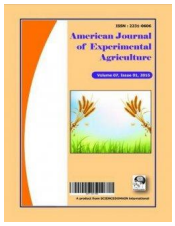

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Gene Pyramiding Improved Resistance to Angular Leaf Spot in Common Bean

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CM and MOS designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors RE, PS and PG reviewed the experimental design and all the drafts of the manuscript. Author GD managed the analyses of the study. Author CM identified the plants. Authors GD and PG performed the statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was conducted to determine the effectiveness of pyramided genes in improving angular leaf spot (ALS) resistance in susceptible common bean cultivars.
Study Design: The experiment was set in randomized block design with three replications.
Place and Duration of Study: The experiment was conducted at International Centre for Tropical Agriculture (CIAT) at Kawanda, Uganda in 2010-2014.
Methodology: Crosses among three *Pseudocercospora griseola* resistant lines of common bean (*Phaseolus vulgaris)* were developed. The crosses involved five inbred lines, AND277, Mexico 54, G5686 and two susceptible cultivars, K132 and Kanyebwa. The resistant lines were crossed in cascading pyramiding scheme to develop triple crosses (TC). The TC F_1 and each of the resistant parents were crossed with each of the two susceptible cultivars to generate four parent crosses

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(FPC) and single crosses (SC), respectively. All the population developed was inoculated with 61:63 *P. griseola* isolate under screenhouse conditions and their reaction was elucidated.
Results: The SC exhibited resistance segregation ratios of 15:1 and 61:3 while TC best fitted for 249:7 and 247:9 ratios. This suggested that two or three genes were present in SC and four genes in the TC. The resistance present in the three sources to ALS race 61:63 is complex; with epistatic mode of inheritance. The four genes in FPC provided more effective resistance against isolate 61:63 than two or three genes in SC.
Conclusion: The FPC lines with combined resistance were more effective than the individual sources for transferring resistance to susceptible cultivars. Future studies needs to be conducted to determine how broad and effective combined resistances in these newly developed lines have against the variability of the ALS pathogen sampled in Uganda.

Keywords: Complex crosses; Pseudocercospora griseola; genes; Phaseolus vulgaris.

1. INTRODUCTION

Angular leaf spot (ALS) caused by *Pseudocercospora griseola* is a major fungal disease limiting common bean (*Phaseolus vulgaris L.*) production [1]. The disease often occurs under mild temperature (16-28°C) and high relative humidity (75-100%). Such conditions, coupled with the use of susceptible cultivars, predispose beans to ALS attack, leading to yield losses of up to 50% among released varieties and popular landraces in Uganda and elsewhere [2,3]. Despite the high prevalence of ALS, the current disease control measures, such as crop rotation, cultivar mixtures and use of fungicides [4] have little or no impact on the disease. Moreover, these control measures cannot be fully practiced due to land shortage and the high cost of fungicides [5]. Use of genetic resistance is so far the most effective control measure; and least expensive and easiest for farmers to adopt and use, because resistance is already embedded in the seed that farmers plant [6].

A few ALS resistant sources such as AND277, Mexico 54, and G5686 have been identified [7]. Genetic studies on these sources revealed that AND277 is resistant to eight races of ALS: 31:17, 31:39, 61:31, 63:19, 63:23, 63:31, 63:35, and 61:41 [7]. Whereas, Mexico 54 and G5686 are resistant to races 63:39 and 31:0 [8,9,5]. These sources of resistance can be useful in facilitating the process of transferring ALS resistance into susceptible farmer preferred Ugandan cultivars. But despite the existence of such ALS-resistant sources, use is limited because many are of Middle-American origin with low adaptability and undesirable traits. The resistant sources are more adapted to conditions in areas where they were developed [10]. For instance, Mexico 54 is a climber and medium-seeded; such a trait is not easily accepted by farmers in Africa [11,12]. Although AND277 and G5686 are medium to large-seeded cultivars, their low yields compared to popular landraces and commercial varieties, limit their use. All these factors limit the direct release of resistant cultivars from available ALS resistance sources in Uganda.

Furthermore, durable resistance within the existing resistance sources is challenged by pathogen variability. Due to *P. griseola* variability, resistance often breaks down as new and more virulent strains of the pathogen evolve and/or the existing strains adapt to the host [13]. Over time resistant cultivars gradually become ineffective. In addition, no single resistant gene is effective against all races of ALS; hence protection conferred by a single gene against a hypervariable pathogen is often short lived [14]. Considering that Uganda has other *P. griseola* races [15] a breeding technique such as gene pyramiding that can address several constraints or diseases races could probably be the right direction in managing ALS in Uganda.

