GENETIC VARIABILITY, PATH COEFFICIENT AND MARKER-TRAIT ASSOCIATION ANALYSIS FOR RESISTANCE TO ROSETTE DISEASE IN GROUNDNUT

By

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DISSERTATION ABSTRACT

Several abiotic, biotic and socio-economic aspects constrain the production of groundnut (*Arachis hypogea* L.). Groundnut rosette disease (GRD) which can cause yield losses of up to 100% in susceptible cultivars, is among the most important biotic stresses. The use of resistant cultivars is the most viable method to control the disease, therefore, breeding for high yielding and GRD resistant cultivars is needed and should be a priority. The present study was conducted to: (i) determine genetic variability for GRD response and yield traits in selected groundnut accessions under natural infestation, (ii) assess the relationship between seed yield and its related traits, and analyse agro-morphological diversity in selected groundnut accessions under natural GRD infestation and (iii) evaluate groundnut recombinant inbred lines for resistance to GRD and perform SNP marker-trait association analysis. Twenty-five groundnut accessions and three controls were evaluated under natural GRD infestation to assess genetic variability for GRD response and yield related traits. Seed yield, number of pods per plant, plant height, GRD incidence and number of secondary branches showed high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), while moderate variation (PCV and GCV) was observed for days to flowering and pod width. A combination of high heritability and genetic advance was recorded for number of secondary branches, plant height, seed yield and GRD incidence, indicating that phenotypic selection based on the mean would be successful in improving these traits. Phenotypic correlations and sequential path analysis indicated that high seed yield was directly associated with taller genotypes, higher number of pods per plant and hundred seed weight, which were a result of higher pod width and lower GRD incidence. Based on morphological traits, the evaluated accessions were grouped into four clusters. Days to flowering and maturity, number of branches, plant height, number of pods per plant, pod width and length, seed yield and GRD incidence, largely influenced this variation. Principal component analysis (PCA) biplot was effective in showing the genetic distance among the accessions with results consistent to those of the cluster analysis. Moreover, Shannon-Weaver diversity indices (0.949-0.9996) for qualitative traits also indicated the existence of high diversity among the accessions. A total of 25 groundnut genotypes, which comprised 21 RILs derived from a bi-parental cross, both parents, and two susceptible controls (CG7 and JL24) were evaluated and used to perform SNP marker-trait association analysis for resistance to GRD. There were significant differences among the lines in all recorded traits, indicating the existence of genetic variability and possibility of effective selection. Interaction of genotype and environment was significant for disease incidence and the glasshouse environment had higher disease pressure, providing the best discrimination among the tested genotypes. ICGV-SM 15605, ICGV-SM 15621,

ICGV-SM 15618, ICGV-SM 15604 and ICGV-SM 15615 were among the resistant and high yielding RILs. Twenty-two highly significant marker-trait associations were identified, which will add to previously reported genomic regions influencing GRD and the aphid vector resistance. Overall, the study showed that taller genotypes, higher number of pods per plant and hundred seed weight can be used to improve seed yield in groundnut, particularly under GRD infestation. The genetic diversity among the accessions provides an opportunity for parent selection that can be used for breeding high yielding and GRD resistant cultivars. In addition, the SNP markers will be useful in classifying groundnut germplasm based on the GRD response and for their use in marker-assisted selection, once validated.

DECLARATION

I, **Nelson Hilário Mubai**, declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original work.
2. The dissertation has not been submitted for any degree or examination at any other university.
3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a) Their words have been re-written but the general information attributed to them has been referenced.
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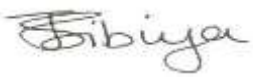
5. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the reference sections.

Signed  Date __08/02/2019_____

Nelson Hilário Mubai (Candidate)

As the candidate's supervisors, we agree the submission of this dissertation:

Signed_____  _____ Date: 11/02/2019_____

Dr Julia Sibiya (Supervisor)

Signed_____  _____ Date __21/02/2019_____

Dr James Mwololo (Co-supervisor)

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DEDICATION

The dissertation is dedicated to my dear parents, Hilário Mubai and Deolinda Mubai.

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ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of Variance
CV	Coefficient of variation
DAS	Days after sowing
DNA	Deoxyribonucleic acid
DTF	Days to flowering
DTM	Days to maturity
ECV	Environmental coefficient of variation
FAOSTAT	Food and Agriculture Organization Statistics
GA	Genetic advance
GAM	Genetic advance as percentage of the mean
GBS	Genotyping by sequencing

GCV	Genotypic coefficient of variation
GLM	General Linear Model
GRAV	Groundnut rosette assistor virus
GRD	Groundnut rosette disease
GRV	Groundnut rosette virus
H^2	Broad-sense heritability
HSW	Hundred seed weight
IBPGR	International Board for Plant Genetic Resources
ICRISAT	International Crops Research Institute for the Semi-arid Tropics
LSD	Least significant difference
MTA	Marker-trait association
NGS	Next-generation sequencing
NPB	Number of primary branches
NPP	Number of pods per plant
NSB	Number of secondary branches
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
PDI	Percentage of disease incidence
PH	Plant height
PL	Pod length
PW	Pod width
RAD-seq	Restriction site associated DNA sequencing
RAPD	Random amplified polymorphic deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
RT-PCR	Reverse transcription polymerase chain reaction
SatRNA	Satellite ribonucleic acid
SED	Standard error of difference
SNP	Single nucleotide polymorphism
SP	Shelling percentage
SSA	Sub-Saharan Africa
SSR	Simple sequence repeat
SYD	Seed yield
SYDP	Seed yield per plant
TAS-ELISA	Triple antibody sandwich enzyme-linked immunosorbent assay

WGS	Whole-genome sequences

CHAPTER 1 GENERAL INTRODUCTION

1.1 Background

Cultivated groundnut (*Arachis hypogea* L., AABB, 2n = 4x = 40) also known as peanut, is a popular allotetraploid legume crop worldwide. The legume originated from South America through hybridization of its diploid ancestors, *Arachis duranensis* (AA) and *Arachis ipaensis* (BB), which was followed by natural chromosome doubling (Talawar, 2004; Bertioli *et al.*, 2015; Zhang *et al.*, 2016). The crop is currently distributed around the tropical, sub-tropical and warm temperate regions of the world, where it plays an important role as both food and cash crop (Maiti, 2002; Nautiyal *et al.*, 2002; Talawar, 2004). It is the sixth and third most important source of vegetable oil and protein, respectively, and ranks 13th among the food crops (Singh and Nigam, 2016). Nautiyal (2002) indicated that the nuts contain 47 to 53% of edible oil, 24 to 35% of protein, 10 to 15% of carbohydrates, and are a good source of minerals (Ca, Mg, P, Fe and Zn), vitamins (E, K and B complex) and fibre. Singh and Nigam (2016) pointed out that in sub-Saharan Africa (SSA), the haulms are used as fodder and the crop is mainly grown by small-scale farmers under low-input production system.

The world annual mean production of unshelled groundnut over the past 10 years is about 42.1 million tonnes (MT) with an average yield of 1.64 t ha^{-1}, produced on an area of 25.67 million ha (FAOSTAT, 2018). Singh and Nigam (2016) indicated that Asia is the major producing continent, with over 58.30% of the world production, followed by Africa (28.34%), America (10%) and Oceania (0.1%) (FAOSTAT, 2018). The African annual mean production of unshelled groundnuts in the past 10 years is about 11.93 MT, grown on 12.57 million ha (equivalent to 48.97% of global area) with a mean yield of 0.95 t ha^{-1} (FAOSTAT, 2018). Although the total groundnut area in Africa is higher, the production is low. The average yield in Africa is about half of the world average (1.64 t ha^{-1}) and it is less than one-third of the yield registered in the major producing countries (3.00 t ha^{-1}) (Singh and Nigam, 2016; FAOSTAT, 2018). However, Chikowo *et al.* (2015) indicated that Africa contributes significantly to the world groundnut production.

1.2 Groundnut production status and constraints in Malawi

Groundnut is a major legume crop in terms of both value and quantity, and has the potential to contribute to food nutrition and income security in Malawi (Chikowo *et al.*, 2015). However, the yields on farmers' fields are below 1.0 t ha^{-1} (Chikowo *et al.*, 2015; FAOSTAT, 2018). Several abiotic, biotic and socio-economic aspects have been shown to be major constraints

to groundnut production (Minde *et al.*, 2008). These include: low plant populations, delayed planting, diseases and pests, use of grain that has been recycled as seed for many years, soil fertility problems, weed competition for nutrients and water, scarcity of labour, lack of technical knowledge and use of unsuitable varieties (Minde *et al.*, 2008; Prasad *et al.*, 2010; Chala *et al.*, 2014; Chikowo *et al.*, 2015). These constraints characterise the low-input production system that has been indicated by Singh and Nigam (2016) to be predominant for groundnut production in Malawi and other developing countries. Moreover, Madhava *et al.* (2003) showed that the crop is predominantly grown by small-scale farmers under rain-fed conditions and in most production areas, rainfall is erratic and insufficient, with negative impact on the groundnut production.

Groundnut is infested by a number of pests and diseases. The most important and widespread diseases are: groundnut rosette disease (GRD), caused by a complex of three agents (*Groundnut rosette assistor virus*-GRAV, *Groundnut rosette virus*-GRV and a *Satellite*-RNA (satRNA) associated with GRV) and transmitted by an aphid (*Aphis craccivora* Koch); early and late leaf spots caused by fungi *Cercospora arachidicola* and *Phaeoisariopsis personata,* respectively and groundnut rust caused by *Puccinia arachidis* Speg (Mace *et al.*, 2006; Minde *et al.*, 2008; Sudini *et al.*, 2015). Minde *et al.* (2008) indicated that over 400 species of pests attack groundnut. Among those pests, *Aphis craccivora* Koch is the most important. According to Waliyar *et al.* (2007), these pests and diseases cause significant economic losses and the situation is worsened by the lack of technical knowledge and support on pest and disease management. Although a range of improved varieties have been developed through breeding, the use of cultivars that are low yielding and susceptible to pests and diseases is another constraint to groundnut production in Malawi (Minde *et al.*, 2008). As such, the yield is still very low (759.77 kg ha^{-1} of unshelled groundnut over the last three seasons). The realized yield is less than half of the world average (1.64 t ha^{-1}), and less than one-third of the potential yield (3.0 t ha^{-1}) (FAOSTAT, 2018). Moreover, the production has suffered from fluctuations since the year 2000, as shown in Figure 1.1.

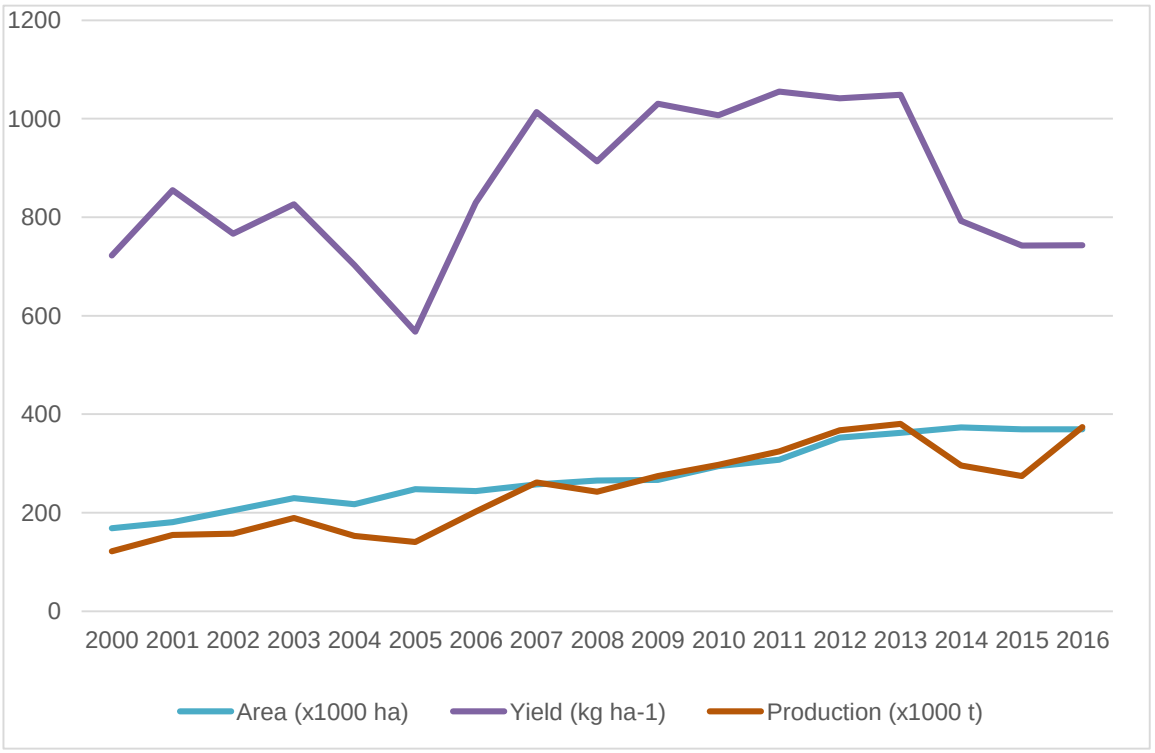

Figure 1.1: Trend of groundnut production in Malawi from 2000-2016

Source: FAOSTAT (2018)

1.3 Problem statement and justification

Groundnut production in Malawi is low and is constrained by many factors (Longwe-Ngwira *et al.*, 2012; Chikowo *et al.*, 2015). GRD is among these factors and is the most destructive groundnut disease in SSA (Subrahmanyam and Merwe, 2000; Thresh, 2003). Olorunju *et al.* (2001) and Minde *et al.* (2008) indicated Malawi as one of the countries in which GRD is a major constraint to groundnut production. According to Anitha *et al.* (2014), GRD has been responsible for devastating losses to groundnut production in Malawi and other African countries. Moreover, Minde *et al.* (2008) reported that whenever GRD occurs, the yield is reduced, and in susceptible cultivars the yield loss can reach 100%. Various epidemics have been reported that resulted in devastating losses, for example in 1975 an epidemic in Nigeria destroyed around 0.7 million ha with an estimated loss of US$250 million; in Zambia, an epidemic occurred in 1995 which affected approximately 43000 ha, causing an estimated loss of US$4.89 million and in the following year in Malawi, the production was reduced by 23% due to the disease (Jackson, 2015; Panguluri and Kumar, 2016). In addition, Waliyar *et al.* (2007) pointed out that GRD is responsible for annual yield losses worth over US$150 million in SSA.

The management of GRD by using insecticides to control the vector and cultural practices such as early sowing at optimum plant density are known (Naidu and Kimmins, 2007; Okello *et al.*, 2014). However, pesticides are expensive to smallholder farmers and the use of resistant cultivars is regarded as the most viable and sustainable control measure (Naidu *et al.*, 1999; Subrahmanyam and Merwe, 2000). Thus there is a need for development of cultivars that have resistance to GRD and other farmer preferred traits. The present study evaluated genetic variability and diversity under natural GRD infestation in selected groundnut accessions in order to identify suitable sources of GRD resistance genes. Recombinant inbred lines (RILs) were also evaluated for resistance and used for marker-trait association analysis to identify SNP markers linked to GRD resistance that can be used in marker-assisted breeding. The resistant lines could be advanced for further evaluations and release, thereby contributing to increased groundnut production in Malawi.

1.4 Objectives

The overall objective of the research was to assess variability and generate new genetic resources and information relevant for GRD resistance breeding and improvement of groundnut production in Malawi.

The specific objectives for the research were:

a) To determine genetic variability for GRD response and yield traits in selected groundnut accessions under natural infestation
b) To assess the relationship between seed yield and its related traits, and analyse agromorphological diversity in selected groundnut accessions under natural GRD infestation
c) To evaluate groundnut recombinant inbred lines for resistance to GRD and conduct SNP marker-trait association analysis.

1.5 Hypotheses

The following hypotheses were tested:

a) There is a great extent of genetic variability for response to GRD and yield traits within the selected groundnut accessions, which can be exploited in breeding new varieties
b) There is a significant relationship between secondary traits and seed yield, and a high genetic diversity among the selected groundnut accessions under GRD infestation
c) The recombinant inbred lines have different levels of resistance to GRD
d) Some SNP markers are associated with response to GRD.

1.6 Overview of the dissertation

The dissertation consists of six chapters, which are condensed into discrete but interdependent chapters according to the University of KwaZulu-Natal's dominant dissertation format. There are some overlaps in terms of references and content among the different chapters and the Crop Science Journal referencing system was used in all chapters.

Chapter 1: General introduction: focuses on introducing the area of study, the significance of the research, scope and justification.

Chapter 2: Literature review: gives an overview of the history, current status and constraints to groundnut production in Malawi. Previous information on the GRD problem, its effect on yield and yield components, and its epidemiology are reviewed. Breeding and screening techniques for GRD resistance are discussed. Correlations, path coefficient and multivariate analysis in groundnuts are also reviewed.

Chapter 3: Objective 1: investigates the variability of a set of groundnut accessions for response to GRD and yield related traits under natural infestation. The genotypic factor was found to be more predominant than the environmental factor for most of the measured traits and GRD resistant accessions were identified. A combination of high heritability and genetic advance was observed for some of the recorded traits.

Chapter 4: Objective 2: measures the relationship between seed yield and secondary traits, and examines genetic diversity in selected groundnut accessions under natural rosette disease infestation. The selection criteria for seed yield was defined through correlations and path analysis. The degree of diversity among the accessions was estimated and the most influential traits were identified through cluster and principal component analysis.

Chapter 5: Objective 3: evaluates the resistance of groundnut recombinant lines to GRD under artificial infestation. Resistant and high yielding lines were identified which can further be advanced and released. Marker-trait association was conducted and identified SNP markers associated to GRD resistance, which can assist future breeding programmes through marker-assisted selection.

Chapter 6: General research overview: presents the summary of research findings, conclusions and recommendations.

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CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This chapter covers a review of literature on several aspects of groundnut (*Arachis hypogea* L.), including the origin, distribution, botany, economic importance and production. It also covers constraints to production, especially in Malawi where the research was conducted. The chapter further describes the groundnut rosette disease, its casual agents, symptoms, effect on yield, epidemiology, diagnosis, management and screening techniques for resistance to the disease. Correlations, path coefficient analysis, heritability, genetic gain, cluster and principal component analysis in groundnut are also discussed, and lastly molecular marker techniques are reviewed.

2.2 Origin and distribution of groundnut

The cultivated groundnut (*Arachis hypogea* L., AABB, 2n = 4x = 40) is an allotetraploid crop species which originated from South America (Simpson *et al.*, 2001). Bertioli *et al.* (2015) and Zhang *et al.* (2016) documented evidence that groundnut originated from hybridization of *Arachis duranensis* Kaprov. and W. C. Gregory (AA) and *Arachis ipaensis* Kaprov. and W. C. Gregory (BB), followed by natural chromosome doubling. Nautiyal (2002) reported that the botanical name of groundnuts was derived from two Greek words, *arachis* meaning legume and *hypogea* meaning below ground, referring to the pod formation in the soil. Hammons *et al.* (2016) indicated that at the time of discovery of America and European expansion into the New World, groundnut was grown widely throughout the tropical and subtropical regions of America. The explorers found in the coastal regions of Peru, where the crop was also extensively grown (before introducing it across the continents), evidence of its cultivation supported by archaeological reports between 300 and 2500 BC (Simpson *et al.*, 2001; Prasad *et al.*, 2010).

Dissemination of groundnut to Asia, Africa, Europe and the Pacific Islands occurred presumably in the sixteenth and seventeenth centuries by Europeans (Isleib *et al.*, 1994; Hammons, 1994). The crop became re-adapted in all these lands and was returned from Africa with slaves to tropical America and United States (Hammons *et al.*, 2016). Currently, the crop is widely distributed and adapted in the tropical, subtropical and warm temperature areas of the world where it is grown for food and vegetable oil production (Maiti, 2002).

2.3 Botany of groundnut

2.3.1 Taxonomy

Groundnut is a member of the family *Fabaceae*, subfamily *Papilionaceae*, tribe *Aeschynomeneae*, subtribe *Stylosanthinae* (Prasad *et al.*, 2010). Three genera of subtribe *Stylosanthinae* are known, and the genus *Arachis* is unique with a peg and geocarpic reproductive growth (Simpson *et al.*, 2001; Hammons *et al.*, 2016). Krapovickas *et al.* (2007) reported that the genus has more than 70 wild species, however only 37 have been named and described, and *Arachis hypogea* is the only domesticated and cultivated species from the genus. *Arachis monticola*, which is also an allotetraploid with AB genome, resembles and is fully cross-compatible with *Arachis hypogea*, and is considered to be the closest wild relative of the cultivated species (Hammons *et al.*, 2016).

