MAKERERE UNIVERSITY

INHERITANCE OF LATE LEAF SPOT (*Phaeoisariopsis personata*) RESISTANCE IN VALENCIA GROUNDNUTS

BY

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DECLARATION

I WAMBI WILBER, declare that this thesis is my original research and no part of it has ever been presented at any University or tertiary institute for any award.

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DEDICATION

To my mother, Namulondo Joyce, Late father Kalumula Christopher, my brothers; Kagolobya Godfrey, Kalumula Ronald, Kalumula Pady, Kalumula Fred, Magero Hennery and sisters; Jane Edikwa, Nambi Justine, Namwembe Monica, Nakairu Judith, Womunafu Juliet and Kaina Flora.

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ABSTRACT

Late leaf spot (LLS) *Phaeoisariopsis personata* (Berk. and Curtis) Deighton is one of the most important foliar diseases of groundnut worldwide. Although fungicide treatment is effective, it is uneconomical to use because of high cost. The deployment of resistant cultivars is a better option to control LLS disease in groundnut. The study was initiated to (i) determine heritability for LLS resistance and (ii) the type of gene action controlling LLS resistance. Breeding materials including F_1, F_2, and F_1backcrosses to susceptible BC_1P_1 and resistant parents BC_1P_2 along with their respective parental lines of crosses between NuMex-M_3× ICGV-SM 02501, Valencia C × ICGV-SM 02501, Valencia C × SGV-07009 and Redbeauty × ICGV-SM 03590 were evaluated in RCBD with three replications at the experimental field at National Semi-Arid Resources Research Institute (NaSARRI), Serere Uganda. Spreader row technique was used to maximize leaf spot inoculum pressure under natural conditions and late leaf spot severity was assessed using a modified nine point scale (1-9). Analysis of variance was performed for generations of each cross, phenotypic and genotypic coefficients of variability, and heritability were estimated using variance components. A joint scaling test was used to determine the nature and magnitude of gene effects controlling LLS resistance. It was observed that all the crosses had highly significant differences among generations for late leaf spot resistance. The Phenotypic coefficients of variability **(**PCV) estimates were high in NuMex-M_3 × ICGV-SM 02501 (28.82%) and Valencia C × ICGV-SM 02501 (24.51%) crosses and moderate in Redbeauty × ICGV-SM 03590 (16.89%) cross. The genetic coefficient of variation (GCV) estimates were high in cross NuMex-M_3 × ICGV-SM 02501 (23.13%), moderate in Valencia C × ICGV-SM 02501 (15.87%) and low in cross Redbeauty × ICGV-SM 03590 (9.50%). Broad-sense heritability estimates for LLS disease score were 32%, 37% and 64%, respectively, for Redbeauty × ICGV-SM 03590, Valencia C × ICGV-SM 02501 and NuMex-M_3 × ICGV-SM 02501crosses. Narrow-sense heritability estimates were 12%, 27% and 36%, for Redbeauty × ICGV-SM 03590, Valencia C × ICGV-SM 02501 and NuMex-M_3 × ICGV-SM 02501 crosses respectively while Genetic advance as percent of mean (GAM) were low in cross Redbeauty × ICGV-SM 03590 (4.17%), moderate in Valencia C × ICGV-SM 02501 (13.63%) and high in NuMex-M_3 × ICGV-SM 02501 (21.37%). A simple additive- dominance model was adequate for the inheritance of resistance to LLS in NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM

02501and Redbeauty × ICGV-SM 03590 crosses. However, the additive effects were more important than dominance. Based on the observed results it can be concluded that selection for LLS resistance disease score is possible. However, the amount of variation and magnitude of gene effects depended on the genetic backgrounds of the materials used in the study. The best strategy for obtaining LLS resistant genotypes is selection of the disease score trait in initial inbreeding generations (F_3 or F_4) for the cross between NuMeX-M_3× ICGV-SM 02501, followed by selection in the following generations with higher inbreeding levels in other crosses.

CHAPTER ONE

INTRODUCTION

1.1Background

The cultivated groundnut (*Arachis hypogaea* L) ($2n = 4x = 40$) is a legume belonging to the family *Leguminosae* (Krapovickas and Gregory, 1994). It is grown primarily as an oil crop, and consumed throughout the tropics and warm temperate regions of the world as fresh or processed nuts. The crop is native to South America Kochert *et al.* (1996), and largely grown in Asia, Africa and America in over 100 countries, with a world total production of 38.6 million metric tons on 21.8 million hectares (FAO, 2013). Africa contributed about 24.4% of world production where Uganda was ranked number eight producing only 175,000 tons on 236,000 ha in 2011(FAO, 2013).

Groundnut in Uganda is the second most important legume after common beans (*Phaseolus vulgaris* L.). The crop is grown in all parts of the country, with largest production in Soroti 19,000 metric turns (MT), Nakasongola (18,000 MT), Amuru (15,000 MT) and Kibaale (12,000 MT) districts of the Eastern, Central, Northern and Western regions respectively. A downward trend from 135,000 MT to 126,000 Mt between 1995/96 and1999/2000, and an upward trend from 126,000 MT to 245,000 MT between 1999/2000 and 2008/9 was registered (UBOS, 2010).

Groundnuts thrive under relatively low rainfall and as a legume, improve soil fertility by fixing atmospheric nitrogen (Janila *et al.,* 2013b). As a cash crop, it gives relatively high returns for limited land area. The crop therefore, requires few inputs, making it appropriate for cultivation in low-input agriculture by smallholder farmers Smartt (1994) and is well adapted to the hot, semi-arid conditions of Uganda. Nutritionally, groundnuts are rich source of energy, because of their high oil (33 to 55%) and protein (19 to 31%) contents (Shilpa *et al*., 2013; Jambunathan, 1991). It is also very good source of minerals (calcium, magnesium and iron) and vitamins (B1, B2 and Niacin) (Savage and Keenan, 1994; Singh and Diwakar, 1993). Groundnut is used for preparation of peanut butter and confectionery products (Janila *et al*., 2013b; Jambunathan, 1991). The groundnut haulms too are very nutritious fodder to animals (Janila *et al*., 2013b; Ozyigit and Bilgen, 2013; Singh and Diwakar, 1993) and can as well be used as compost

(Janila *et al*., 2013b). According to Singh and Diwakar (1993), a variety of industrial products for exportation such as; paint, varnish, lubricating oil, leather dressings, soap, candles, cosmetics, some textile fibres, insecticides, and nitro-glycerine are derived from peanut oil. The shells of groundnut are used in the manufacture of plastic, wallboard, abrasives, fuel and cellulose (Janila *et al*., 2013b).

In spite of increased production as noticed in recent years which was primarily due to increase in area under cultivation, the average yield of groundnut in Uganda (731 kg ha^{-1}) remains well below the yields (3824 kg ha^{-1}) reported from USA (FAO, 2009). The groundnut crop in Uganda is constrained by a combination of factors such as uneven rainfall distribution, continuous cropping without crop rotation Okello *et al*. (2010), limited supply of breeder's seed and poor seed quality, aflatoxin (a toxic substance produced by mould fungi *Aspergillus flavus* and *A.parasticus*), susceptibility to pests (aphids, whiteflies, leaf miners) and diseases (rosette virus, rust, and early and late leaf spots) (Okello *et al*., 2010; Page *et al*., 2002).

1.2 Statement of the problem

Among the foliar diseases, late leaf spot (LLS) is the most devastating fungal disease accounting for the major economic yield loss of Valencia groundnuts in Uganda (Okello *et al*., 2010). The disease occur wherever groundnut is grown and has been reported to cause over 60% yield losses in susceptible cultivars when environmental conditions are conducive for disease development in Uganda (Mugisha *et al*., 2004). There have been efforts to control leaf spot diseases in Uganda using a combination of cultural and chemical measures, however, with limited success (Page *et al*., 2002). The cultural practices that include crop rotation, removal, burning and burying of crop residues after harvest, removal of volunteer groundnuts, deep turning of crop debris are seldom applied by small holder farmers for a number of reasons; such as inadequate land size, lack of information especially in carrying out crop rotation and labor intensiveness. Effective chemical control is heavily reliant upon multiple fungicide applications Jordan *et al*. (2012) which are costly for resource poor farmers in Uganda, and also raises environmental and health concerns. Use of host plant resistance is the most effective and economically viable management option for resource limited farming systems in developing countries. However, use of host resistance is limited by lack of resistant varieties and adequate information on genetics of LLS resistance in the available Valencia breeding

materials, which makes genetic improvement of the crop difficult. Furthermore, many studies have reported quantitative nature of inheritance of LLS resistance (Khedikar *et al*., 2010; Upadhyay *et al*., 2009; Dwivedi *et al*., 2002; Motagi, 2001), suggesting that the inheritance of LLS resistance is rather complicated which could make direct selection rather difficult in the breeding program for LLS resistance. This study was therefore planned to generate genetic information on resistance to LLS in groundnut.

1.3 Justification

Elsewhere in the world efforts have been made in breeding for resistance to groundnut leaf spots and many varieties with improved leaf spot resistance such as; 'York,' 'Georgia-07W', 'Georgia-03L' Southern Runner, Florida MDR98 and 'Georgia Green' are commercially grown in Southeastern part of USA (Culbreath *et al*., 2009). In Uganda, there are no Valencia varieties that are resistant to leaf spot diseases Kalule *et al.* (2010), and yet the Valencia varieties are known for their highest score for sweet taste and more seeds per pod when compared with other botanical varieties (Patte *et al*., 2001). Traders also prefer Valencia due to higher oil content (Kaaya and Warren, 2005).

To broaden the genetic base of Valencia groundnut in Uganda, exotic cultivars, such as Valencia C and NuMex-M_3, commercially grown in USA, were introduced and evaluated for resistance to abiotic (drought) and biotic stresses (leaf spot, rosette and rust diseases and pest) at NaSARRI in Uganda. However, these cultivars were found susceptible to LLS. In contrast, ICGV-SM 03590 and ICGV-SM 02501 from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Malawi and SGV AL (Serere Groundnut Variety, advanced line) from NaSARRI were found resistant to LLS (Kalule *et al.*, 2010).

The introgression of LLS resistance into improved genetic background will be the most effective and economical management option under resource limited farming systems in developing countries like Uganda. Knowledge of inheritance of LLS resistance involving these lines may facilitate the selection of progeny combining resistance into improved genetic background, which will enhance the development and utilization of late leaf spot resistant cultivars to reduce production costs, boost groundnut production in sub-Saharan Africa, improve household income and food security, and promote sustainability of the production environments.

1.4 General objective of the study

To improve Valencia groundnut varieties for resistance to late leaf spot.

1.4.1 Specific objective of the study

(i)To determine heritability for resistance to LLS in Valencia groundnuts

(ii)To determine the gene action controlling resistance to LLS in Valencia groundnuts

1.4.2 Hypothesis

(i) Resistance to LLS is highly heritable

(ii) Additive gene effects predominantly control resistance to LLS

CHAPTER TWO

LITERATURE REVIEW

2.1Botany, origin, diversity and distribution of groundnuts

The cultivated groundnut (*Arachis hypogea*) ($2n = 4x = 40$) is member of genus *Arachis* and belongs to the family *Leguminosae*, subfamily *Fabaceae*, tribe *Aeschynomeneae*, subtribe *Stylosanthenae* (Krapovickas and Gregory, 1994). The genus *Arachis* originates from South America (Kochert *et al*. 1996) and has 80 known species, belonging to nine sections (*Arachis, Caulorrhizae, Erectoides, Extranervosae, Heteranthae, Procumbentes, Rhizomatosae, Trierectoidse and Triseminatae*) (Valls and Simpson, 2005; Krapovickas and Gregory, 1994).

Section *Arachis* has the widest geographical distribution with 31 species known but only *Arachis hypogaea* and *Arachis monticola* are tetrapliods (Kochert *et al*., 1996). The remaining species of section *Arachis* are diploid and grouped into three genomes (A, B and D), each having 20 chromosomes, with exception of three species which have 18 chromosomes (Lavia, 2000; Kochert *et al*. 1996). The cultivated groundnut is a tetraploid, arising from hybridization between A and B diploid species (*A. duranensis and A. ipaensis*) followed by rare spontaneous duplication of the chromosomes (Kochert *et al*., 1996). The resultant allotetraploid plant was probably reproductively isolated from wild relatives leading to low diversity for traits of agricultural interest in cultivated groundnut.

Several marker systems including isozymes, restriction fragment length polymorphisms (RFLPs), random amplified polymorphism DNAs (RAPDs), sequence characterized amplified regions(SCARs), amplified fragment length polymorphisms(AFLPs) and simple sequence repeats(SSRs), have revealed very low level of molecular polymorphism in the cultivated groundnut germplasm compared to other crop species (He and Prakash; 1997; Halward *et al*., 1991; Kochert *et al*., 1991).The wild diploid *Arachis* species however, are genetically very diverse (Halward *et al*., 1991; Kochert *et al*., 1991) and have been selected during evolution by a range of abiotic and biotic stresses, providing a rich source of variation in agronomically important traits.

Six centers of diversity of *A. hypogaea* have been identified in South America (Holbrook and Stalker, 2003). Africa is also an important secondary center with large genetic variation (Hammons, 1982). The archaeological record indicates that *A. hypogaea* was first domesticated in Peru, dated ca .1500 (Banks *et al*., 1993). However, latest molecular data indicated an origin of South America (Kochert *et al*., 1996). The crop was cultivated in many parts of South America, as well as in the Caribbean and Mexico. As a result of explorations by the Spanish and Portuguese, groundnut cultivation spread quickly from America to Africa and Asia (Stalker, 1997). Groundnut is thought to have been introduced in Uganda by early traders and travelers around 1862 after its introduction into east Africa by Portuguese explorers (Busolo-Bulafu, C. M., 1990).

Groundnut is a self-pollinated crop with cleistogamous flowers and the breeding methods used for self-pollinated crops are applied in its breeding (Janila *et al*., 2013b). Singh and Simpson (1994) categorized the *Arachis* gene pool into four: the primary gene pool consisting of *A. hypogaea* and the freely cross-compatible tetraploid *A. monticola*. The cross compatible, diploid species of sections *Arachis* forms the secondary gene pool. The tertiary gene pool comprises the members of the section *Procumbentes* that probably co-evolved with the species of series *Perennes* of the section *Arachis* and are weakly cross–compatible with section *Arachis.* The remaining cross–incompatible species from other sections constitutes the quaternary gene pool.

The morphology, anatomy and reproductive development of peanut has been described by many reporters (Holbrook and Stalker, 2003; Rao, 1988). All members of the genus *Arachis* are distinguished from other plants by flowering above the ground and producing fruits below the ground (Holbrook and Stalker, 2003). The cultivated groundnut (*A. hypogaea*) is an annual herb with two subspecies. The subspecies *hypogaea* has been characterized by absence of flower on the main stem and alternate vegetative and reproductive nodes. It includes two botanical varieties *hypogaea* (*Virginia* bunch *and* Virginia *runner* types) and the less-frequently cultivated *hirsuta.* The *fastigiata* subspecies is typified by flowers on the main stem and sequential reproductive nodes. It has four botanical varieties, *fastigiata* (Valencia type), *vulgaris* (Spanish type), *peruviana,* and *aequatoriana* (Krapovickas and Gregory, 1994). Only *A. hypogaea* has been domesticated Valls and Simpson (2005), although several species have

been cultivated for their edible seed (*A. villosulicarpa* Hoehne and *A. stenosperma* Krapov. and W. C. Gregory) or forage (*A. repens* Handro, *A. pintoi* Krapov. & W. C. Gregory and *A. glabrata* Benth) (Coffelt and Simpson, 1997).

