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Genetic characterization of angular leaf spot resistance in selected common bean landraces from Tanzania

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Angular leaf spot disease (ALS) caused by *Pseudocercospora griseola* is one of the most important bean diseases in Tanzania. The bean landraces Beti-10, Nanka, Nanavala and Nkanamna used in this study have been identified as resistant to ALS but the nature of inheritance and mechanisms of resistance against ALS in those potential sources has not been elucidated. This information is crucial and a necessary first step for a successful breeding programme. The objective of this work was to study the inheritance of ALS resistance in those landraces and to identify the mechanisms of genetic resistance using Simple Sequence Repeat (SSR) markers. Crosses were made between resistant bean landraces and a susceptible bean cv Kablanketi. The parents, F_1, F_2 and backcrosses derived plants were used for inheritance studies and for molecular marker screening using 30 SSR markers. Results indicate that, a single dominant gene control resistance against ALS in each of the four landraces; also the SSR marker Pv-ag004 was found to be polymorphic between Beti-10 and Kablanketi and linked to the disease resistance. The resistance were validated by checking the F_2 population of the cross between Kablanketi × Beti-10. Therefore, since marker Pv-ag004 is polymorphic and linked to ALS resistance, the Beti-10 landrace might be a potential source of ALS resistance. However, a detailed study with more markers need to be done on these landraces with a view to opening the possibilities of identifying new markers linked to ALS resistance and mapping of genes associated with resistance to ALS.

Key words: *Phaseolus vulgaris*, Inheritance, ALS, SSR, *Pseudocercospora griseola*.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important grain legume for direct human consumption and it also provides farm households with both a source of income and food for nutrition (Wortmann et al., 1998). Despite the importance of the common bean crop, diseases especially fungal diseases are among the main problem

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Abbreviations: ALS, Angular leaf spot; **SSR,** simple sequence repeat; **PCR,** polymerase chain reaction.

reducing bean yield worldwide that limit the genetic potential of the crop (de Jesus et al., 2001). The angular leaf spot disease can reduce yield as much as 50 to 80% when susceptible cultivar is grown (de Jesus et al., 2001). It has been estimated that ALS disease cause yield loss of 389 900 t/year in sub-Saharan Africa, 263 600 t/year in East Africa, Tanzania inclusive and 125 300 t/year in Southern Africa (Wortmann et al., 1998). Therefore, ALS is currently the most economically important disease that affects common bean production (de Jesus et al., 2001) and widely distributed disease (Stenglein et al., 2003). In Tanzania the disease is endemic in low to high altitude bean producing zones (Tryphone et al., 2012). Angular leaf spot affects all above ground parts of the bean plant but most notable symptoms are on leaves, where the pathogen induces premature leaf defoliation that results in shrivelled seeds of reduced size and quality (Saettler, 1991). At the same time the lesions on leave causes severe defoliation and decreased leaf area index, which has implication on crop performance.

Breeding for resistance against this disease has been complicated as durable resistance is hardly achievable due to variability of the pathogen and therefore the need to pyramid more than one gene from diverse background to overcome the variability of the pathogen (Pastor-Corrales et al., 1998). Although, the trait is typically vertical as well as horizontally controlled and display a strong interaction with the environment (Kelly and Miklas, 1998; Wortmann et al., 1998). Therefore, selection and identification of resistant cultivar as well as the mechanism of resistance in resistant cultivars is paramount to deal with such a disease.

The large variability of the pathogen necessitates the constant identification and characterization of resistance genes, understanding the genetics of the reaction to the pathogen (Borel et al., 2011) and subsequent development of resistant cultivars so as to minimize the risk associated with epidemics. Information about genetic control is very useful, because it helps breeders to choose the most efficient strategies for development of resistant and productive cultivars. Through bean breeding programme at Sokoine University of Agriculture, local cultivars resistant to ALS pathogen were identified under phenotypic screening in the field. The resistant cultivars include Nkanamna, Beti 10, Nanavala and Nanka. These cultivars are well adapted to Tanzanian bean growing ecologies and succumb less to infection by ALS (Fivawo and Msolla, 2011). Although, sources of resistance ALS have been identified, the nature and inheritance of the resistance in these sources have not been elucidated. This information is very useful in the breeding programmes and as such the need for characterization towards the identification of mechanisms of resistance to ALS is imperative. Furthermore, with the need to simplify the use of these cultivars, molecular markers that are linked to the disease and that are polymorphic should be found, hence the need for molecular marker screening.