The genus *Arachis* has been divided into nine sections, whereby the section *Arachis* comprises an annual and perennial diploid (2n = 2x = 20) and two annual tetraploid species (2n = 4x = 40) (Prasad *et al.*, 2010). Based on the morphological characteristics, which include growth habit, branching pattern, stem colour and pubescence, *Arachis hypogea* is divided into two subspecies, subspecies *hypogea* and subspecies *fastigiata* Waldron (Krapovickas *et al.*, 2007). The subspecies *hypogea* has central and lateral branches, but only the laterals are productive. The seed shows dormancy and the plants are late maturing (120-150 days). The subspecies *hypogea* is further divided into botanical varieties: var. *hypogea* (Virginia: largeseeded, and Runner type: small-seeded) and var. *hirsute* (Prasad *et al.*, 2010). The subspecies *fastigiata* is always erect with a productive central axis, and its seed shows no dormancy and the plants are early maturing (90-120 days). Subspecies *fastigiata* is also divided into botanical varieties: var. *vulgaris* (Spanish), var. *fastigiata* (Valencia), var. *aequatoriana* and var. *peruviana*. Only the subspecies *hypogaea* var. *hypogaea*, subspecies *fastigiata* var. *fastigiata* and var. *vulgaris* are widely grown, especially in America, Asia and Africa (Ferguson *et al.*, 2004; Krapovickas *et al.*, 2007; Prasad *et al.*, 2010).

2.3.2 Morphology

The complex branching pattern, the highly condensed inflorescence and the geocarpic fruit made the crop morphological descriptions confused for a long time (Prasad *et al.*, 2010). Groundnut is a herbaceous crop with a taproot and fairly well developed root system, where the spreading type have a more vigorous root system than the bunch type (Maiti, 2002). Rao and Murty (1994) indicated that most of the root system is found at a depth of 5 to 35 cm and the spreading is confined to a radius of 12 to 14 cm. According to Nigam (2014), at early

stages, the stem is solid, angular and pubescent, but it becomes hollow and cylindrical at later growth stages. In addition, Maiti (2002) reported that the main stem is distinct with height ranging from 12 to 65 cm and a variable number of lateral branches which determine classification of the growth habit into either spreading or erect. The leaves are opposite, pinnate with four leaflets, whereby each leaflet is 1 to 7 cm long and 1 to 3 cm across (Kumar, 2013).

The inflorescence which is a compound monopodium, appears as a cluster of up to three sessile flowers in the leaf axil (Rao and Murty, 1994). The flowering occurs about 25 to 35 days after sowing and the flowers open early in the morning, as soon as they receive sunlight. Groundnut is a self-fertilizing crop and pollination occurs just before the flowers open (Prasad *et al.*, 2010). A peg, stalk-like structure, becomes visible within 4 to 6 days after fertilization under optimum conditions and penetrates the soil developing into pod, the fruit of groundnut (Rao and Murty, 1994; Prasad *et al.*, 2010). A mature pod usually contains up to four seeds and a single-seeded pod may also occur. The groundnut morphology is not fixed and is influenced by genotype and environmental conditions.

2.4 Importance and production of groundnut

Groundnut is a well-known and one of the most important legume crops in the world, grown in tropical and subtropical countries for its high-quality oil (47-53%) and easily digestible protein (24-36%) (Maiti, 2002; Singh and Nigam, 2016). Nautiyal (2002) indicated that the kernels are also rich in carbohydrates (10-15%) and are a good source of minerals (Ca, Mg, P, Fe and Zn), vitamins (E, K and B complex) and fibre. Groundnut is the sixth and third most important source of vegetable oil and protein, respectively, and ranks thirteenth among the food crops (Singh and Nigam, 2016). The crop is mostly grown for food use in North America, West and Southern Africa, Southeast Asia, and Europe while in South America and Southwest Asia it is predominantly grown for edible oil use. Both edible oil and food uses, are important in East Asia and East Africa. Forty-one percent of the global production goes towards food, 49% for oil extraction and the remaining is used as feed and seed (Singh and Nigam, 2016).

In Malawi and other developing countries, apart from food security, groundnut contributes to poverty alleviation as a source of income and the nuts are eaten in various forms (Simtowe *et al.*, 2010; Prasad *et al.*, 2010). Longwe-Ngwira *et al.* (2012) indicated that groundnut is the major legume crop in terms of both value and quantity in Malawi, followed by pigeon pea, common bean, cowpea and soybean. Chikowo *et al.* (2015) reported that in the country, the crop is predominantly grown by small-scale farmers under subsistence system and its

cultivation offers many benefits, such as better family nutrition and incomes, improved soil fertility through nitrogen fixation and fewer diseases on farms with crop rotation involving groundnut, maize and other crops.

Similar to Malawi, in other countries of Africa and Asia, groundnut is mainly grown under lowinput production systems with an average yield ranging from 0.7 to 1.0 t ha^{-1} while in USA, Australia, Brazil, Argentina and China, high-input system is used and higher yields of 2-4 t ha^{1} are obtained (Singh and Nigam, 2016). The annual global mean production of unshelled groundnut over the past ten years is about 42.1 million tonnes (MT) and China is the major producer, with over 37.4% (15.76 MT) of the world production. Africa is the second major producing continent after Asia, with over 28.34% (11.93 MT) of the world production and a yield average of 0.9 t ha^{-1}, which is less than three-quarters of the world average of 1.64 t ha^{1} (FAOSTAT, 2018). A lot of fluctuations have been observed in the production of groundnuts in Malawi from 2002 to 2016 (Table 2.1), and the yield is still very low with a mean of 759.77 kg ha^{-1} over the last three seasons. This yield is less than half of the world average (1.64 t ha^{1}) and one-third of the potential yield (3.00 t ha^{-1}) (Longwe-Ngwira *et al.*, 2012). The large gap between the realized and potential yield is due to several biotic, abiotic and socioeconomic constraints which are further discussed.

Table 2.1: Status of groundnut production over the last 15 years in Malawi

Year	Area (x1000 ha)	Yield (kg ha^{-1})	Production (x1000 t)
2002	205.73	767.4	157.87
2003	230.00	826.6	190.11
2004	218.03	703.6	153.41
2005	248.28	568.2	141.08
2006	244.57	830.3	203.07
2007	258.11	1014.3	261.81
2008	266.12	913.9	243.22
2009	266.95	1030.8	275.18
2010	295.24	1007.6	297.49
2011	308.09	1055.6	325.22
2012	353.19	1042.2	368.08
2013	362.82	1049.5	380.80
2014	373.93	792.9	296.50
2015	369.99	742.9	274.88
2016	369.99	743.5	275.07

Source: FAOSTAT (2018)

2.5 Constraints to groundnut production in Malawi

Groundnut has potential to contribute to food and income security in Malawi, but the yields in farmers' fields are below 1.0 t ha^{-1} (Chikowo *et al.*, 2015; FAOSTAT, 2018). This low

productivity has been reported to be due to several abiotic, biotic and socio-economic aspects which include: untimely rainfall, low plant populations, delayed planting, diseases and pests, use of grain that has been recycled as seed for many years, soil problems, weed competition for water and nutrients, scarcity of labour and lack of technical knowledge (Minde *et al.*, 2008; Prasad *et al.*, 2010; Chala *et al.*, 2014; Chikowo *et al.*, 2015; FAOSTAT, 2018). Although groundnut is known to be drought tolerant, the erratic and insufficient rainfall affects its production negatively (Madhava *et al.*, 2003). Prasad *et al.* (2010) reported that about 90% of yield variation in the semiarid tropics is due to water availability. The production is labour intensive as additional labour is required for stripping, shelling and even grading. About 85% of farm operations are based on manual labour and hand-hoe technologies (Minde *et al.*, 2008).

Quality seed availability is a major constraint since the seed supply is seasonal, and production depends on environmental conditions and price fluctuations. The seed production is mainly done by smallholder farmers and when crisis occurs, they sell or consume what they have put aside as seed (Minde *et al.*, 2008). Currently, there is disbursement of free seed by some institutions from time to time and this coupled with low seed multiplication factor and seed recycling, makes seed companies not invest in groundnut seed production (Chikowo *et al.*, 2015).

Biotic factors include pests and diseases, with the most important and widespread diseases being: groundnut rosette disease, rust, and early and late leaf spot. *Aphis craccivora* Koch is the most important pest and vector of GRD (Minde *et al.*, 2008). Waliyar *et al.* (2007) indicated that these pests and diseases cause significant economic losses when susceptible cultivars are grown. The use of low yielding cultivars that are susceptible to pests and diseases still constrains the groundnut production (Minde *et al.*, 2008). Additionally, in warm climates, kernels are easily infected by *Aspergillus* species which produce aflatoxins (Kumwenda and Madola, 2013; Chala *et al.*, 2014).

2.6 Groundnut rosette disease

Groundnut rosette disease (GRD) is a major groundnut disease endemic to sub-Saharan Africa (SSA). It is caused by a complex of three agents *viz*. *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and a *satellite*-RNA (satRNA) associated with GRV (Naidu *et al.*, 1999; Waliyar *et al.*, 2007; Naidu and Kimmins, 2007). GRAV belongs to the family *Luteoviridade* and replicates autonomously in the cytoplasm of phloem tissue while GRV is a member of the genus *Umbravirus* and has no structural protein. SatRNA is a

singlestranded, linear and non-segmented RNA, which totally depends on GRV for its replication, encapsidation and movement within and between plants (Thresh, 2003; Anitha *et al.*, 2014). Waliyar *et al.* (2007) indicated that groundnut is the only known natural host of GRAV and GRV, and both are transmitted in a persistent manner by an aphid (*Aphis craccivora* Koch) and through grafting, but not via seed, pollen or contact between plants. GRD is also transmitted by mechanical inoculation whereas GRAV is not. Additionally, Olorunju *et al.* (2001) and Hayatu *et al.* (2014) reported that the GRD symptoms are mainly due to SatRNA, and the three agents are dependent on each another, where each plays a crucial role in disease development.

Rosette disease was first reported in 1907 in Tanzania, and has since been observed in several other countries of SSA (Van der Merwe *et al.*, 2001; Ntarea *et al.*, 2003). According to Olorunju *et al.* (2001), the disease occurs mainly in Burkina Faso, Ghana, Nigeria, Côte d'Ivoire, Gambia, Malawi, Mozambique and Uganda. The disease has been also reported in Kenya, Angola, Madagascar, Senegal, Niger, South Africa, Swaziland, Sudan and Democratic Republic of Congo (DRC) (Waliyar *et al.*, 2007). Naidu *et al.* (1999) indicated that symptoms similar to GRD were reported in Asia and South America, but it is generally assumed that the disease is restricted to Africa and its offshore islands such as Madagascar. GRD is regarded as the most destructive groundnut disease in SSA and in susceptible cultivars the yield loss can reach 100% in epidemic years (Olorunju *et al.*, 2001; Van der Merwe *et al.*, 2001; Ntarea *et al.*, 2003). In 1975 an epidemic in Nigeria destroyed around 0.7 million ha with an estimated loss of US$250 million; in Zambia, an epidemic occurred in 1995 which affected approximately 43000 ha, causing an estimated loss of US$4.89 million and in the following year in Malawi, the production was reduced by 23% due to the disease (Jackson, 2015; Panguluri and Kumar, 2016). In addition, Waliyar *et al.* (2007) pointed out that GRD is responsible for annual yield losses worth over US$150 million.

2.6.1 Groundnut rosette disease symptoms and effect on yield

GRD has two main symptom types; chlorotic and green rosette, with variation within each type (Waliyar *et al.*, 2007). Chlorotic rosette is the most predominant while green rosette is only common in west African countries, Uganda, Malawi and Angola (Subrahmanyam *et al.*, 1997; Mugisa *et al.*, 2016). The chlorotic and green variants of satRNA cause the chlorotic and green forms of GRD, respectively and the mosaic rosette is caused by mixed infection with chlorotic and mottle variant of satRNA (Bua and Opio, 2014). In chlorotic rosette, leaves are bright yellow with a few green islands and a curled lamina whereas in green rosette, they appear dark green with light green to dark green mosaic (Nigam and Bock, 1990; Subrahmanyam *et*

al., 2002). Divergence among casual agents, genotypes, plant stage at infestation, climate conditions and mixed infections with other viruses have been reported to contribute to symptom variability (Naidu *et al.*, 1999). Hence, to confirm the presence of the disease and to document the variable symptom types, samples should be collected and tested for the three GRD agents and other viruses.

Subrahmanyam *et al.* (1997) and Waliyar *et al.* (2007) indicated that when GRD infection occurs at the early growth stage, the entire plant is affected, causing severe stunting due to shortened internodes and reduced leaf size, which lead to a bushy appearance while plants infected late may show symptoms in only some branches or parts of branches. GRD affects significantly the agronomic performance of groundnut and its effect on yield depends mainly on the genotype and the growth stage at which infection occurs (Waliyar *et al.*, 2007). When infection occurs prior to flowering, yield loss may reach 100% while in later growth stages (between flowering and pod setting) yield loss depends mainly on infestation severity but is lower, and after pod setting the yield loss is insignificant (Naidu *et al.*, 1999; Subrahmanyam *et al.*, 2002; Waliyar *et al.*, 2007). Van der Merwe *et al.* (1999) and Naidu and Kimmins (2007) indicated that GRAV alone reduces plant height, leaf area index and yield even in symptomless plants. Yield reduction ranging from 28-75% due to GRAV has been reported on genotypes with GRD resistant reaction (Van der Merwe *et al.*, 1999).

2.6.2 Groundnut rosette disease epidemiology and diagnostics

The GRD epidemiology is complex and involves interactions between the two viruses (GRAV and GRV), satRNA, aphid vector, and the host plant in a specific environment of SSA (Naidu and Kimmins, 2007). Waliyar *et al.* (2007) and Panguluri and Kumar (2016) reported that none of the causal agents is seed-borne and the primary infection depends on the survival of infected plants between cropping seasons and the aphid vector. It is assumed that there are native African plants, from which GRD spreads into groundnut. The aphid vector is polyphagous and can survive in around 143 plant species, and one of these species could be the source of the GRD virus. Eighty-three of these species belong to the Leguminosae family, suggesting that the aphid has preference for legume crops (Naidu *et al.*, 1999). Murant (1990) indicated that secondary spread occurs from the primary infection by the aphid movement and considered GRD to be a polycyclic disease, since each infected plant serves as a source of inoculum for initiating subsequent spread. Primary infection at early growth stages allows repeated cycles of infection to occur before the crop matures and vector population declines (Subrahmanyam *et al.*, 1997). Growth stage, genotype, plant population, transmission

efficiency of aphids, proximity to the source of infection and climatic conditions have been reported to influence the GRD spread (Waliyar *et al.*, 2007).

In the field, GRD can be diagnosed based on the characteristic symptoms. According to Waliyar *et al.* (2007), various diagnostic techniques have been developed to confirm the presence of GRD and test for the three casual agents. These techniques are mainly based on biological, serological (protein-based) and genomic properties of the casual agents (Table 2.2).

Table 2.2: Methods for detection of the three GRD agents

Method	GRAV	GRV	SatRNA
Inoculation to indicator plants (biological assay)	No	Yes	Yes (requires GRV)
Enzyme-linked immunosorbent assay (ELISAserological assay)	Yes	No	No
Reverse transcription polymerase chain reaction (RT-PCR: genomic based)	Yes	Yes	Yes

Source: Waliyar *et al.* (2007)

2.6.3 Groundnut rosette disease management

Various methods are available for GRD management and include chemical control to reduce the aphid population, cultural practices and breeding for virus and vector resistance (Naidu *et al.*, 1999; Waliyar *et al.*, 2007; Okello *et al.*, 2014). Subrahmanyam *et al.* (2002) supported the possibility of using chemical control, since the virus is transmitted in a persistent manner. Organophosphorus insecticides have been used and the timing of spray, dosage and type of insecticide used are critical for an effective aphid control (Waliyar *et al.*, 2007; Jackson, 2015). According to Naidu and Kimmins (2007), cultural practices include early planting at optimum plant density which allows ground cover before the aphid's main period of flight activity (aphids prefer widely spaced plantings for landing) and older plants escape from infection because they prefer younger plants. Intercropping groundnuts with cereals such as maize, sorghum, finger millet, beans and cowpea decrease the GRD incidence (Subrahmanyam *et al.*, 2002; Brink and Belay, 2006). Rouging of voluntary sources and early-infected plants prevent the primary and secondary spread of the disease (Waliyar *et al.*, 2007; Naidu and Kimmins, 2007; Jackson, 2015). However, chemical control is not economically practical and cropping practices are difficult for smallholder farmers under subsistence farming conditions (Naidu *et*

al., 1999). In addition, improper use of chemical control may result in development of insecticide-resistant biotypes.

The use of GRD resistant cultivars is the most economical and practical method to control the disease, thus efforts have been made to identify durable resistance sources and also develop resistant cultivars. Various sources of GRD resistance have been identified and the first was a landrace of late-maturing Virginia type from Burkina Faso and Cote d'Ivoire (Naidu *et al.*, 1999; Subrahmanyam *et al.*, 2002). Sources of resistance were also identified in the earlymaturing Spanish type (Nigam and Bock, 1990). Olorunju *et al.* (2001) indicated that these sources were used in breeding programs in SSA to develop resistant cultivars such as RG1 which is known and released in Malawi. Resistance to GRD was also identified in wild *Arachis* species and a high level of resistance was found in a hybrid derived from an interspecific cross between *A. hypogea* and *A. chacoense* (Waliyar *et al.*, 2007).

2.6.4 Screening genotypes for resistance to groundnut rosette disease

Evaluating genotypes for GRD resistance can be carried out under screen house or field conditions with inoculation done by using viruliferous aphids, through grafting or mechanical techniques (Waliyar *et al.*, 2007). Naidu and Kimmins (2007) indicated that the use of viruliferous aphids and grafting allow the evaluation of all the three GRD agents while inoculation through mechanical techniques allows evaluations only for resistance to GRV and SatRNA. Bock and Nigam (1988) developed a satisfactory technique for GRD resistance screening, based on viruliferous aphids inoculation, which resulted in 98% disease incidence in susceptible genotypes. The technique permits rapid field evaluation of a large number of genotypes (Naidu *et al.*, 1999). According to Bock and Nigam (1988), infector rows of a susceptible genotype are planted between two rows of test material and prior to this, infected plants are raised in a greenhouse. One week after emergence, the infected plants are transplanted at 1.5 m spacing within infector rows and subsequently viruliferous aphids are transferred from infected plants in the greenhouse to the infector rows and testing materials on a weekly basis up to 80 days after sowing (Bock and Nigam, 1988; Nigam and Bock, 1990). Waliyar *et al.* (2007) pointed out that diagnostics assays such as TAS-ELISA or RT-PCR should be used to test the presence of the GRD agents during evaluations.

GRD resistance evaluations can be done using either percentage of disease incidence (PDI) or disease severity (Nigam and Bock, 1990; Olorunju *et al.*, 2001; Waliyar *et al.*, 2007). Waliyar *et al.* (2007) indicated that PDI is predominantly used and is recorded at early pod filling stage,

at 80 and 100 days after sowing. The interpretation of resistance level is done as shown in Table 2.3.

Table 2.3: Evaluation of groundnut genotypes for GRD resistance based on percentage of disease incidence

PDI (%)	Inference/ Host response
0-10	Highly resistant
11-30	Resistant
31-50	Moderately resistant
51 and above	Susceptible

Source: Waliyar *et al.* (2007)

When genotypes are evaluated for GRD resistance using severity, Table 2.4 is a guide for data collection and interpretation of the resistance level.

Table 2.4: Disease rating scale for GRD resistance evaluation

Scale	Genotype response	Host response
1	No visible symptoms on the foliage	Highly resistant
2	GRD symptoms on 1 to 20% foliage, but no obvious stunting	Resistant
3	GRD symptoms on 21 to 50% foliage and stunting	Moderately resistant
4	Severe GRD symptoms on 51 to 70% foliage and stunting	Susceptible
5	Severe symptoms on 71 to 100% foliage, stunted or dead plants	Highly susceptible

Source: Waliyar *et al.* (2007)

2.7 Heritability and genetic advance

Heritability is the proportion of phenotypic variance among individuals in a population that is due to genetic effects (Holland *et al.*, 2003; Tada, 2015). Plant breeders mostly select phenotypically superior plants according to the breeding objectives. However, due to environmental factors, genetically inferior plants may appear superior and end up being selected (Behera, 2007; Acquaah, 2009). You *et al.* (2016) indicated that with high heritability estimates, the progeny resembles the selected phenotype and the genetic gain may be materialized. Holland *et al.* (2003) and Acquaah (2009) reported two different heritability estimates, the broad and narrow sense heritability. Broad-sense heritability measures the ratio of total genetic variance (additive, dominance and epistatic effects) to phenotypic variance

while narrow-sense heritability gives a ratio of additive genetic variance to phenotypic variance. The narrow-sense heritability is more useful in breeding and the genetic variance is predominantly additive in self-pollinated crops such as groundnuts (Holland *et al.*, 2003). The trait, population and environment have been reported to influence heritability estimates (Holland *et al.*, 2003; Behera, 2007; Acquaah, 2009).

