudes. There is also information that suggest that moderate temperatures of about 23-28 °C, with three or more consecutive days of wet weather resulting in high relative humidity favour scab disease development (Emechebe, 1980; Emechebe and Shoyinka, 1985), but Iceduna (1993) observed more disease during dry conditions in Uganda. The differences in the incidence and severity recorded under the different conditions is likely due to the prevailing conditions in the environment, under, the biotype and virulence of the fungus occurring in the particular location. At least, two race of the scab fungus have been reported in West Africa where cowpea variety TVx 3236 was resistant to scab in Nigeria but was susceptible in Burkina Faso (Konate and Ouedraogo, 1988).

**Breeding for resistance to scab in Uganda.** Close to a decade ago, National Semi-Arid Resources Research Institute (NaSARRI), Serere, a research institute for the 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 fully tapped. Literature suggests the existence of scab resistant lines within the local germplasm in Uganda (Takan, 1989; Iceduna *et al.*, 1994; Nakawuka and Adipala, 1997; Tumwegamire *et al.*, 1998). Authors such as Tumwegamire *et al.* (1998), indicated that local lines in Uganda (landraces) were less infected by scab than plant introductions. In a recent follow-up study, Afutu *et al.* (2016b) evaluated 100 cowpea lines, all from among the local collections and improved cultivars without any introductions, at two locations (different ecological zones) in Uganda. The authors noted a wide variation among the landraces in response to the scab disease and yield potential and argued that, for the purpose of breeding, different cultivars have to be developed for the different locations due to a highly significant ( $P < 0.001$ ) genotype by location (G×L) interaction effects on the traits. The argument by Afutu *et al.* (2016b) is supported by Acquah (2007) who suggested that a significant G×L signposts inconsistencies in performance or response of genotypes to the particular traits for which they are being evaluated and thus, different genotypes would have to be selected or developed for the different locations. Afutu *et al.* (2016b) reported that 11 cowpea lines, *viz.*, Secow 3B, NE 4, NE 20, NE 32, NE 49, WC 5, WC 7, WC 16, WC 62, and WC 67B were moderately resistant at both locations while only one line, NE 15, was rated resistant to scab at both locations. These lines serve as parents for resistance breeding to scab disease, consistent with earlier reports by authors such as Tumwegamire *et al.* (1998).

Both Sudre *et al.* (2010) and Moulin *et al.* (2012) indicate that the correct characterization of a genotype or isolates of a pathogen are important for crop genetic improvement

programmes. Therefore, in a quest to identify and correctly characterize the causal pathogen of the scab disease occurring in Uganda, two main methods have been employed, morphological (Afutu *et al.*, 2016c) and molecular (Afutu *et al.*, 2016d). In the morphological examinations, Afutu *et al.* (2016c) characterized a total of 495 *Sphaceloma* sp. pure fungal isolates consisting of 419 from infected leaves and 76 from infected pods by employing three different approaches. These include (1) variability in morphological features such as colour of colony, colour 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 selected isolates; and (3) variation in pathogenicity and virulence of selected isolates on 20 promising resistant and high yielding cowpea lines identified in previous studies by Afutu *et al.* (2016b). Based on the first approach, the authors observed 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 different diseases indices (mean incidence, severity, Area Under Disease Progress Curve (AUDPC) and pathogenicity on 20 promising cowpea genotypes. Based on the third approach, the authors confirmed earlier reports by Zeigler and Lozano (1983) and Timmer *et al.* (1996) that one of the characteristic features of the genus of the cowpea scab fungus (*Sphaceloma*) and its related genus *Elsinoe* was its slow growing habit, or media, as most of the Ugandan isolates were slow growing (> 14 days to cover the entire surface of a 90 mm diameter petri dish). Furthermore, among the 20 cowpea genotypes (landraces) reported to have varying levels of resistance and noted that cowpea genotypes 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, 2016c).

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 in literature, 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, authors such as Zeigler and Lozano (1983) reported that different *Sphaceloma* sp. proved impossible to distinguish using colony morphology and colour alone. Consequently, Afutu *et al.* (2016d) used molecular marker techniques to study 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). Thus, Afutu *et al.* (2016d) employed Inter-Simple Sequence Repeat (ISSR) markers 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 previous studies by Afutu *et al.* (2016c). There was no variation detected among the regions or agro-ecological zones but a greater part of the genetic variation existed within populations (96%; PhiPT = 0.040;  $P < 0.001$ ) than among populations (4%; PhiPR = 0.042;  $P < 0.001$ ). Based on the high similarity coefficients ranging from 0.0248 - 0.684, there appeared a high degree of genetic variability among the Ugandan isolates, but both cluster analysis and principal coordinate analysis did not show clear and distinct patterns of clustering of isolates either based on

morphological groups as reported by Afutu *et al.* (2016c). The authors concluded after a Mantel test that there was no significant correlations between geographic distance and genetic distance among populations of the scab fungus occurring in Uganda. This suggests 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.