2.2 Distribution and importance of late leaf spot disease in groundnut

Late leaf spot (LLS) is an important foliar disease of groundnut in Africa, Asia and America. Infections of LLS reduce the photosynthetic area by causing intense lesions on leaves, petioles, and stems that often lead to premature defoliation, loss of integrity of the peg, and hence yield loss. Pattee and Young (1982) reported reduction of leaf area index by 80 percent, carbon dioxide uptake by 85 percent and canopy carbon exchange rate by 93 per cent due to severe leaf spot damage. Photosynthesis of diseased canopies was reduced by defoliation and inefficient fixation of carbondioxide by diseased leaves. Reports have indicated over 50% yield losses from LLS (Kucharek, 2000; Grichar *et al*., 1998; Waliyar, 1991; McDonald *et al*., 1985). Subrahmanyam *et al.* (1992) reported yield reductions of 20 to 100% in South Africa and other parts of the world. Yussif (2010) reported 40-60% yield loss due to leaf spot in Ghana but late leaf spot was reported the most predominant form in all locations of Northern Ghana. Leaf spots in Uganda has been reported to cause over 60% yield losses (Mugisha *et al*., 2004), mostly from LLS. Besides reducing the yield, the disease also has an adverse effect on seed quality and grade characteristics, and quality of fodder which renders it unsuitable as animal feed. Moreover, the control of these disease through application of plant protection measures not only increases the cost of cultivation but also lead to environmental and health hazards. Hence, use of resistant cultivars is the best means of reducing crop losses ((Jordan *et al*., 2012; Shew *et al*., 2010; Page *et al*., 2002).

2.3 The late leaf spot pathogen

The late leaf spot (LLS) fungal pathogen *Phaeoisariopsis personata* (Berk. and Curt) is seen primarily in its imperfect state (Shokes and Culbreath, 1997).The perfect state (*Mycosphaerella berkeleyii* W.A. Jenkins) is classified under the asogeneous fungi and both asci and spermatogonia occur on debris (Pattee and Young, 1982). Ijaz (2011) described the imperfect state as follows: Conidiophores are fasciculate, one to three geniculate with conspicuous conidial scars, mostly arranged in concentric rings on lower surface and darker in colour.

Conidia are olivaceous, obclavate, cylindrical and with one or more septa. The base is shortly tapered with conspicuous hilum and hyaline in colour.

Variation between isolates of leaf spot pathogens has been reported but the races have not been clearly characterized (Ijaz, 2011; Upadhyay *et al*., 2009; Holbrook and Stalker, 2003). Hossain and Ilag (1999) reported variability in virulence among eight *Phaeoisariopsis personata* isolates. Symptoms in plants inoculated with the least virulent isolate appeared later than in those inoculated with the most virulent isolate by an average of 1.25 days in both resistant and susceptible genotypes.

2.4 Epidemiology, pathogenicity and symptoms of late leaf spot disease

The fungi that cause late leaf spots reproduce and infect by means of microscopic spores called conidia. High humidity and temperatures above 19°C promote spore production (Shokes and Culbreath, 1997). Spores produced on infested groundnut residue in the soil during the growing season result to the primary inoculum that causes initial leaf spot infection (Shokes and Culbreath, 1997; McDonald *et al*., 1985). When conditions are favorable the spores develop into germinative tubes that enter the plant cells directly via the epidermis or stomata, allowing intracellular mycelia growth into haustoria that obtain nutrients (Pattee and Young, 1982; Shokes and Culbreath, 1997). Host cell are killed in advance as the hypae penetrates (Upadhyay *et al*., 2009). Lesions develop within 10-14 days and new spores are produced in spots on infected leaves (Shokes and Culbreath, 1997; Shokes and Melouk, 1995). These spores will subsequently infect plants and produce secondary infection after periods of extended leaf wetness and temperatures above 19°C, and the cycle repeats again (Shokes and Culbreath, 1997). Spores are spread by wind, splashing rain, insects and movement of infected crop debris or by movement of pods and seeds that are surface contaminated with conidia (Shokes and Culbreath, 1997; McDonald *et al*., 1985). The pathogen is hemibiotroph of groundnut with no known alternative host (Jordan *et al*., 2012; Upadhyay *et al*., 2009), and may survive from season to season on volunteer groundnut plants and infected crop debris. LLS generally occur later in the season and is often seen as a complex with other leaf spots.

The lesions of LLS are very similar in size and form to those of early leaf spot (ELS) caused by *Cercospora arachidicola*. These lesions are, however, circular in shape, darker brown in color than those of *Cercospora arachidicola* and are without a definite chlorotic halo (Jordan *et al*., 2012; Ijaz, 2011). On the adaxial side of the leaflets, lesions are almost black, in contrast to the lighter colored lesions of ELS. Lesions are rough and black on the lower surface Jyosthna *et al*. (2004), in extreme cases lesions coalesce with each other resulting in premature senescence and shedding of leaflets (McDonald *et al*., 1985). The spots later spreads to the petioles and stems (Jyosthna *et al*., 2004).

In addition to production of Cercosporin, a biologically active red phytotoxin, *P.personata* produced cellulolytic and pectolytic enzymes that altered the starch, sugar and amino acid content of leaf tissue, resulting in reduced leaf efficiency and premature abscission (Pattee and Young, 1982). Mohapatra (1982) also reported association of higher quantities of reducing sugars in infected leaves than healthy ones. Horne *et al*. (1976) reported that the LLS fungus produced haustoria that penetrate individual plant cells. Leaves infected with the fungus showed a marked increase in respiration. Jyosthna *et al*. (2004) reported highest chlorophyll content in resistant cultivar, which decreased upon infection in all cultivars.

2.5 Mechanism of resistance to late leaf spot disease in groundnuts

Medicarpin was reported as the principal phytoalexin of peanut leaves infected with any of four leaf spot pathogens (Strange *et al*., 1988). These antifungal compounds (phytoalexins) are assumed to inhibit pathogen ingress and/or reproduction in peanut tissues. Jyosthna *et al*. (2004) reported higher total phenols, peroxidase and polyphenol oxidase in resistant cultivar, FDRS-10 and upon infection, increased further, in all cultivars under study. Significant differences in banding profiles of phosphatase and esterase isozymes were observed in all cultivars. Shilpa *et al*. (2013) reported higher amounts of proteinase inhibitors in several newly synthesized tetraploids line that were resistant to late leaf spot (LLS).

Several anatomical and morphological characteristics of peanut tissue have been associated with resistance to leaf spot diseases. Resistance to *P. personata* was reported to be associated with formation of pectic substances and thickening of cell walls (Abdou *et al*., 1974). In susceptible cultivars "directed" growth of germ tubes toward stomata has been reported,

whereas no directed growth was detected in resistant genotypes and less on moderately susceptible genotypes (Abdou *et al*., 1974). The stomatal frequency and size was less in resistant cultivar, FDRS-10 and more in susceptible cultivar, TMV-2(Jyosthna *et al*., 2004). However, stomatal size was not found to be a mechanism of resistance in former studies (Cook, 1981).

2.6 Control of late leaf spots

An integrated management of disease, which include host plant resistance, and judicious use of fungicides together with other cultural practices such as crop rotation is important to control LLS (Jordan *et al*., 2012; Ijaz, 2011; Cantonwine *et al*., 2006). Crop rotation can be a useful in delaying initial infections by both leaf spot fungi; which slows down the disease spread for the rest of the growing season. Rotations where groundnuts are grown once every 3 to 4 years are long enough to permit leaf and stem debris from the previous groundnut crop to decompose, thereby minimizing the carry-over of both leaf spots fungi (Jordan *et al*., 2012; Shew *et al*., 2010; Page *et al*., 2002). Deep turning of debris from the previous groundnut crop is also strongly recommended before rotating a field back to groundnut (Page *et al*., 2002). Eliminating volunteer groundnuts in the field or forage crops that follow groundnuts and in the fallow immediately after groundnut harvest is a critical component of a successful rotation program for control of leaf spots.

Host plant resistance is an effective method to control fungal diseases in groundnut. Many varieties with improved leaf spot resistance such as; 'York,' 'Georgia-07W', 'Georgia-03L' Southern Runner, Florida MDR98 and 'Georgia Green' (Culbreath *et al*., 2009), Bailey, Sugg and Florida fancy (Jordan *et al*., 2012; Shew *et al*., 2010) are commercially grown in USA.

There are no varieties known with complete immunity to fungal diseases, however, improved resistance will likely lead to reduction in disease severity (Jordan *et al*., 2012). Selecting groundnut cultivars with partial resistance to late leaf spot disease together with some fungicide protection is an effective disease management strategy as the total fungicide inputs required to maintain optimum yield are greatly reduced (Culbreath *et al*., 2009; Shew *et al*., 2010; Jordan *et al*., 2012).

Biological control of foliar diseases has received less attention, owing to the poor establishment of the introduced biocontrol agents and resulting variations in disease control. Chitin-supplemented application of chitinolytic bacteria; *Bacillus circulans* and *Serratia marcescens* has been proved effective in control of foliar diseases including late leaf spot of groundnut (Kishore *et al*., 2005). *Verticillium lecanii* also has been reported in biocontrol of rust, early and late leaf spot pathogens of peanut (Subrahmanyam *et al*., 1990).

2.7 Components of resistance to *P. personata* leaf spot in groundnut

Recognition of epidemiological components of rate-reducing resistance to late leaf spot disease of groundnut has provided a major strategy for current breeding efforts. Several components contribute to resistance, including initial infection, lesion size, lesion number, sporulation, and defoliation (Anderson *et al.*, 1993; Aquino *et al*., 1995; Dwivedi *et al.*, 2002). Resistance to leaf spots in groundnut has generally been associated with late maturity (Miller *et al.*, 1990; Singh *et al*. 1997). Sporulation, lesion size, lesion number and latent period are important components of resistance to LLS and are highly correlated to each other and with percentage of necrotic area on leaf (Anderson *et al.*, 1990). Lesion diameter, defoliation and sporulation from glasshouse study were correlated with field disease score (Subrahmanyam *et al.,* 1982). Dwivedi *et al.* (2002) observed longer incubation period, longer latent period, lesser lesions per leaf, smaller lesion diameter, lower sporulation index, lesser percentage leaf area damaged, and lower disease scores in the resistant genotype as compared to susceptible genotype and also reported high correlation among components of resistance. According to Pensuk *et al*.(2003) the genotype NC 17135 exhibited a high resistance to late leaf spot because it had the lowest disease score and lesion number per 100 cm^2 of leaf area ,and least sporulation compared to all the commercial cultivars, NC 9, Tainan 9 and Lampang that were highly susceptible. Motagi (2001) reported incubation period, lesion size and lesion on main-stem as the most important components of resistance, having strong association with field disease score, defoliation and remaining green leaf area. Percent defoliation had a highly significant positive association with field disease score (Dwivedi *et al.*, 2002). A detached leaf assay was used to determine components of resistance for Georgia Green, Georganic, and DP-1 peanut cultivars genotypes to infections by *P. personata*. Infection frequency 30 days after inoculation, lesion diameter, and percent necrotic area were greatest for Georgia Green. Latent period was shorter for

Georganic than DP-1, and sporulation per unit lesion area was greatest for Georganic. Enhanced field resistance to late leaf spots reported for DP-1 and Georganic was in part due to lower infection frequencies, smaller lesions, and for DP-1, longer latent periods (Cantonwine *et al*.,2008).

2.8 Sources of resistance and breeding progress for resistance to late leaf spots

Identification of resistance sources and knowledge of components and screening methods to resistance are the prerequisites for the success of disease resistance breeding program. With increased emphasis in screening groundnuts for disease resistance, several sources of resistance to late leaf spot have been identified in *A. hypogaea* in different parts of the world (Holbrook and Isleib, 2001; Holbrook and Anderson, 1995; Anderson *et al*., 1993; Singh *et al*., 1997), and have been used to develop breeding lines with LLS resistance (Holbrook and Stalker, 2003). Holbrook and Isleib (2001) observed that *A. hypogaea* accessions originating from Bolivia were more frequently found with resistance to LLS.

Majority of resistant sources belong to subspecies *fastigiata* var. *fastigiata* and are land races from South America (Subrahmanyam *et al*.,1989), which were reported as moderately resistant to LLS but with many undesirable pods and seed characteristics, not commercially accepted. Wild *Arachis* species in contrast have shown variation ranging from immune to highly resistant reaction to LLS (Subrahmanyam *et al.,* 1985; Abdou *et al.,* 1974).

Some progress has been made to develop LLS resistant groundnut cultivars; however, combining high levels of resistance into improved genetic background with acceptable market traits continues to be a challenge to peanut breeders. The cross compatibility barriers, the linkage drag associated with resistance, the crop duration, the complex nature of resistance to this disease, and the long period required for cultivar development contribute to limited progress realized so far towards developing LLS resistant cultivars with acceptable pod/seed characteristics (Holbrook and Stalker, 2003; Nigam and Dwivedi, 2000).

In spite of these obstacles, elsewhere in the world efforts have been made to breed for resistance to LLS in groundnut, which resulted in release and cultivation of moderately LLS resistant cultivars such as 'York,' 'Georgia-07W', 'Georgia-03L', Southern Runner, Florida MDR98 and 'Georgia Green' in the Southeastern part of USA (Culbreath *et al*., 2009); Bailey,

Sugg and Florida fancy in USA (Jordan *et al.*, 2012; Shew *et al.*, 2010); ICGV-SM 86715 in Mauritius (Moss *et al.*, 1998), and Yue You 223 in China (Liang *et al.*, 1999).

A few interspecific derivatives; ICGV 87165, GPNCW 1, GPNCW 2, GPNCW 3, GPNCW 4, ICGV 86699, and ICGV 87167 possessing high levels of resistance to foliar diseases have also been reported (Moss *et al.*, 1997; Reddy *et al.*, 1996; Nigam *et al.*, 1992). However, these elite germplasm have not been released for cultivation due to agronomically undesirable traits such as late maturity and inferior pod and seed characteristics in comparison with commercially grown cultivars (Khedikar, 2008).

In Uganda breeding for LLS resistance is at infancy stage and none of the Valencia groundnuts currently grown in Uganda are resistant to LLS (Kalule *et al.*, 2010). The National Semi-Arid Resources Research Institute (NaSARRI) in Uganda has screened 75 accessions from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for resistance to leaf spots diseases using infector rows for inoculation at Serere, Eastern Uganda a hot spot location. ICGV-SM 03590, ICGV-SM 02501, ICGV-SM 03703, ICGV-SM 01731, ICGV-SM 02709 and ICGV-SM 02715 were recommended for use in leaf spots disease management.

There is no uniform method for assessing leaf spot resistance. Using infector rows for inoculation, a total of 83 lines of *A. hypogaea* were identified with some resistance and/or tolerance to *Phaeoisariopsis personata* at ICRISAT after the extensive screening of groundnut genotypes for LLS resistance (wynne *et al.*, 1991). Subrahmanyam *et al.* (1985) evaluated ninety-six accessions of wild *Arachis* species for reaction to LLS pathogen at ICRISAT. Inoculation of *C. personatum* was based on detached leaves of TMV2 in a growth chamber. Disease development was assessed 30 days after inoculation, and infection frequency, percent of defoliation, lesion diameter and sporulation components of LLS were evaluated.

A detached leaf assay was also used by Cantonwine *et al*. (2008) to determine components of resistance for Georgia Green, DP-1, and Georganic peanuts genotypes to infections by *C. personatum*. Latent period was shorter for Georganic than DP-1, and sporulation per unit lesion area was greatest for Georganic. Enhanced field resistance to late leaf spots reported for DP-1 and Georganic was in part due to lower infection frequencies, smaller lesions, and for DP-1, longer latent periods. The genotypes were also evaluated in the field for early and late leaf spot

epidemic progress. Severity assessments were made at 7 to 22 day intervals four to five times beginning 89 DAP in 2002, and nine to ten times beginning 59 DAP in 2003. Early leaf spot was the predominant disease in the field study. Late leaf spot occurred late in the season (>100 DAS (Days after sowing) in both years, but the disease never exceeded 10% of the leaf spots combined. Disease severity was monitored over the season using the Florida 1 to 10 scale system (Chiteka *et al*., 1988).

