



Identification of new sources of resistance to angular leaf spot among Uganda common bean landraces

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Abstract

Breeding for resistance to Angular Leaf spot (ALS), a fungal disease caused by *Pseudocercospora griseola* (Sacc), is faced with a challenge of the existence of a few resistance sources that are exotic and not well adapted to environmental conditions in Uganda. In addition, *P. griseola* is a highly variable pathogen that co-evolves with its host, therefore necessitating the continuous identification of new and stronger sources of resistance. Identification of local bean landraces/adapted bean varieties with resistance to ALS would probably quicken the progress of development of resistant cultivars, and reduce yield losses. Seventy four landraces, four commercial varieties, and two controls were screened with four *P. griseola* pathotypes 1:6, 17:39, 21:39, and 61:63 to determine their ALS resistance levels. One landrace, U00297, showed consistent and strong resistance to the four pathotypes. The inheritance of resistance in U00297 was established from three individual populations derived from crosses with three susceptible cultivars, K132, K131, and Kanye bwa, commercially grown in Uganda. The allelic relationships between U00297 and two existing ALS resistance sources, AND277 and G5686 were tested. Segregation ratios of F₂ populations revealed that U00297 resistance to pathotype 17:39 is conferred by a single dominant gene, while digenic epistatic gene interactions were suggested for resistance to other pathotypes. The general and specific combining abilities were significant for resistance revealing the importance of additive and dominant components in the inheritance of ALS resistance. As a resistant parent, U00297 was a good combiner for resistance to pathotype 17:39, and effective resistance source to pathotypes 17:39 and 61:63. The allelic test indicated that the dominant gene in U00297 is independent of resistance genes harboured by resistance sources AND277 and G5686. The information generated is useful to breeding programs targeting developing bean cultivars carrying novel ALS resistance based on genes derived from U00297.

Keywords: *Pseudocercospora griseola*, resistance, combining ability, allelism.

Introduction

Common bean (*Phaseolus vulgaris* L.) is widely grown and consumed in Latin America, Asia and Africa. The crop is a good source of dietary protein consumed wholly without processing compared to other staple crops such as maize, rice and cassava (Buruchara *et al.* 2006). It is also rich in dietary fibre, minerals and vitamins (Gepts *et al.* 2008). Bean production is greatly affected by a number of diseases that occur widely in bean growing areas. Angular leaf spot (ALS), caused by a fungus *Pseudocercospora griseola* (Sacc.) is among the most destructive diseases of common bean. The disease is ranked second among biotic and abiotic factors that constrain bean production in Africa (Aggarwal *et al.* 2003). In the Great Lakes Region, where beans are major sources of protein and calories for several communities, annual bean production is estimated at 3,961,679 MT (FAO, 2012). Of the total metric tonnes of beans produced 374,800 MT are lost due to ALS (Wagara *et al.* 2003). Leaf and stem infections by *P. griseola* result in premature defoliation, shrivelled pods and shrunken seeds, thus reducing the yield potential of

beans (Stenglein *et al.* 2003). In Uganda, yield losses of up to 50% have been reported among commercial varieties (Opio *et al.* 2001). Furthermore, late infection on pods and seeds, also cause scars that reduce on seed quality and market value (Mahuku *et al.* 2003).

Presently, in smallholder farming systems, ALS is managed through cultural practices such as crop rotation and cultivar mixtures. However, these have limited potential in managing the disease, because land scarcity cannot allow crop rotation to be practiced (Stenglein *et al.* 2003). Moreover, effective methods of ALS control like use of fungicide are far beyond the means of low resource endowed farmers. This is because of the high cost and long term consequences fungicide pose to human health and the environment (Mahuku, 2002).

The use of genetic resistance is the most appropriate, safe and cost-effective way to control ALS among smallholder farmers (Wagara *et al.* 2003). A number of exotic sources of ALS resistance do exist and have been utilised in breeding programs targeting ALS and they include

among others Mexico 54, MAR1, MAR2, AND277, G5686, G10909 and G10474 (Mahuku *et al.* 2003, 2009; Caixeta *et al.* 2005). But their limitations are; low adaptability and undesirable traits. Most resistance sources are adapted to environments in which they originated or were developed; this limits their use in other environments where they are not acclimatised to (Holbrook *et al.* 2000). Besides, majority of resistance sources are small-seeded with a climbing growth pattern; such attributes are not readily accepted by farmers in Uganda, and Africa at large (Beebe *et al.* 1981). But landraces maintained by farmers have for a long time been known to have useful agronomic traits. Indeed most existing resistant sources developed elsewhere, have been derived from landraces (Busogoro *et al.* 1999). For instance, G5686, which is a good source of ALS resistance and a member of the ALS differential set, is a landrace that originated from Ecuador (Mahuku *et al.* 2009). Though resistance may exist in some landraces, the high degree of genetic variability of *P. griseola* often compromises the use of ALS resistance derived from landraces (Nietsche *et al.* 2001). This is due to continuous emergency of new races, which break down disease resistance (Young *et al.* 1998). Hence, the need for continuous screening of germplasm to identify new sources of resistance that can regularly be introgressed into commercial cultivars (Young & Kelly, 1996). This will counteract the new emerging races and reinforce resistance in existing ALS resistance sources.

Nonetheless, identifying new sources of resistance alone cannot guarantee full protection of beans against ALS since resistance often breaks down (McDermott, 1993). Moreover, *P. griseola* is a highly variable pathogen with no known single resistance gene that is effective against all races. One way of developing stable resistance against such a variable pathogen, is by pyramiding several identified resistant genes into a single genotype with desirable traits. But pyramiding depends heavily on information related to inheritance and allelic relationship between resistance sources (Namayanja *et al.* 2006). Therefore the study aimed at identifying new sources of resistance to ALS among common bean landraces, determine the mode of inheritance in the identified resistance sources, and the allelic relationship between the new and existing sources of resistance.