Genetic advance also known as response to selection and genetic gain is the change of population mean between generations following selection (Piepho and Möhring, 2007; Acquaah, 2009). According to Nyquist and Baker (1991), genetic gain depends on the amount of variability present in the population from which selection will be conducted, heritability of the target trait and the imposed selection pressure. A large phenotypic variance provides a wide range of variability for selection (Piepho and Möhring, 2007), and if heritability of a trait is high, advancing only top few performers is likely to produce higher genetic gain than selecting many moderate performers (Acquaah, 2009). Ogunniyan and Olakojo (2014) indicated that estimates of both heritability and genetic gain are more reliable and meaningful than individual consideration of the parameters.

The inheritance mode of GRD has been extensively studied by several researchers (Muitia, 2011; Alhassan, 2013; Bua and Opio, 2014; Kayondo *et al.*, 2014; Amoah, 2016; Nalugo *et al.*, 2016). Most of these genetic studies suggested that the inheritance of GRD resistance is quantitative and the additive gene action is predominant. However, dominance and epistasis were also reported to be important in the same studies. Contrary to reports that GRD resistance is mainly controlled by additive genes, Nalugo *et al.* (2016) found dominance gene action to be predominant in Valencia groundnut. High values of broad-sense heritability for GRD resistance have been reported and these were 75.0% (Adu-Dapaah *et al.*, 2007), 95.7% (Alhassan, 2013), 93.0% (Kayondo *et al.*, 2014), 82.9% (Amoah, 2016) and 67.5% (Nalugo *et al.*, 2016). Low to high narrow-sense heritability estimates were also reported by Alhassan (2013) (67.5%), Kayondo *et al.* (2014) (75.0%), Amoah (2016) (43.8%), Kufor (2016) (4.067.0%, depending on the population), and Nalugo *et al.* (2016) (35.4%). The differences in the heritability values are due to differences in populations and environments. For example, AduDapaah *et al.* (2007) used an F4 generation where the non-additive components must have been reduced during selfing, thus recorded lower broad-sense heritability estimates. Alhassan (2013) also reported low value of genetic advance (5.9%) while Nalugo *et al.* (2016) reported moderate to high values which ranged from 13.5-50.7%.

Broad-sense heritability values for days to flowering and maturity of 59.3% and 31.9% (Zaman *et al.*, 2011), 45.5% and 41.2% (Rao *et al.*, 2014), 99.0% and 96.0% (Patil *et al.*, 2015), 75.7%

and 79.4% (Balaraju and Kenchanagoudar, 2016), respectively, were reported in groundnuts. From these studies, low to moderate genetic advance was reported and in contrast with these findings, Patil *et al.* (2015) reported a high genetic advance for days to maturity. Plant height was estimated to have high broad-sense heritability and genetic advance of 66.3% and 30.3% (Meta and Monpara, 2010), 89.4% and 21.0% (Yusuf *et al.*, 2017b), respectively. Number of secondary branches have been indicated to have high values of broad-sense heritability and genetic advance of 97.5% and 183.7% by Korat *et al.* (2009), 96.0% and 145.0% by Patil *et al.* (2015), respectively. Number of primary branches was reported to have a relatively lower heritability (55.8%) and genetic advance (25.2%) (Balaraju and Kenchanagoudar, 2016). Zaman *et al.* (2011) and Rao *et al.* (2014) reported high broad-sense heritability and genetic advance for number of pods (80.3% and 34.9%) and hundred seed weight (87.0% and 28.3%), respectively. Heritability (37.8%-93.2%) and genetic advance (6.1%-46.9%) estimates varying from low to high have been observed for seed yield (Maurya *et al.*, 2014; Rao *et al.*, 2014; Kademani and Renuka, 2017; Yusuf *et al.*, 2017b). Shelling percentage has been indicated to have high heritability (70.0%-93.7%) and low to high genetic advance (3.8%-19.3%) (Korat *et al.*, 2009; Zaman *et al.*, 2011; Balaraju and Kenchanagoudar, 2016).

The reported heritability values which were mostly high, suggest that the genetic effect on resistance to GRD and the other mentioned traits is greater than the environmental effect. The high genetic advance indicates a predominant role of additive genes while moderate values suggest the importance of both additive and non-additive gene action (Wambi *et al.*, 2014).

2.8 Correlations and path analysis in groundnut

Plant breeding aims to improve one or more traits at the same time, but seed yield increase is the most important objective in groundnut breeding programs (Yusuf *et al.*, 2017a; Mandal *et al.*, 2017). Kiranmai *et al*. (2016) indicated that seed yield is a complex quantitative and dependent trait resulting from interplay of various related traits. It is largely influenced by the growing environment and has low heritability (Luz *et al.*, 2011; Mukherjee *et al.*, 2016). Hence, the direct selection for seed yield is less efficient in improving groundnut productivity. Yield improvement efficiency in the crop can be enhanced through exploitation of the relationship between seed yield and its related traits (Zaman *et al.*, 2011; Kiranmai *et al.*, 2016; Mandal *et al.*, 2017). Moreover, Kiranmai *et al.* (2016) reported that trait association analysis is very important in groundnut than other crops since the pods are formed underground and unless association between external plant trait and seed yield are established, it may not be possible to effect proper selection prior to harvest.

Correlation analysis measures association between variables and can either be positive or negative, weak or strong, phenotypic or genotypic, and significant or non-significant (Mohammadi *et al.*, 2003; Mohanan, 2010; Pavlov *et al.*, 2015). The genotypic correlation coefficients have been reported to be higher and more significant than the corresponding phenotypic correlation coefficients, indicating the prevalence of environmental interaction and strong association between traits at genotypic level (Puttha *et al.*, 2008; Zaman *et al.*, 2011; Mandal *et al.*, 2017). Acquaah (2009) and Sabaghnia *et al.* (2010) indicated that correlation between two traits is useful as it indicates the degree of association and provides scope for indirect selection in plant breeding programs. However, it has been reported that correlations are inadequate to describe the importance of each trait associated with seed yield (Mohammadi *et al.*, 2003). Wright (1921) developed a method known as path coefficient analysis which partitions correlation coefficients into direct and indirect effects, allowing the estimates of contribution of each trait to seed yield. Correlation and path coefficient analysis have been used in groundnut breeding to determine selection criteria (Zaman *et al.*, 2011; Kiranmai *et al.*, 2016; Chavadhari *et al.*, 2017; Kademani and Renuka, 2017).

Strong positive correlations between seed yield with number of pods per plant (Rao *et al.*, 2014), number of secondary branches (Patil *et al.*, 2006; Balaraju and Kenchanagoudar, 2016), harvest index, shelling percentage (Mandal *et al.*, 2017) and hundred seed weight (Zaman *et al.*, 2011; Yusuf *et al.*, 2017a) have been reported in groundnut. In contrast, Zaman *et al.* (2011) reported weak negative correlation between seed yield and shelling percentage. Additionally, weak positive correlations were observed between seed yield and the following traits: days to flowering and maturity (Zaman *et al.*, 2011; Mandal *et al.*, 2017), plant height (Balaraju and Kenchanagoudar, 2016; Yusuf *et al.*, 2017a) and number of primary branches (Mandal *et al.*, 2017). Contrary to these reports, Rao *et al.* (2014) observed weak negative correlations between seed yield with days to flowering and maturity. Moreover, seed yield and its components were reported to be negatively correlated to GRD incidence (Van der Merwe *et al.*, 2001; Muitia, 2011; Chintu, 2013; Mohammed *et al.*, 2018). High direct positive effect of number of pods per plant, hundred seed weight, shelling percentage, days to flowering and maturity on seed yield have been reported in groundnut (Zaman *et al.*, 2011; Rao *et al.*, 2014; Kiranmai *et al.*, 2016). Positive direct effects of number of primary and secondary branches on yield were also reported by Patil *et al.* (2006). In addition, Zaman *et al.* (2011) and Kiranmai *et al.* (2016) reported direct negative effects of number of immature pods and rust severity on seed yield.

Traits such as hundred seed weight, number of pods per plant, number of secondary branches, days to maturity and flowering which exhibited positive correlation and high direct positive effects on seed yield, should be considered on selection criteria. Disease related traits, such as GRD incidence and rust severity which were reported to be negatively correlated and exhibit high direct negative effects on seed yield should also be considered and plants or genotypes with susceptible response to these diseases or others should not be selected.

2.9 Multivariate analysis

2.9.1 Cluster analysis

Cluster analysis is a multivariate technique which groups genetically similar genotypes into the same group (Pereira *et al.*, 2015). Mohammadi and Prasanna (2003) indicated that genotypes in the same cluster should exhibit high homogeneity while genotypes in different clusters should show high heterogeneity. Cluster analysis is a useful tool for genetic relationship analysis in plant breeding. It allows identification of genetically diverse genotypes, planning crosses and assigning genotypes into heterotic groups (Subramanian and Subbaraman, 2010; Suryanarayana *et al.*, 2017). It has been reported that the selection of genetically diverse parents is crucial for a successful breeding program, as it provides opportunity for development of new cultivars with desirable traits (Govindaraj *et al.*, 2015; Niveditha *et al.*, 2016).

2.9.2 Principal component analysis in groundnut

Principal component analysis (PCA) is one of the oldest multivariate techniques used for data reduction to clarify the relationship between two or more variables (Mohammadi and Prasanna, 2003). The PCA reduces the dimensionality of a data set consisting of a large number of interrelated variables, retaining as much as possible the variation present in the data set (Jolliffe, 2002). Ali *et al.* (2015) indicated that the total variance present in a data set is divided into a limited number of uncorrelated new variables, the principal components (PCs). These PCs are ordered in such way that the first few retain most of the variation present in all the original variables. Principal components with eigenvalues greater than one are considered meaningful and theoretically have more information than would any single variable alone. Variables with large eigenvectors, either positive or negative, are considered to be contributors to the components (Iezzoni and Pritts, 1991). Additionally, PCA creates two or three dimensional scatter plot of genotypes and the geometrical distances among genotypes in the plot reflect the genetic distances among them with minimum distortion. This allows

visualization of differences among genotypes and identification of possible groups (Jolliffe, 2002; Mohammadi and Prasanna, 2003; Ali *et al.*, 2015).

Aliyu and Zanzam (2011) reported five PCs with eigenvalues greater than one which accounted for about 99% of the total variance in groundnut. In the first principal component, hundred seed weight had the highest positive loading, followed by number of pods per plant, pod yield per plant, days to maturity, shelling percentage and seed yield per plant. Niveditha *et al.* (2016) also reported five PCs which explained 71.46% of the total variability and had eigenvalues greater than one. PC1 had high positive association with haulm yield, followed by shelling percentage, seed yield, number of pods per plant, pod yield, days to flowering and protein content. Number of pods per plant had the highest contribution to PC2, followed by harvest index, days to flowering, shelling percentage and plant height. This suggests that the first component in groundnut groups yield traits and separates the high yielding from low yielding genotypes.

2.10 Molecular markers

Genetic markers are traits which are used to differentiate individuals under study (Singh *et al.*, 2008). In conventional breeding, the variation among individuals is identified by visual assessment through morphological markers and with the development of molecular biology, it is now possible to differentiate individuals based on the DNA differences, through molecular markers (Schlötterer, 2004; Xu, 2010). Tomar (2010) indicated that morphological markers are few in numbers and highly affected by the environment, whereas molecular markers are abundant, robust and independent of environmental conditions. Biochemical markers have also been used in plant breeding but like morphological markers they vary in different environments, limiting their use (Kumar *et al.*, 2009). According to Singh *et al.* (2008) and Xu (2010), an ideal molecular marker should be highly polymorphic, co-dominant in expression (heterozygous loci can be distinguished from homozygous), distributed on the entire genome, without pleiotropic effect, easy to detect, low cost of marker development and genotyping, and high duplicity.

Several types of molecular markers are known and the restriction fragment length polymorphism (RFLP) was the first applied marker in genotyping. It uses restriction enzymes and hybridization with radioactive probes, and it is useful for construction of genetic linkage maps. However, it is time consuming, and has a complicated hybridization and a limited number of available probes (He *et al.*, 2014). Further, PCR based markers were developed and applied in crop improvement. These mainly include the random amplified polymorphic

DNA (RAPD) which uses a single, short (10 nucleotide) and random primer for DNA amplification, amplified fragment length polymorphisms (AFLPs) which combine the RFLP and PCR technology, simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) (Singh *et al.*, 2008). According to Xu (2010), SSRs are tandem repeats motifs of 1 to 6 nucleotides which are abundant in the genome while SNPs refer to an individual nucleotide base difference between two DNA sequences.

The development of genomic tools in groundnut has begun recently and slowly progressed due to tetraploid nature of the crop, low marker polymorphism and lack of genome sequence resources (Janila *et al.*, 2016). Chu *et al.* (2011) indicated that the first groundnut variety developed using molecular techniques was registered in 2003, and since then, China, Japan, USA and India have been using marker-assisted breeding in routine groundnut breeding programs. Both hybridization and PCR based markers have been used in groundnut, mainly for genetic diversity studies (Subramanian *et al.*, 2000; Jiang *et al.*, 2007; Khera *et al.*, 2013), genetic relationships (Kochert *et al.*, 1991; He and Prakash, 2001), population structure (Wang *et al.*, 2011; Ren *et al.*, 2014) and marker-assisted selection (Stalker and Mozingo, 2001; Herselman *et al.*, 2004; Chu *et al.*, 2011). However, Holbrook *et al.* (2011) pointed out that the use of RFLP, RAPD and AFLP markers showed extremely low levels of polymorphism in groundnut while the application of SSR and SNP markers allowed the detection of more frequent polymorphism in the crop. Moreover, Semagn *et al.* (2014) indicated that SNPs are now the markers of choice and have largely replaced the SSRs in plant breeding.

2.10.1 Single nucleotide polymorphisms (SNPs)

SNPs refer to an individual nucleotide base difference between two DNA sequences and a variation is considered SNP if it occurs in at least 1% of the population (Xu, 2010). According to Singh *et al.* (2008), SNPs are the most abundant polymorphic markers throughout the genome. This is because they can be found in both transcribed and non-transcribed regions, and in some cases are the direct cause of phenotypic variation. Moreover, Jehan and Lakhanpaul (2006) pointed out that SNPs are useful for creating high-density genetic maps (which cannot be achieved with other genetic markers) due to their abundance in the genome. Because of their low assay cost, high abundance in the genome, co-dominant inheritance, locus specificity, potential for high-throughput analysis and relatively low genotyping error rates, SNPs are a powerful tool for many applications in plant breeding. These markers have been also applied for germplasm characterization, population structure, genetic relationship, linkage mapping and marker-assisted selection in groundnut (Khera *et al.*, 2013; Zhou *et al.*, 2014; Zhang *et al.*, 2017).

2.10.2 Next generation sequencing and genotyping by sequencing

The availability of whole genome sequences (WGS) has allowed the shift of perspective in DNA markers identification from fragmented based polymorphism to sequence based nucleotide polymorphism (Ray and Satya, 2014). Whole genome sequence based on Sanger sequencing is time consuming, costly and has not been shown to be suitable for processing large number of samples, while next generation sequencing (NGS), also known as deep sequencing has revolutionised genomic research, since it allows the detection of numerous DNA markers and handling of larger numbers of samples at lower cost (He *et al.*, 2014; Vlk and ŘEPKOVÁ, 2017). Several NGS sequencing platforms, such as Roche 454 FLX Titanium, Illumina MiSeq and HiSeq2500, Ion Torrent PGM have been developed and used recently (He *et al.*, 2014). Moreover, many NGS marker discovery technology, such as restriction site associated DNA sequencing (RAD-seq) and genotyping by sequencing (GBS), allow SNP discovery and genotyping simultaneously (Vlk and ŘEPKOVÁ, 2017).

Genotyping by sequencing is a system that constructs reduced representation libraries for the Illumina NGS platform. It generates a large number of SNPs for genetic analysis and genotyping from sequence data at a lower cost than other SNP array platforms (Annicchiarico *et al.*, 2017; Pandey *et al.* (2017). This system is an important cost-effective tool for genomic assisted breeding in most crops and has wide variety of applications (He *et al.*, 2014). Such applications include: marker discovery, genetic diversity studies, linkage mapping for genomic selection, improvement of reference genomes and genomics conservation. GBS is robust across a range of species, thus can be used even without a reference genome for marker alignment (Bhatia *et al.*, 2013; He *et al.*, 2014). The NGS and GBS technology have been also used in groundnut for genetic diversity analysis, population structure, linkage disequilibrium (Zhang *et al.*, 2017), SNP discovery, construction of genetic linkage map (Zhou *et al.*, 2014; Pandey *et al.*, 2017) and analysis of genetic relationship between the diploid ancestors of the crop (Bertioli *et al.,* 2015).

2.10.3 Genome regions controlling groundnut rosette disease resistance

Groundnut breeding programmes have been using phenotyping tools for selecting plants or progenies with desirable characteristics (Pasupuleti *et al.*, 2013). However, conventional breeding has limitations when improving traits with quantitative inheritance which are influenced by genotype by environment interaction, such as GRD resistance (Janila *et al.*, 2016). Moreover, Cobb *et al.* (2013) indicated that even with the best available phenotyping tools, there is a chance of selection bias due to failure of phenotypic screens and escapees.

In contrast, genomic tools are robust, cost-effective, and reliable to enhance genetic gain for specific characters and the whole breeding efficiency (Pasupuleti *et al.*, 2013; Janila *et al.*, 2016).

Efforts have been made in identifying molecular markers linked to trait specific genes/ QTL in groundnuts. These studies identified markers linked to rust and late leaf spot resistance (Hou *et al.*, 2007; Leal-Bertioli *et al.*, 2009; Khedikar *et al.*, 2010; Sujay *et al.*, 2012), aflatoxin contamination and *Aspergillus flavus* resistance (Lei *et al.*, 2006; Yanbin *et al.*, 2009), drought tolerance (Ravi *et al.*, 2011), pod and kernel traits (Gomez Selvaraj *et al.*, 2009; Pandey *et al.*, 2014), protein and high oleic acid content (Sarvamangala *et al.*, 2011; Wang *et al.*, 2011). However, few reports are available on DNA markers linked to GRD and aphid resistance. Herselman *et al.* (2004) identified eight putative AFLP associated with the aphid vector resistance, which explained up to 79.06% of the total phenotypic variation and were located on the chromosomes A01, A02, A03, A04 and at unknown positions. Three of these markers are in coupling with the R allele. Pandey *et al.* (2014) reported two SSR markers linked to GRD resistance, which explained up to 39.29% of the total phenotypic variation and were located on the chromosome B04.

2.11 Conclusion

The reviewed literature provides evidence that GRD is one of the major constraints to groundnut production in SSA. In SSA, extensive research has helped in understanding the disease, identifying sources of resistance and breeding resistant varieties. There are few reports on molecular markers associated with resistance to GRD and its vector. Currently, research has been mainly based on phenotyping tools. Nevertheless, there is a need of continuous research in breeding for resistance to GRD. The research should also consider the development of molecular markers linked to GRD resistance and its vector, which will be used for marker-assisted selection, in order to enhance the breeding efficiency and shorten the breeding cycle.

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CHAPTER 3 ASSESSMENT OF GROUNDNUT ACCESSIONS FOR GENETIC VARIABILITY UNDER NATURAL ROSETTE DISEASE INFESTATION

Abstract

Groundnut is an important oilseed crop and ranks 13th among the food crops in the world. However, production of the crop in Malawi is low (759.77 kg ha^{-1} average yield of unshelled groundnuts) due to several biotic, abiotic, and socio-economic factors. Development of high yielding cultivars, which are resistant or tolerant to these biotic and abiotic stresses is possible, provided variability for the traits is present in the different groundnut germplasm. Therefore, this study was undertaken to determine the extent of variability among selected groundnut accessions for yield and its related traits under natural GRD infestation. The groundnut accessions were planted at ICRISAT Malawi and data were recorded for 13 quantitative traits. Analysis of variance revealed highly significant differences among the accessions. Seed yield, number of pods per plant, plant height, GRD incidence and number of secondary branches showed high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). Moderate variation (PCV and GCV) were observed for days to flowering and pod width while shelling percentage and days to maturity showed low variability. The highest broadsense heritability and genetic advance estimates were observed for seed yield followed by GRD incidence. A combination of high heritability and genetic advance was recorded for the number of secondary branches, plant height, seed yield and GRD incidence. This indicated that phenotypic selection based on the mean would be successful in improving these traits.

Improvement for number of primary branches and shelling percentage based on the evaluated accessions would be limited since they have low genetic potential due to low variability, low heritability and genetic advance.

Keywords: Groundnut, genetic variation, heritability, genetic advance.