Pyramiding resistance genes into a single genotype is one of the practical approaches through which durable resistance can be achieved [16]. Gene pyramiding has been applied successfully in concentrating multiple genes into single cultivars to control diseases such as bacterial blight [17] and blast [18] in rice. The same strategy has successfully been employed to provide durable resistance against soybean mosaic virus in soybean [19]. Nevertheless, gene pyramiding has not been explored in developing durable resistance against ALS in common bean. In other crops, through gene pyramiding, synergistic interactions between genes may occur such that resistance gene combinations are higher than the sum of resistance conditioned by individual genes [20].

Presently, it is not known whether resistance genes from the different ALS resistance sources, once pyramided into a single bean cultivar, would increase the level of resistance in susceptible cultivars. Therefore, the study aim was to determine the effectiveness of pyramided resistance genes in improving levels of ALS resistance in susceptible common bean cultivars and how they interact with each other.

2. MATERIALS AND METHODS

The study was conducted at the International Centre for Tropical Agriculture (CIAT), Kawanda, in Uganda from 2010-2014. Three bean genotypes (Mexico 54, AND277, and G5686) that were previously characterized for ALS resistance and two susceptible parents (K132 and Kanyebwa) were used in this study. Genotype Mexico 54 carries gene *Phg-2*, which is responsible for resistance against race 63:39 [9] and is linked to SCAR marker OPE04 [21]. AND227carries gene *Phg-1*, which is responsible for resistance against eight *P. griseola* races; 31:17, 31:39, 61:31, 63:19, 63:23, 63:31, 63:35, and 61:4 [7] and is linked to SSR marker TGA1.1 on chromosome Pv01 [22]. G5686 is an Andean large-seeded landrace whose origin is Ecuador [5]. The three resistance genes (Phg_{G5686A}, Phg_{G5686B} and Phg_{G5686C}) found in G5686, confer resistance to race 31:0; these genes are linked to SSR markers Pv-ag004 and Pv-cct001 on chromosome Pv04 (opposite ends) and Pv-at007 on chromosome Pv09 [5]. The two genes *Phg-1* and Phg_{G5686A} associated with resistance in AND277 and G5686, respectively, have been mapped on chromosomes Pv01 and Pv04 by markers TGA1.1 at 1.3 cM and Pv-ag004 at 0.0 cM [22,23], respectively. The resistance in AND277 and Mexico 54 [24], [9] is dominant while for G5686 resistance is conditioned by dominant or recessive with complementary or epistatic effects that act alone or in combination genes [25]. The three parents (AND277, Mexico 54 and G5686) used in the pyramiding are also resistant to Uganda *P. griseola* isolates 61:63 and 17:39 [26]. K132 is a large-seeded variety of Andean genepool developed by CIAT and Kanyebwa is a popular Mesoamerican small-seeded landrace in Uganda but both K132 and Kanyebwa are susceptible to ALS [9].

In the process of pyramiding resistance from different sources, Mexico 54 was crossed with AND277 and the F_1 plants were crossed with G5686 to generate triple cross (TC) populations (Fig. 1). The F_1 plants from the TC were grown in screenhouse, harvested and seed bulked. Two sets of one hundred fifty F_2 seeds each were planted in five-litre buckets in the screen house and inoculated with two *P. griseola* isolates (17:39 and 61:63) independently. These two isolates were used because they are the most prevalent and virulent *P. griseola* isolates in Uganda, respectively [15]. The inoculum was applied at a concentration of 2 x 10^4 conidia ml^{-1} at leaf stage V3 (first trifoliate leaf open and the second trifoliate leaf appears) as described by [27]. Disease symptoms on the inoculated plants were evaluated from six to twenty one days after inoculation at three days interval. The disease response was assessed based on 1–9 rating scale, where 1 is immune and 9 is highly susceptible. Ratings of 1-3 are considered resistant, 4-5 intermediate and 6-9 as susceptible [28]. Fifty plants that were resistant to both 17:39 and 61:63 were further screened with molecular markers (OPEO4, TGA1.1, Pv-ctt001, Pv-ag004 and Pv-at007) to confirm the presence of four genes.

