International Journal of Agriculture, Environment and Bioresearch

Vol. 3, No. 01; 2018

ISSN: 2456-8643

INVESTIGATION OF GENE ACTION FOR RESISTANCE TO EARLY LEAF SPOT OF GROUNDNUT

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ABSTRACT

Groundnut (Arachishypogaea L.) is an important global oilseed crop and a major source of protein and vitamins in many rural areas of Africa. In Zambia, the production of groundnut is limited by several factors, among which Early Leaf Spot (ELS) caused by Cercosporaarachidicola Hori, is a major destructive disease. Development of resistant varieties to ELS remains the most viable disease management strategy. The objective of this study was to investigate the type of gene action conditioning resistance to C. arachidicolain order to generate information for breeding of ELS resistant groundnut varieties in Zambia. The field work was conducted at Chitedze Research Station in Malawi which is a known hot spot for groundnut foliar diseases. A recombinant inbred line (RIL) population developed from a biparental cross (Robut 33-1, susceptible and ICGV-SM 95714, resistant) and consisting of 110 F8 RILs was used in the study. Data for analysis was generated by phenotyping of the RIL population and this was conducted during the 2013/14 season under field conditions supplemented by irrigation. To ensure that there was disease infection in the experimental field, diseased debris was used as primary inoculums. The nature of gene action was established by generating a distribution curve while the Chi-square test was used to confirm the generation level of the population. These were done using the area under disease progress curves (AUDPCs) and the results suggested additive gene action. The study thus concluded that the gene action conditioning resistance to ELS was additive and breeding schemes such as pedigree and single seed decent can be used in breeding for resistance to Cercosporaarachidicola.

Keywords: Cercosporaarachidicola Hori, Recombinant Inbred Line, Gene Action, Area Under Disease Progress Curves.

INTRODUCTION

Groundnut (Arachishypogaea L.) is a legume which is grown as an annual crop and is one of the most important food and oilseed crops in the tropical and subtropical areas of the world. In Zambia, groundnut plays an important role in the lives of many rural and urban households. The legume provides a cheap source of edible oil, protein and vitamins B, E and K. The nutrients found in groundnut supplement diets where maize, rice and cassava are the major energy foods (Asiedu, 2010,Monyoet al., 2012). However, the production of groundnut is hampered by diseases such as Early Leaf Spot (ELS) caused by Cercosporaarachidicola Hori (Subrahmanyam,

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1997). The disease is among disastrous diseases affecting groundnut production and causes yield losses of between 32 to 68% (McDonald et al., 1985; Gopal, 2006). The disease causes sub-circular lesions, which appear dark brown on the upper leaflet surface (where most sporulation occurs), and a lighter shade of brown on the lower leaflet surface (Shew, 2012). ELS has been reported in the main groundnut production areas of Zambia including eastern, central and southern regions, where up to 60% loss in kernel yield (Chalabesa2002) has been observed. Besides the yield losses, ELS disease has an adverse effect on seed quality and grade characteristics. It deteriorates the quality of plant biomass and thus renders the fodder unsuitable as animal feed (Nutter and Shokes, 1995).

To minimize losses due to ELS, several methods of control have been developed, which include host plant resistance, cultural control, biological control and chemical control (Ghewandeet al., 1993, Subrahmanyamet al., 1997, Pandeet al., 2001).Although screening for ELS resistance in Zambia begun in 1981, the commonly cultivated and preferred groundnut varieties remain susceptible to the disease (Sandhu et. al., 1985, Kannaiyan and Haciwa, 1990, Ross and Klerk, 2012). Development of resistant varieties is the long term and economical method of managing the disease. Resistant cultivars are also considered the best strategy to surmount additional costs of production and hazardous effect of fungicides on the soil and environment (Okelloet al., 2013;Debele and Ayalew, 2014).Previous breeding efforts towards the development of ELS resistant lines have not been very successful in enhancing genetic resistance, especially due to the complexity of the groundnut genome (Khedikaret al., 2010).Development of high yielding cultivars with resistance to ELS is an important breeding priority to reduce impact of the disease and increase groundnut production. An understanding of the nature of gene action conditioning resistance to Cercosporaarachidicolais likely to contribute in enhancing breeding for the resistance trait(Zongoet al., 2017).