3.1 Introduction

The cultivated groundnut (*Arachis hypogea* L., AABB, 2n = 4x = 40) is a highly self-pollinated crop which originated from South America, where early Spanish and Portuguese explorers found it cultivated extensively (Simpson *et al.*, 2001). It is the sixth and third most important source of vegetable oil and protein, respectively, and ranks 13th among the food crops in the world (Singh and Nigam, 2016). Groundnut is grown worldwide in tropical and subtropical regions mainly for its high-quality oil and easily digestible protein (Maiti, 2002). According to Nautiyal *et al.* (2002) and Talawar (2004), the kernels contain 47-53% of edible oil, 24-36% of vegetable protein, 10-15% of carbohydrates, and are a good source of minerals (Ca, Mg, P, Fe and Zn), vitamins (E, K and B complex) and fibre. Longwe-Ngwira *et al.* (2012) indicated that groundnut is the most important legume crop in terms of value and quantity in Malawi, where it is predominantly grown by smallholder farmers under low-input production system.

Africa is the second major groundnut-producing continent after Asia, with over 28.34% (11.93 MT) of the world production and a yield average of 0.9 t ha^{-1}, which is less than three-quarters of the world average of 1.64 t ha^{-1} (FAOSTAT, 2018). Chikowo *et al*. (2015) indicated that despite the low yields, Africa contributes significantly to the world groundnut. However, groundnut production in Malawi has suffered from fluctuations and the yields are still low with an average of 759.77 kg ha^{-1} over the last three seasons, which is less than half of the yield recorded in the major producing countries (2000-4000 kg ha^{-1}) (Singh and Nigam, 2016). Several biotic, abiotic, and socio-economic factors, constrain the groundnut production in Malawi, and these include the untimely rainfall, lack of quality seed, soil fertility problems and lack of fertilizers, diseases and pests, scarcity of labour and lack of technical knowledge (Minde *et al.*, 2008; Prasad *et al.*, 2010; Chala *et al.*, 2014; Chikowo *et al.*, 2015). Amongst the most important biotic diseases is groundnut rosette disease (GRD). This is a viral disease caused by a complex of three agents (*Groundnut rosette assistor virus* (GRAV), Groundnut rosette virus (GRV) and a *satellite*-RNA (satRNA) associated with GRV) and transmitted by an aphid (*Aphis craccivora* Koch). GRD has been responsible for devastating losses to groundnut production in Malawi and other African countries, with yield losses of up to 100% in susceptible cultivars (Minde *et al.*, 2008; Anitha *et al.*, 2014).

The development of high yielding cultivars which are resistant to both biotic and abiotic stresses, and meet the farmers' preferences should be continuous and a priority activity. It has been indicated that the existence of genetic variability provides opportunities for development of such improved cultivars, because the plant populations and genotypes vary at the genetic level, resulting in different phenotypic performance (Acquaah, 2009; Govindaraj *et al.*, 2015). The knowledge of how variable the populations of interest are is essential, as it allows construction and planning of an ideal genotype. Moreover, Singh (2001) and Zaman *et al.* (2011) reported that existence of variability in traits would greatly benefit groundnut breeding programmes, as it indicates scope for selecting superior genotypes and it is a combined measure of genetic and environmental causes. The genetic variability has to be heritable (Holland *et al.*, 2003).

Genotypic and phenotypic coefficients of variation, broad-sense heritability and genetic advance have been reported for different yield and yield related traits in groundnut (Korat *et al.*, 2009; Zaman *et al.*, 2011; Rao *et al.*, 2014; Yusuf *et al.*, 2017). The coefficients of variation provide a measure to compare variability present in quantitative traits while high heritability coupled with high genetic advance suggest the possibility of effective phenotypic selection (Holland *et al.*, 2003; Acquaah, 2009; You *et al.*, 2016). Therefore, these parameters indicate the genetic potential of a given germplasm and allow to enhance the success in breeding programmes. The characterization based on morphological variability also allows the identification of accessions with valuable and desirable agronomic traits to be used as parents in breeding programmes (Shrestha, 2016). Therefore, the study was undertaken to determine the extent of variability among selected groundnut accessions for yield and its related traits under natural GRD infestation.

3.2 Materials and methods

3.2.1 Plant materials

Genetic variability for yield and its related traits was evaluated under field conditions, and 25 groundnut accessions and three controls (
Table 3.1) were used in the experiment. Cultivars CG7 (susceptible control), ICGV-SM 99568 and ICGV-SM 90704 (resistant controls) which are released and well-known in Malawi, were used as controls. All accessions are maintained at International Crops Research Institute for the Semi-arid Tropics (ICRISAT) in Malawi.

Table 3.1: List of groundnut accessions used in the study

Entry number	Accession	Origin	Botanical group
1	CG 7 (control)	Malawi	Virginia
2	ICG 10384	Nigeria	Spanish
3	ICG 11249	Tanzania	Spanish
4	ICG 11426	India	Virginia
5	ICG 11651	China	Spanish
6	ICG 12509	Unknown	Virginia
7	ICG 12672	Bolivia	Virginia
8	ICG 12697	India	Spanish
9	ICG 12921	Zimbabwe	Spanish
10	ICG 12988	India	Spanish
11	ICG 13942	Unknown	Virginia
12	ICG 13982	USA	Virginia
13	ICG 14985	Unknown	Spanish
14	ICG 15405	Unknown	Valencia
15	ICG 2106	India	Spanish
16	ICG 334	China	Spanish
17	ICG 3584	India	Spanish
18	ICG 3681	USA	Valencia
19	ICG 405	Unknown	Spanish
20	ICG 4955	India	Spanish
21	ICG 5745	Puerto Rico	Virginia
22	ICG 6022	Unknown	Valencia
23	ICG 6057	USA	Virginia
24	ICG 6813	Senegal	Virginia
25	ICG 9507	Philippines	Spanish
26	ICG 9809	Mozambique	Spanish
27	ICGV-SM 90704 (control)	Malawi	Virginia
28	ICGV-SM 99568 (control)	Malawi	Spanish

3.2.2 Experimental site

The accessions were evaluated at ICRISAT Malawi, located at Chitedze Agricultural Research Station ($33^0$38'E and 13^o85'S), from February to June 2018. The station is located 16 km west of Lilongwe (Malawi) with an altitude of 1146 meters above sea level (masl). The accessions were evaluated under natural GRD infestation, since the station is a hotspot area with high GRD pressure during the growing season, especially with late-planted groundnuts. Based on the long-term climatic data, the station has an average minimum and maximum temperature of 16^oC and 24^oC, respectively, with a mean annual rainfall of 892 mm. Weather data for the period of the trial is shown in Table 3.2. The experiment was planted in sandy clay soils under rainfed conditions with supplementary irrigation when necessary.

Table 3.2: Weather data for the period of the trial

Month	Minimum Temperature (°C)	Maximum Temperature (°C)	Rainfall (mm)	Relative Humidity (%)
February	18.57	27.74	160.60	76.57
March	18.09	28.02	209.10	76.13
April	16.03	26.72	4.13	74.00
May	13.90	26.49	3.50	55.56
June	10.54	25.43	0.00	50.40
Average	15.43	26.88	-	66.53
Total	-	-	377.33	-

3.2.3 Experimental design and management

The 28 groundnut genotypes were evaluated using a 7 x 4 alpha lattice design with three replications. Border rows of genotype JL24 that is highly susceptible to GRD were sown around the trial to enhance GRD inoculum build-up. Each genotype was sown in a 3 row-plot of 3 m long, with inter-row spacing of 0.6 m. Intra-row spacing of 0.15 m was used and sowing was done by hand at a rate of two seeds per hill. The seedlings were thinned to one seedling per hole at three weeks after planting, when the plants were fully established and 60 seedlings per plot were allowed to grow. Fertilizers and pesticides were not applied, and the field was kept free of weeds by hand weeding which was done three times. The trial was conducted under rainfed conditions, but supplementary irrigation was applied as necessary. Harvesting and shelling were done manually.

3.2.4 Data collection

Data were recorded on percentage of disease incidence and severity, days to flowering and maturity, number of branches, plant height, yield and its components, and shelling percentage. Disease data were recorded based on Waliyar *et al.* (2007) while yield and agronomic traits were recorded as described for the groundnut descriptors (IBPGR and ICRISAT, 1992). Data, except on percentage of disease incidence and yield, were recorded on five randomly selected plants and 10 mature pods that were also randomly chosen (IBPGR and ICRISAT, 1992; Waliyar *et al.*, 2007).

Percentage of disease incidence (PDI)

Observations on GRD development were recorded visually at 60, 80 and 100 days after sowing (DAS). The number of plants showing GRD symptoms in each plot was determined by counting and PDI was calculated as follows:

$$PDI\ (\%) = \frac{NIP}{TP} * 100$$

Where: PDI is the percentage of disease incidence, NIP is the number of plants showing GRD symptoms and TP is the total number of plants in a plot.

The final PDI was reported and used to reflect the GRD resistance (Iwo and Olorunju, 2009), as shown in Table 3.3. GRD is a viral disease and the method based on PDI for assessment of genotypes for the disease resistance, is the widely used (Waliyar *et al.*, 2007). Severity was also recorded using a 1 to 5 rating scale, where: 1 = no symptoms, 2 = symptoms on 1 to 20% foliage but no stunting, 3 = symptoms on 21 to 50% foliage and stunting, 4 = severe symptoms on 51 to 70% foliage and stunting, and 5 = severe symptoms on 71 to 100% foliage, stunting and dead plants (Waliyar *et al.*, 2007). Severity scores were transformed by ln(x+1) before analysis in order to have residual terms following normal distribution (Gomez and Gomez, 1984).

Table 3.3: Scale of percentage of disease incidence used for evaluation of groundnut genotypes for resistance to GRD

PDI (%)	Inference/ Host response
0-10	Highly resistant
11-30	Resistant
31-50	Moderately resistant
51 and above	Susceptible

Source: Waliyar *et al.* (2007)

Days to flowering (DTF) and days to maturity (DTM)

Days to flowering and maturity were determined as the number of days between sowing date and the date when 50% of plants in a plot had flowered and matured, respectively.

Plant height and number of branches

Plant height (PH), and number of primary (NPB) and secondary branches (NSB) were recorded at 85 DAS. Plant height was taken from the ground to the top of the main stem axis while the branch numbers were measured by counting. These traits were recorded on the five randomly chosen plants in each plot and a mean was calculated.

Yield and yield components

The number of pods per plant (NPP) was recorded during harvesting by counting the mature pods on the five selected plants and a mean was determined for each plot. Pod length (PL) and pod width (PW) were measured on 10 pods randomly chosen, at the lengthiest and widest points, respectively. The pods were sun dried to approximately 8-10% moisture content and

then weighed to determine pod yield per plot. A pod sample of approximately 100 g which was randomly drawn from each plot was shelled, then the seed weighed and the shelling percentage (SP) was determined as follows:

$$SP\ (\%) = \frac{SW}{PWT} * 100$$

Where: SP is the shelling percentage, SW is the seed weight and PWT is the pod weight before shelling.

One hundred seeds were counted and weighed from the shelled samples, and the hundred seed weight (HSW) was recorded and expressed in grams. Seed yield (SYD) was estimated using the formula:

$$SYD\ (kg\ ha^{-1}) = \frac{PY * 10000}{PS} * SP$$

Where: SYD is the seed yield, PY is the pod yield per plot (kg), PS is the plot area (m^2) and SP is the shelling percentage (expressed as a fraction).

3.2.5 Data analysis

3.2.5.1 Analysis of variance

Analysis of variance (ANOVA) was performed on all recorded traits using the General Linear Model (GLM) in SAS version 9.4 (SAS Institute, 2015) and Genstat 18th Edition (Payne, 2014), following the tests of Shapiro-Wilk and Bartlett for residual normality and variance homogeneity, respectively. The ANOVA model was as follows:

$$Y_{ijk} = \mu + G_i + R_j + B(R)_{kj} + \varepsilon_{ijk}$$

Where: Y_{ijk} is the effect of the i^{th} genotype in k^{th} incomplete block in j^{th} replication, μ is the general mean, G_i is the effect of i^{th} genotype, R_j is the effect of j^{th} replication, $B(R)_{kj}$ is the effect of k^{th} incomplete block within j^{th} replication and ε_{ijk} is the error term of i^{th} genotype in k^{th} incomplete bock in j^{th} replication.
Least significant difference (LSD) at 5% significance level, was used for mean separation.

3.2.5.2 Variance components

The analyses of variance were used to estimate the genotypic, environmental and phenotypic variances, using the mean square values, which were equated to their respective expectations

(Singh *et al.*, 1993). The estimates of the variance components of each trait was computed as follows:

$$\sigma e2 = MS_E$$

Where: σ_e^2 is the environmental variance and MS_E is the residual mean square

$$\sigma_g^2 = \frac{MS_G - MS_E}{r}$$

Where: σ_g^2 is the genotypic variance, MS_G and MS_E are the genotypic and residual mean squares, respectively and r is the number of replications

$$\sigma p2 = \sigma g2 + \sigma e2$$

Where: σ_p^2 is the phenotypic variance, σ_g^2 and σ_e^2 are the genotypic and environmental variances, respectively.

3.2.5.3 Genotypic, phenotypic and environmental coefficients of variation

Coefficients of variation were determined based on Johnson *et al.* (1955) as follows:

$$GCV\ (\%) = \frac{\sqrt{\sigma_g^2}}{\bar{X}} * 100$$

Where: GCV is the genotypic coefficient of variation, σ_g^2 is the genotypic variance and $\bar{X}$ is the overall mean.

$$PCV\ (\%) = \frac{\sqrt{\sigma_p^2}}{\bar{X}} * 100$$

Where: PCV is the phenotypic coefficient of variation, σ_p^2 is the phenotypic variance and $\bar{X}$ is the overall mean.

$$ECV\ (\%) = \frac{\sqrt{\sigma_e^2}}{\bar{X}} * 100$$

Where: ECV is the environmental coefficient of variation, σ_e^2 is the environmental variance and $\bar{X}$ is the overall mean.

The genotypic, phenotypic and environmental coefficients of variation were classified according to Sivasubramanian and Menon (1973) as low (0-10%), moderate (11-20%), and high (21% and above).

3.2.5.4 Heritability and genetic advance

Broad-sense heritability was calculated based on Falconer and Mackay (1996) as follows:

$$H^2(\%) = \frac{\sigma_g^2}{\sigma_p^2} * 100$$

Where: H^2 is the broad-sense heritability, $\sigma_g{}^2$ and $\sigma_p{}^2$ are genetic and phenotypic variances, respectively.

The heritability values were classified as indicated by Singh (2001), as low (less than 40%), moderate (41-59%), moderately high (60-79%) and very high (80% and above). Genetic advance was determined according to Acquaah (2009) using the formula:

$$GA = k * H^2 * \sigma_p$$

Where: GA is the genetic advance, k = 1.4 corresponding to 20% of selection pressure, H^2 is the broad-sense heritability and σ_p is the square root of phenotypic variance. Genetic advance was also determined as percentage of the mean, as follows:

$$GAM\ (\%) = \frac{GA}{\bar{X}} * 100$$

Where: GAM is the genetic advance as percentage of the mean and $\bar{X}$ is the overall mean. GAM was categorized as low (0-10%), moderate (11-20%) and high (21% and above), as indicated by Johnson *et al.* (1955).

3.3 Results

3.3.1 Analysis of Variance

The summary of analysis of variance (ANOVA) is shown in Table 3.4. There were highly significant differences ($p < 0.001$) among the accessions for all traits, except for number of primary branches and shelling percentage, where the accessions showed significant differences at $p < 0.01$.

3.3.1.1 Percentage of disease incidence

The environmental conditions were conducive for GRD development and the genotypes reacted differently to the disease. The symptoms appeared early in the susceptible genotypes, which developed progressively from leaf chlorosis to severe stunting and bushy appearance due to shortened internodes. Disease development in resistant and moderately resistant genotypes, was slow with symptoms showing only in some branches or parts of branches. Out of the 28 genotypes evaluated, two were highly resistant, 12 were resistant, 11 were moderately resistant and three were susceptible. The mean values of final disease incidence (PDI) ranged from 4.09% to 69.18% with an average of 31.64% (Table 3.5). The lowest PDI value was recorded in accession ICG 12988, followed by the control ICGV-SM 99568 (7.84%), which were both highly resistant, and accession ICG 11249 (10.20%) which was resistant. The highest PDI value was recorded for accession ICG 12509. The controls CG7 and ICGVSM 90704 were moderately resistant and resistant, with PDI values of 40.17% and 20.81%, respectively. Genotypes with high disease incidence also recorded high severity (Appendix 3.1).

3.3.1.2 Yield and its components

There was significant variation for seed yield and yield components among the evaluated accessions (Table 3.5). Seed yield ranged from 53.60 (ICG 12509) to 1046.40 kg ha^{-1} (ICG 12988) with a mean of 303.11 kg ha^{-1}. The high yielding accession (ICG 12988) out yielded all the controls while accessions ICG 4955 (419.80 kg ha^{-1}) and ICG 334 (403.70 kg ha^{-1}) out yielded only the control CG7 (351.30 kg ha^{-1}). The controls ICGV-SM 99568 and ICGV-SM 90704 were among the five high yielding genotypes with an average of 976.00 kg ha^{-1} and 429.40 kg ha^{-1}, respectively. ICG 12509 (53.60 kg ha^{-1}), ICG 3681 (58.10 kg ha^{-1}) and ICG 3584 (100.10 kg ha^{-1}) were among the lowest yielding accessions. Generally, resistant accessions yielded better than the susceptible ones. Number of pods per plant varied from 3 to 24 with an average of 11. Accessions ICG 3681 and ICG 12509 produced the lowest number of pods while ICG 12988 and control ICGV-SM 99568 recorded the highest number. The control CG7 produced an average of 9 pods per plant while ICGV-SM 90704 produced 15. The mean value for hundred seed weight was 35.58 g with genotypes varying from 23.78 (ICG 3584) to 48.90 g (ICG 5745). Pod length had a mean value of 27.26 mm, with accessions ICG 12697 (20.00 mm) and ICG 6022 (48.25 mm) producing the shortest and longest pods, respectively. A mean value of 12.07 mm was observed for pod width, with genotypes varying from 9.08 mm (ICG 9809) to 15.83 mm (ICG 13942). ICG 9809 and ICG 12697 were among the accessions with the smallest pods while ICG 13942 and ICG 6022 were among the

accessions with the largest pods. Genotypes varied from 57.87% (ICG 12509) to 75.70% (ICG 4955) for shelling percentage and a mean of 67.00% was observed.

3.3.1.3 Agronomic traits

There was wide variation in agronomic traits among the assessed genotypes (Table 3.5). Days to flowering ranged from 30 to 43 with an average of 36. Accessions ICG 12697, ICG 12988 and ICG 9507 which flowered at 30 DAS, ICG 2106 and ICG 4955 which took 31 days to flower, were among the earliest flowering accessions while ICG 13982 (43 days), ICG 11426 (42 days) and ICG 6057 (42 days) were late flowering. The mean days to maturity was 127 with the earliest maturing accessions being ICG 12697 and ICG 10384 which took 116 days to mature, while ICG 6057 and ICG 6813 which matured at 138 DAS, were the latest maturing accessions. The three high yielding accessions matured between 118 and 125 DAS. In terms of plant height, ICG 6813 (46.8 mm) and ICG 3681 (137.6 mm) were the shortest accessions while ICG 12988 (316.7 mm) and ICGV-SM 99568 (344.7 mm), which recorded the highest seed yield, were the tallest genotypes. The number of primary and secondary branches also varied with mean values of 4 and 7 branches per plant, respectively. ICG 12509, ICG 3584 and ICG 14985 produced the lowest number of primary branches (3) while the controls CG7 and ICGV-SM 90704, and accession ICG 6813 produced the highest number (5). The number of secondary branches was as low as 2 (ICG 15045 and ICG 3681) and as high as 15 (ICGVSM 90704).

Table 3.4: Mean squares and significant tests for 13 quantitative traits of 28 groundnut genotypes evaluated under natural GRD infestation

Source of Variation	DF	DTF	DTM	NPB	NSB	PH	NPP	PW	PL	SYD	SYDP	SP	HSW	PDI
Rep	2	0.16ns	75.87***	0.05ns	0.96ns	3960.31*	23.46*	1.85ns	12.65ns	9415.41ns	2.89**	2.44ns	11.89ns	25.33ns
Bloc (Rep.)	9	12.83***	97.63***	1.58***	16.97***	2645.20**	12.95*	7.88***	56.36***	33546.94***	1.58**	75.78*	56.48***	129.50***
Gen	27	49.25***	164.78***	0.70**	44.55***	8924.50***	86.86***	7.70***	89.39***	141575.23***	11.29***	63.92**	158.94***	734.50***
Residual	45	1.87	9.15	0.27	1.81	1078.00	5.00	0.78	4.88	3823.00	0.53	27.70	11.38	25.55

Significant levels: ns, *, **, ***-non-significant differences, significant differences at 5%, 1% and 0.1%, respectively; Rep-replication, Bloc-block, Gen-Genotype, DF- degree of freedom; DTF-days to flowering, DTM-days to maturity, NPB-number of primary branches, NSB-number of secondary branches, PH-plant height, NPP-number of pods per plant, PW-pod width, PL-pod length, SYD-seed yield, SYDP-seed yield per plant, SP-shelling percentage, HSW-hundred seed weight, PDI-final rosette incidence.