2.1 Molecular Analysis

In order to confirm the presence of five genes (*Phg-2, Phg-1,* Phg_{G5686B} $Phg_{G5686A,}$ Phg_{G5686C}), molecular markers SCAR OPE04 (for *Phg-2*), SSR-TGA1.1 (for *Phg-1*) and SSR-Pv-ctt001 (for Phg_{G5686B}), pv-ag004 (for Phg_{G5686A}) and Pv-at007 (for Phg_{G5686C}) were used to tag the pyramided genes in the F_2 progenies. DNA was extracted from young leaves of 50 TC plants following procedures described by [27]. The extracted DNA was quantified using a NanoDrop 2000 c spectrophotometer (Thermo Scientific, Waltham, USA). DNA concentration was adjusted to a standard concentration of 10 ng/ µl before used in the PCR reaction. PCR reactions were carried out in 20 µl volumes containing 1 × DNA polymerase buffer (100 mMTris-HCl, 400 mMKCl, 15 mM $MgCl_2$, pH 9.0), 3mM $MgCl_2$, 0.4mM dNTPs, 1µM of each primer, 0.3U Taq DNA polymerase (Bioneer Inc. Korea) and 50ng of genomic DNA. DNA amplification was performed in a my cycler thermal cycler (BioneerInc, Korea) under a program of one cycle at 94°C for 5 min, followed by 35 cycles at 94°C for 20s, 50°C for 40 s and 65°C for 8 min, and a final 16 min extension at 65°C. The DNA amplicons were electrophoresed in 1.5% agarose gel for 1 h at 90 V in 1XTris–borate–EDTA buffer (89 mM Tris base, 89 mM boric acid–borate and 2mM EDTA pH 8.0) and later stained for 20 min in 0.5 µg/ml ethidium bromide. Gel images were captured using the GeneSnap

gel documentation system (SynGene, Frederick, MD, USA).

Through combined phenotypic and molecular screening, three plants were found to have four genes (*Phg-2, Phg-1*, $Phg_{G5686B,}$ Phg_{G5686A}), all detected by races 17:39 and 61:63 and genes linked with markers OPEO4, TGA 1:1, Pv-ctt001 and Pv-ag004. Nonetheless gene Phg_{G5686C} was not detected because marker Pv-at007 linked to it was polymorphic when evaluated on G5686 and K132 but when it was used to amplify DNA from F_2 individuals of TC the results were not conclusive. The three selected plants with four genes were advanced to F_3 by single seed descend and then crossed with susceptible parents; K132 and KAN to form four-parent cross (FPC) populations: KAN x [(Mexico 54 x AND277 x G5686)] and K132 x [(Mexico 54 x AND277 x G5686)]. In all crosses, the susceptible cultivars were used as female parents. Part of FPC F_1 seeds was retained and the other portion was advanced to the F_2 through selfing.

Besides, in generating single crosses (SC), each resistant parent; AND277, G5686, Mexico 54 used in gene pyramiding were crossed between themselves (AND277 x Mexico 54, AND277 x G5686 and Mexico 54 x G5686) to generate R x R crosses, while each resistant was crossed with each of the two susceptible parents to generate S x R crosses: (K132 x AND277, K132 x Mexico 54, K132 x G5686, Kanyebwa x AND277, Kanyebwa x Mexico 54 and Kanyebwa x G5686. In all crosses involving resistant and susceptible parents, K132 and Kanyebwa were used as female parents. Part of the F1 from SC was retained and the rest was advanced to F2 generation for phenotypic evaluation.

2.2 Phenotypic Screening for Angular Leaf Spot Resistance

The parents (Kanyebwa, K132, AND277, G5686 and Mexico 54) involved in all the populations, SC, TC, FPC, F_1 and F_2 progeny seeds were planted in five-litre buckets in the screenhouse. A randomized complete block design with two replications was adopted for parents and SC F_1. The plants were divided into two equal sets; one set was inoculated with isolate 17:39 and the other with 61:63. The isolates were inoculated at a concentration of 2×10^4 conidia ml^{-1} as described by [27]. Disease symptoms on plants inoculated with 61:63 were evaluated from six to twenty one days after inoculation at three days interval. A 1–9 scale described by [28] was used to score disease symptoms. However, disease symptoms score data on plants inoculated with isolate 17:39 was not reported because no symptoms were observed on plants inoculated with this isolate. Even the susceptible parents (Kanyebwa and K132) inoculated with 17:39 did not express disease symptoms an indication that over time 17:39 isolate could have lost its viability.

2.3 Data Analysis

To estimate the number of pyramided genes among the progeny lines a Mendelian analysis of segregating populations of plants was carried out. The F_1 and F_2 progenies in R x R crosses were categorized into resistant (score of 1-3) and susceptible (score of 4-9). Two, three and four-gene models were developed by taking into consideration the differences in the segregation patterns of the SC F_1 and F_2, as well as TC F_1 and F_2 generations (Table 1).