Materials and Methods

Germplasm

Common bean germplasm used in the study was obtained from Uganda National Bean Programme (UNBP), Namulonge, and the International Centre for Tropical Agriculture (CIAT), Kawanda, in Uganda. A total of 80 bean lines (74 landraces, two checks and four commercial varieties) constituted

the germplasm which was evaluated for *P. griseola* resistance under greenhouse conditions. The landraces that were used had been previously collected from major bean growing areas in Uganda (Okii *et al.*, 2014). The resistance and susceptible checks used included; 1) BAT332 which is a domesticated line, small-seeded and routinely used as one of the differentials for ALS (Mahuku 2002). It is also resistant to race 61:41 (Nietsche *et al.* 2000) and to most Andean and Mesoamerican *P. griseola* races (Buruchara & Buah 1999), and 2) Kanyebe, a popular landrace in Uganda, which is susceptible to ALS (Namayanja *et al.* 2006). Commercial varieties included; K131, K132, NABE4 and NABE 13, which are commonly grown in Uganda. Furthermore, four bean lines (U00297, Mexico 54, AND277 and G5686) were also used for the inheritance and allelic tests. U00297 is a small-sized (25g/100 seeds), cream-seeded landrace with a determinate growth habit, K131 (MCM5001) and K132 (CAL96), are CIAT-bred lines, K131 and K132 belong to the Mesoamerican and Andean gene pools, respectively. G5686, AND277 and Mexico 54 are resistant to races 31:0, 63:31, 63:39 of *P. griseola* with one to three genes that condition resistance and they are inherited in a dominant manner (Carvalho *et al.*, 1998; Caixeta *et al.* 2005 and Mahuku *et al.* 2009).

Screening for *P. griseola* resistance

Fungal isolates: Four *P. griseola* pathotypes (1:6, 21:39, 17:39 and 61:63) sourced from CIAT were used in the screening of bean lines for resistance. The pathotypes had previously been characterised using a set of 12 ALS International bean differential cultivars (CIAT 1995, Ddamulira *et al.*, 2014). The first three isolates were Mesoamerican types while 61:63 was Andean. These isolates also varied in terms of virulence with 1:6 and 61:63 being the least and most virulent pathotypes respectively. On the other hand, 61:63 and 17:39 are some of most virulent and prevalent *P. griseola* pathotypes in major bean growing areas in Uganda, respectively (Ddamulira *et al.*, 2014)

Inoculum preparation: The inoculum used was extracted from monospore cultures of four pathotypes grown on V8 agar media as described by Pastor-Corrales *et al.* (1998). Pure isolates were sub-cultured onto fresh media by adding 100µl of sterile water onto each plate. The spore suspension was spread onto the fresh media containing V8 agar media and incubated for 14 days at 24°C to allow more sporulation. Plates on which isolates were grown were flooded with 100µl of sterile distilled water, and the surface scraped with a glass rod to release the conidia. The dislodged spores in suspension were filtered through a sterile cheese cloth and conidial concentration in the suspension

adjusted to 2×10^4 conidia ml⁻¹ in the final inoculum suspension.

Screenhouse resistance testing: To identify possible sources of resistance, 80 bean lines were evaluated for ALS resistance under screenhouse conditions. Five seeds of each bean line were sown in 5-litre plastic bucket containing forest soil, lake sand, and animal manure in a ratio of 3:1:1. After attaining three trifoliolate leaves, plants were inoculated with spore suspension (2×10^4 spores/ml). The suspension was applied on the lower and upper surface of leaf using a hand sprayer. The inoculated plants were placed in a humid chamber at approximately 22-28 °C with relative humidity of 95 % for 4 days to allow infection to take place. The plants were then transferred into the screenhouse and watered one to two times daily depending on sunshine intensity. The plants were evaluated for ALS resistance according to Schoonhoven and Pastor-Corrales (1987). The area under disease progress curve (AUDPC) was calculated to determine their reaction type.

Confirmation of resistance: The best way to confirm resistance to fungal pathogens is through repeated screening of materials that prove to be resistant and moderately resistant in the preliminary screening stages. Hence, out of 74 landraces, 34 bean lines which were primarily identified to be resistant and moderately resistant to the four pathotypes including, four commercial varieties and two checks (BAT 332 and Kanye bwa) were re-screened twice in replicated trials. Similar isolate preparation, inoculation, plant management procedures and disease assessment done during preliminary evaluation were adhered to in confirmatory evaluation. For statistical data analysis, the area under the diseases progress curve (AUDPC) was calculated for each accession using the midpoint rule method (Campell and Madden, 1990) as shown in Equation .1 below.

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

where “t” is time in days of each evaluation, “y” is the disease percentage representing the infected foliage at each evaluation, and n is the number of evaluations. Means were generated in Genstat (Payne, 2011) and separated by least significant difference at P=0.05.

Inheritance of ALS resistance in U00297

Based on the results from the screening, a study was designed to elucidate the resistance inheritance mechanisms in U00297. A 4 × 4 partial diallel mating design involving four parents (U00297,

K132, K131, and Kanye bwa) was executed according to the Griffings (1956) method I, model I, where the parents were crossed in all possible combinations with reciprocals and ignoring selfs to generate 12 families. U00297 is resistant and the other lines are susceptible to ALS. Different susceptible parents were used to ascertain the nature of resistance genes contained in U00297 under different genetic backgrounds. Part of the generated F₁ seed was used to plant in a backcrossing program to U00297 (BC_R F₁), and to K132, K131 and Kanye bwa (BC_S F₁). Another part of the seed was selfed to generate F₂ seeds. Thereafter, seeds from the parents, F₁, F₂ and backcross populations were planted for evaluation under screenhouse conditions.