Table 3.5: Means of 13 quantitative traits of 28 groundnut genotypes evaluated under natural GRD infestation

Genotype	DTF	DTM	NPB	NSB	PH (mm)	NPP	PW (mm)	PL (mm)	SYD (kg ha^{-1})	SYDP (g)	SP (%)	HSW (g)	PDI (%)	Response
ICG 10384	32	116	4	4	216.2	10	11.42	22.75	196.00	2.92	70.04	30.33	45.88	MR
ICG 11249	33	117	4	3	250.7	19	10.28	23.25	338.60	2.55	60.63	28.13	10.20	R
ICG 11426	42	137	4	8	184.7	7	13.33	28.92	166.40	2.03	67.47	40.03	36.92	MR
ICG 11651	32	121	4	4	199.1	8	11.67	24.17	208.60	2.41	67.17	33.25	55.58	S
ICG 12509	40	137	3	9	143.6	3	13.00	24.08	53.60	0.88	57.87	35.40	69.18	S
ICG 12672	41	135	4	9	193.6	8	13.92	30.92	234.80	2.72	58.62	40.17	29.30	R
ICG 12697	30	116	4	3	207.9	16	9.75	20.00	339.70	2.84	71.90	31.61	18.30	R
ICG 12921	33	122	4	6	250.0	8	11.12	22.83	274.10	2.64	68.39	38.10	16.87	R
ICG 12988	30	119	4	3	316.7	24	9.83	21.75	1046.40	8.08	72.11	30.78	4.09	HR
ICG 13942	40	136	4	13	160.5	9	15.83	33.33	230.60	2.77	70.72	48.27	37.11	MR
ICG 13982	43	130	4	6	177.5	6	11.08	26.25	136.50	2.15	74.28	29.70	68.18	S
ICG 14985	37	120	3	6	179.6	9	13.25	27.58	196.80	2.54	60.59	36.25	38.66	MR
ICG 15405	33	126	4	2	206.4	7	13.53	34.25	176.50	1.97	71.78	31.37	23.46	R
ICG 2106	31	118	4	5	188.9	16	10.25	22.17	339.40	8.50	67.01	28.25	25.37	R
ICG 334	33	125	4	4	273.3	12	10.67	24.83	403.70	3.47	68.66	32.65	23.08	R
ICG 3584	33	122	3	4	195.3	9	10.08	20.42	100.10	1.91	65.98	23.78	46.93	MR
ICG 3681	33	119	4	2	137.6	3	10.85	30.42	58.10	1.20	62.80	27.08	35.15	MR
ICG 405	38	126	4	10	250.3	8	12.20	31.25	164.40	1.93	59.17	32.75	36.99	MR
ICG 4955	31	118	4	5	255.0	16	11.17	21.83	419.80	3.46	75.70	31.22	18.96	R
ICG 5745	37	136	4	10	165.0	10	13.08	31.08	310.00	2.93	71.30	48.90	37.64	MR
ICG 6022	36	130	4	6	238.4	5	15.17	48.25	171.40	1.74	61.70	46.33	26.69	R
ICG 6057	42	138	5	14	197.6	9	14.67	31.92	271.30	2.23	62.78	45.06	20.99	R
ICG 6813	40	138	5	14	46.8	15	10.75	24.33	272.10	2.97	68.09	26.65	35.88	MR
ICG 9507	30	124	4	4	216.1	12	12.00	24.25	369.00	3.18	72.02	37.18	32.06	MR

ICG 9809	33	120	4	3	204.8	12	9.08	22.00	252.40	2.60	68.09	28.26	23.58	R
Genotype	DTF	DTM	NPB	NSB	PH (mm)	NPP	PW (mm)	PL (mm)	SYD (kg ha-^{1})	SYDP (g)	SP (%)	HSW (g)	PDI (%)	Response
Controls														
CG7	38	137	5	13	176.9	9	14.58	31.33	351.30	4.98	71.02	48.26	40.17	MR
ICGV-SM 90704	41	137	5	15	182.7	15	12.67	31.75	429.40	5.42	63.40	38.20	20.81	R
ICGV-SM 99568	37	122	4	4	344.7	24	12.75	27.50	976.00	7.67	66.62	48.33	7.84	HR
Mean	36	127	4	7	205.7	11	12.07	27.26	303.11	3.24	67.00	35.58	31.64	
LSD (5%)	2.25	4.97	0.86	2.21	53.99	3.68	1.45	3.63	101.70	1.20	8.66	5.55	8.31	
SED	1.12	2.47	0.43	1.10	26.81	1.83	0.72	1.80	50.49	0.60	4.30	2.75	4.13	
CV (%)	3.84	2.39	13.09	19.89	15.96	20.25	7.32	8.10	20.40	22.52	7.86	9.48	15.98	
R-Square (%)	94.45	93.01	73.00	94.35	84.90	91.18	88.95	93.06	96.01	93.14	65.94	90.40	94.82	

LSD-least significant difference, SED-standard error of differences, CV-coefficient of variation; DTF-days to flowering, DTM-days to maturity, NPB-number of primary branches, NSB-number of secondary branches, PH-plant height, NPP-number of pods per plant, PW-pod width, PL-pod length, SYD-seed yield, SYDP-seed yield per plant, SP-shelling percentage, HSW-hundred seed weight, PDI-final rosette incidence, HR-highly resistant, R-resistant, MR-moderately resistant, S-susceptible

3.3.2 Variance components and coefficients of variation

The summary of components of variance and coefficients of variation is presented in Table 3.6. All the traits had higher genotypic and phenotypic variances than environmental variance estimates. The phenotypic coefficient of variation (PCV) was higher in magnitude than the genotypic (GCV) and environmental coefficients of variation (ECV). The GCV ranged from 5.19% for shelling percentage to 70.70% for seed yield while PCV varied from 6.17% for days to maturity to 73.58% for seed yield and ECV ranged from 2.39% also for days to maturity to 22.52% for seed yield per plant. Days to maturity and shelling percentage recorded low values of GCV and PCV (5.19% - 9.41%), while number of primary branches had low GCV (9.45%) and moderate PCV (16.14%). Moderate GCV and PCV (11.16% - 14.55%) were observed for days to flowering and pod width. High GCV and PCV (24.86% - 73.58%) were recorded for plant height, percentage of disease incidence, number of secondary branches, number of pods per plant, seed yield and seed yield per plant. Moreover, high ECV values were also recorded for seed yield (20.40%) and number of pods per plant (20.25%).

Table 3.6: Estimates of variance components and coefficients of variation for 13 quantitative traits evaluated under natural GRD infestation

	Variance components estimates			Coefficients of variation		
	σ_g^2	σ_e^2	σ_p^2	GCV (%)	ECV (%)	PCV (%)
	15.79	1.87	17.66	11.16	3.84	11.80
	51.88	9.15	61.02	5.69	2.39	6.17
	0.14	0.27	0.42	9.45	13.09	16.14
	14.25	1.81	16.06	55.87	19.89	59.31
	2615.23	1078.81	3694.04	24.86	15.97	29.55
	27.29	5.00	32.28	47.32	20.25	51.47
	2.31	0.78	3.09	12.58	7.32	14.55
	28.17	4.88	33.05	19.47	8.10	21.09
	45917.41	3823.00	49740.41	70.70	20.40	73.58
		27.70	39.77	5.19	7.86	9.41
PDI	236.32	25.55	261.87	48.59	15.98	51.15
Trait						
DTF						
DTM						
NPB						
NSB						
PH						
NPP						

PW						
PL						
SYD						
SYDP	3.59	0.53	4.12	58.48	22.52	62.66
SP	12.07					
HSW	49.19	11.38	60.57	19.71	9.48	21.87

σ_g^2, σ_e^2 and σ_p^2 are the genotypic, environmental and phenotypic variances, respectively; GCV, ECV and PCV are the genotypic, environmental and phenotypic coefficients of variation; DTF-days to flowering, DTM-days to maturity, NPB and NSB-number of primary and secondary branches, respectively, PHplant height, NPP-number of pods per plant, PW-pod width, PL-pod length, SYD-seed yield, SYDPseed yield per plant, SP-shelling percentage, HSW-hundred seed weight and PDI-percentage of disease incidence.

3.3.3 Heritability and genetic advance

The estimates of broad sense heritability and genetic advance as percentage of the mean are presented in Table 3.7, and these ranged from 30.36 to 92.31% and 4.00 to 95.09%, respectively. Low heritability estimates were observed for shelling percentage (30.36%) and number of primary branches (34.26%) while pod width (74.72%) and plant height (70.80%) had moderately high heritability estimates. Days to maturity and flowering, number of secondary branches, hundred seed weight, number of pods per plant, pod length, percentage of disease incidence, seed yield and seed yield per plant recorded very high broad-sense heritability estimates, which ranged between 81.21 and 92.31%. Genetic advance ranged from 0.31 for number of primary branches to 288.24 for seed yield. Shelling percentage (4.00%), days to maturity (7.35%) and number of primary branches (7.74%) had low estimates of genetic advance as percentage of the mean (GAM), while days to flowering (14.77%) and pod width (15.22%) had moderate GAM, and hundred seed weight (24.87%), pod length (25.16%), plant height (29.28%), number of pods per plant (60.91%), disease incidence (64.62%), number of secondary branches (73.69%), seed yield (95.09%) and seed yield per plant (76.4%) recorded high GAM estimates.

Table 3.7: Estimates of broad-sense heritability, genetic advance and genetic advance as percentage of the mean

Trait	H^2 (%)	GA	GAM (%)
Days to flowering	89.40	5.26	14.77
Days to maturity	85.01	9.30	7.35

Number of primary branches	34.26	0.31	7.74
Number of secondary branches	88.75	4.98	73.69
Plant height	70.80	60.24	29.28
Number of pods per plant	84.53	6.72	60.91
Pod width	74.72	1.84	15.22
Pod length	85.23	6.86	25.16
Seed yield	92.31	288.24	95.09
Seed yield per plant	87.09	2.47	76.40
Shelling percentage	30.36	2.68	4.00
Hundred seed weight	81.21	8.85	24.87
Percentage of disease incidence	90.24	20.44	64.62

H^2-broad-sense heritability, GA-genetic advance and GAM-genetic advance as percentage of mean

3.4 Discussion

3.4.1 Disease development

The environmental conditions during the crop growing period were conducive for GRD development, providing a genetic discrimination for GRD response among the groundnut accessions. The late planting along with a long dry spell which occurred after planting and border rows of a susceptible genotype allowed optimal development of disease. This is in agreement with reports indicating that weather conditions, particularly rainfall, influence GRD development and dry spell favour the aphid population growth, leading to high disease incidences (Naidu *et al.*, 1999; Dwivedi *et al.*, 2003; Waliyar *et al.*, 2007). Moreover, the effect of late planting in GRD development is supported by Naidu and Kimmins (2007), as it allows the occurrence of the aphid's main period of flight activity before the ground cover, leading to high aphid population, since they prefer wide space for landing. There was differential response to GRD among the groundnut accessions, and the susceptible accessions manifested the disease symptoms rapidly from chlorosis in some branches to stunting and bushy appearance. Similar results were reported for susceptible genotypes in previous studies (Subrahmanyam *et al.*, 1991; Subrahmanyam *et al.*, 1997; Bua and Opio, 2014). However, disease development was slow in resistant accessions and plants showed mild symptoms in only some branches or parts of branches. Moreover, the symptomless plants may have been infected by one of the casual agents (either GRAV or GRV), but not by SatRNA which is responsible for GRD symptoms. The presence of GRAV in symptomless

plants grown under an environment conducive to GRD development has been confirmed in similar studies (Waliyar *et al.*, 2007; Anitha *et al.*, 2014). However, in the current study its presence was not confirmed.

Ideally, genotypes should combine good levels of disease resistance, desired agronomic traits and high yielding capacity. An example of such genotypes was accession ICG 12988 which out yielded all the controls and recorded the lowest disease incidence, followed by ICG 4955 and ICG 334, which yielded relatively low but demonstrated good levels of resistance. The control ICGV-SM 99568, which combines GRD resistance, drought tolerance and high yielding ability was also an example of such genotypes. Most of the susceptible accessions produced low seed, indicating that the disease affected the seed yield. The effect of GRD on seed yield could be explained by the reported negative correlations between GRD incidence with seed yield and number of pods per plant (Van der Merwe *et al.*, 2001; Muitia, 2011; Chintu, 2013). Additionally, this is in line with Thresh (2003) and Panguluri and Kumar (2016) who indicated that GRD affects the yield significantly in susceptible genotypes. Such yield reduction is due to reduction of leaf size and internodes, fewer pod number of which most of them do not produce seed, and reduced seed weight. Accession ICG 12988 was reported to be resistant and high yielding under both natural and artificial infestation in previous studies, agreeing with the current study (Van der Merwe and Subrahmanyam, 1997; Kapewa and Chiyembekeza, 2002; Chintu, 2013). The controls ICGV-SM 99568 and ICVG-SM 90704 were also reported to be GRD resistant in previous studies (Waliyar *et al.*, 2007; Monyo *et al.*, 2007; Chattopadhyay *et al.*, 2015). This indicates that this accession and the two controls have stable GRD resistance and can be used to develop resistant varieties.

3.4.2 Agronomic performance

The significant differences among the accessions in seed yield and yield components, indicated the existence of variability in their genetic makeup. Apart from the differences in disease response exhibited by the accessions, the divergence in terms of agronomic traits was also a reason of variation in yield. Seed yield was affected by the disease and in symptomless plants, yield reduction may have occurred due to GRAV infection which does not cause symptoms. This is supported by Van der Merwe *et al.* (1999) and Naidu and Kimmins (2007), who reported yield reduction varying from 28 to 75% due to GRAV in groundnut symptomless plants evaluated under GRD environment. Okello *et al.* (2013) and Engels (2014) indicated that temperatures ranging from 24 to 30^{o}C are required for good growth and yield in groundnut. However, a minimum average of 15.43^{o}C and maximum average of 26.88^{o}C occurred during the experiment, which may have affected the production

of photo-assimilates, leading to low yields. High seed yield (966 kg ha^{-1}) was reported for accession ICG 12988 by Van der Merwe and Subrahmanyam (1997), agreeing with the current results. The controls ICGV-SM 99568 and ICGV-SM 90704 were also reported to be high yielding even under GRD environment (Van der Merwe and Subrahmanyam, 1997; Monyo *et al.*, 2007; Chintu, 2013). However, in the study ICGV-SM 90704 had a low yield with an average of 429.4 kg ha^{-1}. These discrepancies could be a result of differences in environmental conditions among the studies. Moreover, the control CG7 was low yielding, confirming the reports of Monyo *et al.* (2007) and Chintu (2013), which indicated that although CG7 yields well under GRD-free environment, it yields low to moderate under GRD environment due to its susceptible response.

3.4.3 Coefficients of variation, heritability and genetic advance

Generally, the phenotypic coefficient of variation (PCV) was higher in magnitude than the genotypic coefficient of variation (GCV) for all the traits, indicating the influence of environment upon these traits. However, differences between PCV and GCV were small for most of the traits. Similar findings were reported by Zaman *et al.* (2011) and Yusuf *et al.* (2017). High GCV and PCV were recorded for plant height, number of secondary branches, number of pods per plant, seed yield, seed yield per plant and final GRD incidence, indicating high degree of genetic and phenotypic variability from which selection can be implemented. Such high variation for above traits have also been reported earlier (Korat *et al.*, 2009; Zaman *et al.*, 2011; Rao *et al.*, 2014; Yusuf *et al.*, 2017). Shelling percentage and days to maturity showed low GCV and PCV, indicating the narrow range of variability for these traits among the evaluated groundnut accessions and a restricted scope of selection. Similar findings were reported by Maurya *et al.* (2014), and Balaraju and Kenchanagoudar (2016) for shelling percentage, John *et al.* (2012) and Patil *et al.* (2015) for days to maturity. The highest environmental influence on the phenotype was observed for seed yield per plant, followed by seed yield and number of pods per plant, which recorded the greatest environmental coefficients of variation. This phenomenon may be due to the polygenic nature of these traits and are supported by Behera (2007) and Acquaah (2009), who also reported high environmental influence for yield traits.

Heritability is a measure of proportion of phenotypic variance caused by gene effects and its estimates along with genetic advance would be more meaningful and useful in predicting a trait under phenotypic selection than individual consideration of the parameters (Johnson *et al.*, 1955; Holland *et al.*, 2003; Acquaah, 2009). The combination of high broad-sense heritability and genetic advance were observed for plant height, number of secondary

branches, number of pods per plant, pod length, seed yield, hundred seed weight and final GRD incidence. Such combinations indicate the predominant role of additive gene action and the possibility of effective phenotypic selection for improvement of these traits while high heritability alone indicate high correlation between genotype and phenotype, and low environmental contribution to the phenotype (Holland *et al.*, 2003; Acquaah, 2009; You *et al.*, 2016). These combinations have been reported in similar studies by Korat *et al.* (2009) and Patil *et al.* (2015) for number of secondary branches, Meta and Monpara (2010) and Yusuf *et al.* (2017) for plant height, Rao *et al.* (2014) and Rathod and Toprope (2018) for number of pods per plant, Zaman *et al.* (2011) and Narasimhulu *et al.* (2012) for hundred seed weight, Khan *et al.* (2000) and Yusuf *et al.* (2017) for seed yield, and Alhassan (2013) for GRD incidence. Contrary to this study, low heritability estimates were reported for seed yield (John *et al.*, 2012; Rathod and Toprope, 2018). Differences in heritability values among the studies could be a result of differences in either the environment and/or population used.

Days to flowering and pod width recorded high heritability and moderate genetic advance while days to maturity had high heritability and low genetic advance. Similar findings were reported by John *et al.* (2012) and Patil *et al.* (2014) for days to flowering and maturity. The low broadsense heritability and low genetic advance for number of primary branches and shelling percentage indicate low genetic potential and selection may not be effective for these traits. These results are in accordance with Korat *et al.* (2009), Parameshwarappa *et al.* (2010) and Rao *et al.* (2014). Moreover, the low heritability for these traits could be explained by their low genotypic and phenotypic variability existing in the evaluated groundnut accessions.

3.5 Conclusions

The results from this study revealed the presence of a wide genetic variability among the evaluated accessions which can be exploited in groundnut breeding programs. Analysis of variance also revealed highly significant differences among the accessions for all the recorded traits. High variability (GCV and PCV) coupled with high broad-sense heritability and genetic advance were observed for plant height, number of secondary branches, seed yield and final GRD incidence, indicating the possibility of effective phenotypic selection for improvement of these traits. Improvement for number of primary branches and shelling percentage based on the evaluated accessions would be limited since they have low genetic potential due to low variability, low heritability and genetic advance.

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Appendix 3.1: Means of transformed severity of 28 groundnut genotypes evaluated under natural GRD infestation

Genotype	Severity	Genotype	Severity	Genotype	Severity
ICG 10384	1.57	ICG 13982	1.42	ICG 6022	1.10
ICG 11249	1.05	ICG 14985	1.23	ICG 6057	1.14
ICG 11426	1.39	ICG 15405	1.10	ICG 6813	1.28
ICG 11651	1.47	ICG 2106	1.20	ICG 9507	1.24
ICG 12509	1.27	ICG 334	1.26	ICG 9809	1.16
ICG 12672	1.23	ICG 3584	1.37	*Controls*	
ICG 12697	1.04	ICG 3681	1.18	CG7	1.26
ICG 12921	1.26	ICG 405	1.25	ICGV-SM 90704	1.21
ICG 12988	0.96	ICG 4955	1.31	ICGV-SM 99568	1.00
ICG 13942	1.23	ICG 5745	1.24		
Genotype MS	0.05***				
Mean	1.23				
LSD (5%)	0.17				

SED	0.08
CV (%)	8.26
R-Square (%)	78.33

Significant levels: ***- significant differences at 0.1%, MS-mean square, LSD-least significant difference, SED-standard error of differences and CV-coefficient of variation

CHAPTER 4 CORRELATION, PATH COEFFICIENT AND GENETIC DIVERSITY ANALYSIS IN SELECTED GROUNDNUT ACCESSIONS UNDER NATURAL ROSETTE INFESTATION

Abstract

Yield is a complex quantitative trait largely influenced by the environment and generally has low heritability. Hence, direct selection for seed yield is less efficient in improving groundnut productivity. However, the efficiency can be enhanced by exploiting the relationship between seed yield and its related traits. Moreover, the use of genetically diverse parents is essential to generate genetic variation for successful selection of genotypes in a breeding program. Therefore, the study aimed to analyse the relationship between seed yield and its related traits through correlation and path coefficient analysis, and determine the morphological diversity among selected groundnut accessions under natural GRD infestation. The accessions were planted at ICRISAT Malawi and data were recorded on 13 quantitative and 10 qualitative traits. Results showed that seed yield was positively correlated with number of pods per plant, shelling percentage, hundred seed weight, plant height and number of primary branches. A strong negative correlation was observed between seed yield and GRD incidence. Sequential path analysis revealed that high seed yield was directly associated with taller plant types, higher number of pods per plant and hundred seed weight, which were a result of higher pod width, lower GRD incidence and number of secondary branches. Therefore, more weight should be given to these traits when improving seed yield in groundnut, particularly under GRD infestation. Cluster analysis revealed existence of diversity among the evaluated groundnut accessions and geographical origin did not have any influence on the clustering pattern. Three principal components were generated which cumulatively explained 77.44% of the total variation and Principal Components Analysis (PCA) biplot was effective in showing the genetic distance among the accessions with results consistent to those of the cluster analysis. Moreover, Shannon-Weaver diversity indices (0.949-0.9996) for qualitative traits also indicated the existence of high diversity among the accessions.