Before conducting the χ^2 goodness-of-fit tests, homogeneity of ratios test was performed to assess the difference in segregation between the two replications. The χ^2 test of homogeneity was based on the Mather model [29]. Where the homogeneity of ratios test indicated no difference in the segregation pattern of a cross between the two replications, then data from the replications were pooled prior to χ^2 goodness of-fit test. The χ^2 value for goodness-of-fit test was calculated using the Mather model. Means of parents and progenies were compared to provide insight in the types of gene action conditioning ALS resistance in both the R x R and S x R populations. Comparisons between means of FPC and SC (S x R) populations were used to determine the effect of pyramided resistance genes. Means were computed using the restricted (residual) maximum likelihood (ReML) analysis in GenStat [30]. Where the mean squares from ReML analysis indicated significant genotype effects, means were compared using a Student t-test for each pairwise comparison of interest, based on the standard error of the difference (SED) for that specific pair of entries. The Student t-test was used due to unequal number of individuals among genotypes tested [31].

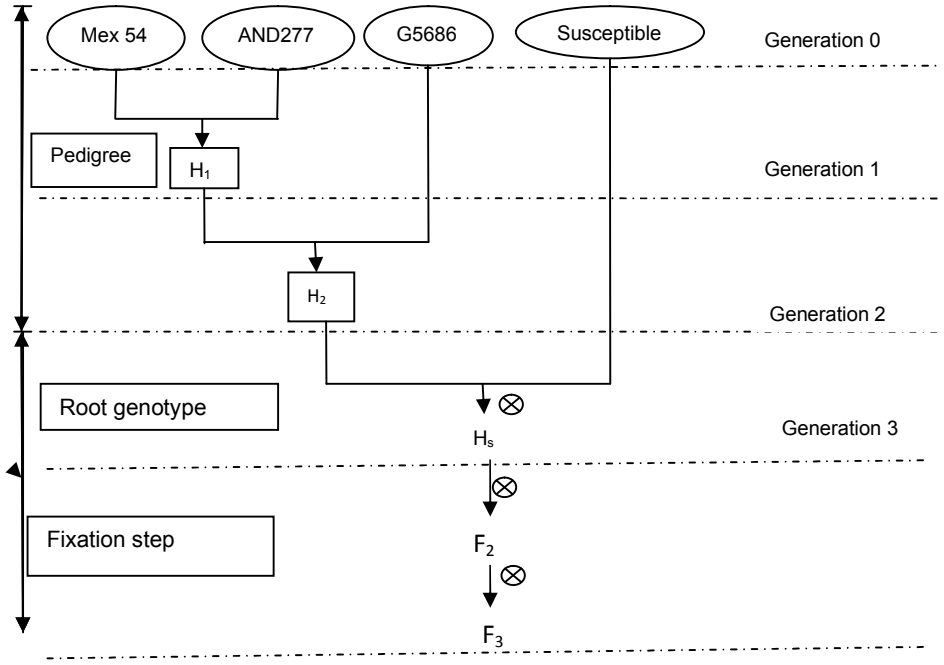

Fig. 1. Cascading pedigree gene pyramiding scheme. H refers to hybrid created by the cross

Table 1. Parents and F_2 population with and without angular leaf spot resistant genes

No. entries	Pedigree	Molecular makers				
		OPE04	***TGA1.1***	***Pv-ctt001***	***Pv-ag004***	***Pv-at007***
3	F_2TC	+	+	+	+	-
37	F_2TC	+	-	+	-	-
10	F_2TC	-	+	+	+	-
15	Mexico 54	+	-	-	-	-
15	G5686	-	-	+	+	+
15	AND277	-	+	-	-	-
15	K132	-	-	-	-	+
15	Kanyebwa	-	-	-	-	-

(+) prescence, (-) absence of the gene, TC triple cross

3. RESULTS AND DISCUSSION

3.1 Estimate of Resistance Genes in R x R Crosses

In single (SC) and triple (TC) crosses the distribution was normal, though skewed towards resistance (Fig. 2). In all the crosses the distribution of plant resistance against isolate 61:63 grouped the tested plants in two distinct phenotypic classes.

Bean plants were categorized into resistant (R) and susceptible (S) classes (Table 2). The F_2 populations of AND277 x G5686 and Mexico 54 x G5686 single crosses fitted 15:1 and 61:3 segregation ratios; except Mexico 54 x AND277 population which did not fit either ratio (Table 2). On the other hand, F_2 population of [(Mexico 54 x AND277) x G5686)] fitted 247:9 and 249:7 segregation ratios but did not fit 15:1 and 63:1 ratios (Table 2).