The seeds were sown in 5-litre plastic pots containing forest soil, lake sand, and animal manure in a ratio of 3:1:1. The experiment was replicated three times and watered to provide moisture. Between 14-30 seeds of each parent and F₁ individuals were evaluated depending on seed availability. The number of F₂ individuals evaluated also ranged from 98 to 166 for each cross and 16 to 97 for each backcross population depending on seed availability. To determine the inheritance pattern, a Chi squared goodness-of-fit test was performed on data from crosses between U00297, K131, K132, and Kanye bwa to verify if observed segregation ratios of resistant and susceptible plants fitted the expected Mendelian 3:1, or epistatic 9:7 and 15:1 phenotypic ratios, respectively. Furtherstill, combining ability effects and variance were also calculated according to Griffing’s (1956) method 1 model 1. Parents and crosses were considered fixed effects, while replications were considered as random factors. The following model was used:

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + r_{ij} + e_{ijk} \dots\dots\dots (2)$$

where y_{ij} is the mean phenotypic value; μ is general mean; g_i and g_j are the GCA effects of the i^{th} and j^{th} parents respectively; s_{ij} is SCA effects of the ij^{th} cross; r_{ij} is the reciprocal effect associated with the ij^{th} cross and e_{ijk} is the residual effect.

Allelism of identified resistance

U00297 was crossed with G5686, AND277, BAT332 and Mexico 54 to generate F₁ and F₂ populations. These four bean genotypes possess resistant and complementary genes, which are responsible for their resistance action. The resistance is controlled by one, two or three dominant genes (*Phg_{G5686A}*, *Phg_{G5686B}*, *Phg_{5686C}*, *Phg-1*, and *Phg-2*) depending on the genotype (Carvalho *et al.* 1998; Caixeta *et al.* 2005; Namayanja *et al.*, 2006, and Mahuku *et al.*, 2009). Most of these genes are inherited in monogenic and dominant manner (Caixeta *et al.* 2003 and Namayanja *et al.*, 2006). In the course of crossing,

the crosses involving Mexico 54 and BAT332 failed and only F₁s of U00297 × G5686 and U00297 × AND 277 were obtained. It is probable that Mexico 54 and BAT 332 were not compatible with U00297. Seeds from parents, F₁, F₂ and backcross populations were planted for evaluation under greenhouse conditions. The seeds were sown in 5-litre plastic pots containing forest soil, lake sand, and animal manure in a ratio of 3:1:1. The experiment was replicated and watered regularly to provide the required moisture for proper growth. Thirty to forty plants of each parent, BC₁F₁ and F₁ individuals were evaluated. The number of F₂ individual plants ranged from 50-157 depending on seed availability. To test for allelic relationship between resistance sources, segregation ratios for each R × R progeny were computed. Genetic hypotheses were tested for significance for each population using the chi-squared goodness-of-fit test to determine the deviation of observed frequencies from the hypothesized ratios.

Data collection

Data for resistance, inheritance, and allelic studies was collected for 21 days at an interval of three days, using the CIAT 1–9 visual scale (Schoonhoven and Pastor-Corrales 1987), described as follows: 1, plants with no visible disease symptoms; 3, presence of a few small non-sporulating lesions that cover approximately 2% of the leaf surface; 5, plants with several small lesions with limited sporulation and covering approximately 5% of leaf surface; 7, plants with

abundant and generally large sporulating lesions covering approximately 10% of leaf surface and associated with chlorosis and necrosis; 9, 25% or more of leaf surface with large sporulating and often coalescing lesions, frequently associated with chlorosis resulting in severe and premature defoliation. The area under disease progressive curves (AUDPC) was calculated from the disease scores and symptom intensity determined by the reaction type. Individual plants for each bean line were considered resistant (R) when AUDPC value symptom score ≤ 13.5, intermediate resistant (IR) AUDPC 13.5-27 and susceptible (S) AUDPC > 27.

RESULTS

Screening for *P. griseola* resistance

In a first screening, AUDPC ranged from 30.2-40.5 among 74 landraces. The analysis of variance for AUDPC among these landraces indicated that there were significant AUDPC differences ($P < 0.05$) among these landraces for each of the four *P. griseola* pathotypes (1:6, 17:39, 21:39 and 61:63) (Table 1). Out of 74 landraces screened, 14% were rated as resistant (< 13.5) with no symptoms observed on the leaves, 22% were moderately resistant (13.5- 27.0) having small lesions on leaves with limited sporulation, while 54% were considered to be susceptible (> 27.0) to *P. griseola*. On the other hand, significant ($P < 0.05$) differences for AUDPC among commercial varieties were observed only for two pathotypes, 1:6 and 17:39 (Table 1).