This research made use of phenotyping data to investigate the type of gene action (i.e. the action and interaction of genes) conditioning resistance to Cercosporaarachidicolain groundnut. A good knowledge on the genetics of resistance will enable groundnut breeders to design an efficient breeding strategy in order to develop early leaf spot resistant groundnut varieties.

2. Materials And Methods

2.1Experiment Site and Plant Material

The research was conducted at Chitedze Agricultural Research Station in Malawi. The station is located on longitude 13o 85' S and latitude 33o 38' E and lies at an altitude of 1146 m above sea level. It has a mean annual temperature of 20oC and receives a mean annual rainfall of 892 mm with 85% falling between November and March. A recombinant inbred line (RIL) mapping population of 110families was developed at ICRISAT Malawi between ELS resistant line, ICGV-SM 95714 and a susceptible genotype, Robut 33-1. Both parents are early maturingValencia types but Robut 33-1 was obtained from India while ICGV-SM 95714 was developed at ICRISAT-Malawi and was used as the pollen parent. A summary of the parental traits is provided in Table 1. The F1s were selfed to produce F2s, which were advanced using single seed descent

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(SSD) until F8 and this was done between 2005 and 2014. For initial crosses, parental lines were grown under glasshouse conditions at Chitedze Agricultural Research Station in Malawi.

Table 1: Summary of traits of the parents used to create the mapping population

	Trait/Aspect	Robut 33-1	ICGV-SM 95714
1	Origin	India	Breeding line developed in Malawi
2	Type	Virginia	Valencia
3	Morphological description	Profuse branching, alternate flowering pattern, medium sized pods	3 seeded
4	Yield	1,200-1,500 kg/ha	1,700-2,000kg/ha
5	Seed colour	Tan	Tan
6	Maturity	Early (115 days)	Early (90-100 days)
7	Disease resistance	Bud necrosis	Early leaf spot
8	Disease susceptibility	Early leaf spot	-
9	Seed dormancy	Long	No seed dormancy
10	Sex in Cross	Female	Male
11	Countries in which released as a variety	India as Kadiri 3.	Not yet released but used as an ELS resistant source

2.2 Phenotyping of RIL Population for ELS Resistance

Both RILs and parental lines were grown under natural field conditions at a known hotspot for foliar diseases in Chitedze, Malawi during the 2013/14 rainy seasons. After randomization, seeds of each of the 110 RIL families were sown in single 3-m rows, alongside the parental lines using intra-row spacing of 20cm and a rate of 2 seeds per station. Weeding was done by hand and the plants were regularly irrigated to maintain the required soil moisture throughout the growing season.

Diseased plant debris from the previous season was used as inoculum. The inoculum production was done in a nursery using a highly ELS susceptible variety, JL24. The plant debris that constituted the inoculum was obtained from diseased plants that were harvested at 4 months after planting and kept in a structure with high humidity and temperature to ensure viability of the pathogen. Before application, the plant debris containing the inoculum was cut into small pieces to facilitate uniform spreading. The materials were then uniformly spread along the rows in equal measures. The inoculation was done 25 days after planting. Irrigating the field using sprinklers created favorable conditions for disease development.

2.3 Data Collection

Data collection on response to ELS infection commenced 60 days after planting. Disease scoring was conducted by visual assessment using a 1-9 scale(Chiteka, 1988) as shown in Table 2, where 1 refers to highly resistant and 9 is highly susceptible. Scoring was done at 60, 75, 90 and 100

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days after planting. For each RIL family, initial plant stand was noted and four plants were selected randomly and tagged. Data was collected from the tagged plantsfor each RIL family. A rating of three or lower was regarded as an indication of resistance, a rating of between three and four as an indication of tolerance and a rating of five to nine as susceptible (Pretorius, 2006).