3.2 Interaction of Pyramided Resistance Genes

It was observed that all the R x R crosses showed non-significant deviations (P >.05) of the F_1 mean from MP, the F_2 mean from MP, and the F_2 mean from the average of MP and F_1 (Table 3). But S x R crosses exhibited both

insignificant (P >.05) and significant (*P*=.05) deviations from the means (Table 4). The F_2 populations of KAN x Mexico 54, KAN x AND277 and KAN x G5686 exhibited non significant deviation (*P* >.05) of F_1 mean from MP and F_2 mean from the average of MP and F_1 while F_2 populations of K132 x Mexico 54, K132 x AND277 and K132 x G5686 - exhibited a significant (*P* =.05) deviations of the F_1 mean from MP and F_2 mean from the average of MP and F_1 (Table 4). In the same way, the four-parent crosses- FPC exhibited a significant negative deviation (*P* <.001) of F_1 mean from MP and F_2 mean from the averages of MP and F_1 (Table 4).

3.3 Effect of Pyramided Resistance Genes in S x R Crosses

In both KAN (Kanyebwa) and K132 populations the F_2 mean of both FPC had significant negative deviation from the SC means, indicating lower ALS symptom severity in the FPC than in the SC (Table 4). The F_2 frequency distributions also showed that FPC in both KAN and K132 populations had higher proportions of resistant plants than any of the SC in the respective populations (Figs. 3 and 4).

4. DISCUSSION

In this study, three different angular leaf spot resistant bean genotypes were used to improve the level of resistance against isolate 61:63 in two common bean cultivars; K132 and Kanyebwa through gene pyramiding. The study revealed that combining resistance genes from different sources increased the level of resistance against *P. griseola* isolate 61:63. This supports earlier observation made by [32] and [20] that combining resistance gene from different resistance sources provide better resistance against common bean diseases. Thus pyramiding resistance through hybridization of different ALS resistance sources possibly is one of the strategies that can enhance resistance against ALS and also increase the genetic base of ALS resistance in common bean.

In developing disease resistance, it is important to ascertain the number of genes responsible for resistance for a particular kind of disease you are dealing with. In this study, segregation for ALS resistance in single cross (SC) showed that F_2 population of AND277 x G5686 cross best fitted a 15:1 ratio. This indicated that the SC segregated for two genes with duplicate dominant epitasis gene action and one dominant gene present in each parent [33]. On the other hand, the F_2 population of Mexico 54 x G5686 best fitted 63:1 suggesting that most likely the F_2 population segregated for at least two dominant genes and one recessive gene for resistance. However, the F_2 population of Mexico 54 x AND277 did not fit for both segregation ratios, indicating that either genes in AND77 and Mexico 54 are possibly found on the same locus or closely linked to each other. This concurred with earlier studies by [7] which indicated that genes in Mexico 54 and AND277 co-segregate upon inoculation with *P. griseola races* 63:23 and 63:19. On the other hand, F_2 population of [(Mexico 54 x AND 277) x G5686)] best fitted to ratio 249:7. This suggested that the F_2 population segregated for four genes, two dominant and two complementary genes.

In all R x R crosses, results indicated insignificant deviations of F_2 means from the mid-parent means and F_1. This implied that ALS resistance in such crosses was primarily additive in nature. The results concurred with earlier findings by [34], who showed that genetic control of angular leaf spot reaction in common bean leaves and pods of cross Carioca MG x ESAL 686, was dominated by additive gene effects. Furthermore, populations of KAN x Mexico 54, KAN x AND277 and KAN x G5686 also exhibited additive gene action. Similar results were reported from Tanzania in crosses between resistant genotypes (Mexico 54 and G5686) and susceptible local genotypes Kablanketi and Spenjeli [35].

In several previous studies it has been reported that alleles that interact at a single locus in an additive manner are responsible for resistance in most of the ALS resistance sources [36]. However, in this study significant deviations of the F_1 means from MP, and F_2 means from the average of MP and F_1 for K132 x Mexico 54, K132 x AND277 and K132 x G5686 and FPC populations, reflected that epistatic interaction was responsible for resistance in the single crosses involving K132 as a susceptible parent and in the complex crosses. The inheritance for the three sources was different in the Kanyebwa and K132 parents which indicated that inheritance was sensitive to genetic background. This concurred with earlier work by [9] which showed that inheritance of ALS resistance depends on the genetic background of the parents used in a cross. This phenomenon is

further explained by [21] who reported that, resistance to pathotype 63-19 was due to a dominant allele at a single locus when Mexico 54 was crossed with the Rudá cultivar (Mesoamerican), but when the same parent was crossed with a snap bean cultivar, [37] observed that resistance was due to a recessive allele at a different single locus.

