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Genetics of angular leaf spot (ALS) resistance in South African market class dry bean (*Phaseolus vulgaris*) cultivars

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Abstract

Angular leaf spot (ALS) caused by Pseudocercospora griseola (Sacc.) is one of the serious fungal diseases affecting dry bean in Africa, including South Africa. Host plant resistance is the best management strategy, of which its effectiveness requires knowledge of the genetics underlying the resistance in guiding breeding efforts. In this study, the inheritance of ALS resistance was studied through the generation mean analysis biometrical procedure. Six generations consisting of the two parents Ukulinga and Gadra, and its cross derived filial progenies (F1), second-generation (F2), and backcrosses of F1 to Ukulinga (BCP1) and Gadra (BCP2) were planted in a net-house and later inoculated using a mixture of P. griseola isolates. Leaf lesions (% disease severity) were rated using a CIAT 1-9 scale and analysed using SAS macros in Proc GLM of SAS version 9.3. Results of ANOVA for a full model displayed significant additive effects ($P<0.05$) and highly significant ($P<0.001$) additive x dominance effects. Segregation analysis indicated 9:7 ratio, implying the involvement of complementary gene effects. The number of genes was estimated to be 1.79; however, due to epistasis two or more genes possibly govern the resistance in this cross. Broad and narrow sense heritabilities were 0.40 and 0.33, respectively. Therefore, the estimated narrow-sense heritability, additive gene effects, and epistatic interaction imply that it is prudent to delay selections until later stages, in which homozygosity could be achieved and additive effects fixed.

Keywords: Angular leaf spot, generation mean analysis, heritability, inheritance, selection.

Introduction

Angular leaf spot (ALS) caused by Pseudocercospora griseola (Sacc.) (Crous et al., 2006), is one of the serious fungal diseases affecting the production of dry bean in the warm and humid parts of South Africa. Dry bean is one of the country's widely grown field crops, mainly produced by commercial farmers throughout the country's production regions, except in KwaZulu-Natal and the Eastern Cape, where the crop is also grown primarily by small scale farmers for direct consumption due to its high nutrient composition, as a cheap source of dietary protein and for its agronomic importance (Liebenberg et al., 2002). Locally, there is a ready market for the large-seeded red speckled sugar beans of the Andean genepool, which approximately occupy 65–75% of the production (Aarathon and Thombela, 2016). However, the co-evolution of ALS causal organism with its host (López et al., 2006; Chilagane et al., 2016), has translated into the vulnerability of the predominantly grown red speckled beans to the ALS disease.

Angular leaf spot is known to affect all aerial plant parts, with initial symptom expression usually coinciding with the onset of flowering and often continues until maturity (Mongi, 2016). Factors such as alternating cool and wet environmental conditions, a ready source of inoculum and a susceptible variety, results in high severity of the disease, leading to massive yield losses of up to 80% (Singh and Schwartz, 2010). Therefore, to prevent crop losses disease management measures such as the use of fungicides, disease-free seed, and cultural practices are required. With the use of fungicides specifically, consideration should be placed on the marginal rate of return, as this impacts on the profitability of the bean enterprise, as well as on the technical capabilities of the farmer to determine the optimum calibration rate that can reduce the disease infestation. In Tanzania, recent findings revealed reduced yield losses on common bean due to the use of fungicides, except that the current calibration rates currently used by the bean farmers are ineffective towards preventing yield losses, thus prompting the need to exploit genetic resistance to manage the disease (Mongi et al., 2018).

Worldwide, breeding for ALS resistance is complicated by the highly variable nature and virulence of P.griseola, with pathotype 63-63 possessing the ability to infect all the

resistant sources of ALS (Silva et al., 2008). Since the inheritance pattern of ALS resistance is complex, as it can be both quantitative or qualitative, and tends to be conditioned by 1, 2, or 3 recessive or dominant genes, breeding efforts can target single-gene resistance through the backcross strategy or accumulation of favourable alleles through recurrent breeding (Miklas et al., 2006). The backcross strategy may provide a short term resistance to ALS, but the resistance can easily be broken down by the emergence of virulent strains (Pereira et al., 2015). On the other hand, the recurrent selection offers the possibility to develop durable or horizontal resistance (Ramalho et al., 2012; Pereira et al., 2019), but may prolong the breeding cycle under conventional breeding unless if supported by marker-assisted selection.