Table2: ELS Scoring Scale

Leaf spot Score[1]	Description	Disease severity (%)
1	No disease	0
2	Lesions largely on lower leaves; no defoliation	1-5
3	Lesions largely on lower leaves; very few lesions on middle leaves; defoliation of some leaflets evident on lower leaves	6-10
4	Lesions on lower and middle leaves, but severe on lower leaves; defoliation of some leaflets evident on lower leaves	11-20
5	Lesions on all lower and middle leaves; over 50% defoliation of lower leaves	21-30
6	Lesions severe on lower and middle leaves; lesions on top leaves but less severe; extensive defoliation of lower leaves; defoliation of some leaflets evident on middle leaves	31-40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and some middle leaves	41-60
8	Defoliation of all lower and middle leaves; lesions severe on top leaves and some defoliation of top leaves evident	61-80
9	Defoliation of almost all leaves leaving bare stems; some leaflets may be present, but with severe leaf spots.	80-100

[1]≤3 = resistance, 3 - 4 = tolerance, 5 - 9 = susceptible

2.4 Data Analysis

Analysis of phenotypic data involved the calculation of the area under disease progress curves (AUDPCs) and the generation of the distribution curve. AUDPCs for each RIL family and the parental lines was calculated using the trapezoidal method below:

$$AUDPC = \sum_{i=1}^{n-1} \left[\frac{X_i + X_{i+1}}{2}\right](t_{i+1} - t_1)$$

whereX_i is the disease incidence, n is the number of evaluations and $(t_{i+1} - t_i)$ is the time interval between two consecutive evaluations (Campbell and Madden, 1990).

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To verify the generation level of the biparental population, a Chi-square test was performed using the equation stated below:

$$\chi^2 = \sum \frac{(fo - fe)^2}{fe}$$

where*fo* = observed sample frequency and *fe* = expected frequency (Acquaah, 2007). The Chi-square test was used to determine whether the phenotypic data conforms to the expected Mendelian ratios. In a biparental population under consideration (F_8), segregation would have stabilized and the expected genotype ratio is 1:1 (Collard*et al.*, 2005). In performing the test, the two extreme ends of the distribution were categorized as resistant and susceptible while the categories in-between were either medium susceptible or medium resistant. This was done on the basis that the RIL population was developed from two parents contrasting for resistance to the disease.

In order to generate a distribution curve, the AUDPCs were put into categories and the shape of the distribution curve was used to suggest the nature of gene inheritance for resistance to the pathogen.

3.0 RESULTS

Average disease scores of the RILs ranged from 2 to 8 suggesting the presence of both susceptible and resistant phenotypes. The AUDPCs which were calculated from mean scores ranged from 72.5 to 225 score-days with a mean of 147.75 and a population standard deviation of 36.69. Based on the AUDPCs, the RIL families were categorized as resistant or susceptible to facilitate performance of the Chi-square Test (Table 3). The results showed that the segregation of the F8 RILs to ELS followed the Mendelian ratio of 1:1.Frequency distribution of the AUDPCs indicated a near-normal distribution pattern (Figure 1).

Table 3: Observed categories of areas under Disease Progress Curves (AUDPCs)

AUDPC Range	Frequency	Phenotype Category	Chi-square Category
70_90	10	Resistant	57 (Resistant)
91_110	12		
111_130	12	Medium Resistant	
131_150	23		
151_170	24	Medium Susceptible	53 (Susceptible)
171_190	17		
191_210	6	Susceptible	
211_230	6		