Keywords: Groundnut, correlation, path analysis, diversity, cluster and principal component analysis

4.1 Introduction

Cultivated groundnut (*Arachis hypogea* L., AABB, 2n = 4x = 40) also known as peanut, is a legume crop that originated in South America through hybridization of its diploid ancestors, *Arachis duranensis* (AA) and *Arachis ipaensis* (BB), followed by spontaneous chromosome

doubling (Talawar, 2004; Bertioli *et al.*, 2015; Zhang *et al.*, 2016). Currently, it is grown in tropical and subtropical countries for its high-quality oil (47-53%) and easily digestible protein (24-36%) (Maiti, 2002; Singh and Nigam, 2016). The crop is the sixth and third most important source of vegetable oil and protein, respectively, and ranks 13th among the food crops in the world (Singh and Nigam, 2016). However, several biotic, abiotic, and socio-economic factors constrain groundnut production in Malawi and other developing countries (Chala *et al.*, 2014; Chikowo *et al.*, 2015). Groundnut rosette disease (GRD), which is a viral disease caused by a complex of three agents (*Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and a *satellite*-RNA (satRNA) associated with GRV) and transmitted by an aphid (*Aphis craccivora* Koch) is among the major constraints. Therefore, the development of high yielding cultivars which are resistant to both biotic and abiotic stresses, and meet farmers` preferences should be continuous and a priority activity.

Plant breeding aims to improve one or more traits at the same time, with seed yield increase being the most important objective in groundnut breeding programs (Yusuf *et al.*, 2017; Mandal *et al.*, 2017). Acquaah (2009) and Kiranmai *et al.* (2016) indicated that seed yield is a complex quantitative trait, resulting from an interplay of various related traits. It is largely influenced by the growing environment and generally has low heritability (Luz *et al.*, 2011; Mukherjee *et al.*, 2016). Hence, direct selection for seed yield is less efficient in improving groundnut productivity. Nevertheless, yield improvement efficiency in the crop can be enhanced by exploiting the relationship between seed yield and its related traits through correlation and path coefficient analysis (Zaman *et al.*, 2011; Kiranmai *et al.*, 2016; Mandal *et al.*, 2017). Kiranmai *et al.* (2016) reported that trait association studies are very important in groundnut than other crops, because the pods are formed underground and unless association between external plant traits and seed yield are established, it may not be possible to effect proper selection prior to harvest. Correlation and path coefficient analysis have been reported in groundnut (Patil *et al.*, 2006; Zaman *et al.*, 2011; Rao *et al.*, 2014). However, it has been indicated that their estimates are influenced by the environment and/or the genotypes used (Kiranmai *et al.*, 2016).

The selection of genetically diverse parents is essential for a successful breeding program, as it provides opportunity for the development of new improved cultivars with desirable traits (Govindaraj *et al.*, 2015; Niveditha *et al.*, 2016). Cluster and principal component analysis (PCA) are useful tools for genetic relationship analysis in plant breeding. This is because they group genetically similar genotypes into the same group and create a scatter plot of genotypes with the geometrical distances among them reflecting their genetic distances with minimum

distortion, respectively (Jolliffe, 2002; Mohammadi and Prasanna, 2003; Ali *et al.*, 2015; Pereira *et al.*, 2015). This study, therefore, aimed to analyse the relationship between seed yield and its related traits through correlation and path analysis, and determine the morphological diversity among selected groundnut accessions under natural GRD infestation, to identify traits contributing the most to seed yield and the genetically diverse accessions, which would assist future groundnut breeding programs.

4.2 Materials and methods

4.2.1 Plant materials, experimental site and data collection

Twenty-eight groundnut genotypes, comprising 25 accessions and three released cultivars, were evaluated under natural GRD infestation (germplasm given in Chapter 3, Table 3.1). Data on 13 quantitative traits, which comprised days to flowering (DTF), days to maturity (DTM), number of primary (NPB) and secondary branches (NSB), plant height (PH), number of pods per plant (NPP), pod width (PW), pod length (PL), shelling percentage (SP), seed yield (SYD), seed yield per plant (SYDP), hundred seed weight (HSW) and percentage of rosette incidence (PDI) were collected as explained in Chapter 3, section 3.2.4.

Qualitative data were recorded on 10 traits (

Table 4.1, Appendix 4.1, 4.2, 4.3 and 4.4), following the groundnut descriptors (IBPGR and ICRISAT, 1992). The recorded qualitative data included growth habit and branching type (recorded at podding stage), stem surface, leaf shape, leaf colour and flower colour (recorded at flowering), pod constriction (recorded at harvest), seed colour, primary seed colour and seed size (recorded after shelling).

Table 4.1: Descriptors used for evaluation of qualitative traits on groundnut accessions

Descriptor	Key	Description and code
Growth habit	GH	1-procumbent 1, 2-procumbent 2, 3-decumbent 1, 4-decumbent 2, 5 decumbent 3, 6-erect and 7-other
Branching type	BT	1-alternate, 2-sequencial, 3-irregular with flowers on main stem, 4irregular without flowers on main stem, 5-other
Stem surface	STS	1-glabrous, 2-sub-glabrous (hair in one or two rows along the main stem), 3-moderately hairy (three or four rows of hairs along the main stem), 4-very hairy (stem surface mostly covered with hairs), 5woolly (as in 4 but with long hairs)
Leaf shape	LS	1-cuneate, 2-obcuneate, 3-elliptic, 4-oblong-ellipic, 5-narrow-ellipic, 6-wide-ellipic, 7-suborbicular, 8-orbicular, 9-ovate, 10-obvate, 11oblong, 12-oblong-lanceolate, 13-lanceolate, 14- linear-lanceolate, 15-other
Leaf colour	LC	1-yellow/ yellow-green, 2-light green, 3-green, 4-dark green, 5-bluish green, 6-other
Flower colour (petal)	FC	1-white, 2-lemon, 3-yellow, 4-orange-yellow/ yellow-orange, 5orange, 6-dark orange, 7-garnet/brink red, 8-other
Pod constriction	PC	1-none, 2-slight, 3-moderate, 4-deep, 5-very deep
Seed colour	SC	1-one colour, 2-variegated
Primary seed colour	PSC	1-white, 2-off-white, 3-yellow, 4-very pale tan, 5-pale tan, 6-light tan, 7-tan, 8-dark tan, 9-greyed orange, 10-rose, 11-salmon, 12-light red, 13-red, 14-dark red, 15-purlish red/ reddish purple, 16-lihgth purple, 17-purple, 18-dark purple, 19-very dark purple, 20-other
Seed size	SDS	1-very small, 2-small, 3-medium, 4-large, 5-very large

Source: IBPGR and ICRISAT (1992)

4.2.2 Data analysis

4.2.2.1 Correlation analysis

To determine the degree of relationship among the 13 quantitative traits, phenotypic correlation analysis was performed following Pearson's method and using PROC CORR in

SAS version 9.4 (SAS Institute, 2015). Correlation coefficients were categorized according to Belsley *et al.* (2005) as weak (0.0-0.4), moderate (0.4-0.6) and strong (0.6-1.0).

4.2.2.2 Path coefficient analysis

Path coefficient analysis was carried out using two procedures, which were conventional and sequential path analysis. For conventional path analysis, all the traits were used as first-order predictors with seed yield as response variable where the correlation coefficients were partitioned into direct and indirect effects in Microsoft Excel 2016, as indicated by Dewey and Lu (1959). For sequential path analysis, sequential stepwise multiple regressions were used, in SAS version 9.4 (SAS Institute, 2015), to organize the traits into first and second-order predictors, based on their contribution to the variation in seed yield and minimum collinearity (Mohammadi *et al.*, 2003). Tolerance (TOL) and variance inflation factor (VIF) were used to measure the level of multicollinearity for each predictor trait. Tolerance ($TOL = 1 - R_j^2$, where R_j^2 is the coefficient of determination for the prediction of j^{th} variable by the predictor variables) is the amount of variance of the selected independent variable not explained by other independent variable while variance inflation factor is the inverse of tolerance ($VIF = 1/TOL$) and designates the extent of effects of other independent variables on the variability of the selected independent variable (Hair *et al.*, 1995; Paul, 2006). Generally, variance inflation factor greater than five is an evidence of excessive multicollinearity (Belsley *et al.*, 2005; Akinwande *et al.*, 2015). Therefore, plant height, number of pods per plant, seed yield per plant and hundred seed weight were considered as first-order predictors due to their high contribution to total seed yield variation and low multicollinearity. This procedure was repeated, taking each first-order predictor as dependent variable to find their first-order predictors, which were second-order predictors for seed yield. The direct and indirect effects in the different path orders were estimated as described by Dewey and Lu (1959) and classified based on Lenka and Misra (1973) as negligible (0.00-0.09), low (0.1-0.19), moderate (0.2-0.29) and high (0.3-0.99).

4.2.2.3 Cluster analysis

The measured variables were standardized to unit variance as indicated by Gan *et al.* (2007), by dividing each observation by the standard deviation of the trait. The standardized values were used for cluster analysis using PROC CLUSTER in SAS version 9.4 (SAS Institute, 2015) with average linkage method based on Euclidean distance. The dendrogram was constructed using PROC TREE in the same software.

4.2.2.4 Principal component analysis

The standardized values were also used to perform principal component analysis (PCA) based on the correlation matrix in SPSS version 25 (Bryman and Cramer, 2012) and the PCA biplot was plotted using Genstat 18th Edition (Payne *et al*., 2014). Only the principal components with eigenvalues greater than one were considered in determining variability among the accessions, as indicated by Iezzoni and Pritts (1991).

4.2.2.5 Shannon-Weaver diversity index

The diversity index of Shannon-Weaver (H') was calculated in Microsoft Excel 2016 as described by Hutcheson (1970). The index was used as a measure of phenotypic diversity of each qualitative trait and was determined as follows:

$$H' = \sum_{i=1}^{n} pi \log_e pi$$

Where: n is the number of phenotypic classes for a trait and pi is the proportion of accessions in the ith class of an n-class trait. Each value of diversity index was divided by its maximum value ($\log_e n$) to keep the values between zero and one.

4.3 Results

4.3.1 Correlation and path coefficient analysis

Table 4.2 shows the magnitude of relationship among the quantitative traits. The results showed that there was high degree of association between some of the traits. Seed yield was strongly positive and significantly correlated ($p < 0.001$) with plant height (r = 0.66) and number of pods per plant (r = 0.87). However, it was weakly negative correlated ($p > 0.05$) with days to flowering (r = -0.26), days to maturity (r = -0.21), number of secondary branches (r = -0.12), pod width (r = -0.17) and pod length (r = -0.20). Further, it showed weak positive and nonsignificant correlations with number of primary branches (r = 0.15) and hundred seed weight (r = 0.19), but strong negative correlation with GRD incidence ($p < 0.01$, r = -0.66). The number of pods per plant had moderate positive correlation with plant height ($p < 0.01$, r = 0.51), but weak positive correlation with shelling percentage ($p > 0.05$, r = 0.27). Positive correlation coefficients were also recorded between hundred seed weight with days to flowering ($p < 0.05$, r = 0.47), days to maturity ($p < 0.01$, r = 0.57), number of primary branches ($p > 0.05$, r = 0.32), number of secondary branches ($p < 0.01$, r = 0.52), pod width ($p < 0.001$, r = 0.82) and pod length ($p < 0.001$, r = 0.61), while negative correlation coefficient was observed with percentage of disease incidence ($p > 0.05$, r = -0.09). Days to maturity

demonstrated strong positive correlation with days to flowering ($p < 0.001$, r = 0.86) and number of secondary branches ($p < 0.001$, r = 0.84), but moderate positive correlation with number of primary branches ($p < 0.001$, r = 0.49) and moderate negative correlation with plant height ($p < 0.01$, r = -0.49).

4.3.1.1 Conventional path analysis

The estimates of direct and indirect effects of yield related traits on seed yield by conventional path analysis are shown in Table 4.3. High levels of multicollinearity were observed for some predictor traits. The indirect effects were mostly lower in magnitude than the direct effects. Number of pods per plant recorded the highest positive direct effect on seed yield of 0.586, followed by days to maturity (0.332), plant height (0.281), seed yield per plant (0.259) and hundred seed weight (0.155). Percentage of disease incidence (0.019), shelling percentage (0.018) and number of primary branches (0.079) showed the lowest and negligible positive direct effects on seed yield. The most negative direct effect of the examined traits on seed yield was found for number of secondary branches (-0.271) and was moderate while pod length (-0.047), pod width (-0.020) and days to flowering (-0.012) showed negligible negative direct effects on seed yield. The highest positive indirect effect on seed yield was found for seed yield per plant via number of pods per plant (0.451) while the most negative indirect effect was recorded for GRD incidence through number of pods per plant (-0.410).

4.3.1.2 Sequential path analysis

The sequential path analysis (Table 4.4 and Figure 4.1) had low multicollinearity for all the predictor traits. These and the ordering of the predictor traits into first and second-order predictors, provided a better understanding of their interrelationships and relative contribution to seed yield. Plant height, number of pods per plant, seed yield per plant and hundred seed weight were considered first-order predictors, which accounted for about 88% of the variation in seed yield. These traits showed low to high positive direct effects on seed yield, with the highest effect being observed for number of pods per plant (0.552), followed by seed yield per plant (0.276), plant height (0.236) and hundred seed weight (0.177). The indirect effects of seed yield per plant (0.425) and plant height (0.282) on seed yield through number of pods per plant were the highest positive. These indirect effects were higher in magnitude than the corresponding direct effects while the remaining were lower.

The path analysis of the second-order predictors over the first-order predictors, revealed that nearly 44% of the variation for plant height was due to number of secondary branches and GRD incidence, which had high negative direct effects on plant height of -0.388 and -0.510,

respectively and negligible indirect effects. In the same order path, GRD incidence (-0.698) and pod length (-0.405) had high negative direct effects on number of pods per plant and together accounted for about 63% of the variation in number of pods per plant. Pod width and GRD incidence explained nearly 70% of the variation in hundred seed weight, where pod width had high positive direct effect (0.859) while GRD incidence showed moderate negative direct effect (-0.231). These two second-order predictors had lower indirect effects on hundred seed weight.

Table 4.2: Phenotypic correlation among 13 quantitative traits of groundnut accessions evaluated under natural GRD infestation

	DTM	NPB	NSB	PH	NPP	PW	PL	SYD	SYDP	SP	HSW	PDI
DTF	0.86***	0.35	0.75***	-0.42*	-0.37	0.62***	0.45*	-0.26	-0.24	-0.34	0.47*	0.39*
DTM		0.49***	0.84***	-0.49**	-0.35	0.68***	0.52**	-0.21	-0.19	-0.19	0.57**	0.31
NPB			0.67***	-0.28	0.11	0.28	0.29	0.15	0.30	0.19	0.32	-0.10
NSB				-0.48*	-0.15	0.58**	0.37	-0.12	-0.01	-0.19	0.52**	0.17
PH					0.51**	-0.15	-0.08	0.66***	0.43*	0.11	0.12	-0.58**
NPP						-0.41*	-0.41*	0.87***	0.77***	0.27	-0.08	-0.70***
PW							0.79***	-0.17	-0.18	-0.24	0.82***	0.16
PL								-0.20	-0.22	-0.30	0.61***	0.00
SYD									0.82***	0.29	0.19	-0.66**
SYDP										0.26	0.10	-0.51**
SP											-0.05	-0.08
HSW												-0.09

Significant levels: *, **, *** indicate significant correlations at 5, 1 and 0.1% probability, respectively; DTF-days to flowering, DTM-days to maturity; NPB and NSB-number of primary and secondary branches, respectively, PH-plant height, NPP-number of pods per plant, PW-pod width, PL-pod length, SYD-seed yield, SYDP-seed yield per plant, SP-shelling percentage, HSW-hundred seed weight and PDI-percentage of disease incidence.

Table

4.3: Direct and indirect effects with all traits as first-order predictors on seed yield and measures of multicollinearity

Trait	Direct effect	Indirect effect by													
		DTF	DTM	NPB	NSB	PH	NPP	PW	PL	SYDP	SP	HSW	PDI	TC (SYD)	TOL	VIF
DTF	-0.012	-	0.285	0.028	-0.203	-0.119	-0.216	-0.012	-0.021	-0.063	-0.006	0.072	0.007	-0.260	0.194	5.161
DTM	0.332	-0.010	-	0.038	-0.228	-0.137	-0.208	-0.014	-0.024	-0.050	-0.003	0.089	0.006	-0.209	0.127	7.859
NPB	0.079	-0.004	0.161	-	-0.181	-0.080	0.063	-0.006	-0.014	0.077	0.003	0.050	-0.002	0.148	0.278	3.594
NSB	-0.271	-0.009	0.279	0.052	-	-0.134	-0.087	-0.012	-0.017	-0.002	-0.004	0.081	0.003	-0.119	0.128	7.839
PH	0.281	0.005	-0.162	-0.022	0.129	-	0.299	0.003	0.004	0.112	0.002	0.019	-0.011	0.659	0.321	3.112
NPP	0.586	0.004	-0.118	0.009	0.040	0.144	-	0.008	0.019	0.199	0.005	-0.012	-0.013	0.871	0.173	5.767
PW	-0.020	-0.007	0.224	0.022	-0.157	-0.041	-0.237	-	-0.037	-0.048	-0.004	0.128	0.003	-0.175	0.122	8.183
PL	-0.047	-0.005	0.171	0.023	-0.099	-0.023	-0.239	-0.016	-	-0.056	-0.006	0.095	0.000	-0.202	0.220	4.551
SYDP	0.259	0.003	-0.064	0.023	0.002	0.121	0.451	0.004	0.010	-	0.005	0.016	-0.009	0.821	0.310	3.227
SP	0.018	0.004	-0.062	0.015	0.052	0.030	0.158	0.005	0.014	0.068	-	-0.008	-0.002	0.293	0.539	1.856
HSW	0.155	-0.005	0.190	0.025	-0.141	0.035	-0.046	-0.016	-0.029	0.027	-0.001	-	-0.002	0.193	0.190	5.250
PDI	0.019	-0.005	0.103	-0.008	-0.047	-0.162	-0.410	-0.003	0.000	-0.131	-0.002	-0.014	-	-0.662	0.287	3.479

TOL-tolerance, VIF-variance inflation factor, TC (SYD)- total correlation to seed yield; DTF-days to flowering, DTM-days to maturity, NPB and NSB-number of primary and secondary branches, respectively, PH-plant height, NPP-number of pods per plant, PW-pod width, PL-pod length, SYDP-seed yield per plant, SPshelling percentage, HSW-hundred seed weight and PDI-percentage of disease incidence.

Table

Table 4.4: Direct and indirect effects for yield related traits grouped into first and secondorder predictors

Response Trait	Predictor Trait	Direct effect	Indirect effect by				Adjusted R^2	TOL	VIF
			PH	NPP	SYDP	HSW			
SYD	PH	0.236	-	0.282	0.119	0.022		0.713	1.403
	NPP	0.552	0.120	-	0.213	-0.014		0.338	2.958
	SYDP	0.276	0.102	0.425	-	0.019		0.381	2.625
	HSW	0.177	0.029	-0.043	0.029	-	87.970	0.898	1.113
			NSB	PDI					
PH	NSB	-0.388	-	-0.088	-	-		0.970	1.031
	PDI	-0.510	-0.067	-	-	-	43.730	0.970	1.031
			PDI	PL					
NPP	PDI	-0.698	-	-0.002	-	-		1.000	1.000
SYDP	PDI			-	-	-	22.800	1.000	
			PW	PDI					
HSW	PW	0.859	-	-0.038	-	-	70.450	0.974	1.027
	PDI	-0.231	0.140	-	-	-		0.974	1.027
		-0.405	-0.003						1.000
	PL	-0.507	-						1.000 -
	-					-	62.710		1.000

TOL-tolerance, VIF-variance inflation factor, SYD-seed yield, PH-plant height, NPP-number of pods per plant, SYDP-seed yield per plant, HSW-hundred seed weight, NSB-number of secondary branches, PDI-percentage of disease incidence, PL-pod length and PW-pod width.