Jyosthna *et al*. (2004) screened thirteen groundnut (*Arachis hypogaea*) cultivars for resistance to *Phaeoisariopsis personata* under natural disease pressure at hot spot location. They were classified into resistant (cultivar FDRS-10), moderately resistant (cultivars K - 134, TCGS-l56 and Tpt-3) and susceptible (Tpt-l, Tpt-2, TCGS-29, TCGS-91, TCGSl50, TCGS-341, Tpt-4 JL-24 and TMV-2), based on a disease severity score of 1-9 scale.

Pensuk *et al*. (2003) also compared seven peanut cultivars for their resistances to late leaf spot diseases under field conditions at hot spot location. At 80 DAS, disease score based on a disease severity score of 1-9 scale, sporulation index and lesion number per 100 cm^2 of leaf area were recorded as the resistance parameters of the disease. The genotype NC 17135 exhibited a high resistance because it had the lowest disease score and lesion number per 100 cm^2 of leaf area ,and least sporulation compared to all the commercial cultivars, NC 9, Tainan 9 and Lampang that were highly susceptible. In 2008 Kumari screened groundnut mutant lines for LLS resistance on a scale of 1-9. Higher LLS severity was observed at harvest (5.76) than at 90 days after planting (4.4).

In a genetic analysis study for LLS resistance by Janila *et al*. (2013a), artificial inoculation was done at 50 DAS by spraying the test plants and infector rows with conidial suspension of LLS pathogen and perfo-irrigation was provided daily for 15 minutes in the evening hours for 30 days to promote disease development. Resistance parameters including disease score, defoliation percentage and leaf area damage (LAD) on 78, 89 and 104 DAS were recorded on each plant in each generation. A 9-point scale, as described by Subrahmanyam et al. (1995), was followed to record disease scores in the field. Area under the disease progress curve (AUDPC) was calculated based on the defoliation percentage and LAD on 78, 89 and 104 DAS. Susceptible parent had an average disease score of 5.9 at 78 days, while both the resistant parents had a disease score of < 2.3. In a span of 26 days, the disease score of JL 24

increased from 5.9 to 8.5 , while that of the resistant parents rose from <2.3 to <3.6 only. Consequently, the resistant parents exhibited lower AUDPC than the susceptible parent.

In daillel analysis involving eight parental lines by Vishnuvardhan *et al.* (2011), late leaf spot severity was scored on 1–9 point scale at harvest, i.e., 105 days after sowing (DAS) as described by (Subrahmanyam *et al*. 1995). Incidence and spread of foliar diseases was promoted through spraying of spore suspension from 60 DAS and humid environment was created through sprinkler system of irrigation in the absence of wet weather. Spore suspension was prepared by soaking the infected leaf debris overnight in water.

2.9 Genetics of resistance to late leaf spot disease in groundnuts

Genetic studies on late leaf spot (LLS) resistance suggest that resistance is complex and polygenic in nature and probably controlled by several recessive genes (Khedikar *et al*., 2010; Upadhyay *et al*., 2009; Dwivedi *et al*., 2002; Motagi, 2001; Vasanthi and Reddy, 1997; Nevill, 1982). Motagi (2001) reported duplicate recessive genes controlling resistance to LLS with favorable resistance alleles coming from inter-specific derivative CS 16 (ICGV 86855). Resistance to LLS has been reported to be determined by two genes (Tiwari *et al.,* 1984) and five-locus recessive genes in the crosses involving cultivated groundnut and wild *Arachis* species (Sharief *et al.,* 1978). A QTL study of 268 recombinant inbred lines of a mapping population TAG 24 × GPBD 4 segregating for late leaf spot resistance identified 11 QTLs in the individual environments and four QTLs across environments associated with resistance to LLS. However, all these QTLs were minor contributing 1.70–6.50% of the phenotypic variation. The several QTLs with small effects (1.70–6.50%) indicated that resistance to LLS is controlled by many and small effect loci (Khedikar *et al*., 2010). Luo *et al.* (2005) identified 56 genes in response to late leaf resistance using microarray and real-time PCR.

Although gene action of LLS resistance has been reported by many workers but the genetics of a particular trait may vary with variation in plant material and environment in which the materials are evaluated from. A generation mean analysis involving three crosses Jogoly *et al*. (1999b) and a diallel analysis involving six parents Jogoly *et al*. (1999a) indicated that additive gene action was important for LLS disease score. Dominance and epistatic effects were less important for most parameters except for disease score in one cross (Jogoly *et al*., 1999b). The

results indicated that single plant selection for those resistant traits in F_2 generation was possible. Further study involving eight parent diallel analysis revealed the importance of additive gene action in controlling LLS severity, however, the variance ratio (GCA/SCA) was less than one which indicated influence of non-additive gene action (Vishnuvardhan *et al*., 2011).

In addition to additive and dominance variation, it has been suggested by Shoba *et al*. (2010) that epistasis may also be involved in the inheritance of many quantitative characters in groundnut including LLS resistance. Janila *et al*. (2013a) reported that resistance to LLS was controlled by a combination of both, nuclear and maternal gene effects in addition to epistatic effects (additive x additive, additive x dominance and dominance x dominance). While variation due to dominance effects and their interactions cannot be exploited effectively in groundnut, additive x additive epistatic variation is potentially useful, as it can be fixed in homozygous cultivars (Singh and Oswalt, 1991). The exploitation of the additive x dominance and dominance x dominance types of interactions require the maintenance of heterozygosity. Kaczamarek *et al*. (2002) reported that heterozygote populations are more adaptable than homozygote populations to varied environmental conditions. Maintaining production under conditions of climate and environmental change will require the breeding of new crop varieties better adapted to these conditions. So it is necessary to study gene action of quantitative traits before starting any breeding program.

2.10 Genetic variability and heritability for late leaf spot resistance

Wynne and Gregory (1981) stated that improving traits in groundnuts requires sufficient variability, an understanding of genetic control, techniques for assuring desired traits, and a breeding strategy for effective use of variability. Phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) have been reported moderate for late leaf spot in 28 F_2 populations involving eight parents Vishnuvardhan *et al*. (2012). Kumari, (2008) reported high PCV (29.96-36.070) and GCV (27.71-32.96) in a population involving mutant lines that were evaluated in the field for LLS severity at two stages under natural condition. However, Khedikar, (2008) observed high PCV (21.71-33.5) and moderate to high (14.6 – 24.76) GCV for late leaf spot.

Success of a breeder in changing the characteristic of a population depends on the degree of correspondence between the phenotypic and genotypic values. Heritability estimates provides information about the correspondence between the phenotypic and genotypic variances. Thus heritability estimates provide information on which to base a breeding procedure (Dabholkar, 1992). Heritability estimates and genetic parameters are useful when comparing the expected genetic gain from selection based on alternative selection strategies. Hence heritability estimates can be used to predict gain from selection (Falconer and Mackay, 1996; Kearsey and pooni, 1996).

Effective selection in early generation of segregating materials can be achieved only when additive genetic effects are substantial and heritability is high (Kearsey and pooni, 1996). Heritability (h^2_n) estimates from variance components were found to be low for most components of resistances to late leaf spot except for lesion size (mm) in Lampang × RLRS 15 (98%) and Khon Kaen 60-1 × RLRS 15(91%) crosses and sporulation in Lampang × RLRS 15 (99%) cross in groundnut, suggesting that selection in early generation based on phenotype of individual plants would be ineffective (Kormsa-art *et al.*, 2002). According to Anderson *et al*. (1991) estimates of narrow sense heritability have ranged from low to high (0.18 to 0.74) for all components of resistance from parent-offspring regression estimates. Khedikar *et al*. (2010) however, reported high to very high (40.87 to 82.81%) heritability (broad sense) of LLS resistance in groundnut.

Higher estimate of heritability do not necessarily provide high values of genetic advance Vishnuvardhan *et al*. (2012) and heritability alone provides no indication for the amount of genetic progress in the trait that can be achieved through selection. The effectiveness of selection depends upon genetic advance of the character selected along with heritability **(**Manju and Sreelathakumary, 2002). The information on genetic advance help in understanding the type of gene action involved in the expression of various polygenic characters (Singh and Narayanan, 1993).

According to Dabholkar, (1992) and Falconer and Mackay (1996) heritability is a property of a population being studied and the environment circumstances to which the individuals are subjected. Therefore, it's important to get information on heritability of LLS resistance on the

available Valencia breeding material before starting any breeding progress.The knowledge about heritability and nature of genetic effects prevailing in the breeding material is necessary to decide on a breeding procedure to be chosen.

According to the literature reviewed, late leaf spot (LLS) is an important foliar disease of groundnut worldwide. With increased emphasis in screening groundnuts for disease resistance, several sources of resistance to late leaf spot have been identified in *A. hypogaea* in different parts of the world and some progress has been made to develop LLS resistant groundnut cultivars elsewhere in the world. In Uganda breeding for LLS resistance is at infancy stage and none of the Valencia groundnuts currently grown in Uganda are resistant to LLS.

Many studies have reported quantitative nature of inheritance of LLS resistance suggesting that the inheritance of LLS resistance is rather complicated and this could contribute to limited progress realized towards developing LLS resistant cultivars in groundnut breeding programs for LLS resistance. Information on heritability and gene effects of LLS resistance has been reported by many workers, however, it should noted that the genetics of a particular trait may vary with variation in plant material and environment in which the materials are evaluated from. Therefore, it's important to understand the genetics of LLS resistance using the available Valencia breeding materials before starting any breeding progress. The knowledge about heritability and nature of genetic effects prevailing in the breeding material is necessary to decide on a breeding procedure to be chosen. This study was therefore planned (1) to determine heritability for resistance to LLS in Valencia groundnuts and (ii) to determine the gene effects controlling resistance to LLS in Valencia groundnuts.

CHAPTER THREE

Determination of heritability of late leaf spot resistance

3.1 Introduction

In Uganda groundnuts are the second most important legume crop after common beans (*Phaseolus vulgaris* L.), grown in all parts of the country (UBOS, 2010). Nutritionally, groundnut kernel is a rich source of energy, because of its high oil content and protein content (Asibuo *et al*., 2008; Jambunathan, 1991). It is also a very good source of minerals (calcium, magnesium and iron) and vitamins (B1, B2 and Niacin) (Singh and Diwakar, 1993). Groundnuts thrive under low rainfall and as a legume, improve soil fertility by fixing nitrogen.

Among the foliar diseases of groundnuts , late leaf spots is said to be the most devastating fungal disease accounting for the major economic yield loss of Valencia groundnuts in Uganda (Okello *et al*., 2010). The disease occur wherever groundnuts are grown and has been reported to cause over 60% yield losses in susceptible cultivars when environmental conditions are conducive for disease development (Mugisha *et al*., 2004). Effective chemical control is heavily reliant on multiple fungicide applications (Jordan *et al*., 2012) which are costly for resource poor famers in Uganda, and raises environmental and health concerns.

The deployment of resistant cultivars to LLS disease in groundnut could be effective in decreasing the production costs, improving production quality and reducing detrimental effects of the chemicals on ecosystems. This emphasizes the breeders to exploit the available genetic resources through plant improvement techniques. However, this is limited due to lack of information on genetic variability and heritability of LLS resistance. This information is available elsewhere, but limited in Uganda and yet Uganda wants to start a breeding program on LLS resistance. Furthermore, it has been reported that LLS resistance is quantitatively inherited (Khedikar *et al*., 2010; Upadhyay *et al*., 2009; Dwivedi *et al*., 2002; Motagi, 2001), therefore, for the success of such a breeding program on LLS resistance, the breeders are required to collect information about the genetic variability and heritability in the population before starting any improvement work.

According to Wynne and Gregory (1981), improving traits in groundnuts requires sufficient variability, an understanding of genetic control, techniques for assuring desired traits, and a breeding strategy for effective use of variability. Khedikar, (2008) observed high PCV (21.71-33.5) and moderate to high (14.6 – 24.76) GCV for late leaf spot.

The genetic variability is heritable from generation to generation. Therefore, the effectiveness with which selection can be based on phenotypic performance to exploit genetic variability can be known from heritability estimates. Though information on heritability of LLS resistance has been provided by many authors, Dabholkar, (1992) and Falconer and Mackay (1996) concluded that heritability is a property of a population being studied and the environment circumstances to which the individuals are subjected. According Anderson *et al*. (1991) estimates of narrow sense heritability of LLS resistance ranged from low to high (0.18 to 0.74) while Khedikar *et al*. (2010) reported high to very high (40.87 to 82.81%) heritability (broad sense) of LLS resistance in groundnut. Heritability estimates along with genetic advance provide better prediction of expected gain under selection instead of heritability alone. Presence of high variability and heritability coupled with high genetic advance for a trait offers much scope for its improvement. In this study genetic variability, heritability and genetic advance of LLS disease score were estimated and information obtained will be used in suggesting a future breeding program strategy for LLS resistant groundnut genotypes.

3.2 Materials and methods

3.2.1 Study area

The research was conducted at the National Semi-Arid Resources Research Institute (NaSARRI) of the National Agricultural Research Organization (NARO) located 01^0 30 00N and 33^0 33 00E in Serere district, Uganda. This location represents a humid and hot climate that receives an annual rainfall 1,000–1,200mm.

3.2.2 Materials

In the study seven groundnut genotypes (Table 1), with varying levels of response to LLS were used. The genotype had been characterized for resistance to LLS by the Groundnut Improvement Program at NaSARRI.

Table 1: Origin, pedigree, and response to LLS of six groundnut lines

Genotype	Pedigree	Country of origin	Botanical name	Response to LLS
Redbeauty	Landrace	Uganda	Valencia	Susceptible
Valencia C	Selection from Colorado Manfredi	USA	Valencia	Susceptible
NuMex-M_3	Valencia C × ICGV 87157	USA	Valencia	Susceptible
JL 24[a]	----------	India	Spanish	Highly susceptible
ICVG-SM O3590	----------	Malawi ICRISAT	Virginia	Resistant
ICGV-SM 02501	----------	Malawi ICRISAT	Spanish	Resistant
SGV 07009	SGV 91707× Serenut 1	Uganda	Virginia	Resistant

[a]=Not included in the study, but used as the spreader to augment disease pressure

3.2.3 Generation of first filial generations (F_1 progeny).

Valencia C, NuMex-M_3 and Redbeauty were used as female (susceptible lines) while SGV-07009, ICVG-SM 03590 and ICGV-SM 02501 were the male parents (Resistant lines). In July 2011 three seeds from each of the parents were planted in plastic pots of diameter 45 cm and height 15 cm containing loam and sandy soil. Parents were grown in a glass house and later

thinned to two. Staggered planting of parents was done where the male parents were planted one week earlier the female parents in order to synchronize flowering, and to ensure continuous availability of flowers and floral buds for making crosses. Plants were watered after every two weeks until they reached physiological maturity.

At flowering the female parents were emasculated with forceps in the evening (4.00-6.00 pm) and crossing was done in the following morning (between 8.00 and 10.00 am) by rubbing the pollen from donor parents on the stigma of the emasculated plants carefully by using hands. The nodes of the flowers that were crossed were tagged with labels, and on the label the female parent was written first followed by the male parent. Bi-parental mating design was employed where four crosses were made between NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, Redbeauty × ICGV-SM 03590 and Valencia C × SGV-07009 parental lines. In each cross 15 female flowers were pollinated. At physiological maturity the pods of the parents and crosses (F_1s) were harvested separately, dried, and packed in labeled envelops, and stored.