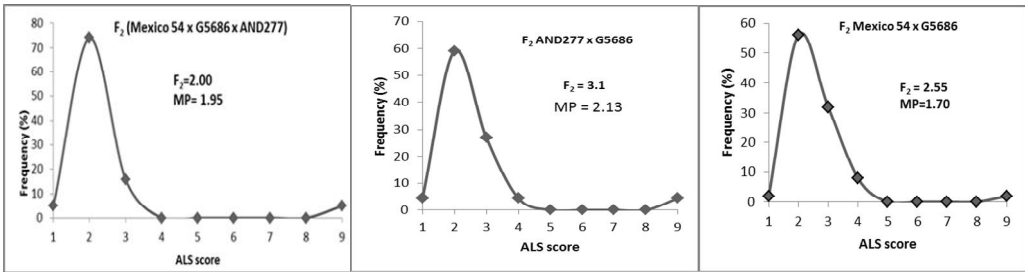

Fig. 2. Frequency distribution of angular leaf spot scores in populations of TC and SC cross mating of common bean genotypes resistant to angular leaf spot

Table 2. Observed vs. hypothesized phenotypic class frequencies for resistant and susceptible reaction to *P. griseola* race 61: 63 in F_1 and F_2 single and triple R x R cross populations

Cross	Number of plants			Observed ratio R:S	Expected ratio R:S	Number of R genes	Goodness of fit	
	Total	R	S				X^2	P-value
F_2SC_1	220	190	15	13.6:1	15:1	2 genes with duplicate dominant epistasis	1.40	0.24
					61:3	2 dominant genes and 1 recessive gene	4.00	0.05
F_2SC_2	240	220	10	23.0:1	15:1	2 genes with duplicate dominant epistasis	1.80	0.18
					61:3	2 dominant and one recessive gene	0.50	0.49
F_2SC_3	97	97	0	12.0:1	15:1	2 genes with duplicate dominant epistasis	6.46	0.01
					61:3	2 dominant and one recessive gene	5.66	0.02
F_1TC	150	140	10	14.0:1	15:1	2 genes with duplicate dominant epistasis	0.00	0.83
					61:3	2 dominant and one recessive gene	1.30	0.25
					247:9	2 dominant and 2 recessive genes	4.4	0.04
					249:7	2 dominant and 2 complementary genes	8.7	0.03
F_2TC	485	460	25	18.4:1	15:1	2 genes with duplicate dominant epistasis	9.9	0.02
					61:3	2 dominant and one recessive gene	4.2	0.04
					247:9	2 dominant and 2 recessive genes	1.1	0.29
					249:7	2 dominant and 2 complementary genes	0.3	0.59

SC_1= (AND277 x G5686), SC_2= (Mexico 54 x G5686), SC_3 = (Mexico 54 x AND277), TC = [(Mexico 54 x AND 277) x G5686)]; R= resistance and S = Susceptible. Chi-square P-values greater than .05 indicate that the observed values were not significantly different from the expected

Table 3. Angular leaf spot symptom severity means scores of parental, F_1 and F_2 genotypes and their comparisons in R x R crosses

Crosses	P_1	P_2	P_3	MP	F_1	F_2	F_1-MP	F_2-MP	F_2-(MP +F_1)/2)
SC_1	1.60	1.79	-	1.70	2.20	2.55	-0.50^{ns}	0.85^{ns}	0.60^{ns}
SC_2	-	1.79	2.46	2.13	2.40	3.10	0.27^{ns}	0.97^{ns}	0.83^{ns}
SC_3	1.60	-	2.46	2.03	1.99	2.12	-0.04^{ns}	0.09^{ns}	0.11^{ns}
TC_1	1.60	1.79	2.46	1.95	1.79	2.00	-0.16^{ns}	0.05^{ns}	0.13^{ns}

P_1 =Mexico 54, P_2 =G5686, P_3 =AND277, SC_1 = G5686 x Mexico 54, SC_2 = (AND 277 x G5686), SC_3 =Mexico 54 x AND277, TC_1 = [(Mexico 54 x AND 277) x G5686]