The successful development of resistant cultivars heavily relies on understanding the underlying pattern of inheritance, the number of genes involved and the availability of diverse sources of resistance to ALS. However, the absence of such critical information disrupts the progress and effectiveness of host plant resistance as a disease control strategy (Mahuku et al., 2004). Several approaches can be exploited to unravel the genetic analysis of quantitative traits, and the generation mean analysis (GMA) is one of the biometrical techniques that has been widely used in estimating additive, dominance and epistatic effects in both self- and cross-pollinating species (Hallauer et al., 2010). Therefore, from these estimates, a breeder can compute and draw inferences on the significance of genetic and environmental variances and in particular, the proportion of additive variance out of the total genetic variance which is crucial in improving complex traits and serves as a reference to improved variety selection (Vanderplank, 1984).

The genetic basis is known to vary with a trait, each particular cross, the susceptible parent and race used in the study, this compels the need to study the inheritance mechanism for each specific population (Namayanja et al., 2006). Therefore, the objectives of this study were to establish the mode of gene action conditioning ALS resistance, estimate the number of genes contributing to the ALS resistance trait, and the narrow sense and broad sense heritabilities to inform the breeding strategies.

Results

Means analysis

The cultivars Ukulinga and Gadra used as parents in this study reacted differently to ALS infestation and results of ANOVA indicated that the six generations reacted significantly different to ALS (P<0.0001, Table 1). Means separation by the least square difference (LSD P<0.05), showed that the F1, F2 and BCP1 reaction to ALS was not significantly different from each other, with their mean ALS severities between those of the parental values, although skewed towards the resistant parent. On the other hand, the P1, P2, and BCP2 had significantly varied reactions from each other (Table 2).

Frequency, segregation analysis and model information

The frequency distributions of the ALS severity of the segregating and non-segregating generations are shown in Figure 1. Generally, the Ukulinga plants (P1) were resistant and the Gadra plants (P2) were susceptible. The F1 plants showed an intermediate reaction, with a lower severity than the mid-parent mean in their reactions to ALS. The frequency distribution of the F2 displayed that more than 60% of the plants were resistant. The backcross to Ukulinga plants had a bimodal distribution, with almost equal proportions of resistant: susceptible plants, and skewed towards the resistant parent. The reaction of plants to the mixture of ALS strains is based on the following classification, 0 - 3 resistant, while >4 highly susceptible. Plants that were scored as zero had no visible symptoms, while those with 1 - 3 scores had few visible symptoms, and plant with symptoms above 4 had many visible symptoms with those above 7 showing signs of sporulating.

The chi-square distribution of the F2 generation indicated that the resistant and susceptible plants followed a 9:7 ratio. Results of the Wald type F-test, which is synonymous with the joint scaling test proposed by Mather and Jinks in 1971 for a lack of fit (Piepho and Möhring, 2010), provided the relevant information on the adequacy of the additive–dominance model. In this study, the simple additive-dominance model could not provide better estimates of the genetic effects, as indicated by a highly significant reaction (F value 10.99, <.0001) for a full model (5 df), and therefore a full model was adopted in drawing up statistics of this population. Findings of the t-tests and regression analysis on the coefficients deployed in the establishments of significance and the estimation of the values of the genetic effects are displayed in Table 3. The additive effects were significant and positive, additive x dominance effects were positive and highly significant, dominance effects were non-significant and negative, additive x additive were positive and not significant and dominance x dominance were non-significant and negative.

Variance components, heritabilities and minimum number of genes

The variance components, heritabilities, and the number of genes conditioning resistance are shown in Table 4.