Table 1. Analysis of angular leaf spot severity on 74 landraces and 4 commercial bean varieties under greenhouse conditions at Kawanda based on four *Pseudocercospora griseola* isolates.

| Pathotype | Landraces | | | | | Commercial varieties | | | | |
|-----------|-----------|-----------------------------|-------|-----------|------|----------------------|-----------------------------|-------|-----------|------|
| | DF | MS (ALS severity on leaves) | AUDPC | SED =0.05 | CV% | DF | MS (ALS severity on leaves) | AUDPC | SED =0.05 | CV% |
| 1:6 | 73 | 688.8** | 30.2 | 3.39 | 13.8 | 3 | 689.1** | 28.4 | 2.90 | 12.5 |
| 17:39 | 73 | 381.6** | 32.7 | 8.29 | 31.1 | 3 | 111.1** | 42.2 | 2.34 | 20.1 |
| 21:39 | 73 | 334.1** | 34.5 | 5.53 | 19.6 | 3 | NS | 29.3 | 9.70 | 40.5 |
| 61:63 | 73 | 169.6** | 40.5 | 6.45 | 19.5 | 3 | NS | 28.1 | 6.20 | 25.0 |

** $P < 0.01$, NS- not significant $P > 0.05$, CV-coefficient of variation, MS- Mean square, DF- Degrees of freedom, SED-standard error of difference

The reaction of 34 landraces (which were resistant or moderately resistant to four pathotypes in the first screening trial) to inoculation of individual pathotypes was significantly ($P < 0.05$) different (Table 2). The AUDPC values for pathotypes 1:6, 21:39, 17:39 and 61:63 ranged from 4.5-40.5, 9-32.8, 5.8-36.9 and 12.9-35.2, respectively. Most landraces (62.5%) were resistant (< 13.5) to

pathotype 1:6; in contrast majority were susceptible (70%) to pathotype 61:63 (Table 2), which is among the most virulent pathotype in Uganda. Forty-seven percent of the screened bean lines were moderately resistant (rating 13.5 - 27.0) to pathotype 21:39, while the smallest percentage (17.5%) of screened bean lines was moderately resistant to 1:6.

Table 2. Reaction of 40 common bean lines to inoculation with four *P. griseola* pathotypes under screenhouse conditions at Kawanda.

| Bean lines | Landraces | Seed size | Growth habit | ALS REACTION | | | | | | | |
|-----------------------------|-----------|-----------|--------------|--------------|----|-------|----|-------|----|-------|----|
| | | | | 1:6 | | 21:39 | | 17:39 | | 61:63 | |
| | | | | AUDPC | RC | AUDPC | RC | AUDPC | RC | AUDPC | RC |
| U0041 | | Small | Bush | 9.0 | R | 22.5 | I | 14.0 | I | 27.6 | S |
| U0074 | | Large | Bush | 4.5 | R | 9.0 | R | 9.0 | R | 34.2 | S |
| U351 | | Large | bush | 9.0 | R | 13.5 | R | 13.5 | R | 28.3 | S |
| U0066 | | Large | Climber | 9.0 | R | 13.5 | R | 31.5 | S | 28.7 | S |
| U1-9 | | Large | Bush | 7.2 | R | 13.5 | R | 10.8 | R | 33.1 | S |
| U0077 | | Large | Bush | 9.0 | R | 18.0 | I | 18.9 | I | 33.2 | S |
| U614 | | Large | Bush | 13.5 | R | 14.8 | I | 21.6 | I | 31.6 | S |
| U620 | | Large | Bush | 9.0 | R | 25.2 | I | 14.4 | I | 29.2 | S |
| U0082 | | Large | Climber | 14.8 | I | 13.5 | R | 9.0 | R | 22.3 | I |
| U204 | | Large | Bush | 19.3 | I | 25.2 | I | 31.5 | S | 32.3 | S |
| U00335 | | Medium | Bush | 9.0 | R | 13.5 | R | 15.5 | I | 28.4 | S |
| U0043 | | Medium | Bush | 11.7 | R | 19.5 | I | 8.55 | R | 18.9 | I |
| U284 | | Medium | Bush | 5.8 | R | 20.7 | I | 8.55 | R | 17.4 | I |
| U608 | | Medium | Climber | 10.4 | R | 20.7 | I | 30.1 | S | 30.8 | S |
| Masindi yellow | | Medium | Bush | 7.2 | R | 32.8 | S | 14.4 | R | 23.1 | I |
| U650 | | Medium | Bush | 38.7 | S | 16.2 | I | 36.9 | S | 32.4 | S |
| U342 | | Medium | Bush | 13.5 | R | 23.8 | I | 27.5 | S | 24.4 | I |
| U00297 | | Small | Bush | 9.0 | R | 13.5 | R | 7.6 | R | 12.9 | R |
| U00101 | | Medium | Bush | 5.9 | R | 31.5 | S | 19.8 | I | 32.4 | S |
| U274 | | Medium | Bush | 14.8 | I | 23.8 | I | 24.7 | I | 28.9 | S |
| U0049 | | Small | Bush | 14.4 | I | 14.9 | I | 12.2 | R | 14.7 | I |
| U0068 | | Small | Bush | 36 | S | 30.1 | S | 20.3 | I | 32.2 | S |
| U0070 | | Small | Climber | 9.0 | R | 37.3 | S | 18.0 | I | 35.2 | S |
| U0080 | | Small | Bush | 11.7 | R | 27.0 | I | 24.3 | I | 33.2 | S |
| U0083 | | Small | Bush | 13.5 | R | 28.3 | S | 29.7 | S | 32.7 | S |
| U0085 | | Small | Bush | 16.2 | I | 30.1 | S | 5.82 | R | 20.8 | I |
| U00212 | | Small | Bush | 9.0 | R | 16.2 | R | 16.2 | R | 15.7 | I |
| U609 | | Small | Bush | 13.5 | R | 22.5 | I | 27.0 | I | 27.8 | S |
| U653 | | Small | Climber | 19.4 | I | 34.2 | S | 24.7 | I | 29.1 | S |
| U659 | | Small | Climber | 40.5 | S | 31.5 | S | 27.5 | S | 33.3 | S |
| U0010 | | Small | Climber | 40.5 | S | 14.8 | I | 29.3 | S | 34.8 | S |
| U635 | | Small | Climber | 11.7 | R | 19.3 | I | 17.6 | I | 29.1 | S |
| U0053 | | Small | Climber | 4.5 | R | 18.0 | I | 17.1 | I | 24.4 | I |
| U1-5 | | Small | Bush | 25.2 | I | 23.8 | I | 36.4 | S | 32.4 | S |
| Checks | | | | | | | | | | | |
| BAT332 | | Small | Bush | 4.5 | R | 4.5 | R | 5.5 | R | 4.0 | R |
| Kanyebwa | | Medium | Bush | 27.4 | S | 30.2 | S | 37.8 | S | 32.2 | S |
| Commercial varieties | | | | | | | | | | | |
| K131 | | Small | Bush | 37.3 | S | 27.4 | S | 28.5 | S | 32.7 | S |
| K132 | | Large | Bush | 28.4 | S | 29.7 | S | 28.8 | S | 33.2 | S |
| NABE13 | | Large | Bush | 10.8 | R | 16.7 | I | 12.6 | R | 18.4 | I |
| NABE4 | | Large | Bush | 28 | S | 31.2 | S | 32.9 | S | 33.7 | S |
| Mean | | | | 15.7 | | 21.8 | | 20.5 | | 27.5 | |
| LSD_(0.05) | | | | 6.3 | | 12.1 | | 15.3 | | 16.0 | |
| CV% | | | | 26.4 | | 37.6 | | 34.8 | | 46.2 | |