3.2.4 Generation of F_1, F_2, BC_1P_1 and BC_1P_2 populations

In December 2011, 15 F_1 seeds generated above from each cross along with their respective parents were planted in plastic pots containing loam and sandy soil and grown in a glass house. The F_1 seed were planted alongside with their respective parents to identify the successful crosses. These parents were also used to generate more F_1 seeds as described above. At flowering, five F_1 plants were selfed to generate F_2 seeds while five plants were backcrossed to susceptible parents (P_1) and five plants to donor plants (P_2) to produce $BC_1P_1$and BC_1P_2 seeds respectively. The parents of the respective crosses were used as male parents and the F_1 generation as female parents in generation of BC_1P_1 and BC_1P_2 seeds. Emasculation and hybridization process was done as described for generation of F_1 above. The crossing scheme used is summarized in Figure1.

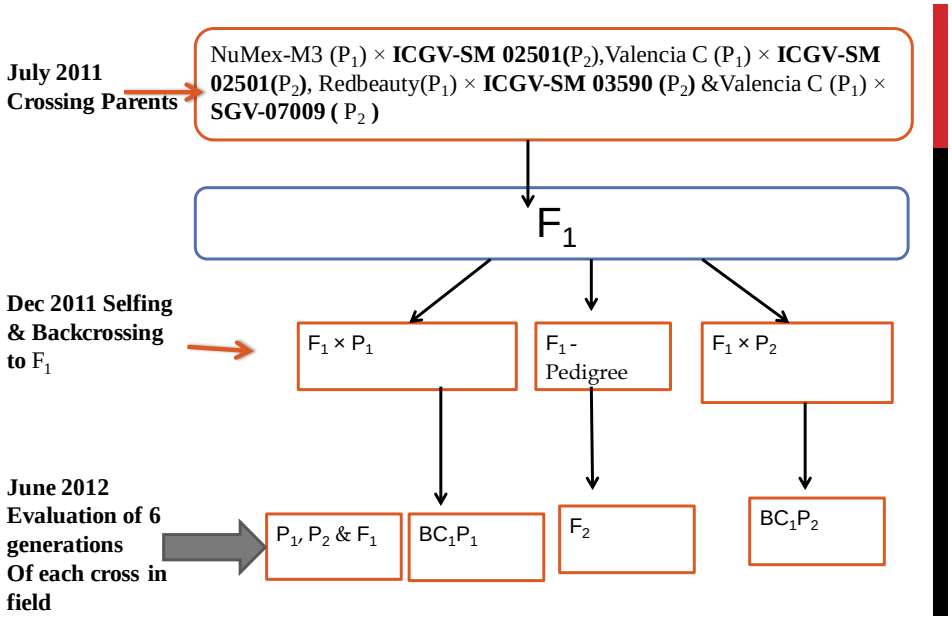


Figure 1: Crossing Scheme used to generate F_1, F_2, BC_1P_1 & BC_1P_2

3.2.5 Evaluation of the six generations of each cross of the four crosses

3.2.5.1 Field Layout

The generations of the four crosses were evaluated in the experimental field at NaSARRI, a known hotspot for LLS disease. Six generations, namely P_1, P_2, F_1, F_2 and $BC_1P_1$and BC_1P_2 of each cross of the four crosses *viz.*,NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, Redbeauty × ICGV-SM 03590 and Valencia C × SGV-07009 were set in a randomized complete block design (RCBD) in three replicates with 2-row-plots of ten plants each. The populations and parental lines were planted in the field at a spacing of 45 cm ×15 cm in June 2012, and the experiment was kept free of weeds throughout the cropping season.

3.2.5.2 Inoculation

To maximize leaf spot inoculum pressure under natural conditions spreader row technique was used. The groundnut line JL 24 which is highly susceptible to late leaf spot was provided by the groundnut improvement Program NaSARRI and used as a spreader row for LLS disease.

Spreader rows were planted after every two rows of test materials and at the border of the experiments to maintain the effective inoculum load.These rows were planted two weeks before planting the experimental materials.

3.2.5.3 Data collection

Late leaf spot disease severity scoring was done at 60 and 115 days after sowing (DAS) (at harvesting maturity) using a modified nine point scale (Subrahmanyam *et al*., 1995) (Table 2 and Figure 2), where a score of 1 was rated as highly resistant (HR), 2 to 4 as resistant (R), 5 and 6 as moderately resistant (MR), 7 and 8 as susceptible, and 9 as highly susceptible (HS).

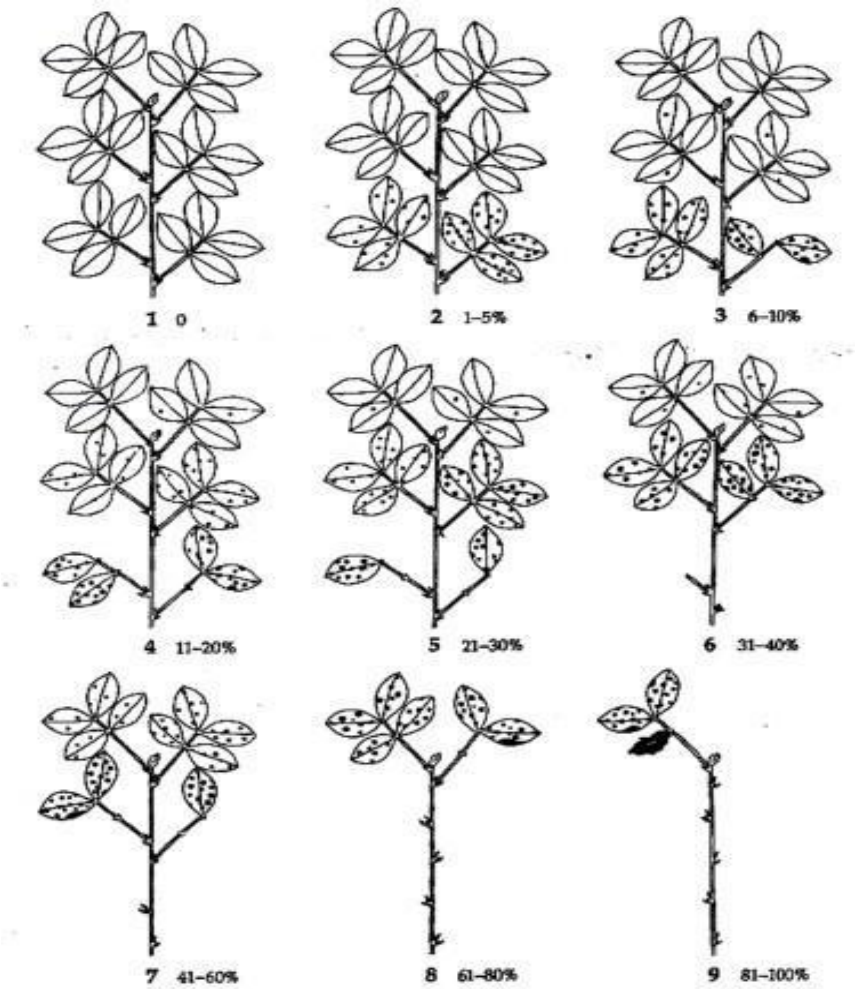


Figure 2: The modified 9-point scale for field evaluation of late leaf spot (Subrahmanyam *et al*., 1995)

Table 2: Modified 9-point scale used for field screening groundnut genotypes for resistance to late leaf spot.

Disease Score	Description	Disease severity(%)[1]
1	No disease	0
2	Lesions present largely on lower leaves; no defoliation	1-5
3	Lesions present largely on lower leaves, very few 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 present on all lower and middle leaves; over 50% defoliation of lower leaves	21-30
6	Severe lesions on lower and middle leaves; lesions present but less severe on top leaves; 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; severe lesions on top leaves; some defoliation of top leaves evident	61-80
9	Almost all leaves defoliated, leaving bare stems; some leaflets may remain, but show severe leaf spots	81-100

(Subrahmanyam *et al.*, 1995)

Table 3: Details on the number of plants per generation that were evaluated for each cross

Generations	NuMeX-M_3 × ICGV-SM 02501	Valencia C × ICGV-SM 02501	Valencia C × SGV 07009	Redbeauty× ICGV-SM 03590
P_1 (S)	30	30	30	30
P_2(R)	30	30	30	30
F_1	35	40	48	42
F_2	60	55	58	54
BC_1P_1	50	40	44	50
BC_1P_2	45	50	55	53

P_1(S) =Susceptible parent, P_2(R) =resistant parent, F_1=first filial generation, F_2= Second filial generation, BC_1P_1 = Backcross to susceptible parents (P_1) and BC_1P_2 =Backcross to resistant parent (P_2),

3.3 Statistical Analysis

3.3.1 Analysis of Variance (ANOVA)

Data on disease severity at harvesting maturity (115 DAS) on the generations of each cross were subjected to ANOVA using a computer program Genstat version 13 to test for the significance of the differences between the generations of each cross for LLS disease score. The ANOVA was based on the linear mathematical model: $Y_{ij} = \mu + r_i + g_j + e_{ij}$; where Yij= observed effect for i^{th} replication and j^{th} genotype, μ = grand mean of the experiment, r_i = effect of the i^{th} replication, g_i = effect of the j^{th} genotype, e_{ij} = residual effect.The generation means were compared using Fisher's protected least significant difference test at 5% level of probability where ANOVA showed significant difference (Payne *et al*., 2010).

3.3.2 Estimation of variance components

In order to determine phenotypic and genotypic coefficients of variability, heritability and genetic advance for LLS resistance, variance components (environmental, genotypic, additive and dominance) were obtained following (Kearsey and pooni, 1996) method for the three

crosses (NuMeX-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, and Redbeauty × ICGV-SM 03590) as detailed below.

Environmental variance or error (σ^2e) = (σ^2P_1+ σ^2P_2 +2 σ^2F_1)/4……..Equation 1

Where; σ^2P_1 =variance of susceptible parents, σ^2P_2=variance of resistant parents and σ^2F_1=variance of first filial generations

Phenotypic Variance [σ^2F_2] = variance of F_2 generation…….... Equation 2

Genotypic variance in F_2[$\sigma^2G(F_2)$]= σ^2F_2-σ^2e……Equation 3

Where; σ^2F_2=variance of F_2 generation and σ^2e = Environmental variance

Additive variance in F_2[$\sigma^2A(F_2)$]=(2σ^2F_2)- [$\sigma^2BC_1P_1$ + $\sigma^2BC_1P_2$]……...Equation 4

Where; σ^2F_2 = variance of F_2 generation, and $\sigma^2BC_1P_1$and$\sigma^2BC_1P_2$ = variance of backcross to female and male parents respectively.

Dominance variance in F_2[$\sigma^2D(F_2)$] = $\sigma^2G(F_2)$- $\sigma^2A(F_2)$……..Equation 5

Where; [$\sigma^2G(F_2)$]= Genotypic variance in F_2 and $\sigma^2A(F_2)$=Additive variance in F_2

3.3.3 Coefficient of variability

Phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were estimated following the method suggested by Singh and Chaudhury (1985), and the PCV and GCV values were classified as described by Sivasubramanian and Menon (1973) as low (0-10), medium (10-20) and high (20 and above).

Phenotypic coefficient variation (PCV) = ($\sqrt{V_P}$)/$\overline{X}$)*100

Genotypic coefficient variation (GCV) = ($\sqrt{V_G}$)/$\overline{X}$)*100

Where,

V_P= Phenotypic variance as described in equation2

V_G=Genotypic variance as described in equation 3

$\overline{X}$=Grand mean of the character under evaluation

3.3.4 Heritability

Variance components (environmental, genotypic, additive and dominance) obtained above were used to determine broad sense (h^2_b) and narrow sense (h^2_n) heritability (Kearsey and pooni, 1996) in the three crosses (NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501and Redbeauty × ICGV-SM 03590) as detailed below.

Broad-sense (h^2_b) = $[\sigma^2G(F_2)/V_{F2}]$ *100

Where; $\sigma^2G(F_2)$= Genotypic variance in F_2 and V_{F2}= phenotypic variance of F_2 generation

Narrow-sense heritability (h^2n) = $100[\sigma^2A(F_2)/V_{F2}]$

Where; $\sigma^2A(F_2)$= Additive variance in F_2 and V_{F2}= Phenotypic variance of F_2 generation

3.3.5 Genetic advance (GA)

Genetic advance was estimated following Singh and Chaudhury (1985) method.

Genetic advance (GA) = $h^2_n \times k \times \sigma_p$

Where,

h^2_n = Narrow sense heritability estimate

σ_p = Phenotypic standard deviation

K = Selection intensity at 5% is equal to 2.06

3.3.6 Genetic advance as percent of mean (GAM)

GAM% = $(GA/\overline{X})$*100

Where $\overline{X}$= Grand mean of the trait

GA = Genetic advance

The Genetic Advance as percent of Mean (GAM %) was categorized as described by Johnson *et al.* (1955) as low (0-10), medium (10-20) and high (20 and above).

3.4 Results

3.4.1 ANOVA results

The results of ANOVA are shown in Table 4. The mean disease scores of the donor parents ICGV-SM 02501and ICGV-SM 03590 was low except SGV-07009 (another donor parent) which had very high disease scores like the susceptible parents (NuMeX-M_3, Redbeauty and Valencia C). Moderate to high levels of LLS resistance was observed in all populations of the crosses, which showed highly significant differences among their generations for LLS disease score (Table 4).

Table 4: Results of LLS mean score and standard error for the six generations of the 4 crosses

Generation	NuMeX-M_3 × ICGV-SM 02501	Valencia C × ICGV-SM 02501	Valencia C × SGV 07009	Redbeauty× ICGV-SM 03590
P_1 (S)	6.79±0.25c	7.44±0.38d	7.29±0.61b	7.00±0.41c
P_2(R)	3.42±0.18a	3.40±0.16a	8.36±0.31b	3.50±0.50a
F_1	3.50±0.50ab	3.83±0.40ab	7.52±0.78b	4.50±0.50a
F_2	5.33±0.88b	5.22±0.40c	5.00±0.38a	4.60±0.40a
BC_1P_1	5.25±0.75b	4.75±0.48bc	8.17±0.65b	5.00±0.58ab
BC_1P_2	4.75±0.63ab	4.25±0.63abc	5.25±0.37a	4.50±0.50a
MS	**27.32**	**20.14**	**21.20**	**4.03**
F	**19.93****	**20.80****	**13.66****	**4.35****
CV %	**22.1**	**20.6**	**18.2**	**18.1**

P_1(S) =Susceptible parent, P_2(R) =resistant parent, F_1=first filial generation, F_2= Second filial generation, BC_1P_1 = Backcross to susceptible parents (P_1) and BC_1P_2 =Backcross to resistant parent (P_2), MS=Mean sum of square, F = Variation ratio, **=significant at P<0.01, CV=Coefficient of variation,

In all the four crosses, the means of the parents (P_1 and P_2) showed a tendency to be more extreme and contrasting for LLS resistance except for Valencia C × SGV-07009 cross which had scores of 7.29 and 8.36 for the susceptible and resistant parents respectively (Table 4). Therefore, this cross was not considered for further analysis. With exception of Valencia C ×

SGV-07009 cross, the backcrosses BC_1P_1 and BC_1P_2 showed the mean leaf disease score close to their respective recurrent parents (Table 4). The segregants in F_2 generation of the cross Valencia C × ICGV-SM 02501, NuMex-M_3× ICGV-SM 02501 and Valencia C × SGV-07009 showed moderate severity for LLS disease score, while that of Redbeauty× ICGV-SM 03590 cross were highly resistant to LLS. In general the grand mean result indicated that resistance was higher in populations of cross Valencia C × ICGV-SM 02501(4.77) than NuMex-M_3 × ICGV-SM 02501(5.30) and Redbeauty × ICGV-SM 03590 (5.30) (Table 5 and plate 1&2).