Discussion

Results of ANOVA revealed a highly significant variation among the parents for ALS severity, and therefore it was possible to perform the GMA. The means and standard errors for the generations (Table 2) and the potency ratio or F (-0.48) (Table 4), which is a measure of the relative importance of the dominance over additive effects, was less than 1, indicating partial dominance for ALS resistance, and the negative sign implies that recessive alleles were in greater abundance than the dominant alleles. The chi-square indicated a 9:7 ratio, suggesting the involvement of complementary epistasis, thus the expectation is that each gene makes an essential contribution towards the improved levels of resistance (Darbeshwar, 2012). Previous findings in Mahuku et al. (2009) established the involvement of three complementary genes responsible for ALS resistance.

In previous studies, the genetic effects for ALS resistance were commonly estimated on an additive-dominance model. In this study, on the contrary, the simple additive-dominance model was inadequate to provide estimates of the genetic effects, which possibly suggest the involvement of higher-order interactions and involvement of two or more genes in the

Table 1. Analysis of variance for angular leaf spot severity for six basic generations.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value	Pr>F
Replicate	1	115.6	115.6	3.13	0.0787
Plant(Generation)	174	7595.7	43.7	1.18	0.1348
Generation	5	2224.4	444.9	12.04	<.0001

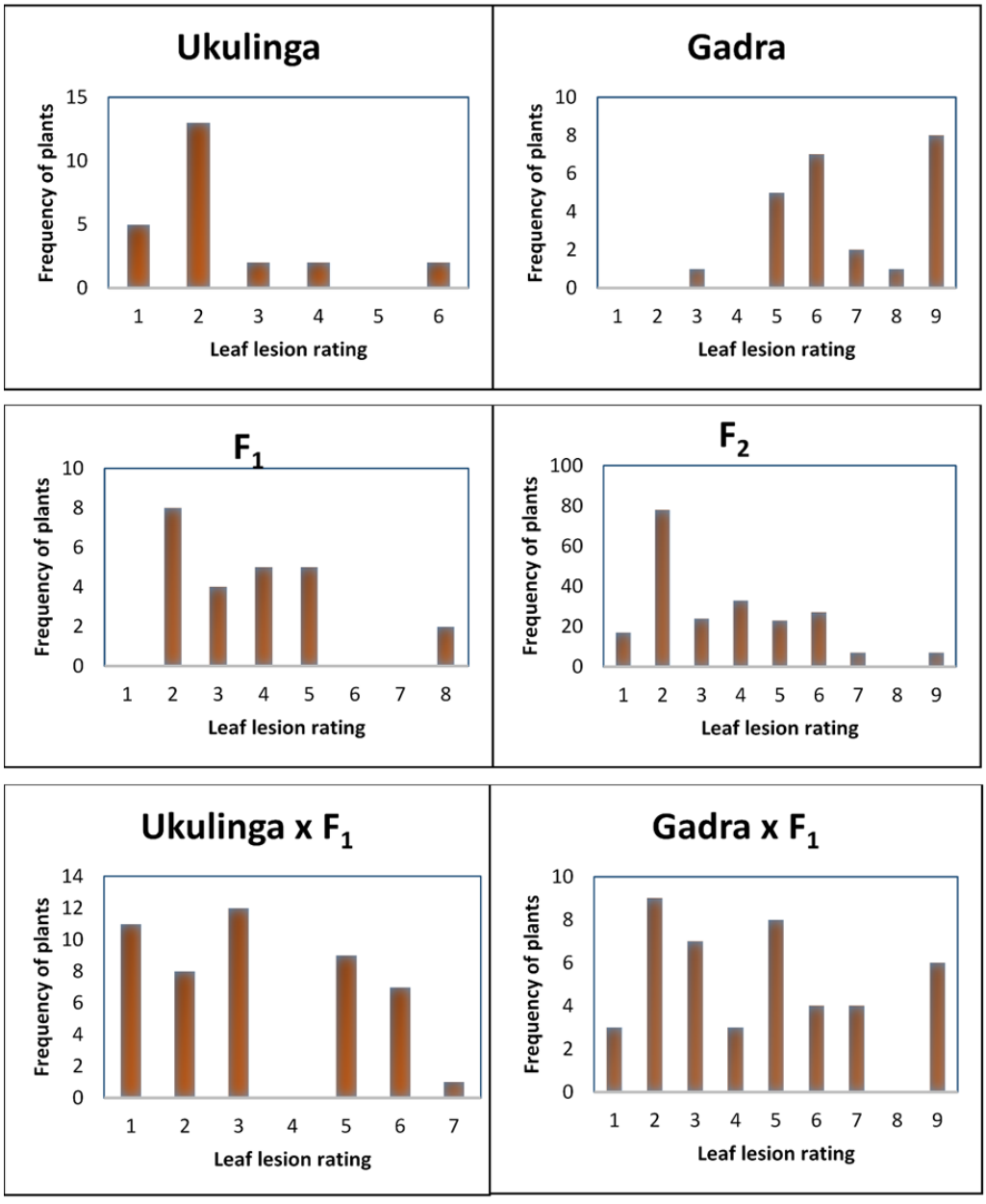

Fig 1. Frequency distributions of the six basic generations of Ukulinga x Gadra on a 1-9 disease severity assessment scale (1= resistant – 9 = susceptible).

Table 2. Generation means (%) of the six generations, LSD (0.05) and standard errors.

Generation	Mean	S.E
P1	2.5c	±1.9
P2	13.5a	±9.5
F_1	3.7cb	±1.8
F_2	4.4cb	±6.4
BCP1	3.8cb	±2.6
BCP2	6.9b	±9.2

Mean values followed by the same letter are not significantly different. based on LSD (0.05).