AUDPC= Area under disease progressive curve, RC = resistance category.

Apart from the resistant check (BAT332), only landrace U00297 was resistant to all the four pathotypes. Three landraces (U0074, U351 and U1-9) were resistant to three pathotypes but susceptible to the most virulent pathotype 61:63. It was also observed that most commercial varieties were susceptible to the four pathotypes except one recently released commercial variety NABE13

which was resistant to 1:6 and 17:39, moderately resistant to 21:39, but susceptible to 61:63.

Inheritance of resistance to *P. griseola*

U00297 was resistant (AUDPC < 13.5) to pathotypes 17:39, 21:39 and 61:63 while parents K131, K132 and Kanyebwa were all susceptible (AUDPC >13.5) to the same pathotypes (Table 3). Pathotype 1:6 was excluded from those used for

inheritance study due to loss of viability that led to no observable disease symptoms appearing on plants inoculated with it. Most F₁ plants grew healthy with no visible diseases symptoms', suggesting that ALS resistance is inherited in a

dominant manner. Nonetheless, the F₁ cross U00297 × K131 was susceptible to 61:63 and U00297 × K131 and U00297 × Kanye bwa where susceptible to 21:39 (Table 3).

Table 3. Reaction of parents and F₁ progenies to inoculation of 61:63, 17:39 and 21:39 *P. griseola* pathotypes under greenhouse conditions at Kawanda.

| Parents | Pathotype | Resistant | Susceptible | Total |
|--------------------------------|-----------|-----------|-------------|-------|
| U00297 | 61:63 | 30 | 0 | 30 |
| K131 | 61:63 | 0 | 29 | 29 |
| K132 | 61:63 | 0 | 30 | 30 |
| Kanyebwa (KB) | 61:63 | 0 | 31 | 31 |
| U00297 | 17:39 | 30 | 0 | 30 |
| K131 | 17:39 | 0 | 29 | 29 |
| Kanyebwa (KB) | 17:39 | 0 | 29 | 29 |
| U00297 | 21:39 | 30 | 0 | 30 |
| K131 | 21:39 | 0 | 29 | 29 |
| Kanyebwa (KB) | 21:39 | 0 | 30 | 30 |
| F ₁ (K131 × U00297) | 61:63 | 0 | 26 | 26 |
| F ₁ (K132 × U00297) | 61:63 | 23 | 0 | 23 |
| F ₁ (KB × U00297) | 61:63 | 19 | 0 | 19 |
| F ₁ (K131 × U00297) | 17:39 | 17 | 0 | 17 |
| F ₁ (K132 × U00297) | 17:39 | 24 | 0 | 24 |
| F ₁ (KB × U00297) | 17:39 | 26 | 0 | 26 |
| F ₁ (K131 × U00297) | 21:39 | 0 | 14 | 14 |
| F ₁ (KB × U00297) | 21:39 | 0 | 17 | 17 |

The chi-squared test indicated that segregation of ALS resistance in F₂ population of crosses KB × U00297 and K131 × U00297 when inoculated with 61:63 and 17:39 fitted the tested ratio 9:7, respectively (Table 4). The best fit to 9:7 in these crosses suggests that they segregated for atleast two genes. In contrast crosses K132 × U00297 and K131 × U00297 when inoculated with 61:63 and 21:39, respectively, exhibited segregation ratio of 7:9, suggesting the presence of complementary epistatic gene interactions (Table 4). F₂ populations (K132 × U00297 and KB × U00297) fitted the test ratio of 3:1 when inoculated with 17:39. But cross KB × U00297 failed to fit the same test ratio when it was inoculated with 21:39 (Table 4). This possibly suggests test ratios differs depending on the pathotype being used. The segregation ratios in the backcross populations fitted the expected segregation ratios 1:1 and 1:0 respectively, except for the back cross with resistant parent (U00297) when it was inoculated with 17:39 (Table 4).

The results indicated both general combining ability (GCA) and specific combining ability (SCA) were highly significant (P<0.001). The GCA effects associated with resistant parent revealed that the effect of U00297 was negative and highly significant for resistance to both 61:63 and 17:39 (Table 5). U00297 exhibited good combining ability for resistance to both pathotypes. Crosses K131 × U00297 and K132 × U00297 had significant negative SCA effects for resistance to

61:63 and 17:39 pathotype (Table 5), which confirmed their tendency to resist the two pathotypes. On the other hand, cross Kanye bwa × U00297 was a specific cross for resistance to 61:63 and 17:39 as evident from its significant and positive SCA effects (Table 5).