3.4.2 Estimation of coefficients of variability and heritability

The results demonstrating phenotypic and genotypic coefficients of variation, heritability and genetic advance estimates for resistance to LLS are presented in (Table 5).The PCV estimates were high in NuMex-M_3 × ICGV-SM 02501(28.82%) and Valencia C × ICGV-SM 02501 (25.21%) crosses and moderate in Redbeauty× ICGV-SM 03590(16.89%) cross. The genetic coefficient of variation (GCV) estimates were high in cross NuMex-M_3× ICGV-SM 02501(23.13%), moderate in Valencia C × ICGV-SM 02501(15.87%) and low in cross Redbeauty × ICGV-SM 03590 (9.50%).

Broad-sense heritability estimates for LLS disease score were 32%, 37% and 64%, respectively, for Redbeauty × ICGV-SM 03590, Valencia C × ICGV-SM 02501 and NuMex-M_3 × ICGV-SM 02501. Narrow-sense heritability estimates were 12%, 27% and 36%, for Redbeauty × ICGV-SM 03590, Valencia C × ICGV-SM 02501 and NuMex-M_3 × ICGV-SM 02501 crosses respectively while GAM were low in cross Redbeauty × ICGV-SM 03590 (4.17%), moderate in Valencia C × ICGV-SM 02501 (13.63%) and high in NuMex-M_3× ICGV-SM 02501 (21.37%).

Table 5: Genetic variance components and parameters for resistance to late leaf spot in groundnut

CROSS	NuMex-M_3 × ICGV-SM 02501	Valencia C × ICGV-SM 02501	Redbeauty × ICGV-SM 03590
V_E	0.83	0.90	0.54
V_G	1.50	0.54	0.25
V_A	0.83	0.39	0.10
V_D	0.67	0.15	0.16
PCV	28.82	25.21	16.89
GCV	23.13	15.43	9.50
h^2_b(%)	64.00	37.00	32.00
h^2_n(%)	36.00	27.00	12.00
$\overline{X}$	5.30	4.77	5.30
GA	1.13	0.67	0.22
GAM%	21.37	13.63	4.17

V_E = Environmental variance, V_G=Genotypic variance, V_A=Additive variance, V_D=Dominance variance, PCV and GCV=Phenotypic and Genotypic Coefficient of Variation respectively, $h^2_b$and h^2_n=Broad and narrow sense heritability respectively, $\overline{X}$=Grand mean, GA=Genetic advance and GAM=Genetic advance as percent of mean.

a. Valencia C

b. ICGV-SM 02501

c. BC_1P_1 (Valencia Cx Valencia C)

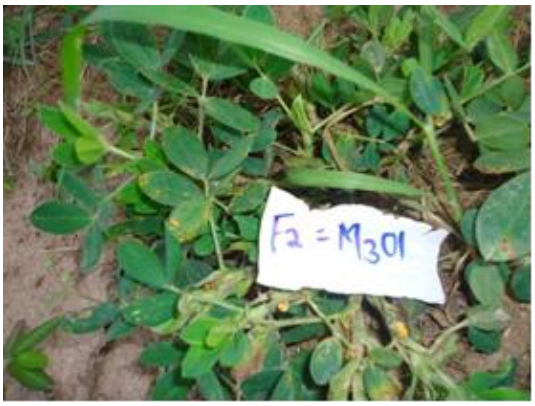
d. BC_1P_2 (Valencia C x ICGV-SM 02501)

e.ICGV-SM 03590

f. F_2(Valencia C X ICGV-SM 02501)

Plates 1:Variability for LLS resistance among the different generations of the crosses (severity at 6o DAP)

a. F_2 (NuMex-M_3× ICGV-SM 02501)



b.BC_1P_2 (NuMex-M_3 × ICGV-SM 02501)

c.NuMex-M_3

d.BC_1P_2 (NuMex-M_3 × ICGV-SM 02501)

e.F_1(Redbeauty × ICGV-SM 03590)

f.BC_1P_2(Redbeauty × ICGV-SM 03590)

Plates 2: Variability for LLS resistance among the different generations of the crosses (severity at 6o DAP)

3.5 Discussion

The ANOVA for the 4 crosses showed highly significant differences ($P \leq 0.01$) among generations for late leaf spot resistance (Table 4), suggesting presence of genetic variability for LLS disease score in the generations. Variability for LLS resistance was also reported by Vishnuvardhan *et al*. (2012) inexperimental materials that comprised of 28 F_2 populations. In the present study genotypes ICVG-SM 02501 and ICGV-SM 03590 showed high resistance to LLS (Plate1b&e), and are recommended for use in breeding program as sources of resistance to late leaf spot. An earlier report by Kalule *et al.* (2010) demonstrated that these lines were the best parents for LLS resistance. Moderate to high levels of LLS resistance was observed in the populations of the 4crosses (Table 4), indicating that the trait under study was heritable. The results partly agree with that of John *et al*. (2008) who reported moderate incidence of LLS in F_2 population of the Kadiri-3 × ICGV-88083. Kornegay *et al*. (1980) also observed minimal leaf defoliation in F_1 and F_2 generations of the cultivated Virginia.

The mean of F_1s of NuMex-M_3 × ICGV-SM 02501,Valencia C × ICGV-SM 02501 and Redbeauty × ICGV-SM 03590 crosses tended towards the mean of ICGV-SM 02501 and ICGV-SM 03590 the resistant parents respectively (Table 4). Such hybrid vigour (relative heterosis) in F_1s could be due dominance effects or epistatic gene action. In groundnut however, commercial production of F_1 seed is not feasible currently because it's predominately self-pollinated. In addition due to its tetraploid nature heterosis is unstable in groundnut. According to John *et al*. (2012), heterotic crosses in self-pollinated crops help breeders to select appropriate crosses that could lead to desirable transgressive segregants in advanced generations. Therefore, breeding methods such as recurrent selection and single seed descent may be used in exploitation of such heterosis if it's due dominance effects or epistatic gene action in future breeding programs for these crosses.

Several reports indicate that resistance to LLS in groundnut is controlled by several recessive genes (Khedikar *et al*., 2010; Upadhyay *et al*., 2009; Dwivedi *et al*., 2002; Motagi, 2001; Nevill 1982), however, the F_1's in the present study exhibited partial resistance owing to the low severity scores observed (3.5 to 4.5) as compared to the susceptible parents with scores >6. Walls and Wynne (1985) concluded that partial resistance in F_1 could not be explained solely

by completely recessive genes. They believed that modifier genes were affecting the phenotypic expression of genes at loci controlling resistance. Anderson *et al*. (1990) also reported recessive as well as modifier genes to be involved in resistance to LLS in groundnut.

The other donor line SGV-07009 was highly susceptible to late leaf spot, inspite of the fact that segregating populations F_2 and BC_1P_1 of the cross Valencia C ×SGV-07009 were moderately resistant (Table 4). Natarajan *et al*. (2001) reported that crosses involving susceptible parents may tend to produce resistant progeny with stable resistance due to additive genetic action. Babu (2010) recommended that such transgressive segregants that arise from the susceptible parents on both sides can also be used as potential genetic stocks in resistance breeding programs.

Moderate to high level of genetic coefficients of variability **(**GCV) (15.43 to 23.13) and phenotypic coefficients of variability **(**PCV)(16.89 to 28.82) was noticed for LLS resistance in all crosses, except for the cross Redbeauty× ICGV-SM 03590 which showed low GCV (9.50) (Table 5).The results are comparable with Vishnuvardhan *et al*. (2012) observation of moderate PCV(19.04) and GCV (16.48).The results of the study also partly agree with those of Khedikar *et al*.(2010) which indicated high PCV (21.71 to 33.55), Khedikar, (2008) which indicated moderate to high GVC (14.46 to 24.76) and Kumari (2008) which indicated high PCV (29.96 to 36.07) and GCV(27.71 to 32.96) for late leaf spot resistance. High PCV and moderate to high levels of GCV revealed high magnitude of heritable variation for LLS resistance in these crosses.

Generally the magnitude of PCV (16.89 to 28.82) was superior over that of GCV (9.50 to 23.13) indicating greater influence of environment on the trait thereby reducing possible response to selection for LLS resistance on phenotypic basis. Similar observations were also reported by Korat *et al.* (2009) and Vishnuvardhan *et al*., (2012). Nonetheless, in the current study, high GCV (23.13) was exhibited in the cross NuMex-M_3 × ICGV-SM 02501. A high GCV indicated that the character had high variability which can be attributed to genotype and with very little effect of the environment. According to Oyiga and Iguru (2011), when the magnitude of GCV is higher, it indicates that the genetic component is the major contributor to the total variance of the trait under study. High PCV and GCV of a trait may result in high heritability which suggests that the improvement of this trait by simple selection method could

be possible. Vishnuvardhan *et al*. (2012) concluded that high GCV may indicate a predominant role of additive gene actions and amenability for phenotypic selection in early generations.

Heritability is defined as the extent to which a trait variation is heritable. All the three crosses in the present study showed moderate to high broad-sense heritability (32-64%) and low to high GAM (4.17-21.37%) while low to moderate narrow-sense heritability (12-36%) was noted for LLS disease score (Table 5). The results are in a close agreement to earlier studies by Kormsa-art *et al.* (2002) and Jogloy *et al*. (1987) which indicated low h^2_n (0.0–0.13) for LLS resistance and Anderson *et al*. (1991) which indicated low to high h^2_n (0.18 to 0.74) for LLS resistance. Moderate to very high h^2_b (40.87 to 82.81) estimates were reported for leaf spot disease severity in groundnut Khedikar *et al*. (2010), and Kumari, (2008) also report very high h^2_b (83.50 to 85.50). Vishnuvardhan *et al*. (2012) reported moderate GCV (16.48), high broad-sense heritability (74.91) and GAM (29.38) while Kumari (2008) observed high GCV (27.71 to 32.96), broad-sense heritability (83.50 to 85.50) and GAM (52.78 to 62.05) for late leaf spot severity. The discrepancy of the results is not unexpected because such quantitative traits are often affected by several environmental factors and the genetic background of the parental materials. Falconer and Mackay (1996) concluded that heritability values depend on the population and environmental conditions in which the materials are evaluated.

Moderate to high h^2_b revealed the existence of inherent variability among the genotypes, which is more useful for exploitation in selection and hybridization programs. High h^2_b (64.41%) estimates was observed in cross NuMex-M_3× ICGV-SM 02501 indicating a high responses to selection due to reduced environment influence thereby validating the results obtained with the high GCV (23.13%) value for this cross. Anderson *et al*. (1986a) also observed high heritability for LLS resistance and concluded that individual plant selection for LLS would be effective in early generations. Moderate h^2_b estimates were observed in Redbeauty × ICGV-SM 03590(32%) and Valencia C × ICGV-SM 02501 (37%) crosses suggested a high influence of the environment on the trait in these crosses. Thus selection of genotypes from initial generations for LLS disease score in these crosses may be difficult. Singh (1993) concluded that low to moderate heritability estimates makes selection considerably difficult or virtually impractical due to the masking effect of the environment on the genotypic effect. The high environmental variation could have been as result of variation in relative humidity within the

micro-climates. Furthermore, LLS resistance is polygenically controlled, and cumulative environmental effects on this polygenically controlled trait could have given poor heritabilities for this trait. In such cases simple selection may not be rewarding. Breeding efforts to increase resistance will require good control over environmental variance. Adequate experimental design, accurate phenotyping are key interventions that could increase the heritability of such a polygenic trait.

In addition, relatively few individuals and different number of individuals/generation were analysed for each cross (ref Table 3), this variation also could have had bearing on the heritability estimates that were obtained for the different crosses. It's likely that the error variance increase while working with small populations and this could have resulted to low heritability estimates observed some crosses. For instance in the study 30-60 plants were used, in other studies the number ranged from 40-400 Janila *et al*.(2013a) but also reported moderate to high (0.37- 0.82) broad sense heritability for LLS disease score which is comparable to the observations made in the current study. Though this study was done on relatively few individuals due limited time and low success rate of the crosses after hybridization, it provides quick trends on the genetics of LLS resistance, which are of practical relevance to groundnut breeders.

Broad sense heritability provide us with useful information on genetic variability, however, it should be noted that the coefficient comprises all the genetic influences in its expression, instead of only the additive effects of additive genes. Thus, except for conditions where dominance effects are null, this cannot be used as a precise indicator for obtaining a precise estimation of selection gains. In other words, estimates of selection gains may be over-estimated by the use of this coefficient. Therefore, h^2_n coefficient is more important, because it comprises only additive effect of additive genes.

In this study low to moderate values for narrow-sense heritability were observed in all crosses, which indicated either greater dominance effects on the trait or the environment that contributed a large effect on LLS resistance than the genotypes. It is normally verified that an increase in magnitude of dominance component of the variance (V_D) implies a decrease in h^2_n in the reference F_2 generation. Thus selection of genotypes from initial generations for LLS disease score in these crosses may be difficult due to the higher influence of dominance effects.

According to (Kearsey and Pooni, 1996; Kormsa-art *et al.*, 2002) selection for low heritability traits, or those controlled by dominance, is ineffective when carried out in early generations. For this reason, selection based on individual plants for LLS resistance is more effective when undertaken in subsequent generations in all crosses. In this way, the occurrence of heterozygotes is reduced and the available additive variance for selection is increased, thereby providing higher possibilities of selection gains for the trait. According to Silva *et al.* (2004), in theory, it is considered that an F_5 generation individual presents enough homozygosis levels to allow for selection, mainly due to the absence of significant additions to the level of homozygous individuals in future generations which would imply longer periods for selection.

According to Jinks and Pooni (1984), if selection is delayed further into the inbreeding program for low heritability traits, there will be an increase in h^2_n and hence increase in response to selection, however, it would be appropriate to use family rather than individual, if selection is to be based on early generations. Kearsey and Pooni (1996) recommended that selection in F_2 and other generations of the population to be improved should be based on family means in order to get high genetic gain among the progeny because the environmental variation is reduced by working with means. For characters with low h^2_n estimates, Kearsey and Pooni (1996) and Oeveren and Stam (1993) recommended that bulk and single seed descent (SSD) breeding methods, followed by selection on family mean can ensure high genetic gain among the progeny.

In the present study, the estimate of h^2_n was 36% for the LLS disease score in NuMex-M_3× ICGV-SM 02501 cross. Ali *et al*. (1999) and Brogin *et al.* (2003) reported that traits with heritability estimates higher than 30% allow for genetic gains through selection in initial generations of inbreeding, such as F_3 or F_4 generations.

Heritability estimates along with genetic advance provide better prediction of expected gain under selection instead of heritability alone. Higher estimate of heritability do not necessarily provide high values of genetic advance (Vishnuvardhan *et al*., 2012) and heritability alone provides no indication for the amount of genetic progress in the trait that can be achieved through selection. The effectiveness of selection depends upon genetic advance of the character selected along with heritability ie. Genetic advance (GA) = $h^2_n \times k \times \sigma_p$ **(**Manju and Sreelathakumary, 2002).

Moderate (36%) heritability (h^2_n) coupled with high genetic advance (21.37%) was observed for LLS resistance in NuMex-M_3 X ICGV-SM 02501 cross, indicating significant role of additive gene action for its inheritance. Therefore, simple selection methods would be effective for improvement of this trait. The results are comparable with reports of Vishnuvardhan *et al*. (2012) which indicated high GA for LLS resistance. Low (27%) heritability (h^2_n) estimates along with moderate genetic advance (13.63%) was observed in Valencia C × ICGV-SM 02501 cross, which indicated that additive and non-additive gene actions have a role in the inheritance, and phenotypic selection would be effective to some extent. The successful breeding methods will be the ones, which exploit additive and non-additive gene effect such as recurrent selection (Nidagundi *et al*., 2012), diallel selective mating (Jensen, 1978) and use of biparental mating (Soomro *et al*., 2010). Low (12%) heritability (h^2_n) estimates coupled with low genetic advance (4.17%) was observed in Redbeauty × ICGV-SM 03590 cross, indicating that the trait was poorly heritable and could be due to either predominance of non-additive gene activity, presence of large environmental effects or a relatively small population that was used. In such a case simple selection methods may not be rewarding, suggesting maintenance of heterozygosity in the population.