Table 3. Estimates of genetic effects, standard errors and significance for ALS severity.

Parameter	M	A	d	aa	ad	Dd
ALS	4.4***	3.1±1.3*	-0.7±3.6ns	3.6±3.2ns	-8.6±1.6***	-1.6±6.4ns

m=constant (intercept); a= additive, d= dominance, aa= additive x additive; aa= additive x dominance; dd= dominance x dominance.* significant (P< 0.1); *** highly significant (P< 0.001); ns = not significant (P< 0.05).

Table 4. Variance components and heritabilities of ALS resistance.

Parameter	VP	VE	VG	F (potency)	h^2b	h^2n	Genes
Value	40.7	24.2	16.5	-0.48	0.40	0.33	1.79

Table 5. Generation means, variances and additive-dominance model for linear mixed models.

Generations	[a] coefficient xi1	[d] coefficient xi2	σ^2a	σ^2d	Σad
P1	1	0	0	0	0
P2	-1	0	0	0	0
F_1	0	1	0	0	0
F_2	0	0.5	1	1	0
BCP1	0.5	0.5	0.5	1	-1
BCP2	-0.5	0.5	0.5	1	1

[a]: additive effect; [d]: dominance effect; ad: interaction effect of [a] and [d]; xi1 and xi2 each generation's coefficient.

inheritance of resistance to ALS in this population. Whilst, additive genetic components were significant, the additive x dominance (ad) was highly significant (Table 3) indicating the preponderance of non-additive effects for ALS resistance in this population, which should be taken into consideration when formulating breeding programs. The significance and positive component of additive genetic effects, in turn, suggest that population improvement through selections is feasible in this population. Seemingly, the significance of these epistatic components (ad) provides an insight into the importance of hybridisation, as the results portray that a higher level of ALS resistance can be derived in the form of hybrids. However, since common bean is a self-pollinating crop whereby pure lines are chiefly developed as a consequence of the inadequacy of F1 seed to produce hybrids (Matzinger, 1963; Kelly, 2010), this renders the exploitation of non-additive genetic effects in dry bean of limited applicability.