Testing allelic relationship between resistance genotypes

The allelic relationship between angular leaf spot resistance gene in landrace U00297 and other resistance genes previously characterised in cultivars G5686, AND277 and Mexico 54 are presented in Table 6. The segregation of ALS resistance in the allelism test fitted 15 resistant: 1 susceptible and 63 resistant: 1 susceptible ratio, which exhibited the action of dominant genes conferring resistance to 17:39, 21:39 and 61:63. The chi-square χ^2 values showed a good fit for a segregation ratio of 15 resistant to 1 susceptible in three F₂ populations from crosses U00297 × G5686, U00297 × AND 277 and G5686 × AND 277 (Table 6), which demonstrates the presence of two dominant genes that confer resistance to pathotypes 17:39 and 21:39 of *P. griseola*. These results support the hypothesis that the gene conferring resistance to pathotypes 17:39 and 21:39 of this fungal pathogen, present in U00297, is independent from other genes (*Phg-1*, *Phg_{G5686A}*), harboured in AND 277 and G5686, respectively.

In addition, the allelism test applied to the cross AND277 × G5686 had a segregation ratio of 63R:1S, which exhibited the action of three dominant genes that confer resistance to pathotype 61:39. This also indicated independence of AND277 genes from *Phg_{G5686A}*, *Phg_{G5686B}* and *Phg_{G5686C}* genes. No susceptible plants were observed in the population from the cross Mexico

54 × AND277 which indicated that the resistance gene in the two cultivars co-segregate and are either in same locus or are closely linked genes. On the other hand, all the G5686 × Mexico 54 crossed flowers aborted probably due to incompatibility as described by Shii *et al.* (1980) and Gepts and Bliss (1985).

Table 4. Reaction of F₂, back cross progenies to inoculation of three *P. griseola* pathotypes under screenhouse conditions at Kawanda.

| Populations | Pathotypes | Total no. of plants | Observed plants | | Expected ratio | χ^2 | P-value |
|--------------------------------|------------|---------------------|-----------------|----|----------------|----------|---------|
| | | | R | S | R:S | | |
| F ₂ (K131 × U00297) | 61:63 | 157 | 72 | 85 | 7:9 | 0.2839 | 0.5940 |
| F ₂ (K132 × U00297) | 61:63 | 166 | 74 | 92 | 7:9 | 0.0462 | 0.8296 |
| F ₂ (KB × U00297) | 61:63 | 77 | 47 | 30 | 9:7 | 0.7176 | 0.3969 |
| BC _{K132} | 61:63 | 58 | 27 | 31 | 1:1 | 0.2759 | 0.5994 |
| BC _{K131} | 61:63 | 53 | 25 | 28 | 1:1 | 0.1698 | 0.6803 |
| BC _{KB} | 61:63 | 61 | 32 | 29 | 1:1 | 0.8251 | 0.3637 |
| BC _{U00297} | 61:63 | 47 | 45 | 2 | 1:0 | 0.0957 | 0.1915 |
| F ₂ (K131 × U00297) | 17:39 | 98 | 62 | 36 | 9:7 | 1.9598 | 0.1615 |
| F ₂ (K132 × U00297) | 17:39 | 98 | 70 | 28 | 3:1 | 0.6663 | 0.4142 |
| F ₂ (KB × U00297) | 17:39 | 157 | 123 | 34 | 3:1 | 0.9363 | 0.3332 |
| BC _{K132} | 17:39 | 59 | 28 | 31 | 1:1 | 0.0763 | 0.1525 |
| BC _{K131} | 17:39 | 67 | 35 | 32 | 1:1 | 0.1343 | 0.7140 |
| BC _{KB} | 17:39 | 54 | 29 | 25 | 1:1 | 0.2963 | 0.5862 |
| BC _{U00297} | 17:39 | 97 | 95 | 2 | 1:0 | 0.0000 | 0.0412 |
| F ₂ (K131 × U00297) | 21:39 | 102 | 41 | 61 | 7:9 | 0.5234 | 0.4693 |
| F ₂ (KB × U00297) | 21:39 | 111 | 72 | 39 | 3:1 | 6.0810 | 0.0136 |
| BC _{K132} | 21:39 | 47 | 25 | 22 | 1:1 | 0.0000 | 0.0851 |
| BC _{K131} | 21:39 | 64 | 30 | 34 | 1:1 | 0.2500 | 0.6171 |
| BC _{KB} | 21:39 | 81 | 42 | 39 | 1:1 | 0.1111 | 0.7389 |
| BC _{U00297} | 21:39 | 16 | 16 | 0 | 1:0 | 0.0000 | 1.0000 |

R: resistant, S: susceptible, Chi-square P values greater than 0.05 indicate that the observed values were not significantly different from the expected value.