Molecular markers linked to genes that control resistance to ALS disease are available (Nietsche et al., 2000; Queiroz et al., 2004; Miklas, 2005). Among the useful molecular markers is the Simple Sequence Repeats (SSR) which has been extensively used to identify angular leaf spot disease resistance genes in common bean (Blair et al., 2000). They provide several advantages over other markers when applied in plant breeding programmes as they are based on the polymerase chain reaction (PCR) technique; they are co-dominant, represent single loci and can detect high level of polymorphism and reproducibility. The SSR are also closer to the resistance alleles of some angular leaf spot genes (Collard and Mackill, 2008). The relatively low recombination frequency between the SSR and the locus can be characterized as useful marker for indirect selection. For example, the expected frequency of susceptible plants selected as resistant in F_2 population is 4.5% (Silva et al., 2003). Therefore, parents and the F_2 populations can be screened to ascertain the resistance and identification of promising markers for ALS resistance. The objective of this work was to study the inheritance of resistance to ALS in four landraces and identify genetic factors for resistance using DNA molecular markers.

MATERIALS AND METHODS

Plant materials

The susceptible but adapted bean parent, Kablanketi was collected from farmers' fields in Mbeya, Region. Landraces Nanka, Nanavala, Beti 10 and Mkanamna were obtained from SUA bean germplasm collection and were characterized as ALS resistant both in screen house and in the field (Fivawo and Msolla, 2011).

Population development

The bean cv Kablanketi was crossed with the resistant parents; Nanka, Beti 10, Nkanamna and Nanavala to generate F_1 populations. The F_1 plants were backcrossed to the susceptible and resistant landraces to generate backcrosses. The remaining F_1 plants were self pollinated to produce the F_2 population.

Plant evaluation

The seeds from the parents, F_1, F_2, and backcross populations were planted in greenhouse in pots containing sterilized forest soil for evaluation of their resistance to ALS. Local isolates of *Pseudocercospora griseola* were obtained from bean leaves collected from bean plants growing at the SUAs-Crop Museum, and isolation, inoculum preparation and inoculation were done as described by Tryphone et al. (2012). The F2 populations from each cross of the susceptible x resistant parents were planted in the screen house and inoculated with *P. griseola* suspensions for ALS disease evaluation. The plants were evaluated for the reaction to ALS in the screen house following the CIAT evaluation scale of 1-9 (Van Schoonhoven and Pastor-Corrales, 1987).

Table 1. Segregation for ALS resistance of F_2 populations derived from the crosses between Kablanketi and resistant parents.

Crosses	No. of plants assessed	Expected ratio	Observed ratio	χ^2	*P*
Kablanketi x Nanka	135	3:1	98:37	0.417	0.518
Kablanketi x Beti 10	120	3:1	87:33	0.400	0.527
Kablanketi x Nanavala	140	3:1	102:38	0.343	0.558
Kablanketi x Nkanamna	138	3:1	105:33	0.087	0.768

Extraction and amplification of DNA

Total genomic DNA extraction was done as described by Delapotar et al. (2003) with some modifications as follows. Sodium acetate was used instead of potassium acetate, incubation stage was done at 60°C for 30 min. instead of 65°C for 45 min., centrifuging was done at 16 000 g for 10 min instead of 20 min at 3 000 g. For DNA amplification, PCR reaction was performed and different annealing temperatures were used depending on the primer used.

Gel electrophoresis and documentation

Amplification products were separated through electrophoresis migration in a 3% agarose gel, 1X TBE (Tris-Borate EDTA) buffer. The gel was stained in Ethidium Bromide at 0.5 µg/ml for 30 min and de-stained for 30 min by using distilled water. The bands present on the gel were observed and the mounted digital camera was used to capture the amplified fragments for documentation and scoring.