The low-medium narrow sense (0.33) and moderate broad sense (0.40) heritabilities (Table 4) detected in this study can be attributed to the predominance of non-additive genetic effects, which are not easily fixable, and therefore cannot be passed from parent to offspring, hence the need to delay selections to later generations. The heritability (h2n) values for ALS resistance in previous studies have been reported to be medium-high ranging from 0.45 to ± 0.70 (Tryphone et al., 2012). However, in the populations in those studies, no epistasis were reported and the inheritance was regulated by monogenic and dominant genes, with predominance of additive gene effects.

The number of genes conditioning resistance in this cross was two. Similar results have been obtained in other inheritance studies (Ng'ayu-Wanjau, 2013). Contrary, in some cases a single dominant gene has been reported in ALS inheritance, whilst two or more genes have also been reported (Miklas et al., 2006; Mahuku et al., 2009). In the management of ALS, varieties with durable (intermediate reactions) resistance derived from the accumulation of minor genes, with small additive effects, have been recommended (Ng'ayu-Wanjau et al., 2016). In this study, the results point towards the possibility of exploiting the recurrent backcross strategy, followed by selections in later generations to accumulate favourable alleles in a cultivar. Alternatively, marker-assisted selection would also facilitate effective selections in early generations (Tryphone et al., 2012).

Materials and methods

Plant genetic materials

The experiment was conducted as a preliminary genetic study. Crosses were developed from two parents, Ukulinga (P1) and Gadra (P2), in the greenhouse at CERU, University of Kwazulu-Natal, South Africa (latitude: -29 37' 33.60" S_ longitude: +30 24' 14.70" E). The genetic material investigated in this study are market class bean varieties bred by a local seed company Pro-seed in South Africa and are candidate parental lines for improvement of several agronomic traits, including ALS. The parents are protected by plant breeder's rights and were chosen based on their known levels of resistance and

susceptibility to ALS. Ukulinga cultivar (P1) possesses a high level of resistance to ALS, whilst cultivar Gadra (P2) is susceptible, thus befitting one of the requirements of the generation mean analysis method for divergent parents. Both cultivars are large-seeded red speckled beans, with a determinate bush growth habit type (Type I). To develop the experimental population, seeds from the parents were sown in pots in soil consisting of composted pine bark in a glass house and crosses were made following the hooking or rubbing method described by Muimui (2007). Thereafter, the derived first filial (F1) progeny and its parents, P1 and P2, were planted in August to December 2015 to generate the backcrosses, referred to as BCP1 (Ukulinga x F1) and BCP2 (Gadra x F1), respectively, and the selfed F1 resulted in the second filial (F2) generation. The number of seeds was adequate to perform the GMA, and the seeds of P1, P2, F1, F2, BCP1, and BCP2 were planted in a net-house in July 2016.

Inoculum preparation, application, and disease evaluation

The monosporic cultures of P. griseola were isolated from naturally infected bean leaves, which were collected from the field, placed in moistened petri dishes and incubated to promote sporulation. The isolation and multiplication were done following the standardised method developed by Castellanos et al. (2016). To obtain an adequate amount of inoculum, each monosporic isolate was increased on potato dextrose agar (PDA) (39 g/l) medium and incubated for 12 days. Sterile distilled water was poured on the petri dishes, which were gently scraped with a glass rod to harvest the conidia. The isolates were finally mixed, and a hemacytometer was used to establish the concentration of conidia, which was adjusted to 2 x 104 conidia/ml-1 before their use as conidia suspension for artificial inoculation (Mahuku et al., 2003).
Initially, the inoculum was applied on two sets of fully developed trifoliate leaves using a knapsack sprayer, and the application was repeated twice at the pre-flowering and flowering stages to obtain an even inoculum distribution. Plants were covered in a greenhouse with plastic sheeting for four days after the application to promote humidity and better symptom development. Thereafter, the net-house was kept humid through a fogging system at 65% relative humidity. The disease evaluation was done on the leaves by using a diagrammatic 1-9 CIAT visual rating scale as follows 1 = >1%, 3 = 2%, 5 = 5%, 7 = 10% and 9 = >25%. Plants scored as 1 - 3 were considered resistant, whilst those >4 were susceptible (Pastor-Corrales and Schoonhoven, 1987). Two evaluations were conducted, one at the flowering stage and the other at pod filling, however, only the last rating (scored as percentage leaf area affected) was considered for analysis in this study.