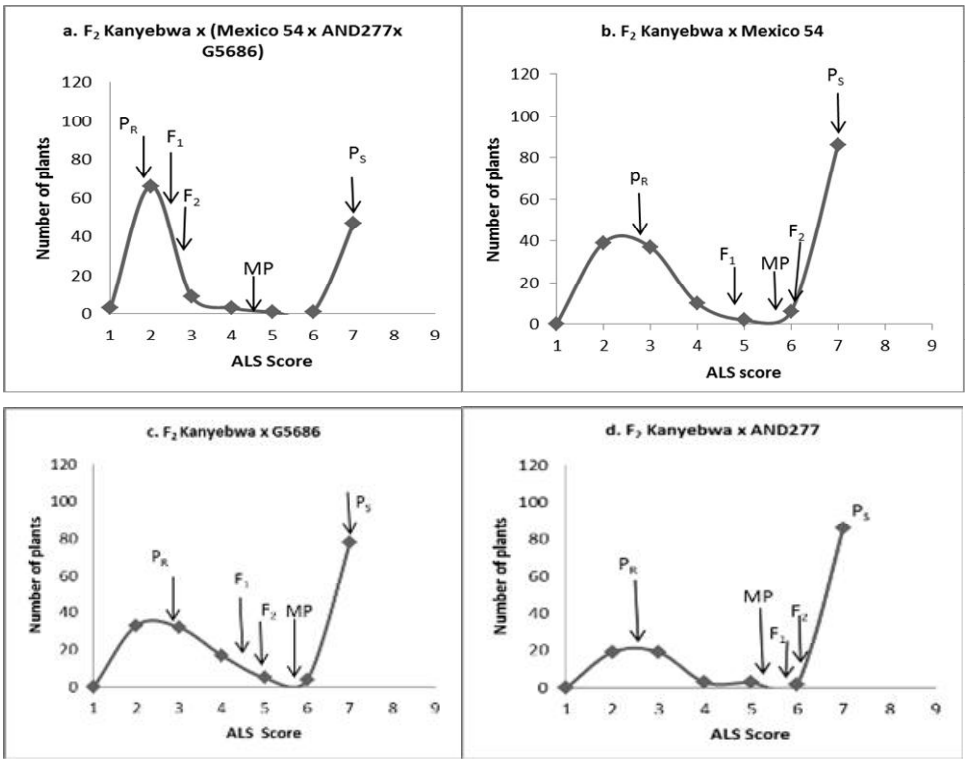

Fig. 3. Frequency distribution of F_2 Kanyebwa population inoculated with *P. griseola* isolate 61:63, MP-mid parent, P_R-resistant parent, P_S- susceptible parent

Table 4. Angular leaf spot symptom severity mean scores of parental and four-parent cross F_1, F_2 and their comparisons after inoculation with *P. griseola* race 61:63

	P_S	P_R	MP	F_1	F_2	F_1-MP	F_2-(MP +F1)/2	FPC_{F1}-SC_{F1}	FPC_{F2}-SC_{F2}
KAN x Mexico 54	7.97	2.38	5.18	5.62	4.76	-0.45^{ns}	-0.64^{ns}	-2.29**	-2.04*
KAN x AND277	7.97	2.46	5.18	6.12	6.10	-0.91^{ns}	0.45^{ns}	-1.03**	-3.38*
KAN x G5686	7.97	2.66	5.78	4.98	4.49	-0.34^{ns}	-0.89^{ns}	-2.38**	-1.77*
FPC_{KAN}	7.97	1.41	4.37	2.61	2.72	-1.76**	-0.77**		
K132 x Mexico 54	7.33	2.38	4.85	3.52	4.81	-2.53**	0.63*	-0.06^{ns}	-2.15*
K132 x AND277	7.33	2.46	4.89	3.53	5.00	-1.36**	0.79*	-3.51^{ns}	-2.34*
K132 x G5686	7.33	2.66	4.98	3.55	4.93	-1.43**	-0.67*	0.04^{ns}	-2.27**
FPC_{K132}	7.33	1.41	4.69	2.49	2.66	-2.20**	-0.93**		

*FPC = Four -parent cross; FPC_{Kan} = Kan x [(AND 277 x G5686) x Mexico 54]; FPC_{K132}= K132 x [(AND 277 x G5686) x Mexico 54)]; Kan =Kanyebwa; P_R and P_S = means of resistant and susceptible parents, respectively; P_S for the FPC was the mean for the triple-cross F_1; F_1 and F_2 = means of F_1 and F_2 generations, respectively; MP = mid-parent value; F_1-MP = F_1 deviation from MP; F_2-(MP+F_1)/2 = mean deviation of F_2 from the average of MP, ns = not significant at P >.05; * and ** = significant at P = .05 and P < .001, respectively*