Overall, the study has provided insight into the genetics of LLS resistance. The key findings are that: (a) Considerable amount of heritable variation for LLS disease scores was revealed.

(b) Heritability estimates depended on the genetic background of the parents that were used.

Based on the observed results, it can be concluded that the best strategy for obtaining LLS resistant genotypes is for selection to be carried out in initial inbreeding generations (F_3 or F_4) for the Cross between NuMex-M_3× ICGV-SM 02501, followed by selection in the following generations with higher inbreeding levels in other crosses.

This information is extremely valuable and should thus guide the breeding programs which wish loam or work more on the student's thesis.

CHAPTER FOUR

4.0 Determination of gene action controlling late leaf spot resistance

4.1 Introduction

Inheritance studies on late leaf spot (LLS) suggest that resistance is quantitatively inherited (Khedikar *et al*., 2010). A good knowledge of the genetic systems controlling expression of these quantitative traits facilitates the choice of adopting the most efficient breeding and selection procedure. In addition to additive and dominance variation, it has been suggested that epistasis may also be involved in the inheritance of LLS resistance in groundnut (Shoba *et al*., 2010). However, the information on non-allelic interactions for LLS resistance in groundnut is very limited. While variation due to dominance effects and their interactions cannot be exploited effectively in groundnut, additive x additive epistatic variation is potentially useful, as it can be fixed in homozygous cultivars (Singh and Oswalt, 1991). Although additive gene actions of LLS resistance have been predominantly reported in the control of LLS resistance (Anderson *et al*., 1986 a and b and Walls and Wynne, 1985), the genetics of LLS resistance may vary with the genetic background of the parental lines used in the study and environmental conditions in which they are evaluated (Shoba *et al*., 2010). In the present study, the generation mean analysis was employed to obtain information on the type and magnitude of gene actions controlling LLS in groundnut in order to provide a basis for an evaluation of selection methods for the improvement of the groundnut population.

4.2 Materials and methods

The six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) of crosses between NuMex-M_3× ICGV-SM 02501, Redbeauty × ICGV-SM 03590 and Valencia C × ICGV-SM 02501were used for this study. The generations were developed as illustrated earlier in chapter 3.

4.2.1 Data collection and Analysis

Late leaf spot disease severity scoring was done 115 days after planting using a modified nine point scale (1-9) (Subrahmanyam *et al*., 1995).Visual score of disease reaction on individual plants from each of the six generations (P_1,P_2,F_1,F_2,BC_1P_1 and BC_1P_2) was used to calculate the generation means and variances. The means and variances of the six generations from the three crosses (NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501 and Redbeauty × ICGV-SM 03590) were subjected to scaling tests A, B and C (Mather and Jinks, 1982) to test for the adequacy of additive-dominance model. The A and B scaling tests provide the evidence for the presence of additive x additive (*i*), additive x dominance (*j*) and dominance x dominance (*l*) type gene interactions, while the C scaling test provide a test for type dominance x dominance (*l*) epistasis.

The scales were tested for their significance by *t*- test at 5% level of significance as, t_A =A-0/SE_A, t_B =B-0/SE_B and t_C =C-0/SE_C where SE_A, SE_B and SE_C are standard errors of A,B and C scaling tests, respectively. The null hypothesis for test of significance (H_o) was that A=0 or B and C in place of A of the scaling test. The additive-dominance model was considered adequate when the *t*- test of any one of the three scales was found not significant. The following assumptions were made while performing the scaling test; (1) all generations have been raised in the same environment,(2) only autosomal inheritance is considered (3) non-allelic interaction is absent and (4) no differential fertility and viability.

To estimate the gene effects a joint scaling test was performed following the method described by Kearsey and Pooni (1996) which uses the weighted least squares analysis, whereby weighting factors is the inverted ratio of the variance of the means for each generation evaluated and the inverse of the matrix of the parameters. The variance of the means of the generations was obtained by dividing the treatment mean variances by their respective number of individuals on which observations were recorded in each generation. The weighted analysis was used due to the fact that the estimates of the means are obtained with distinct precision among the different generations (Dabholkar, 1992; Kearsey and pooni, 1996). Genetic models were adjusted to means of the parent lines P_1 and P_2, $F_1$and their segregating generations F_2 and the respective backcrosses BC_1P_1 and BC_1P_2. Initially, it was predicted to use a simple genetic model additive- dominance type involving m, [a] and [d] parameters. The components m

represents the average value between parents, [a] represents the algebraic sum of the additive effects of all distinct loci between the parents, and [d] represents the algebraic sum of dominance effects of all distinct loci between the parents. The expectations of the six generations on an additive-dominance model are shown in (Table 6). Accuracy of the model was verified by a chi-square (χ^2) test and components within each model were evaluated for significance by *t*-test.The adequate model was obtained only when all the components estimated were significant by a *t*-test and non-significant at the chi-square (χ^2) test. If the three parameter models prove to be unsatisfactory (significant χ^2 value) for explaining genetic mechanisms controlling the trait being investigated, an alternative six parameter model, including non-allelic interaction parameters between pairs of loci as additive by additive [aa], additive by dominance [ad], and dominance by dominance [dd] is used.

Table 6: Expectations of the six generations on an additive-dominance model

Generations	M	[a]	[d]
P_1	1	1	0
P_2	1	-1	0
F_1	1	0	1
F_2	1	0	0.5
BC_1P_1	1	0.5	0.5
BC_1P_2	1	-0.5	0.5

Source: Kearsey and Pooni, 1996

4.3 Results

The results of the scaling tests and estimates of gene effects along with their standard error are presented in Table 7 and 8 (a &b) respectively. In the present study all values of A, B and C scaling tests were not significantly different from zero by *t*-test (Table 7), implying that genetic effect was either additive or dominance or both additive and dominance gene action, and hence additive - dominance model was adequate for explaining resistance to LLS.

On the joint scaling test, the initial 3-parameter model [m, a &d] (Table 8a) that was employed was found to be adequate for all crosses as revealed by non-significance of the χ^2 values, confirming absence of any epistatic interactions in these crosses as revealed by results of the

scaling tests. Therefore, the interacting terms (additive by additive [aa], additive by dominance [ad], and dominance by dominance [dd]) were not computed.

Table 7: Scaling test estimates along with their standard errors and *t* test for the scaling tests of the 3 crosses

CROSS	Scaling test	Scaling test values observed	*t*' value
NuMex-M_3×ICGV-SM 02501	A	2.58 ± 1.59	1.62n.s.
	B	0.21 ± 1.37	0.16n.s.
	C	4.00 ± 3.68	1.09n.s.
Redbeauty × ICGV-SxM 03590	A	1.00 ± 1.08	0.93n.s.
	B	-1.50 ± 1.55	-0.97n.s.
	C	-1.10 ± 1.38	-0.80n.s.
Valencia C × ICGV-SM 02501	A	-1.78 ± 1.10	-1.61n.s.
	B	1.27 ±1 .33	0.95n.s.
	C	2.38 ± 1.84	1.29n.s.

A=Scaling test A, B=Scaling test B and C=Scaling test C, and *t* =calculated *t* values and n.s. = P >0.05

Table 8a: Genetic parameters for LLS disease score for the three crosses on a three parameter model.

3 parameter model	NuMex-M_3 × ICGV-SM 02501	Redbeauty × ICGV-SM 03590	Valencia C × ICGV-SM 02501
M	5.13**±0.15	5.23**±0.30	5.37**±0.19
[a]	-1.66**±0.15	-1.57**±0.30	-1.93**±0.93
[d]	-1.20**±0.47	-0.87±0.57	-1.44**±0.42
$\chi 2$	4.45ns	5.99ns	6.374ns
DF	3	3	3

M= mid- parental value,[a]= additive gene effects,[d]=dominance gene effects, DF=degree of freedom and $\chi 2$=chi-square value

Table 8b: Genetic parameters for LLS disease score for the three crosses on a two parameter model.

2 parameter model	NuMeX-M_3× ICGV-SM 02501	Redbeauty × ICGV-SM 03590	Valencia C × ICGV-SM 02501
M	4.98** ± 0.14	4.89** ± 0.19	4.95**±0.15
[a]	1.62** ± 0.16	-1.63** ± 0.29	-1.65**±0.18
$\chi 2$	10.97*	6.02ns	26.11**

M= mid- parental value,[a]= additive gene effects, DF=degree of freedom and $\chi 2$=chi-square value

However, in the crosses NuMeX-M_3× ICGV-SM 02501 and Valencia C × ICGV-SM 02501, the trait showed adequate fit to an additive–dominance model [m, a &d] than in Redbeauty × ICGV-SM 02501 cross as evident from the estimates [d] of this cross that was not significant (Table 8a). Therefore, it was suggested to refit a 2-parameter model, with m and [a] parameters only so that more precise estimates are obtained in Redbeauty × ICGV-SM 02501. On a 2-parameter model (Table 8b), the trait showed adequate fit in only Redbeauty × ICGV-SM 02501 cross. The results revealed that both additive and dominance gene effects contributed significantly to the inheritance of LLS resistance in all the crosses except in Redbeauty × ICGV-SM 02501 cross where the effects of dominance were not significant. However, both additive and dominance gene effects were negative but the magnitudes of additive effects were higher than that of the dominance effects. The mid-parental effects (m) were significant and positive for all the crosses in all the models.

4.4 Discussion

Fisher's protected LSD test of means demonstrated that differences between the parents of each population were indeed real and significant (Table 4). The backcrosses BC_1P_1 and BC_1P_2 showed means that were close to those of their respective recurrent parents. These results confirmed the choice of parents for the present study as contrasting for LLS resistance which is a prerequisite for generation means analysis as proposed by Mather and Jinks (1980).

The results showed that there were no epistatic effects involved in the expression of LLS resistance in these crosses, which partly agree with previous findings by Nevill, (1982) and

Jogoly *et al*. (1999b) which indicated that both additive and dominant effects are involved in the expression of LLS resistance. Many authors however, have reported predominantly additive gene effects for most of the components of resistance to LLS including; latent period, lesion number, lesion size, defoliation, incubation period, leaf area damage, reduced spore production, area under disease progress curve and disease score (Kornegay *et al.,* 1980; Anderson *et al.,* 1986b; Jogoly *et al*., 1999a and b; Vishnuvardhan *et al*., 2011; Janila *et al*., 2013a) which is also comparable to results of the current study. In this study, additive component [a] contributed more to the inheritance of LLS disease score than the dominance component in all the 3 crosses, which is comparable to earlier reports by (Anderson *et al*., 1986 a and b; Walls and Wynne, 1985; Jogloy *et al.,* 1987; Vishnuvardhan *et al*., 2011), suggesting that selection would be effective to some extent. Shoba *et al*. (2010), in contrast reported that in addition to predominance of non-additive component [d], the epistatic effects (additive by additive and dominance by dominance) also contributed resistance to LLS in groundnut. In addition to epistatic effects (additive x additive, additive x dominance and dominance x dominance), Janila *et al*. (2013a) reported that resistance to LLS was controlled by a combination of both, nuclear and maternal gene effects. Such variation in the results is probably due to genetic background of the parents and variation in environmental conditions in which the populations were evaluated.

The presence of significant additive effects in NuMex-M_3 × ICGV-SM 02501 and Valencia C × ICGV-SM 02501 crosses suggest that selection for LLS resistance disease is possible but presence of significant dominance effects suggest that selection should be practiced in later inbreeding generations. The breeding method that exploits both additive and non-additive gene effects may be suitable for the improvement of the groundnuts for LLS resistance. Singh and Oswalt (1991), Nidagundi *et al*. (2012) and Janila *et al*. (2013a) recommended that for traits controlled by additive and dominance gene effects recurrent selection may be a useful breeding strategy while Janila *et al*. (2013a) suggested use of reciprocal recurrent selection, and Dabholkar (1992) recommended biparental mating to improve the traits when both additive and non-additive effects are involved in trait expression.

The results for Redbeauty × ICGV-SM 02501 showed that additive gene action was most important for disease score of LLS while dominance effects were less important which

indicated that genetic improvement of the populations of this cross could be easier for LLS resistance. However, Ayele, (2011), and Ali and Khan (2007) concluded that effective selection in early generations of segregating materials can be accomplished only when additive genetic effects are substantial and heritability is high. Earlier studies by Kormsa-art *et al.*2002; Anderson *et al*., 1991; Jogloy *et al*., 1987) reported low to high narrow sense heritability for LLS resistance. Therefore, in Redbeauty × ICGV-SM 02501 cross selection in early generations of segregating materials could be effective only when the heritability of LLS resistance is high.

Negative sign of additive effect indicated that ICGV-SM 02501 and ICGV-SM 03590 were the source of LLS resistance which took low value on the scale while the negative sign of dominance effects indicated that dominance was in the direction of susceptibility.

Based on the result of this study, the additive and dominance gene effects were important for LLS resistance in groundnuts. However, the estimate obtained varied depending on the parental backgrounds used in making cross. Overall, the additive effects were predominant for LLS resistance. Therefore, genetic improvement of this trait is possible in all the crosses.

CHAPTER FIVE

5.1 General discussion, conclusions and recommendations

The significant differences among generations of the crosses in the investigation indicated presence of genetic variability in the material used and provide a good opportunity for improving groundnut for LLS resistance. ICVG-SM 02501 and ICGV-SM 03590 lines in the present study showed high resistance to LLS, and should be involved in breeding program as a source of resistance to late leaf spot which is in line with Kalule *et al.*(2010) report that indicated that these lines were the best parents for LLS resistance. The GCV estimates were high in cross NuMex-M_3 × ICGV-SM 02501(23.13%), moderate in Valencia C × ICGV-SM 02501(15.43%) and low in cross Redbeauty × ICGV-SM 03590 (9.50%). According to Mulualem *et al*. (2013), high GCV value of a character suggests the possibility of improving the trait through selection.

Although the genotypic coefficient of variation revealed substantial genetic variability present in the genotypes for LLS resistance, it does not provide full scope to assess the variation that is heritable. Heritability estimates would be helpful to the breeder to estimate genetic advance and to predict percentage of genetic advance in the population(s) under study (Dabholkar, 1992 and Mulualem *et al*., 2013).

In the current study, a high GCV (23.13), moderate h^2_n (36%) coupled with high GAM (21.37%) was exhibited in NuMex-M_3 × ICGV-SM 02501cross which might indicate a reduced environmental influence on the trait and a predominant role of additive gene actions and thus amenability for phenotypic selection in early generations. A moderate GCV (15.43), low h^2_n (27%) along with moderate GAM (13.63%) was exhibited in Valencia C × ICGV-SM 02501 cross which suggests that the additive and non-additive gene actions have role in inheritance of LLS resistance. Thus selection for LLS resistance would be effective to some extent. Low GCV (9.50), h^2_n (12%) coupled with low GAM (4.17%) was exhibited in Redbeauty × ICGV-SM 03590 cross suggesting the predominance of non-additive gene actions or higher environmental effects in the inheritance which would make selection not effective in early generations for this cross. The poor estimates also could be attributed to the few individuals that were used in the study.

Additive-dominance model in the present study was adequate for LLS resistance in NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501 and Redbeauty × ICGV-SM 03590 crosses. The results of the study partly agree with those of other reports (Vishnuvardhan *et al*., 2011; Jogoly *et al*., 1999b; Nevill, 1982). In the study, additive component [a] contributed more to the inheritance of LLS disease score, which is comparable to earlier reports by (Anderson et al., 1986 a and b; Walls and Wynne, 1985). Shoba *et al*. (2010) however, reported in addition to predominance of non-additive component [d], the epistatic effects (additive by additive and dominance by dominance) also contributed resistance to LLS in groundnut. Such variation in results is probably due to genetic background of the parents and variation in environmental conditions in which the populations were evaluated.