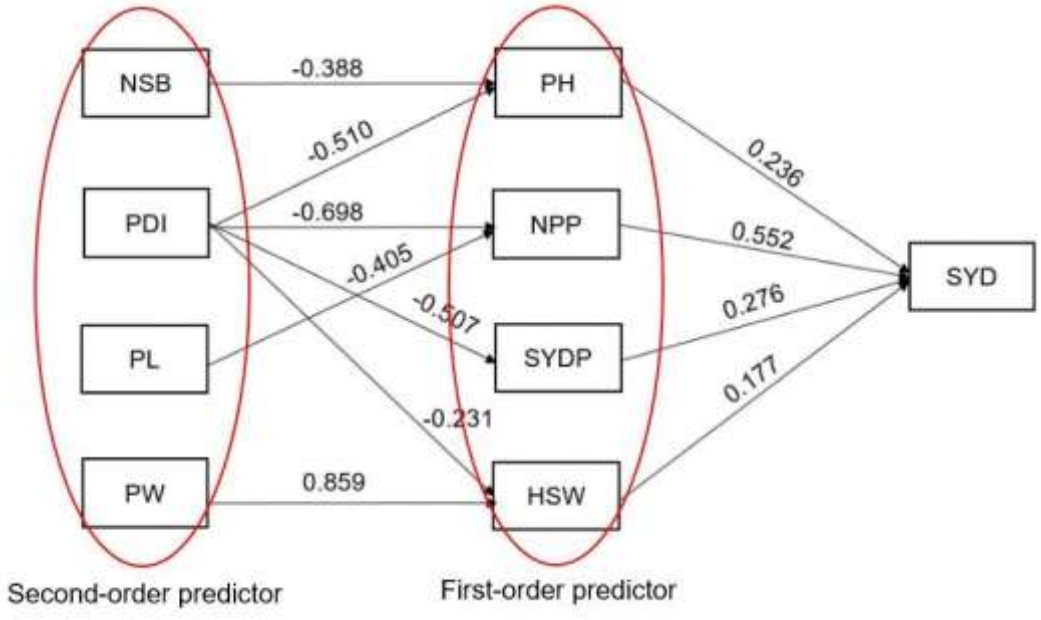

Figure 4.1: Sequential path diagram showing the interrelationships among the first and second-order predictors contributing to seed yield

SYD-seed yield, PH-plant height, NPP-number of pods per plant, SYDP-seed yield per plant, HSWhundred seed weight, NSB-number of secondary branches, PDI-percentage of disease incidence, PLpod length and PW-pod width.

4.3.2 Cluster analysis

Cluster analysis showed a clear variation among the evaluated groundnut accessions (Figure 4.2). At truncation level of 0.85 in the coefficient scale, the genotypes were grouped into four clusters and the cluster means for the recorded quantitative traits are shown in Table 4.5. Apart from other differences among the clusters, botanical group was predominant. Cluster II was the largest with 13 accessions (46.43% of the total germplasm) which were mostly Spanish and Valencia with low hundred seed weight and yields. Cluster I and III had seven (25.00%) and three (10.71%) accessions, respectively. Most of these accessions were Virginia and cluster I recorded a higher hundred seed weight. Cluster IV was the smallest with two genotypes (7.14% of the total germplasm) which were Spanish, high yielding and GRD resistant. Accessions ICG 11249 and ICG 9809 were the most similar. Accession ICG 6813 was a singleton near the first cluster while ICG 14985 and ICG 12509 were singletons near the fourth cluster.

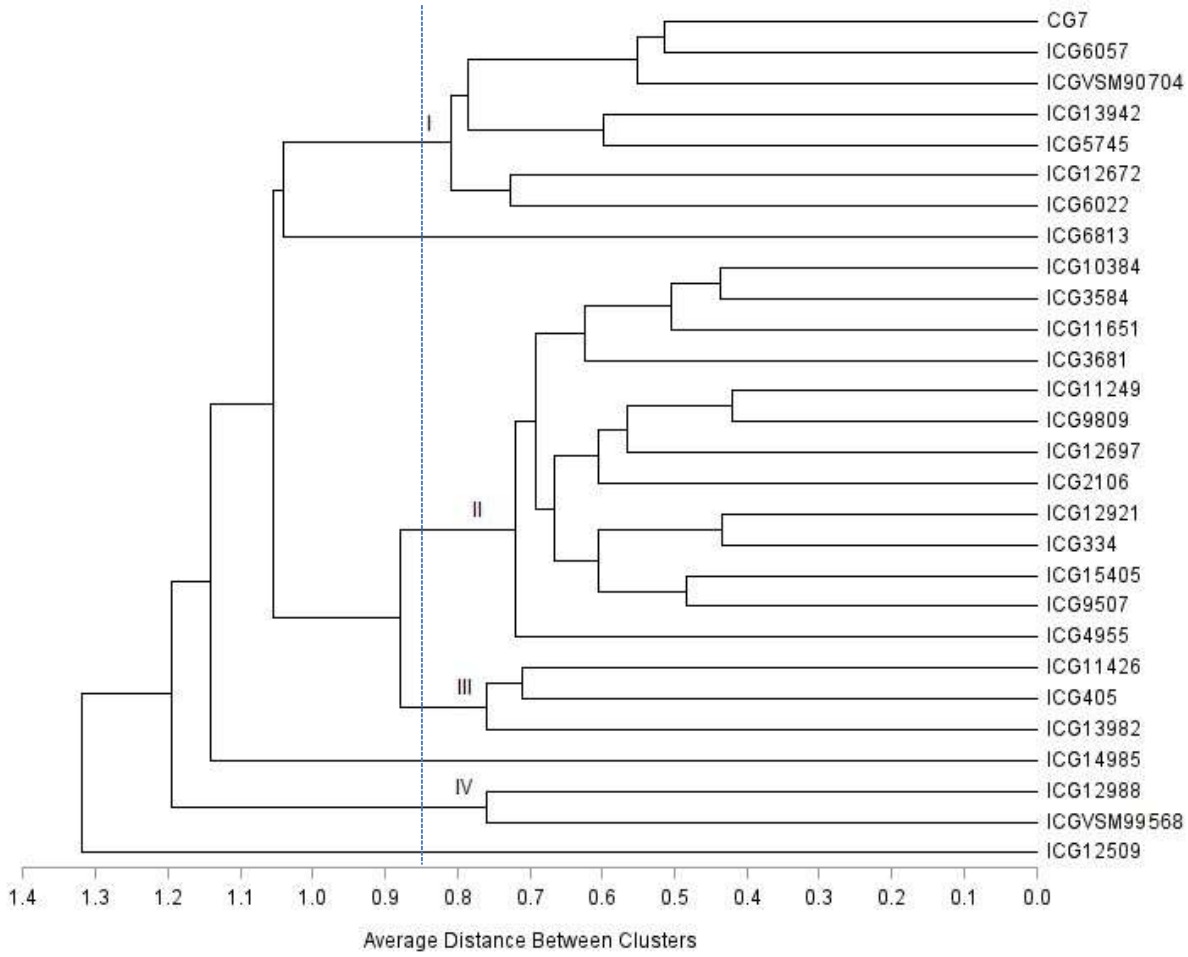

Figure 4.2: Dendrogram of 28 groundnut genotypes generated based on average linkage cluster analysis using phenotypic traits

Table 4.5: Cluster means for 13 quantitative traits measured in 28 groundnut accessions under natural GRD infestation

Trait	Cluster I	Cluster II	Cluster III	Cluster IV
Days to flowering	39	32	41	33
Days to maturity	136	120	131	121
Number of primary branches	4	4	4	4
Number of secondary branches	12	4	8	4
Plant height (mm)	187.81	215.48	204.17	330.70
Number of pods per plant	9	11	7	24
Pod width (mm)	14.27	10.91	12.20	11.29
Pod length (mm)	34.08	24.09	28.81	24.63
Seed yield (kg ha^{-1})	285.54	267.38	155.77	1011.20
Seed yield per plant (g)	3.26	3.05	2.04	7.88

Shelling percentage (%)	65.65	68.47	66.97	69.37
Hundred seed weight (g)	45.03	30.86	34.16	39.56
Percentage of disease incidence (%)	30.39	28.88	47.36	5.97

4.3.3 Principal component analysis

Three principal components with eigenvalues greater than one were generated (Table 4.6). These accounted for most of the variation observed and cumulatively explained 77.44% of the total variation among the 13 quantitative traits. The first component (PC1) alone had an eigenvalue of 5.27 and explained 40.51% of the total variation, mainly due to seed yield, seed yield per plant, number of pods per plant, plant height which had positive contribution and GRD incidence with negative contribuition to the component. This component can be called productivity and GRD response dimension, and separates the genotypes according to their yielding ability and GRD response. The second principal component (PC2) accounted for 24.84% of the total variation, with most of the variation being attributed to days to flowering and maturity, number of primary and secondary branches. This component can be called physiological dimension, which separates the genotypes based on their botanical groups (Spanish, Valencia and Virginia). The traits that contributed most to the third principal component (PC3), which accounted for 12.09% of the total variation were pod width and pod length.

Table 4.6: Principal component analysis showing eigenvalues, eigenvectors and percentage of variation explained by the first three principal components

	Eigenvectors		
Trait	PC1	PC2	PC3
Days to flowering	-0.31	0.66	0.46
Days to maturity	-0.24	0.78	0.47
Number of primary branches	0.25	0.82	0.02
Number of secondary branches	-0.08	0.88	0.32
Plant height	0.68	-0.57	0.19
Number of pods per plant	0.88	-0.03	-0.32
Pod width	-0.12	0.37	0.87
Pod length	-0.11	0.19	0.84
Seed yield	0.95	-0.01	-0.06

Seed yield per plant	0.85	0.16	-0.19
Shelling percentage	0.31	0.13	-0.46
Hundred seed weight	0.25	0.36	0.79
Percentage of disease incidence	-0.79	0.21	-0.08
Eigenvalue	5.27	3.32	1.57
Proportion of total variance (%)	40.51	24.84	12.09
Cumulative variance (%)	40.51	65.35	77.44

4.3.3.1 Principal component analysis biplot

The PCA biplot (Figure 4.3) shows the relationship among the different variables and accessions with respect to the first two principal components. The geometrical distances among accessions in the biplot reflect the genetic distances among them. Smaller angles between dimension vectors in the same direction indicated high correlation of the traits in terms of discriminating genotypes, and an example of such traits are days to maturity and flowering. Genotypes excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull. The cultivar ICGV-SM 99568 and accession ICG 12988 excelled in seed yield, which was contributed mostly by number of pods per plant, shelling percentage and plant height. Accessions ICG 13942 and ICG 6057, which matured late were plotted in the direction of late maturing as expected. The other two cultivars ICGV-SM 90704 and CG7 were clustered together in the direction of high hundred seed weight and high number of secondary branches, while the accession ICG 12509 was plotted in the same direction of high disease incidence and recorded the highest incidence value. The first principal component (PC1) which represents the productivity and GRD related traits separated the accessions in such way that most of the higher yielding and less diseased (lower incidence values) were plotted at the positive side of the component. On the other hand, the second component (PC2) which represents the physiological traits, scattered most of the Virginia accessions (which mature late and have high number of branches) at the positive side.

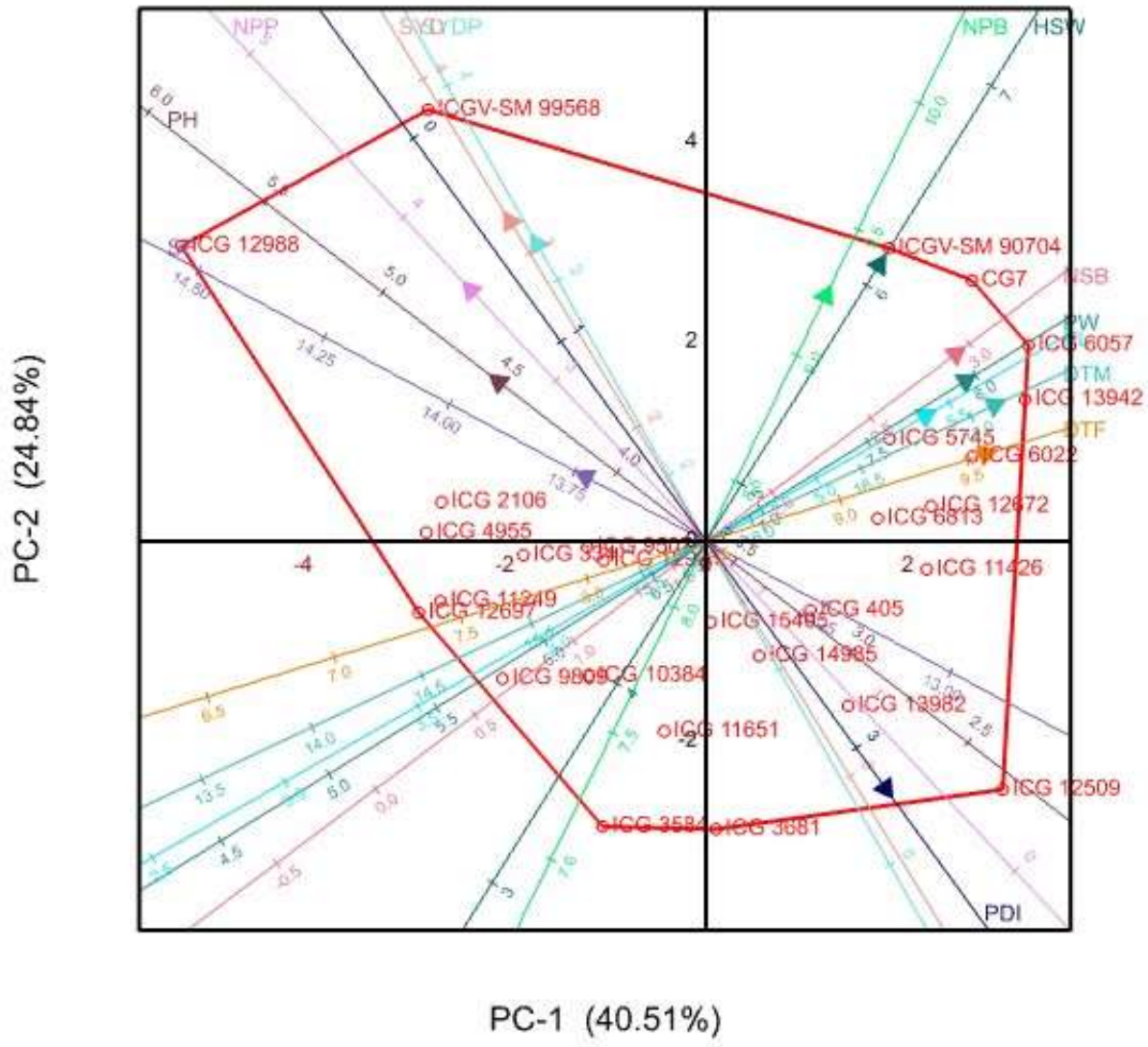

Figure 4.3: Principal component biplot showing the overall genetic variation among the groundnut accessions

DTF-days to flowering, DTM-days to maturity, NPB and NSB-number of primary and secondary branches, respectively, PH-plant height, NPP-number of pods per plant, PW-pod width, PL-pod length, SYD-seed yield, SYDP-seed yield per plant, SP-shelling percentage, HSW-hundred seed weight and PDI-percentage of disease incidence

4.3.4 Shannon-Weaver diversity index

The diversity indices (H`) were determined to compare phenotypic diversity among the 10 qualitative traits in the groundnut accessions (Table 4.7). Generally, high diversity indices were observed, which ranged between 0.949 for leaf colour and 0.9996 for flower colour.

Table 4.7: Shannon-Weaver diversity indices for the 10 qualitative traits

Trait	GH	BT	STS	LS	FC	LC	SC	PSC	PC	SDS
H`	0.985	0.984	0.972	0.973	0.9996	0.949	0.996	0.979	0.993	0.966

H`- Shannon-Weaver index, GH-growth habit, BT-branching type, STS-stem surface, LS-leaf shape, FC-flower colour, LC-leaf colour, SC-seed colour, PSC-primary seed colour, PC-pod constriction, SDSseed size.

4.4 Discussion

4.4.1 Correlation analysis

Seed yield had positive correlations with number of pods per plant, plant height, shelling percentage, hundred seed weight and number of primary branches. Similar associations have been reported in previous studies (Zaman *et al.*, 2011; Rao *et al.*, 2014; Mandal *et al.*, 2017; Yusuf *et al.*, 2017). These positive associations suggest that selecting for these traits would simultaneously bring improvement to seed yield. The very strong positive correlation between seed yield and number of pods per plant may suggest that these traits share some common genes (Almeida *et al.*, 2014; Kozak and Azevedo, 2014). Moreover, Gomez Selvaraj *et al.* (2009) reported one SSR marker that was linked to both traits, and another marker which was linked to pod length and hundred seed weight, agreeing with the observed strong positive correlation between the last two traits. The positive correlation between seed yield and plant height may indicate that tall genotypes have more capacity to accumulate photo-assimilates, resulting in higher seed yields.

Seed yield showed strong negative correlation with GRD incidence, confirming the previous reports of Van der Merwe *et al.* (2001), Muitia (2011) and Mohammed *et al.* (2018). This further confirms the negative effect that the GRD has on seed yield. Seed yield also showed weak negative correlations with days to flowering and maturity, agreeing with the previous reports of Khan *et al.* (2000), Rao *et al.* (2014), and Rathod and Toprope (2018). However, weak positive correlations between seed yield with days to flowering and maturity were reported earlier by Mandal *et al.* (2017) and Reddy *et al.* (2017), which suggested that late flowering and maturing genotypes have enough time to accumulate photo-assimilates, resulting in higher yields. The number of secondary branches per plant had a weak negative correlation with seed yield, contradicting the previous strong positive correlations reported by Patil *et al.* (2006) and Balaraju and Kenchanagoudar (2016). The divergence in correlation coefficients could be a result of differences in either genotypes and/or environment used in these studies.

4.4.2 Path analysis

The correlation analysis may not provide a clear picture of the importance of each secondary trait in determining seed yield (Dewey and Lu, 1959; Kozak and Azevedo, 2014). Wright (1921) developed path coefficient analysis, which partitions the correlation coefficients into direct and indirect effects, allowing the estimates of contribution of each trait to seed yield. Several researchers have used the conventional path analysis (all the traits used as first-order predictors) in groundnut, and the traits often highlighted in this regard were number of pods per plant (Patil *et al.*, 2006; Rao *et al.*, 2014), plant height (Mandal *et al.*, 2017; Reddy *et al.*, 2017), hundred seed weight (Zaman *et al.*, 2011; Rao *et al.*, 2014), days to maturity (Rao *et al.*, 2014; Rathod and Toprope, 2018) and number of secondary branches (Patil *et al.*, 2006). The conventional path analysis in the current study, recorded the highest positive direct effect on seed yield for number of pods per plant, followed by days to maturity, plant height and hundred seed weight, agreeing with most of the earlier reports.

Although conventional path analysis easily identifies the direct and indirect effects of secondary traits on seed yield, it usually leads to high levels of multicollinearity, which confound the detection and interpretation of the actual contribution of each of these traits on seed yield (Blalock Jr, 1963; Mohammadi *et al.*, 2003). Similarly, high levels of multicollinearity were observed for some predictor traits in the conventional path analysis in the current study. The use of sequential path analysis, resulted in low multicollinearity for all the predictor traits and allowed ordering of these traits into first and second-order predictors through sequential stepwise multiple regression. These provided a better understanding of the interrelationships among the traits and their relative contribution to seed yield (Kozak and Azevedo, 2014; Olivoto *et al.*, 2017). The magnitude of contribution of the secondary traits on seed yield was influenced in different ways, which should be considered for more efficient selection (Figure 4.1). The sequential path analysis clearly indicated that high seed yield was directly associated with taller plant types, higher number of pods per plant and hundred seed weight, which were a result of higher pod width, lower GRD incidence and number of secondary branches. Hence, more weight should be given to these traits when selecting for seed yield in groundnut, particularly under GRD infestation.

Kiranmai *et al.* (2016) indicated that path analysis is influenced by the environment and/or the genotypes used, supporting some of the divergence between the current and the earlier reports. Contrary to the observations from this study, the number of secondary branches and pod length, were reported to have positive contribution on seed yield (Patil *et al.*, 2006). However, Zaman *et al.* (2011), and Vange and Maga (2014) reported negative direct effect of number of secondary branches on seed yield, agreeing with results from the current study and

supporting the influence of genotype and/or environment in path analysis. The divergence between the current and previous studies, could be explained by the genotypes used and their GRD response, since the Virginia (which generally produce high number of secondary branches) and Valencia (which have long pods) accessions, were low yielding, mainly due to their susceptible response to GRD. Hence, more studies should be conducted, particularly under both GRD and GRD free-environments, to ascertain the contribution of these traits on seed yield across the three botanical groups in groundnut. Moreover, the number of pods per plant and hundred seed weight have been reported consistently to have positive direct contribution on seed yield (Zaman *et al.*, 2011; Rao *et al.*, 2014; Mandal *et al.*, 2017; Reddy *et al.*, 2017).