ALS characterization

Screening for the markers closely linked to the ALS resistance in common bean landraces (Beti-10, Nanka, Nanavala and Nkanamna) was done by genotyping the parents using 30 SSR markers. Two DNA pools of ALS resistant and ALS susceptible individuals [landrace cultivar (resistant) × Kablanketi (susceptible)] F_2 population were constituted using 10 plants of each pool to make two samples. Then, the individual DNA samples were made for both susceptible and resistant plants. Those DNA samples were used to detect the linked markers to the ALS resistance. Thirty microsatellites markers were screened in for polymorphism among parents and the F_2 populations.

Data collection and analysis

Data obtained on disease score were processed and analysed by the 14th Edition GenStat statistical package. The chi-square test was used to test the phenotypic segregation of the populations from the crosses between Kablanketi and the respective parents for the inheritance study. The heritability was calculated from variances as the distribution of the score data for ALS disease.

RESULTS AND DISCUSSION

Inheritance of ALS resistance

Segregation analysis of the F_2 generation for the crosses between Kablanketi and Beti 10, Nanka, Nkanamna and Nanavala landraces, showed a ratio of three resistant plants to one susceptible (3:1). For all cases, the Chi-square test supported the occurrence of monogenic inheritance where the dominant allele is responsible for the resistance (Table 1). Analysis of F_2 and Backcross generations resulted from the crosses using these resistant parents showed that, genes for resistance have been successfully transferred to the adapted susceptible parent in different proportions for the different populations. Having single and dominant gene control for the disease resistance, it is possible to introgress these genes for resistance into susceptible preferred varieties. The F_2 populations segregated in 3: 1 (Resistance to Susceptible) ratio showing monogenic inheritance, with resistance being due to one dominant allele. Similar results have been observed from other resistance sources like Mexico 54 (Sartorato et al., 1999; Mahuku et al., 2004; Tryphone et al., 2012); AND 277 (Carvalho et al., 1998) and MAR 2 (Ferreira et al., 2000). The identification of these genes is extremely important to bean breeding programmes aiming at developing cultivars with durable resistance to this pathogen.

However, inheritance of the common bean resistance to ALS has shown to be complex in some situations. The genetic control of reaction in some lines was observed to vary according to the susceptible parent used. A single gene with dominant allele was observed for the resistance to the pathotype 63-19 when bean line Mexico 54 was crossed with the Ruda cultivar (Mesoamerican) (Sartorato et al., 1999). Mahuku et al. (2002) described the resistance of Mexico 54 to pathotype 31-55 as a single gene with resistance being due to the recessive allele, when crossed with a snap bean cultivar. Another fact that stands out is the continuous response to phenotypic recurrent selection for resistance to the angular leaf spot (Amaro et al., 2007). In the genetic control of resistance to the angular leaf spot minor genes could be involved that were environmentally influenced, in addition to major genes or modifier genes.

Heritability studies for ALS resistance

Narrow sense heritability was estimated for the crosses and results were as shown in Table 2. The heritability in narrow sense ranged from 0.46 for a cross between Kablanketi x Nkanamna to 0.73 for Kablanketi x Nanka. Estimating narrow sense heritability among crosses made

Table 2. Heritability in narrow sense estimated for the crosses between Kablanketi × 4 landraces.

Population	Organ assessed	Estimated narrow sense heritability (h^2)
Kablanketi x Nanka	Leaves	0.73
Kablanketi x Beti 10	Leaves	0.68
Kablanketi x Nanavala	Leaves	0.53
Kablanketi x Nkanamuna	Leaves	0.46