The presence of significant additive effects in NuMex-M_3× ICGV-SM 02501 and Valencia C × ICGV-SM 02501crosses suggest that selection for LLS resistance disease is possible but presence of significant dominance effects suggests that selection should be practiced in later generations. Nidagundi *et al*. (2012) recommended that for traits controlled by additive and dominance gene effects recurrent selection may be a useful breeding strategy while Soomro *et al*. (2010) suggested development of transgressive segregants through hybridization and utilization of hybridization system that exploits both additive and non-additive effects. The results for Redbeauty × ICGV-SM 02501 cross showed that additive gene action was the most important for LLS score of while non-additive effects (dominance and epistatic effects) were less important which suggests that selection for individual plants could be possible in early generations. However, Ayele (2011) and Ali and Khan (2007) reported that effective selection in early generations of segregating materials can be accomplished only when additive genetic effects are substantial and heritability is high. Therefore, in Redbeauty × ICGV-SM 02501 cross selection in early generations of segregating materials may not be effective due to high environmental influence on the trait which could have resulted to low heritability. In such a case, breeding efforts to increase resistance will require effective control of environmental variance. This can be achieved through using appropriate experimental design and accurate phenotyping of LLS.

Conclusion and Recommendations

The present investigation revealed considerable amount of variation for LLS disease scores. Such variation was a demonstration of prospects for improving groundnut for resistance to LLS. Additive-dominance model was adequate for LLS resistance in NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501 and Redbeauty × ICGV-SM 03590 crosses implying there was no epistatic effects involved in the expression of LLS resistance in these crosses. The additive component [a] contributed more to the inheritance of LLS than the dominance component. However, the amount of variation and magnitude of gene effects depended on the genetic backgrounds of the materials used in the study.

The environment contributed greater effect on variation for LLS resistance than the genotype in some crosses, suggesting that breeding efforts to increase resistance to LLS will require effective control over environmental variance and this can be achieved through using appropriate experimental design and accurate phenotyping of LLS.

Based on the observed results, it can be recommended that selection for LLS resistance is possible using the available materials. The best strategy for obtaining LLS resistant genotypes is for selection to be carried in subsequent generations (F_3 or F_4) for NuMex-M_3× ICGV-SM 02501 cross, followed by selection in the following generations with higher inbreeding levels in other crosses. Use of recurrent selection, reciprocal recurrent selection, biparental mating may be useful breeding strategies to improve such a trait which is controlled by both additive and non-additive gene effects. Bulk and single seed descent (SSD) breeding methods, followed by selection on family mean can also ensure high genetic gain among the progenies of such trait with low heritability.

A more detailed heritability study is recommended at advanced generations, other than F_2 to yield more accurate heritability estimates.

REFERENCES

Abdou, Y. A.M., Gregory, W. C., and Cooper, W. E. (1974). Sources and nature of resistance to *Cercospora arachidicola* and *Cercosporidium personatum* in *Arachis* spp. *Peanut Science*, 1:6-11.

Ali, M.A. and Khan, I.A. (2007). Assessment of genetic variation and inheritance mode of some metric traits in cotton (*Gossypium hirsutum* L.).*Journal of Agriculture & Social Science* 3: 112–116.

Ali, N., Wynne, J,C. And Murphy, J.P. (1999). Estimation of genetic effects and heritability for early maturity and agronomic traits in peanuts (Arachis hypogaea L.,).Pak.J.Bot., 31:323-335

Anderson, W. F., Beute, M. K., Wynne, J.C. and Wongkaew, S. (1990). Statistical procedures for assessment of resistance in a multiple foliar disease complex of peanut. *Phytopathology*, 80:1451-1459.

Anderson, W. F., Holbrook, C. C. and Brenneman, T. B. (1993). Resistance to *Cercosporidium personatum* within groundnut germplasm. *Groundnut Science* 20:53–57.

Anderson, W. F., Holbrook, C. C., and Wynne. J. C. (1991). Heritability and early-generation selection for resistance to early and late leaf spot in peanut. *Crop Science* 31:588–593.

Anderson, W. F., Wynne, .J. C., and Green, C. C. (1986a). Potential for incorporation of early and late leafspot resistance in peanut. *Plant Breeding* 97:163-170.

Anderson, W. F., Wynne, .J. C., Green, C. C., and Beute, M. K. (1986b). Combining ability and heritability of resistance to early and late leaf spot of peanut. *Peanut Science*, 13: 10-14.

Aquino,V.M., Shokes, F.M., Gorbet, D.W., and Nutter,F.W.,Jr. (1995).Late leaf spot progression on peanut as affected by components of partial resistance. *Plant Disease*, 79:74-78.

Asibuo, J.Y., Akromah, R., Safo-Kantanka, O., Adu-Dapaah, H.K., Ohemeng-Dapaah, S.,Agyeman, A. (2008). Chemical composition of groundnut, *Arachis hypogaea* (L) landraces. *Afr. J. Biotechnol* 7: 2203-2208.

Ayele .A.G. (2011).Heritability and genetic advance in recombinant inbred lines for drought tolerance and other related traits in sorghum (*Sorghum bicolor*). *Continental Journal. Agricultural Science* 5: 1 – 9.

Babu.C. (2010). Pre-breeding in sugarcane (*Saccharum* sp. hybrids) for red rot resistance and economic traits. *Electronic Journal of Plant Breeding*, 1: 1024-1034.

Banks, D. J., Pozorski, T., Pozorski, S., and Donnan, C. B. (1993). Origin and evolution of peanut from archaeological evidence.Proc. Am. Peanut Res. Educ. Soc. 25:34

Brogin, R.L., Arias, C.A.A., and Toledo, J.F.F. (2003). Genetic control of soybean resistance to brown spot (*Septoria glycines*): First studies. *Crop Breeding and Applied Biotechnology*, 3:35-44.

Busolo-Bulafu, C. M. (1990). Groundnut improvement program in Uganda.Fourth Regional Groundnut Workshop for Southern Africa, Arusha, Tanzania. ICRISAT, Pp 55–59.

Cantonwine, E.G., Culbreath, A.K., Holbrook C.C., and Gorbet. D.W. (2008). Disease progress of early leaf spot and components of resistance to *Cercospora arachidicola* and *Cercosporidium personatum* in runner-type peanut cultivars. *Peanut Science*, 35:1–10.

Cantonwine, E.G., Culbreath, A.K., Stevenson, K.L., Kemerait, R.C., Brenneman, T.B.,Smith, N.B. and Mullinix, B.G. (2006). Integrated disease management of leaf spot and spotted wilt of peanut. *Plant disease*, 90:493-500.

Chiteka, Z.A., D.W. Gorbet, F.M. Shokes, T.A. Kucharek, and D.A.Knauft.(1988). Components of resistance to late leaf spot in peanut.I. Levels of variability-implications for selection. *Peanut Science*, 15:25-30.

Coffelt, T. A. and Simpson, C. E. (1997). Origin of groundnut. In: Compendium of groundnut Diseases. Eds: Kokalis-Burelle, N., Porter, D. M., Rodriguez-Kabana, R., Smith D.H. and Subrahmanyam P. *The American Phytopathological Society*. St. Paul, Minnesota USA. pp. 1-6

Cook, M. (1981). Susceptibility of peanut leaves to *Cercosporidium personatum*. *Phytopathology*,71:787-91

Culbreath.A., Beasley.J., Kemerait.R., Prostko.E., Brenneman.T., Smith.N., Tubbs.S., Paz, Rabiu.J Olatinwo, Tillman.B and Gevens.A, Weeks.R and Hagan.A. (2009). Minimizing diseases of groundnut in the southeastern united states; the 2009 Version of the Groundnut Disease Risk Inde.Pp 41-56. In: E. Prostko. Ed., 2009 Peanut Update. The University of Georgia, Cooperative Extension Publication CSS-09-0114.

Dabholkar .A. R. (1992). Elements of biometrical genetics. New Delhi 110059, Pages 38-165.

Dwivedi, S.L., Pande. S., Rao, J.N. and Nigam S.N., 2002. Components of resistance to late leaf spot and rust among interspecific derivatives and their significance in a foliar disease resistance breeding in groundnut (*Arachis hypogaea* L.). *Euphytica*, 125:81–88.

Falconer, D.S. and Mackay, F.C.T. (1996). Introduction to Quantitative Genetic 4th Edition Longman Group Limited. Pages,108-183

FAOSTAT.2009.Crop Productivity. http://faostat.fao.org.

FAOSTAT.2013.Crop Productivity. http://faostat.fao.org.

Grichar, W.J., Besler, B.A., and Jaks, A.J. (1998). Peanut (*Arachis hypogaea* L.) cultivar response to leaf spot disease development under four disease management programs. *Peanut Science*, 25: 35-39.

Halward, T. M., Stalker, H. T., Larue, E. A. and Kochert, G. (1991). Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. *Genome*, 34: 1013–1020

Hammons, R . O. (1982). Origin and early history of the peanut. In: Peanut Science and Technology, ed, H. E. Pattee, C. T. Young, pp. 1-20. Yoakum , TX: Am. Peanut Res. Educ. Soc. Inc.

He, G., and C. S. Prakash. (1997). Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). *Euphytica*, 97:143–149.

Holbrook, C. C., and Isleib,T. G. (2001). Geographical distribution of genetic diversity in *Arachis hypogaea*. *Peanut Science*, 28: 80–84.

Holbrook, C. C., and W. F. Anderson. 1995. Evaluation of a core collection to identify resistance to late leaf spot in peanut. *Crop Science*, 35:1700–1702.

Holbrook, C. and stalker H.T. (2003).Peanut breeding and plant genetic resources.*Plant Breeding Reviews*, 22:297-338.

Horne, C.W., Lee, Jr. T.A. and Philley, G.L.(1976). A system for improving control of foliage disease on peanuts through weather monitoring.Texas Agricultural Extension Service, College Station, Texas.

Hossain, M. and Ilag , L.L. (1999). Pathogenic variability *Phaeoisariopsis personata* (Berk. and Curt) in resistant and susceptible peanut (*Arachis hypogaea L.*) genotypes. *Philippine Agricultural Scientist* 82:434-442.

Ijaz,M. (2011). Epidemiology and management of *Cercospora* leaf spot of peanut (*Arachis hypogaea* l.) in Punjab.PhD.Thesis,Pir Mehr Ali Shah Agriculture University Rawalpindi, Pakistan Pages 1-150

Jambunathan, R. (1991). Groundnut quality characteristics. Pages 267-275 in:Uses of tropical grain legumes: Proceedings of a Consultants Meeting, 27 -30 Mar 1989 (Patancheru :ICRISAT) 267–275.

Janila,P., Ramaiah,V., Rathore.A., Upakula.A., Reddy.R.K., Waliyar.F.& Nigam.S.N .2013a. Genetic analysis of resistance to late leaf spot in interspecific groundnuts.*Euphytica*. ICRISAT. Pp 1-11

Janila,P., Nigam S.N., Pandey.M.K., Nagesh.P. & Varshney.R.K. (2013b). Groundnut improvement: use of genetic and genomic tools. *Plant Science*.4:1-12.

Jensen, N.F. (1978).Composite breeding methods and the diallel selective mating system in cereals. *Crop Science*.9:622-626

Jinks, J.L. and Poon,H.S. (1984). Comparison of inbredlines produced by single descent and pedigree inbreeding.*Heridity*.53:299-308.

Jogloy, S., Pensuk, V., Patanothai, A. and Wongkaew, S. (1999a). Combining ability of resistance to late leafspot and rust of peanut. *Thai Journal Agricultural Science*. 32: 281-287.

Jogloy, S., Pensuk, V., Patanothai, A. and Wongkaew, S. (1999b). Generation mean analyses of late leafspot and rust resistance in peanut (*Arachis hypogaea* L.) *Thai Journal Agricultural Science.* 32: 423-433.

Jogloy, S., Wynne, J. C. and Beute, M. K. (1987). Inheritance of late leaf spot resistance and agronomic traits in peanut. *Peanut Science*, 14: 86-90.

John, K., Reddy.R. P., Reddy,K.H., Sudhakar.P. and Reddy, N.P.E. (2012). Identification of best heterotic crosses for yield and water use efficiency traits in groundnut (*Arachis hypogaea* L.). *Journal of Plant Breeding and Crop Science*. 4 :17-24.

John.k, R.P. Vasanthi, O., Venkateswarlu, T., Krishna, M. and Naidu, P. H.(2008). Genetic analysis of pod yield and resistance to biotic stresses in groundnut (*Arachis hypogaea l.*). *Legume Research*, 31: 227 – 229

Johnson, H. W., Robinson, H. F. and Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybeans. *Agronomy Journal*, 47: 314-318.

Jordan.D.L., Brandenburg, R.L., Brown, A.B., Bullen, G.S., Roberson, G.T., Shew, B. and Spears,F.J. (2012). Peanut Information. North Carolina Cooperative Extension Service,

College of Agriculture & Life Sciences North Carolina State University. Pages, 100-127

Jyosthna, M. K., Eswara Reddy, N. P., Chalam, T. V. and Reddy, G. L. K. (2004). Morphological and biochemical characterization of *Phaeoisariopsis personata* resistant and susceptible cultivars of groundnut (*Arachis hypogaea*). *Plant Pathology Bulletin.* 13: 243-250.

Kaaya, N.A and Warren, H.L. (2005). A review of past and present research on aflatoxin in Uganda. *African Journal of Food, Agriculture, Nutrition and Development*.5:1-3.

Kaczamarek, Z., Surma, M., Adamski, T., Jezowski, S., Madajewski, R., Krystkowiak, K. And Kuczynska, A. (2002). Interaction of gene effects with environments for malting quality of barley doubled haploids. *Theoretical and Applied Genetics*. 43: 33-42.

Kalule.D., Deom.C. and Puppala,N. (2010).Screening groundnut acessions for Rossette and Leaf spot Diseases in Uganda.Annual Meetings of ASA, CSSA, SSA, Long Beach, CA – Oct 31 – Nov (abstr.).

Keasey,J.M. and Pooni,S.H.(1996). The genetic analysis of quantitive Traits.1st Edn. Chapman and hall.

Khedikar, P.Y. (2008).Molecular Tagging and Mapping of Resistance to Late Leaf Spot and Rust in Groundnut (*Arachis hypogaea* L.).PhD Thesis *University of Agricultural Sciences, Dharwad.*Pages 8-116

Khedikar, Y. P., Gowda, M. V. C., Sarvamangala, C., Patgar, K. V., Upadhyaya, H. D and Varshney, R. K. (2010). A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). *Theoretical and Applied Genetics*. 121:971–984.

Kishore, G. K., Pande, S. and Podile, A. R. (2005). Biological control of late leaf spot of peanut (*Arachis hypogaea*) with chitinolytic bacteria. *Phytopathology* 95:1157-1165.

Kochert G, Stalker,T.H., Gimenes, M., Galgaro, L., Romero Lopes, C. and Moore, K. (1996). RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). *American Journal of Botany* 83:1282-1291

Kochert GD, Halward T, Branch WD, Simpson CE. (1991).RFLP variability in peanut (*Arachis hypogaea* L.) cultivars and wild species.*Theoretical and Applied genetics,* 81 : 565-570.

Korat .V.P., Pithia, M.S., Savaliya, J.J., Pansuriya, A.G. and Sodavadiya P.R. (2009).Studies on genetic variability in different genotypes of groundnut (Arachis hypogaea L.). *Legume Research*. 32: 224-226.

Kormsa-art, T., Jogloy, S., Wongkaew, S. and Lertrat, K. (2002). Heritabilities and correlations for late leafspot resistance and agronomic traits in groundnut (*Arachishypogaea L*). *Songklanakarin J. Sci. Technol.* 24: 555-560.

Kornegay, J. L., Beute, M. K. and Wynne, J. C. (1980). Inheritance of resistance to *Cercospora arachidicola* and *Cercosporidium personatum* in six Viginia-type peanut lines. Peanut Science, 7: 4-9.