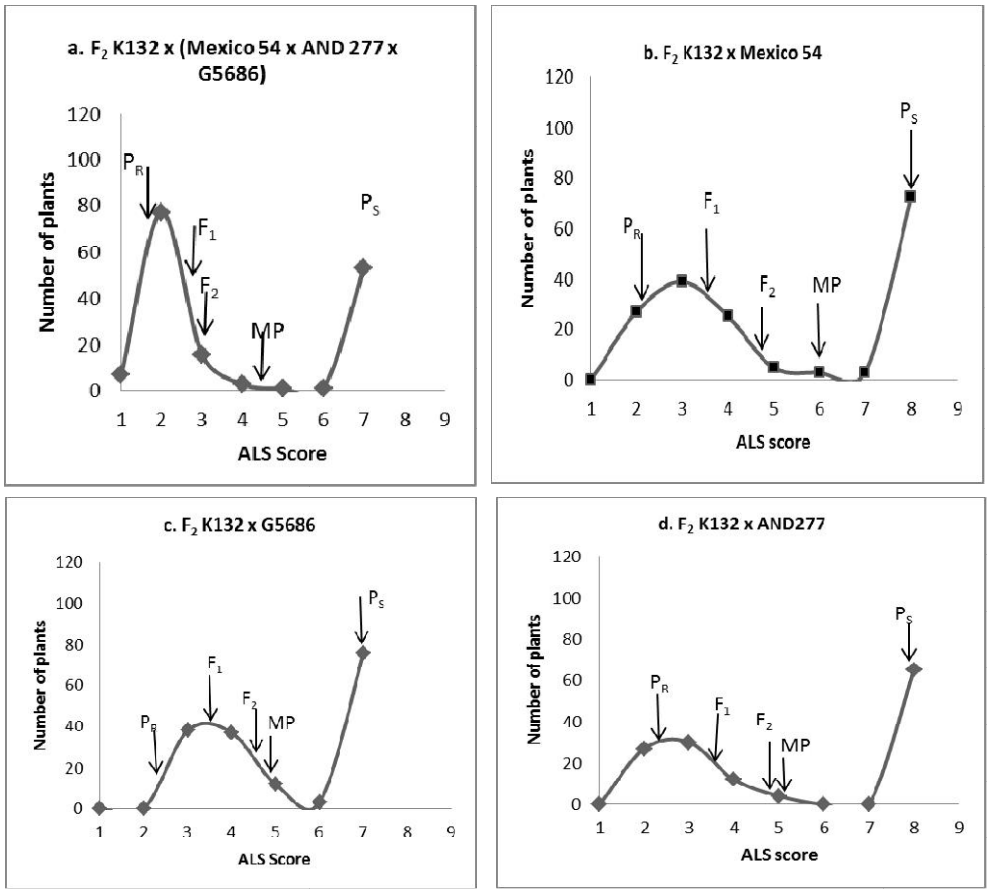

Fig. 4. Frequency distribution of F_2 K132 population inoculated with *P. griseola* isolate 61:63 Pr-resistant parents, Ps –susceptible parent, MP- mid parent

In terms of effectiveness of pyramided genes, FPC plant population had low ALS symptom severity compared to SC populations. The low disease severity in FPC was attributed to epistatic interaction because in Kanyebwa population, FPC was the only cross with significant (P =.05) negative F_2 deviation from the average of MP and F_1. Similarly though all crosses in K132 population had significant (P =.05) F_2 deviations, it was only FPC that showed a significant negative deviation (P < .001) of the F_2 mean from the average of the MP and F_1. Most times the effectiveness of epistatic interactions depends on whether the F_2 deviation from the average of MP and F_1 is positive or negative [20]. The negative deviation of F_2 from the average of MP and F_1, in FPC implied that epistatic interaction had beneficial effects, which contributed to effective resistance in FPC crosses while the positive deviations of F_2 mean from the average of MP and F_1 observed for most of the SC indicated that epistatic interaction had detrimental effect to ALS resistance through favouring susceptibility [38]. From these observations it has been seen that epistatic effects contributed to better resistance of FPC population against isolate 61:63 than SC. This was likely due to more beneficially interacting loci in FPC than SC. The result also further confirms, and is consistent with, the additive nature of resistance indicated in the R x R crosses. The better performance of FPC over SC demonstrates that combining resistance genes from different ALS resistance sources can improve resistance in susceptible cultivars than when single sources of resistance are used.

4. CONCLUSION

Resistance present in the three sources to ALS race 61:63 is complex; the sources of resistance did not exhibit dominant inheritance against this race but rather epistatic inheritance. The inheritance or resistance for the three sources

was different in the Kanyebwa and K132 parents which indicated that inheritance was sensitive to genetic background. Susceptible F_2 individual plants observed from the F_2 crosses between resistance sources confirmed independence of the resistance genes from among the different sources. The combined resistance genes in SC and TC crosses exhibited additive effects within the cross and slightly increased level of resistance to an individual ALS race when all three sources were combined. Markers and resistance to isolate 61:63 was used to identify F_2 plants with all four putative genes (*Phg-2, Phg-1*, $Phg_{G5686B,}$ $Phg_{G5686A)}$. The F_3 lines from these F_2 plants exhibited the highest level of resistance to an individual race compared to the original resistance sources. The F_3 lines with combined resistance were more effective than the individual sources for transferring resistance to susceptible cultivars of major importance in Uganda. Future studies needs to be conducted to determine how broad and effective combined resistances in these newly developed lines have against the variability of the ALS pathogen sampled in Uganda.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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