with Kablanketi and Beti 10, Nanka, Nkanamna and Nanavala cutivars are moderate to high with regard to ALS resistance implying that these donor parents are suitable parents to be used for improvement of ALS resistance breeding programmes compared to the other parents. In a study conducted by Borel et al. (2011) heritability in narrow sense estimates ranged from 0.19 to 0.68 in common bean. The heritability is not immutable; it depends on the population and environmental conditions in which individuals were grown. The degree to which the variability of a quantitative character may be transmitted to the progeny is referred to as heritability. Heritability is among the most important genetic parameters in plant breeding (Allard, 1999). Falconer and Mackay (1996) categorized heritability estimates as low or weak (0 to 0.2), moderate or medium (0.21 to 0.39) and high or strong (0.4 to 1.0). However, low heritability estimates suggest that selection in early generation would not be effective since no improvement would result for the trait, this could be caused by environmental effects (Amaro et al., 2007). High heritability estimates indicate that the additive gene effects play an important role for that trait. It implies that this trait or character was not largely influenced by environment. Traits with relatively high heritability or additive gene variance have been reported to respond highly to selection and cross breeding (Falconer and Mackay, 1996). The potential of a cross in common bean can be predicted on the basis of the performance of parents or that of the progeny of early generation. Thus, estimating heritability is important because it enables the breeder to base selection on the phenotypic performance for improving a particular trait.

Angular leaf spot resistance and molecular markers

Among the four resistant landraces viz. Nanka, Beti-10, Nanavala and Nkanamna, Beti-10 was selected for molecular screening for ALS resistance. The choice of Beti 10 was due to the results from marker validation among the parents that showed Beti 10 to be more polymorphic than others Out of 30 SSR primer pairs, one SSR (Pv-ag004) was polymorphic in Beti-10 and Kablanketi (Figure 1). Co-segregation analysis of the polymorphic marker and disease reaction in the F_2 population derived from Kablanketi x Beti-10 confirmed that this SSR marker is associated with resistance to ALS in landrace Beti-10 and F2 individuals. There is a consistence of phenotypic expression and marker based on Pv-ag004. This SSR marker is polymorphic in parents with 270 bp for resistant parent and 240 bp for susceptible parent (Figure 1). The SSR Pv-ag004 segregates with resistant individuals. From previous studies by Mahuku et al. (2009), the microsatellite marker Pv-ag004 segregated with resistant gene Phg_{G5686A}, linked with ALS resistance on linkage group B04 of the consensus molecular linkage map of common bean (Mahuku et al., 2009). This marker is co-dominant and polymorphic to both resistant and susceptible parent. Pv-ag004 marker amplified and it happened to be polymorphic between Beti-10 and Kablanketi, this can be explained that the Beti-10 may carry the gene Phg_{G5686A} or a similar gene to *P. griseola* which is resistance to ALS. Therefore, Beti-10 is identified as the potential donor of resistance to ALS and this is explained by 68% heritability (Table 2).

However, it is not apparent whether these genes are the same or different from those present in Mexico 54 or MAR 2. It has been observed that Beti-10 was resistant to many isolates of *P. griseola* that were to cause disease on other genotypes (Fivawo and Msolla, 2011), signifying that the resistance genes in the two cultivars are distinct. Nevertheless, mapping of marker linked to the resistant parent will provide a better understanding of the relationship between of ALS resistance from these sources. This information will allow breeders to make an informed selection of resistant parents to use in their programmes and avoid over deployment of a single locus from different sources for resistance genes. Also, to avoid the loss of these genes during selection, it is essential to identify molecular markers tightly linked to the genes of interest that will permit the identification and detection of these genes and permit their use in marker-assisted selection (Kelly, 1995). In absence of allelism tests, locating the markers on the linkage map would help to determine gene independence and their relationship to genes from other sources of resistance (Miklas et al., 2006). Such information will help breeders to make informed selection of resistance genes to use in their breeding programmes.