Krapovickas, A. and Gregory W. C. (1994). Taxonomy of the genus *Arachis* (Leguminosae). *Bonplandia* 8:1–186.

Kucharek, T. (2000). Groundnut leaf spot and rust. Plant Pathology Fact Sheet. Florida Cooperative Extension Service/Institute of Food and Agricultural Sciences/ University of Florida.

Kumari, V. (2008). Morphological and molecular characterization of induced mutants in groundnut.M.Sc.Thesis University of Agricultural Sciences, Dharwad.Pages 1-110

Lavia, G.I. (2000). Chromosome studies in wild *Arachis* (Leguminosae). Institute de Botanica del Nordeste. 53: 277-281

Liang, X., Yicong, L., Shaoxiong, L. and Guiyuan, Z. (1999). Yue You 223: A high yielding Chinese cultivar with good resistance to rust. *International Arachis Newsletter*, 19 : 16.

Luo, M., Dang, P., Bausher, M.G. and Holbrook, C.C. (2005). Identification of transcripts involved in resistance responses to leaf spot disease caused by *Cercosporidium personatum* in peanut *(Arachis hypogaea). Phytopathology* 95:381-387.

Manju, P. R. and Sreelathakumary, I. (2002). Genetic variability, heritability and genetic advance in hot chilli *(capsicum chinense* jacq.). *Journal of Tropical Agriculture* 40:4-6.

Mather, K and J.L Jinks .(1980). Biometrical Genetics: 3rd Edn, Chapman and Hall Ltd., London.

Mather, K. and Jinks, J. L. (1982). Biometrical genetics, cornell Iniversity Press. Thaca. N.Y

McDonald,D.,Subrahmanyam,P., Gibbons, R.W. and Smith,D.H.(1985).Early and Late leaf spots of groundnuts.Information Bulletin No.21.International Crops Research, Institute for the Semi-Arid Tropics Patancheru, A.P.502324,India.Page 1-16

Miller, I. L., Norden, A. J., Knauft, D. A. and Gorbet D. W. (1990). Influence of maturity and fruit yield on susceptibility of peanut to *Cercosporidium personatum* (late leaf spot pathogen). *Peanut Science*. 17:52–58.

Mohapatra, N.K. (1982). Post-infection changes in sugar content of groundnut leaves infected with *Cercospora personata. Geobios* 9: 246-248.

Moss, J. P., Singh, A. K., Nigam, S. N., Hildebrand, G. L., Govinden, N., Ismael, F. M., Subrahmanyam, P. and Reddy, L. J. (1998). Registration of ICGV-SM 86715. *Crop Science*. 38 : 572.

Moss, J. P., Singh, A. K., Reddy, L. J., Nigam, S. N., Subrahmanyam, P., Mcdonald, D. and Reddy, A. G. S.(1997). Registration of ICGV 87165 peanut germplasm with multiple resistance. *Crop Science*, 37: 1028.

Motagi. (2001). Genetic analysis of resistance to late leaf spot and rust vis-à-vis productivity in groundnut (*Arachis hypogaea* L.).PhD. Thesis, University of Agricultural Sciences, Dharwad, India, Page, 1-165.

Mugisha.J., Ogwalo,R., Ekere,W. and Ekiyar, V. (2004). Adoption of IPM groundnut production technologies in eastern Uganda.*African Crop Science Journal*. 12: 383-391.

Mulualem,T., Dessalegn,T. and Dessalegn,Y.(2013). Genetic variability, heritability and correlation in some faba bean genotypes (*Vicia faba* L.) grown in Northwestern Ethiopia. *International Journal of Genetics and Molecular Biology*. 5: 8-12.

Natarajan, U.S., Balasundaram, N., Ramana Rao, T.C., Padmanaban, P., Mohanraj, D., Karthigeyan, S. and Damodaran, S. (2001). Role of *Saccharum spontaneum* in imparting stable resistance against sugarcane red rot. Sugarcane International, pp:17-20.

Nevill, D.J. (1982). Inheritance of resistance to *Cercosporidium personatum*in groundnuts: A genetic model and its implications for selection. *Oleagineux* 37:355–366.

Nidagundi, J. M., Patil, S. S., Salimath, P. M., Kajjidoni, S. T., Patil, B. C. and Hegde, M. G. (2012). Genetic analysis of seed cotton yield and its component traits in *Gossypium hirsutum* L.*Karnataka J. Agric. Sci* 25: 260-261.

Nigam, S. N., Reddy, L. J., Subrahmaniyam, P., Reddy, A. G. S., Mcdonald, D. and Gibbons, R. W. (1992). Registration of ICGV 87157, an elite peanut germplasm with multiple resistances to disease. *Crop Science*, 32 : 837.

Nigam, S.N. and Dwivedi, S.L. (2000). Enhanced resistance to leaf spot in cultivated groundnut (*Arachis hypogaea* L.) on a partially-resistant peanut (*Arachis hypogaea L.*) cultivar. ICRISAT-Patancheru, India. Soil and Crop Sci. Soc. Fla. Proc. 50:37-40.

Oeveren, A.J.Van and Stam, P. (1993). Comperative simulation studies on the effect of selection for quantitative traits in autogamous crops: early selection vurses single seed descent. *Heridity*.69:342-351.

Okello D. K., Biruma, M. and Deom, C. M. (2010). Overview of groundnuts research in Uganda: Past,present and future. *African Journal of Biotechnology* 9:6448-6459.

Oyiga, B.C and Iguru.M.I. (2011). Genetic variation and contributions of same floral traits to pod yield in bambara groundnut (Vigna subterranean L.verdc) under two cropping seasons in the derived Savana of South-East Nigeria. *International Journal of Plant Breeding* 1:58-63.

Ozyigit, Y. and Bilgen.M. (2013). Forage Potential of some groundnut (*Arachis hypogaea L.*) Cultivars. *Romanian Agricultural Research*. 30:1-6.

Page, W. W., Busolo-Bulafu, C.M., vander Merwe, P.J.A. and Chancellor, T.C.B . (2002). Groundnut manual for Uganda: Recommended groundnut production practices for smallholder farmers in Uganda. Chatham, UK: Natural Resources Institute. Pages 1-12

Pattee, H. and Young, G.T. (1982).Peanut Science and Technology. Yoakum, Texas 77995, USA.

Pattee, H.E., Isleib, T.G., Gorbet, D.W., Giesbrecht, F.G. and Cui, Z. (2001). Parental selection in breeding for roasted peanut flavor quality. *Peanut Science.* 28: 51–58.

Payne,.R., WS.A. Harding, D.A. Murray, D.M. Soutar, D.B. Baird, A.I. Glaser, I.C. Channing, S.J. Welham, A.R. Gilmour, R. Thompson, R. Webster.(2010).A Guide to ANOVA and Design in GenStat. 13th Edn. VSN International ltd.pp1-103

Pensuk, V., Patanothai, A., Jogloy, S., Wongkaew, S., Akkasaeng C., and Vorasoot, N. (2003).Reaction of peanut cultivars to late leafspot and rust.*Songklanakarin J. Sci. Technol*., 25: 289-295.

Rao, V. R. (1988). Botany. p. 24–64. In: P. S. Reddy (ed.), Groundnut. Indian Council Agr. Res., New Delhi, India. Rucker, K. S., C. K. Kvien,

Reddy, L. J., Nigam, S. N., Moss, J. P., Singh, A. K., Subrahmanyam, P., Mcdonald, D. and Reddy, A. G. S. (1996). Registration of ICGV86699 peanut germplasm line with multiple disease and insect resistance. *Crop Science*36: 821.

Savage, G. P. and Keenan, J. I. 1994. The composition and nutritive value of groundnut kernels. In: The Groundnut crop: Scientific basis for improvement. (Eds.) Smart J., Chapman and Hall London. Page 173-213.

Sharief, Y., Rawlings, J.O. and Gregory, W.C. (1978). Estimates of leafspot resistance in three interspecific hybrids of *Arachis*. *Euphytica* 27:741-751.

Shew, B., Lassiter, B., Wilkerson.G. and Phipps, P. (2010). NC Peanut Information Guide, (AG 331), North Carolina Cooperative Extension Service, and the 2010 Virginia Peanut Production Guide, (432-101), Communications and Marketing,VPI.North Carolina State University & Virginia Tech University.

Shilpa,K., Sunkad.G., Kurella.S., Marri.S., Padmashree.K., Jadhav.D.R., Sahrawat.K.and Mallikarjuna.N. (2013). biochemical composition and disease resistance in newly synthesized amphidiploid and autotetraploid Peanuts.*Food and Nutrition Sciences* 4:169-176

Shoba.D., Manivannan, N. and Vindhiyavarman, P. (2010). Gene effects of pod yield and its components in three crosses of groundnut (*Arachis hypogaea L.).* Dept. of Oilseeds, Tamil Nadu Agricultural University, Coimbatore- 641003

Shokes, F. M. and Culbreath, A. K. (1997). Early and late leaf spots. pp. 17–20. In: N. Kokalis-Burelle, D. M. Porter, R. Rodriguez-Kabana, D. H. Smith, and P.Subrahmanyam (eds.).Compendium of groundnut diseases. 2nd Edn. American Phytopathological Society Press, St. Paul, MN.

Shokes, F. M., and Melouk, H. A. 1995. Plant health management in peanut production. Pages 1-6 in: Peanut Health Management, H. A. Melouk and F. M. Shokes, eds. American Phytopathological Society Press, St. Paul, MN.

Silva NO, Ramalho MAP, Abreu AFB and Carneiro J.E. (2004).Performance of common bean families after different generations under natural selection.*Genet Mol Biol* 27:574-578.

Singh, A.K. and Simpson, C.E.(1994). Biosystemic and genetic resources. In: The Groundnut Crop:A Scientific Basis for Improvement(Ed. J. Smartt)Chapman and Hall, London, pp 96–137

Singh, P. and Narayanan, S.S. (1993). Biometrical techniques in plant breeding. Kalyani, Publishers New Delhi.

Singh, R.K. and Chaudhary, B.D. (1985). Biometrical Methods in Quantitative Analysis. Kalayani Publishers. New Delhi.P.318

Singh,A.K., Mehan,V.K., and Nigam, S. N.(1997). Sources of resistance to groundnut fungal and bacterial diseases:An Update and Appraisal. Information Bulletin no.50.Patancheru: ICRISAT.

Singh.B.d.1993. Plant Breeding 5th Edn, Kalyani Publshers,Rajender Nagar, PP 102,104

Singh.F and B. Diwakar.(1993).Nutritive value and uses of pigeonpea and groundnut. Human Resource Development Program. ICRISAT. Patancheru, Andhra Pradesh 502-324, India.

Singh.F. and Oswalt.D.L.(1991). Genetics and breeding of groundnut. ICRISAT. Human Resource Development Program. Patancheru, Andhra Pradesh 502 324, India

Sivasubramanian, S. and Menon, M.(1973). Heterosis and inbreeding depression in rice. *Madras Agricultural Journal*, 60: 1139.

Smartt, J.(1994). The groundnut in farming systems and the rural economy a global view.In: The Groundnut Crop: A Scientific Basis for Improvement. J. Smartt, Edn. Chapman & Hall, London pp. 664- 699.

Soomro.Z.A., Kumbhar.M.B. Larik.A.S., Imran.M. and Brohi.S.A.(2010). Heritability and Selection Response in Segregating Generation of Upland Cotton. *Pakistan J. Agric.Res* 23:25-29.

Stalker,H.T. (1997). Peanut (*Arachis hypogaea*).Field Crop Research.53:205-217

Strange, R. N., Edwards, C. and Richards, S. (1988). Phytoalexins of groundnuts and their role in resistance to fungal parasites. *Phytoparasitica* 16:202-3

Subrahmanyam, P., Bose, J.P., Hassane, H., Smith, D.H., Mounkaila, A., Nandguru, B.J. and Sankara, P.H. (1992). Groundnut diseases in Niger and Burkina faso. *Oleagineux*, 47: 119-129.

Subrahmanyam, P., D. McDonald, R.W. Gibbons, S.N. Nigam, and D.J. Nevill. (1982). Resistance to rust and late leaf spot diseases in some genotypes of *Arachis hypogaea*. *Peanut Science*.9:6-10.

Subrahmanyam, P., McDonald, D., Waliyar, F., Reddy, L.J., Nigam, S.N., Gibbons, R.W., Rao, V. R., Singh, A.K., Pande, S., Reddy, P.M. and SubbaRao, P.V.S. (1995). Screening methods and sources of resistance to rust and late leaf spot of groundnut. Information Bulletin No: 47 ICRISAT.Page 24

Subrahmanyam, P., Moss, J. P., Mcdonald, D., Rao, P. V. S. and Rao, V. R. (1985). Resistance to leaf spot caused by *Cercosporidium personatum* in wild *Arachis* species. *Plant Disease.* 69: 951-954.

Subrahmanyam, P., Rao, V. R., Mcdonald, D., Moss, J. P. and Gibbons, R. W. (1989). Origins of resistance to rust and late leaf spot in peanut (*Arachis hypogaea* L.). *Economic Botany.* 43: 444-455.

Subrahmanyam.P., Reddy, P. M. and McDonald, D. (1990). Parasitism of rust, early and late leaf spot pathogens of peanut by *Verticillium Lecanii*.*Peanut Science* 17:1-4.

Tiwari, S. P., Ghewande, M. P. and Mishra, D. P. (1984). Inheritance of resistance to rust and late leaf spot in groundnut (*A. hypogaea*). *Journal of Cytology and Genetics*19: 97-101.

Uganda Bureau of Statistics (UBOS). (2010).Uganda census of agriculture 2008/2009. Volume IV: Crop Area and Production Report. Page 49.

Upadhyay.R.K., Mukherji, K.G., Chamola, B.P. and Dubey. O.P. (2009). Intergrated pest and disease management. APH Publishing Cooperation .New Delhi, pages 303-324.

Valls J.F. and Simpson. C.E. (2005). New species of *Arachis* (Leguminosac) from Brazil, Paraguay and Bolivia. *Bonplandia* 14:35-63

Vasanthi .R.P. and Reddy, C.R. (1997). Inheritance of testa colour and resistance to late leaf spot and rust in groundnut (*Arachis hypogaea* L.). *Journal of Oilseeds Research.* 14:244–248.

Vishnuvardhan K, M., Vasanthi R.P. and Reddy K.H. (2011). Combining ability of yield, yield traits and resistance to late leaf spot and rust in groundnut. *Journal of SAT Agricultural Research*.9:1-5

Vishnuvardhan.K.M., Vasanthi.R.P., Reddy K. H.P., and Reddy, B.V. (2012).Genetic variability studies for yield attributes and resistance to foliar diseases in groundnut *(Arachis hypogaea* l.). *International Journal of Applied Biology and Pharmaceutical Technology*.3: 390-394.

Waliyar, F. (1991). Evaluation of yield losses due to groundnut leaf diseases in west africa. Summary Proceedings of the second ICRISAT regional groundnut meeting for West Africa, 11–14 September 1990. ICRISAT Sahelian Centre, Niamey, Niger. ICRISAT) Patanecheru, Andhra Pradesh India. pp. 32–33.

Walls, S. B.and Wynne, J. C. (1985). Combining ability for resistance to *(Cercosporidium personatum)* for five late leaf spot-resistant peanut germplasm lines.*Oleagineux*. 40:389-396.

Wynne, J. C. and Gregory, W. C. (1981). Plant breeding. *Advances in Agronomy*. 34: 39-71.

Wynne, J. C., M. K. Beute and S. N. Nigam.(1991). Breeding for disease resistance in peanut (*Arachis hypogaea* L.). Annu. Rev. Phytopathol. 29:279-303

Yussif,I. (2010). Screening of groundnuts (*Arachis hypogaea* L.) for resistance to early and late leaf spots. M.Sc.Thesis Kwame Nkrumah University of Science and Technology, page 1-83