Table 5. GCA and SCA effects of parental lines of crosses with their reciprocal values for resistance to angular leaf spot.

| | Pathotype 61:63 | | | | Pathotype 17:39 | | | |
|-----------------------|-----------------|--------------|---------------|---------------|-----------------|---------------|---------------|---------------|
| | Male U00297 | K131 | K132 | Kanyebwa | U00297 | K131 | K132 | Kanyebwa |
| Female | | | | | | | | |
| Parent means | 2.00 | 6.50 | 6.17 | 6.67 | 2.00 | 5.92 | 5.42 | 6.33 |
| U00297 | <i>0.58</i> | <i>0.65*</i> | <i>0.04**</i> | <i>0.03**</i> | <i>0.92</i> | <i>0.88*</i> | <i>-0.33*</i> | <i>0.24**</i> |
| K131 | <i>0.04</i> | <i>0.58</i> | <i>0.65</i> | <i>0.03</i> | <i>-0.13</i> | <i>0.92</i> | <i>-0.88</i> | <i>0.24</i> |
| K132 | <i>-0.04</i> | <i>0.04</i> | <i>0.51</i> | <i>0.04</i> | <i>0.34</i> | <i>-0.13</i> | <i>-0.03</i> | <i>0.33</i> |
| Kanyebwa | <i>-0.08</i> | <i>0.13</i> | <i>0.04</i> | <i>0.51</i> | <i>-0.13</i> | <i>0.13</i> | <i>-0.34</i> | <i>0.03</i> |
| Parental GCA's | <i>1.68**</i> | <i>0.23</i> | <i>0.07</i> | <i>0.25</i> | <i>-1.92**</i> | <i>1.36**</i> | <i>0.07</i> | <i>4.40</i> |

SCA and reciprocal values appear in the upper and lower triangles in *italics*, respectively.

*, ** Significance of the effect from zero at 0.05 and 0.01 levels of probability.

Table 6. Reaction of F₂ progenies derived from resistant parents to inoculation of 61:63, 17:39 and 21:39 *P. griseola* pathotypes under greenhouse conditions at Kawanda.

| F ₂ populations | Pathotypes | Total No. plants | Observed plants | | Expected ratio | x ² | P-value |
|----------------------------|------------|------------------|-----------------|---|----------------|----------------|---------|
| | | | R | S | | | |
| G5686 × U00297 | 61:63 | 104 | 102 | 2 | 63:1 | 0.1049 | 0.7460 |
| AND 277 × U00297 | 61:63 | 111 | 105 | 6 | 15:1 | 0.0567 | 0.8119 |
| AND 277 × Mexico 54 | 61:63 | 85 | 85 | 0 | 15:1 | 5.6667 | 0.0173 |
| AND 277 × G5686 | 61:63 | 103 | 97 | 6 | 15:1 | 0.0026 | 0.9791 |
| G5686 × U00297 | 17:39 | 100 | 92 | 8 | 15:1 | 0.9131 | 0.3393 |
| AND 277 × U00297 | 17:39 | 94 | 88 | 6 | 15:1 | 0.0456 | 0.8308 |
| AND 277 × Mexico 54 | 17:39 | 97 | 97 | 0 | 15:1 | 6.4667 | 0.0110 |
| AND 277 × G5686 | 17:39 | 98 | 92 | 6 | 15:1 | 0.0110 | 0.9163 |
| G5686 × U00297 | 21:39 | 56 | 51 | 5 | 15:1 | 1.0735 | 0.3002 |
| AND 277 × U00297 | 21:39 | 108 | 101 | 7 | 15:1 | 0.0735 | 0.7835 |
| AND 277 × Mexico 54 | 21:39 | 96 | 96 | 0 | 15:1 | 6.4000 | 0.0114 |
| AND 277 × G5686 | 21:39 | 105 | 98 | 7 | 15:1 | 0.1269 | 0.7216 |

R: resistant, S: susceptible, Chi-square P values greater than 0.05 indicate that the observed values were not significantly different from the expected value.

DISCUSSION

Developing resistant bean cultivars partly depends on variability expressed by the disease-causing pathogen. Since most fungal diseases are spread by highly variable pathogens, it is important to continuously diversify sources of resistance as a strategy to control angular leaf spot and rationalise the breeding process. We identified landrace U00297 to be resistant to four *P. griseola* pathotypes 1:6, 17:39, 21:39 and 61:63 under greenhouse conditions. In some genetic backgrounds, resistance in U00297 is conferred by a single dominant gene, which is independent of resistance genes found in cultivars AND277 and G5686, while in others, resistance, is due to epistatic gene interaction involving two or three genes. Resistance in U00297 has been successfully transmitted into certain F₂ progenies. This is evident in our study by F₂ plants which were resistant to *P. griseola* pathotype 17:39.

The screening process revealed variation in reaction of bean lines to Ugandan *P. griseola* pathotypes. Only U00297 was resistant to four pathotypes, indicating low levels of resistance among the other bean lines evaluated and the complexity of managing *P. griseola* in bean-growing areas. Nonetheless, the resistant line identified could be a good source of resistance, which can supplement other existing resistance sources to develop durable ALS resistance. Given the fact that U00297 is resistant to pathotypes 17:39 and 61:63, which are among the most prevalent and virulent pathotypes in Uganda, it constitutes a resistant source that can provide desired resistance to commercial bean varieties in Uganda that are known to be susceptible to ALS (Opio *et al.* 2001).

Our findings were in line with earlier studies by Mahuku *et al.* (2002), which also identified four bean accessions in a core bean collection that were resistant to pathotype 63-63 (one of the most virulent pathotypes that overcomes resistance in

differentials) under greenhouse conditions. Similarly, Wagara *et al.* (2007) identified 13 bean genotypes that were resistant to at least 40 *P. griseola* pathotypes in Kenya. Similarly, U00297 has a potential of being used to improve resistance against ALS among susceptible commercial bean varieties in Uganda.