In a subsequent study in Uganda (Afutu *et al.*, 2016b, 2016c), 11 cowpea genotypes exhibiting resistance to scab were crossed using a diallel mating design in a quest to study the heritability, gene action and combining ability effects for scab disease resistance, yield and yield related traits. The 11 parents together with 55 resultant progenies were evaluated at the two locations in Uganda used by Afutu *et al.* (2016b) for evaluation of the initial 100 cowpea lines. Following a combined analysis of data from the two locations, the authors noted that there was no significant genotype by environment ( $G \times E$ ) interaction effects for any of the traits studied and suggested that the selected parent and resultant progenies performed consistently at both locations. The authors suggested that, non-additive gene effects were more important for scab disease resistance, yield and related traits except for the number of pods per plant and seeds per pod. Nakawuka and Adipala (1997) also suggested that additive gene effects were more important for inheritance to scab disease resistance while Tumwegamire *et al.* (1998) suggested that both additive and non-additive gene effects were important for inheritance resistance to scab. It is important to recognize that the interpretations of the implications of the conclusions made from inheritance studies by any author including authors such as Nakawuka and Adipala (1997), Tumwegamire *et al.* (1998), are dependent on whether the parents were considered as fixed effects or random effects (Griffing, 1956a and 1956b). If based as fixed effects, the interpretations are limited to the specific set of parents involved in the crossing and evaluations. On the other hand, when the parents are considered as random effects, it allows for the interpretations to be applicable to the wider population represented by those parents. Three parents, Alegi, NE15 and NE48, had significant negative GCA effects for scab disease severity suggesting that these parents transmitted genes for resistance and could therefore be selected for breeding for resistance to scab disease in Uganda.

## Discussion and conclusion

The literature reviewed above on occurrence of scab disease in Uganda suggest that human practices such as the particular cowpea cultivars chosen for cultivation, the cropping system adopted, the particular crop chosen to precede cowpea in rotation, and natural causes such as the altitude (> 1200 m.a.s.l.) at which the cowpea field lies, greatly affect the occurrence of the disease in Uganda and probably elsewhere. The literature reviewed strongly suggest that in selecting land for cowpea production, fields previously cultivated with other scab (*Sphaceloma* sp.) host crops such as cassava and beans should be avoided as these practices encourage the buildup of fungal inocula or increase the possibilities of cross infections as demonstrated by Alvarez *et al.* (2003) in which isolate of the *Sphaceloma* from the weed *E. heterophylla* was shown to cross infect cassava.

Indeed, it appears that to manage the disease, cowpeas should be sole cropped, or where mixed cropping is to be practiced, other host crops of the scab fungus such as cassava and other leguminous crops such as common beans should be excluded. The literature further suggests that the most promising approach to managing the occurrence of scab at locations lying at altitudes above 1200 m.a.s.l would be by the use of resistant cultivars.

The reviewed literature also suggest that sources of resistance to the scab disease exist among the Ugandan local germplasm collections (Nakawuka and Adipala, 1997; Tumwegamire *et al.*, 1998; Afutu *et al.*, 2016b). Further the landraces were less infected by the scab fungus than the introductions. Therefore the wide diversity in the cowpea landraces and the existence of genes for resistance to the scab fungus should be exploited to develop cultivars with resistance to the scab disease and achieve high yields. It is also apparent from the literature that based on the both morphological and molecular characterization of the scab fungus occurring in Uganda there is high variation within the isolates, implying that breeding should target broad rather than specific resistance breeding. On the other hand, the information suggest there is no variation among the three main agro-ecological zones in the country where the cowpea crop is cultivated as there was no spatial structure or distinct clustering of isolates based on the agro-ecological zones of origin. These findings, therefore, postulate that, for the purpose of breeding for resistance to the scab fungus occurring in the country, there will be no need to develop different cultivars for the different agro-ecological zones. Additionally, available literature on the genetics of inheritance of resistance to scab disease among the landraces suggest that both additive and non-additive gene actions are important and these genes can be exploited to develop resistance to scab disease.

In conclusion, the literature reviewed suggest that breeding for cowpea resistance to the scab fungus and high yields in Uganda, can be achieved using Ugandan landraces as the sources of genes. Future breeding efforts can therefore concentrate on these local collections available within the country to achieve the breeding objective.

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