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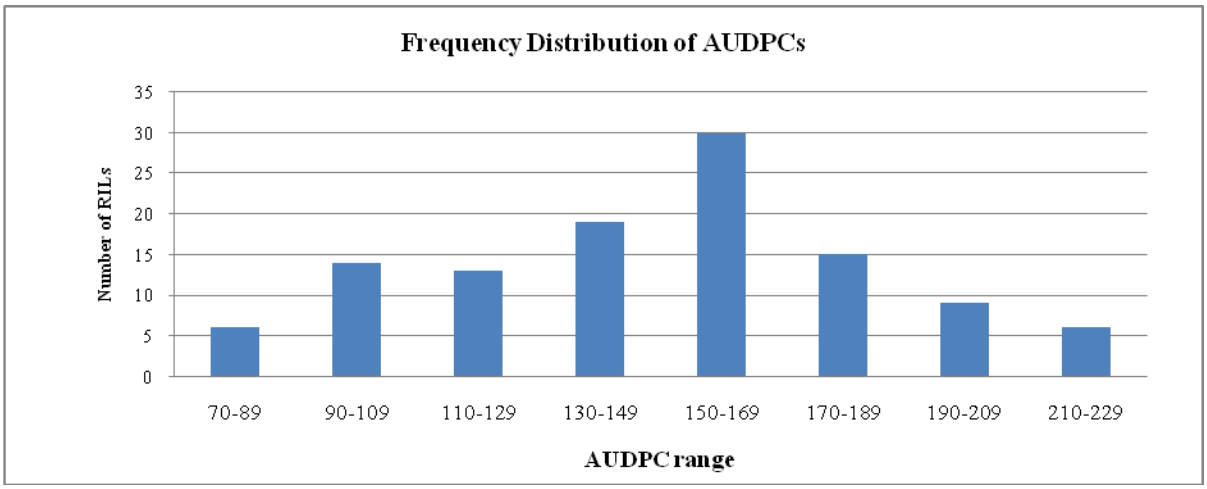

Figure 1: Frequency distribution of Area Under Disease Progress Curve (AUDPCs)in the RIL population

4.0 DISCUSSION

In this study, the nature of gene action conditioning resistance to Cercosporaarachidicola, the pathogen that causes Early Leaf Spot (ELS) disease in groundnut was investigated. Although ELS occurs naturally in the field, it does not spread uniformly. Therefore, artificial inoculation which was done 25 days after planting was aimed at ensuring that there is uniform spreading of the disease so as to produce reliable phenotypic data. Average disease scores of the RILs ranged from 2 to 8 on a scale of 1 to 9 with 1 being highly resistant and 9 being highly susceptible (Chitekaet al., 1988). The presence of average scores that are close to the two extremes (resistant and susceptible) indicates the presence of both susceptible and resistant phenotypes in the population. This is expected as the population is from a biparental cross of parents contrasting in disease resistance. Furthermore, with the hypothesis that resistance to the disease is quantitative, the phenotypic data was expected to be a continuous variation and not discrete classes of resistant and susceptible lines (Conner and Hartl, 2004). In this study, the phenotypic data was put into classes purely for the purpose of conducting the Chisquare test so as to verify the generation level of the biparental population.

The phenotypic data obtained as AUDPCs, had a range of 72.5 to 225 score-days with a mean of 147.75. The data showed that the RILs were distributed continuously over the range of the AUDPC values in an approximately normal shape suggesting a quantitative nature of resistance in the (Robut 33-1 × ICGV-SM 95714) population. This suggests that the trait may be controlled by polygenes. Earlier studies using generation means to investigate gene action indicated that additive gene action was significant for both early and late leaf spot disease resistance (Anderson, 1991). Similar results on importance of additive gene action were reported for leaf spot diseases of groundnut including ELS (Padmajaetal., 2013; Wambiet al., 2015). Zongo (2017) also found that additive gene action played a significant role for inheritance of components of ELS resistance and that the environment had less influence in expression of ELS.

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The phenotypic results also showed that the segregation of the F8 RILs to the disease followed the Mendelian ratio of 1:1. This is the expected ratio in RILs at such a generation because segregation would have stabilized (Collard et al., 2005). This confirmed that the population was at a higher generation and therefore, the generation was ideal for investigating gene action using phenotypic data (Pretorius, 2006). However, the ratio of 1:1 which indicates the presence of distinct groups may not be used to interpret the gene action as being dominant because the categorization was done for the purpose of performing the Chi-square test.

The fact that additive gene action was found to determine the nature of gene action, implies that this trait can be transferred from parent to offspring. Breeding schemes such as pedigree and single seed decent can be used (Singh, 2009; Acquaah, 2007) in breeding for resistance to Cercosporaarachidicola.

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