Experimental design

For the GMA experiment, two seeds were sown in each pot filled with composted pine bark and arranged in a randomized complete block design (RCBD) with two replicates in the net-house. To capture as much variation as possible and to increase the precision of the estimates, the experimental size consisted of a large number of F2 plants, and the backcrosses and a small number of the non-segregating (F1 and the parents) populations (Piepho and Möhring, 2010). Darbeshwar (2000) proposed a ratio of 20:50:30 for the non-segregating, F2 and BC generations respectively. Therefore, the number of plants evaluated for each replicate of a generation were: F1 =12; P1 =12; P2 =12; (non-segregating); F2=96 (segregating) and BCP1=48 and BCP2 =48 (segregating).

Genetic and statistical analysis

The analysis of variance, mean comparison and estimations of genetic effects were done using the mixed model approach as outlined in Piepho and Möhring, (2010), using SAS macros in Proc GLM of SAS version 9.3 (SAS Institute Inc., 2011). The segregation analysis was performed in Microsoft Excel based on a classification of either resistant or susceptible to ALS. A full model (including epistasis terms) below and Table 5, adopted from Kearsey and Pooni (1996) and Piepho and Möhring (2010), was used in the mixed model to compute generation means and variances:
$\mu i = m + |a|xi1 + |d|xi2 + |aa|x2i1 + |dd| x2i2 + |ad| x2i1 x2i2$; where:
μ = the genetic value of the ith generation; m is the intercept (constant); a, d, aa, dd, and ad are the genetic effects, while x2i1 and x2i2 are the corresponding coefficients.
The estimates of the additive, dominance and epistatic terms were calculated using the covtest option in SAS. The broad and narrow sense heritabilities were calculated from the estimates of the genetic, additive and phenotypic variances. The following refers: P1 = parent 1, P2 = parent 2 and F1 = first filial generation, F2 = second filial generation, BCP1 backcross to parent 1, BCP2 = backcross to parent 2. Estimates of the following variance components were computed from the formulas as outlined in Gusmini et al. (2007):
Environmental variance σ^2e or $VE=(\sigma^2 (P1) + \sigma^2 (P2) +2[\sigma^2 (F1))/4$
Phenotypic variance $\sigma^2 p=\sigma^2F2$
Genotypic variance $\sigma^2 g$ of $VG=\sigma^2p - \sigma^2e$
Additive variance $VA=[2\sigma^2(F2)] – [\sigma^2(BCP1) + \sigma^2(BCP1)]$
Dominance variance $VD=\sigma^2BCP1+\sigma^2BCP1-\sigma^2F1-\sigma^2e$
Additive x dominance $VAD=½(\sigma^2BCP1 – \sigma^2BCP2)$
Broad sense heritability $h^2b=VG/(VG + VE)$
Narrow sense heritability $h^2n=VA/(VA+VD+VE)$
Average degree of dominance or potency test(F)=(VD/VA) ½
Gene numbers were estimated from Mather and Jinks, (1982) as $((\mu P1-\mu P2)^2)/VA$

Conclusion

In conclusion, the additive and additive x dominance effects were significant in this population and it was established that two complementary genes were involved in the inheritance pattern of ALS. Therefore, varieties with partial and durable resistance to ALS can be developed. Narrow sense heritability was low, which might be partially explained by the involvement of higher-order genetic effects or environmental influences, and therefore it is not prudent to conduct early generation selections. Complementary epistasis was involved in conditioning ALS resistance, however, as a limitation of the GMA, the magnitude of epistasis on the additive and dominance effects could not be estimated under this model, indicating the need for a more complex model.

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