Integrating molecular markers in plant breeding has the potential to increase the efficiency of crop breeding above that reached by classical breeding methods alone. The

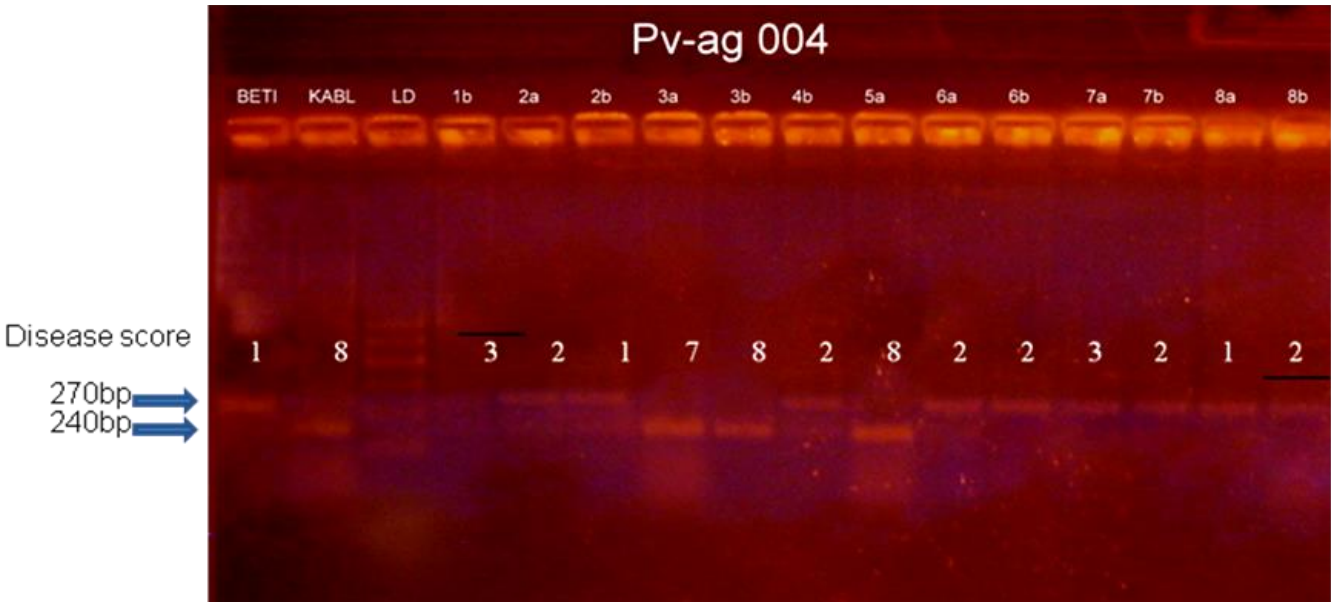

Figure 1. Amplified products for two parents and F_2 individuals with Pv-ag004 SSR marker. Lane 1, resistant parent (Beti-10), Lane 2, susceptible parent (Kablanketi) Lane 100bp ladder and Lane 4-16 are the segregating F_2 individuals.

efficiency of MAS is a function of the distance between the gene of interest and the markers (Mahuku et al., 2009). For a marker to be useful in molecular marker assisted breeding, it must be located within 5 cM of the resistance gene but ideally, a marker that is <1 cM is the most useful (Kelly and Miklas, 1998). The SSR Pv-ag004 linked to Phg_{G5686A} has the greatest potential for molecular marker assisted breeding as it is mapped 0.0cM from the resistance gene (Mahuku et al., 2009).

Conclusion

The inheritance of angular leaf spot disease resistance in common bean landraces, Beti-10, Nanka, Nanavala and Nkanamna were established. The results indicate that one single dominant gene controls the resistance in the studied landraces. Also, the heritability for angular leaf spot trait in the landraces was high indicating the additive genetic effect for that trait. SSR marker Pv-ag004 was found to be linked to resistance in Beti-10. This landrace can be used as resistant parent in pyramiding ALS resistant genes in the farmers preferred varieties. This calls for detailed analysis of the mapping of all the identified resistant parents for resistance with many and robust markers. Furthermore, this may come-up with more new markers because few molecular markers have been developed for a few ALS resistance genes. At the same time, to come up with the clear picture of the genes present, whether they are similar or distinct to already existing ones, allelism test is important for resistant parents. Also, there should be a way of comparing the identified landrace resistant parents with Mexico 54 because it is known to be resistant to all African isolates of ALS.

Conflict of interests

The author(s) did not declare any conflict of interest.

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