Though commercial varieties are routinely screened for ALS resistance during variety development, our findings revealed that most of them were susceptible to *P. griseola*. This was in support of earlier work by Opio *et al.* (2001), which indicated that 50% of commercial varieties in Uganda were susceptible to ALS. Susceptibility among commercial bean varieties is probably attributed to breakdown of host resistance by the pathogen as commercial varieties become increasingly used by farmers (McDermott 1993). Because of the inherent evolutionary variability of *P. griseola*, over time new strains develop that overcome the resistance in commercial varieties (Pedro *et al.* 2006). This was reflected in our findings when the newly released variety NABE13 was resistant to two pathotypes, while popular varieties, such K132, K131 and NABE 4, which were released much earlier and are commonly used by farmers (Kalyebara *et al.* 2005), were all susceptible to the four pathotypes. This implied that even with newly released varieties, resistance breakdown is likely to be experienced over time. Hence, the need for regular monitoring of disease resistance in released varieties. This could facilitate the process of genetic improvement of newly released bean varieties for resistance against ALS.

One approach to ensure continued improvement of ALS resistance in bean varieties is through understanding the inheritance and segregation pattern in new sources of resistance. This is pertinent in breeding because it offers breeders an opportunity to design strategies that maximises efficiency in developing improved resistant cultivars. Our findings showed that F₁ plants were

resistant to most pathotypes, suggesting that resistance in U00297 is inherited as a dominant trait. The monogenic inheritance of resistance indicates that pedigree or backcross breeding would be adequate to transfer resistance to susceptible lines. Similarly, segregation for resistance in F₂ K132 × U00297 and KB × U00297 populations were consistent with a ratio 3:1 as resistant: susceptible, which further confirmed that U00297 resistance to pathotype 17:39 was due to a single dominant gene.

The dominant nature of resistance in U00297 cultivar revealed that resistance transfer into KB and K132 is possible through conventional breeding provided that both alleles to be transferred are dominant alleles. Muthoni *et al.* 2011 and Caixeta *et al.* 2003 reported similar resistance inheritance pattern in other ALS resistant sources. Similarly, inheritance to *P. griseola* in Mexico 54 and BAT332 is also reported to be monogenic with a single dominant gene effect. Mahuku *et al.* (2009) also reported that ALS resistance in bean cultivar G5686 to pathotypes 31-0 was conditioned by a single or three dominant genes. However, previous inheritance studies have revealed that resistance to *P. griseola* is conditioned by few genes that can either be recessive or dominant depending on the cultivar used as a susceptible parent (Carvalho *et al.* 1998). In our study it was observed that segregation ratios in F₂ population deviated from the expected ratios indicating that resistance of U00297 to pathotypes 21:39 and 61:39 involved digenic epistatic gene interactions.

The GCA and SCA were significant determinants of resistance for some parents. This indicated the critical role both additive and dominance or epistatic components play in the inheritance of ALS resistance. GCA was more pronounced than SCA for resistance, thus procedures that emphasise use of additive effects for the incorporation of resistance should enhance genetic gain from selection during bean improvement. But it should be noted that both additive and dominance appear to be effective in transmitting genes conditioning ALS resistance.

Analysis of GCA for parents provides breeders with useful information on the average performance of a line in hybrid combinations (Ana and Staub, 2002). Such analyses are important because they provide an indication of genetic difference that exist among lines being evaluated and the importance of genes with largely additive effects. Earlier studies have shown the influence of additive and dominance effects on ALS resistance expression in bean cultivars BAT322 and KBT (Fivawo *et al.* 2013). The same authors reported the predominance of additive variance over dominance variance in ALS resistance expression, which concurs with our findings.

Furthermore, parents with higher GCA estimates for other traits such as yield are used for the constitution of new populations, aiming at attaining high genetic progress in breeding programs. However, for the case of disease resistance evaluation, the interest concentrates on genotypes with lower severity of the disease, or either, genotypes that contribute to diminish the expression of the character and, consequently, show negative estimates of GCA estimates (Cruz and Regazzi, 2001). In our study, the negative GCA values indicate the contribution for the resistance to *P. griseola* in common bean, which were observed for resistant parent U00297. In contrast, positive estimates were observed on susceptible (K132, K131 and Kanye bwa) parents. Kanye bwa presented the most unfavorable general combining abilities estimate. It is therefore one of the parents with the lowest capacity to contribute to resistance alleles to the genetic pool under study.

Specific combining ability is the deviation from the performance predicted on the basis of general combining ability. The SCA effects are an important criterion for the evaluation of crosses that will eventually be used to develop hybrids. Two crosses had high negative and significant SCA effects: K131 × U00297 and K132 × U00297, which indicated the presence of non-additive gene effects for 61:63 and 17:39 resistances, respectively. It is probable that either one of the parents in these crosses possesses some dominant resistance genes or that epistasis among disease resistance loci was involved. U00297 possesses a dominant gene for resistance in these crosses as exhibited earlier by the segregation ratios observed in F₂ plants. The SCA effects for Kanye bwa × U00297 were positive and significant, indicating non-additive, epistatic gene action governing susceptibility to 61:63 and 17:39 resistance.

In our study, G5686 × U00297 (R × R) yielded a ratio of 63R:1S in the F₂ generation, when inoculated with pathotypes 61:63, suggested a segregation of three unlinked resistance genes. Because Mahuku *et al.* (2009) posited the existence of three resistance genes in G5686 and our current, one of them being shared between G5686 and U00297.

Conclusion

The study identified landrace U00297 as a potential source of resistance to four *P. griseola* pathotypes. Resistance to 17:39 in U00297 is inherited in a dominant manner. It is possible to adequately transfer resistance in U00297 into K132 and Kanye bwa using pedigree breeding. Based on GCA results, U00297 is a good combiner and effective source of resistance to 17:39 and 61:63, while SCA values for U00297 crosses with K131 and K132 indicated presence of non-additive gene effects for resistance to 61:63 and 17:39. Resistance gene that

confers resistance in U00297 is independent of resistance genes harboured by AND-277 and G5686. This information will aid breeding programs targeting improving resistance to ALS using U00297 as the parent.

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