4.4.3 Cluster and principal component analysis

Clustering genotypes based on their agro-morphological characters is useful as it assists in identification and selection of best performers and genetically diverse parents for hybridisation (Govindaraj *et al.*, 2015; Niveditha *et al.*, 2016). The study indicated the presence of diversity among the evaluated groundnut accessions. Groundnut accessions grouped in different clusters could be evaluated for combining ability. These findings are consistent with the high genotypic and phenotypic coefficients of variation for most of the characters reported in Chapter 3 of this study and are supported by Siddiquey *et al.* (2006) and Banerjee *et al.* (2007), who indicated that there is abundant genetic divergence in groundnut germplasm. The distribution of the accessions indicated that geographical origin did not have any influence on clustering pattern. Moreover, this indicates that geographical diversity is not a measure of genotypic diversity. Similar results were reported by Ariyo (1987) and Makinde and Ariyo (2010) in groundnut, and Subramanian and Subbaraman (2010) in maize. The high ShannonWeaver diversity indices, which indicated the existence of high diversity for the qualitative traits among the accessions, are consistent with results of the cluster analysis. Moreover, these findings are also consistent with previous studies that reported high diversity indices for qualitative traits in groundnut (Upadhyaya *et al.*, 2002; Upadhyaya, 2003; Gokidi, 2005).

Principal component analysis under natural GRD infestation revealed three components with eigenvalues greater than one. Iezzoni and Pritts (1991) indicated that components with eigenvalues greater than one are meaningful and theoretically have more information than any single variable alone. The traits correlated with the three meaningful principal components are important as they contributed the most towards divergence of the groundnut accessions. The first and the second component explained most of the variation among the accessions. Similar results were reported in groundnut (Makinde and Ariyo, 2010; Aliyu and Zanzam, 2011;

Niveditha *et al.*, 2016) and in soybean (Aondover *et al.*, 2013; El-Hashash, 2016). The first component had eigenvalue of 5.27, and grouped yield and GRD related traits. This component can be called productivity and GRD response dimension, and separates the genotypes according to their seed yield and response to GRD. The second component was correlated with days to flowering, days to maturity, number of primary and secondary branches, and separated the accessions in such way that most of the Virginia types (which mature late and have high number of branches) were plotted together at the positive side of the component. The third component had an association with pod width and pod length, suggesting that it represents the pod size. These findings are in agreement with Aliyu and Zanzam (2011), and Niveditha *et al.* (2016) who found the first component correlated with yield related traits in groundnut. Moreover, PCA biplot was effective in showing the genetic distance among the accessions with results consistent to those of the cluster analysis. For instance, ICGV-SM 99568 and ICG 12988 were clustered together in both analysis. Similar trend was reported earlier by Niveditha *et al.* (2016) in groundnut.

4.5 Conclusions

Results from the current study revealed that seed yield was positively correlated with number of pods per plant, shelling percentage, hundred seed weight, plant height and number of primary branches. Strong negative correlation was observed between seed yield and GRD incidence. Sequential path analysis clearly indicated that high seed yield was directly associated with taller plant types, higher number of pods per plant and hundred seed weight, which were a result of higher pod width, lower GRD incidence and number of secondary branches. Therefore, more weight should be given to these traits when improving seed yield in groundnut, particularly under GRD infestation. Cluster analysis revealed existence of diversity among the evaluated groundnut accessions and geographical origin did not have any influence on clustering pattern. The first PC from the principal component analysis explained 40.51% of the total variation, mainly due to yield and GRD related traits. The second component accounted for 24.84% of the total variation, with most of the variation being attributed to days to flowering, days to maturity, number of primary and secondary branches while traits which mostly contributed to the third component that accounted for 12.09% of the total variation were pod width and pod length. PCA biplot was effective in showing the genetic distance among the accessions with results consistent to those of the cluster analysis. Moreover, diversity indices of Shannon-Weaver also revealed existence of high diversity among the accessions.

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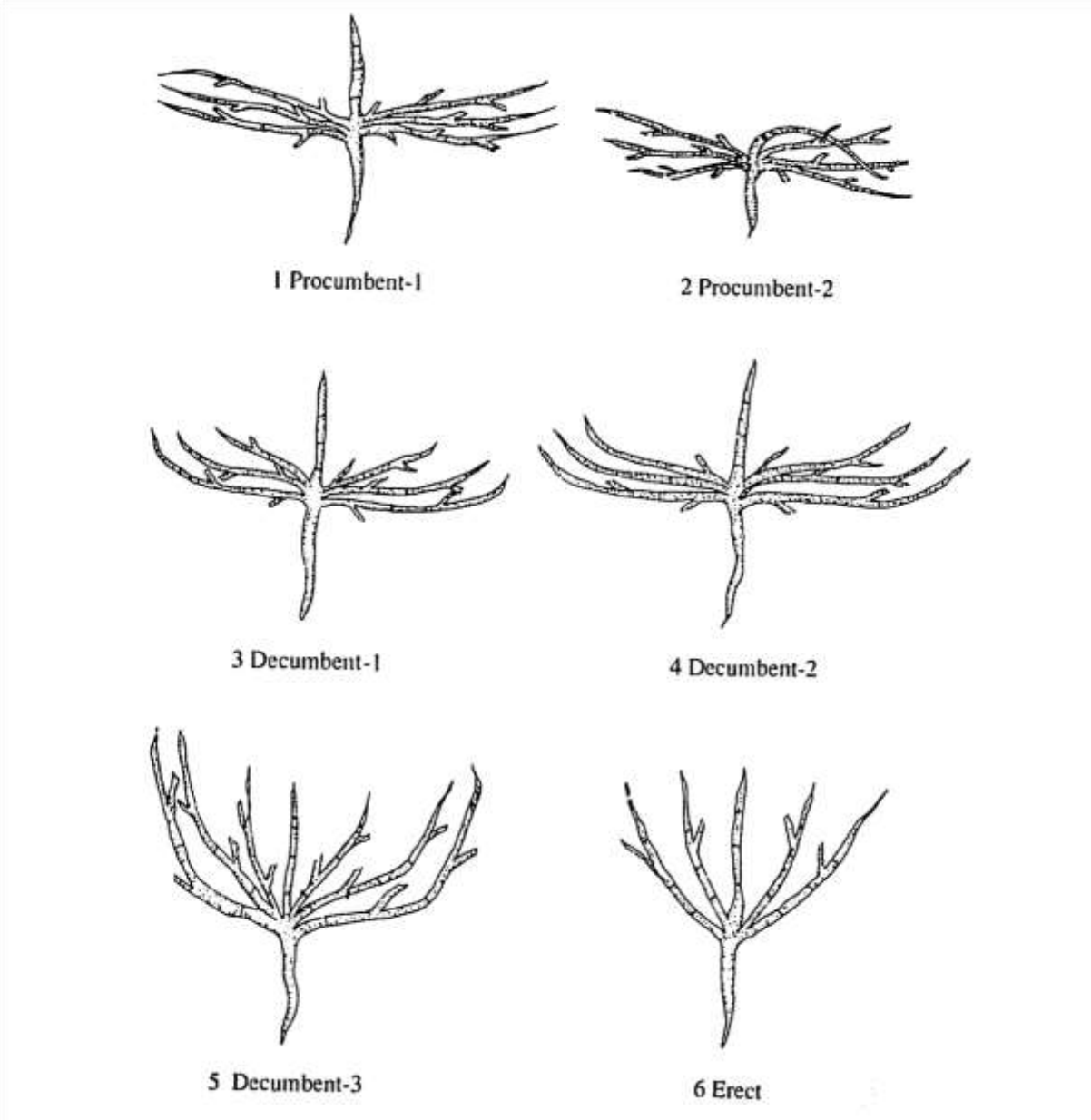
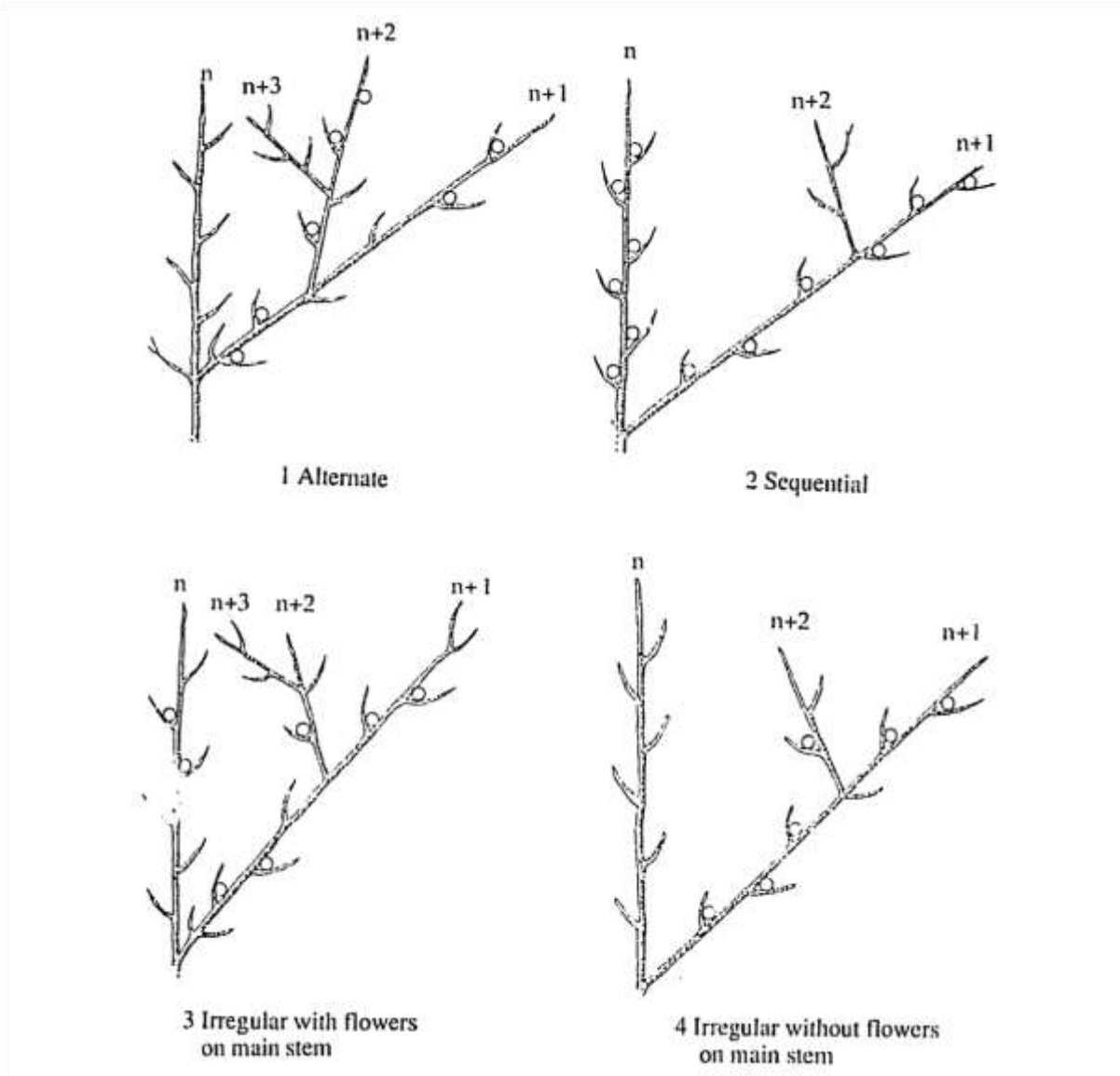
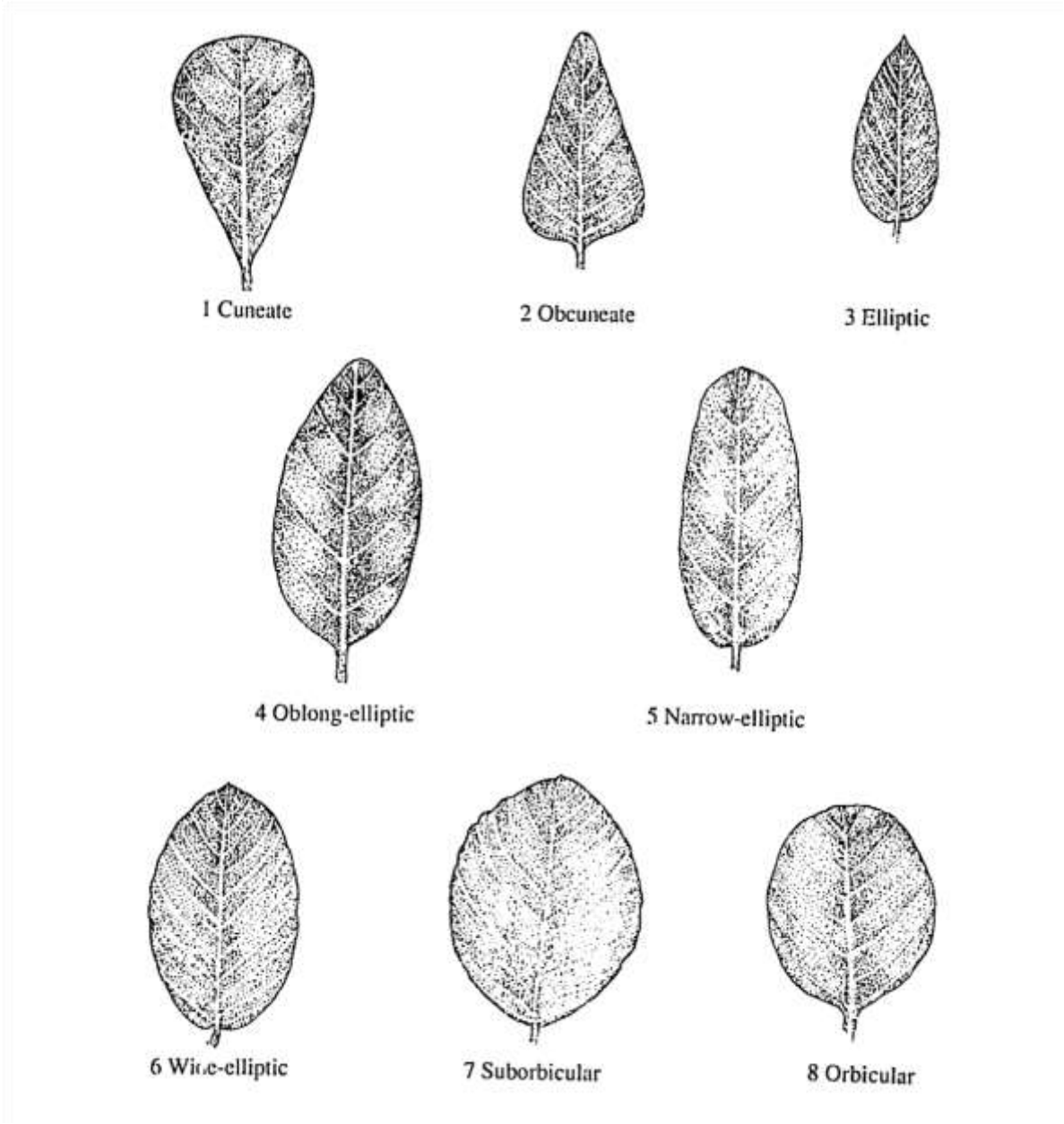
Appendix 4.1: Groundnut descripotrs used for growth habit



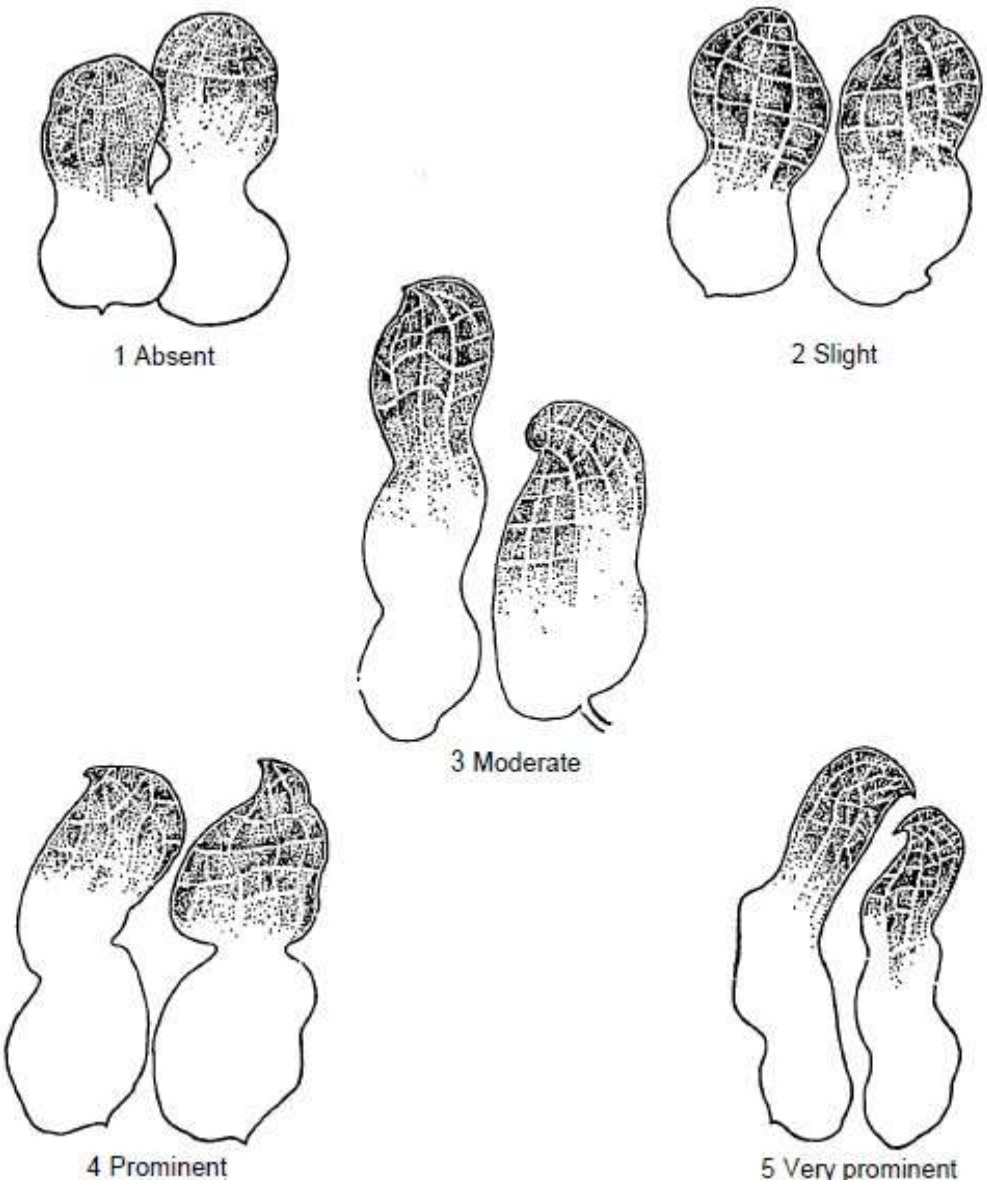
Appendix 4.2: Groundnut descriptors used for branching type



Appendix 4.3: Groundnut descritors used for leaf shape



Appendix 4.4: Groundnut descriptors used for pod constriction



CHAPTER 5 EVALUATION OF GROUNDNUT RECOMBINANT INBRED LINES AND SNP-BASED MARKER-TRAIT ASSOCIATION ANALYSIS FOR RESISTANCE TO ROSETTE DISEASE

Abstract

Groundnut rosette disease (GRD) is among the major constraints limiting groundnut productivity in sub-Saharan Africa and has resulted in yield losses of up to 100% in epidemic

years. The use of resistant cultivars is the most viable method to control the disease and the application of marker-assisted selection during breeding programmes is cost effective and enhances genetic gain. Therefore, the current study aimed at evaluating recombinant inbred lines (RILs) for resistance to GRD and implementing single nucleotide polymorphism (SNP) based marker-trait association to identify resistant lines and markers linked to GRD resistance, respectively. The RILs were assessed under field and glasshouse conditions at ICRISAT Malawi and data were recorded on yield and GRD related traits. ANOVA revealed significant differences among the lines in all recorded traits, indicating the existence of genetic variability and possibility of effective selection. Interaction of genotype and environment was significant for disease incidence and the glasshouse environment had higher disease pressure. ICGVSM 15605, ICGV-SM 15621, ICGV-SM 15618, ICGV-SM 15604 and ICGV-SM 15615 were among the resistant and high yielding RILs. The study identified 22 highly significant markertrait associations, which will add to previously reported genomic regions influencing GRD and the aphid vector resistance, to be used for marker-assisted selection in groundnut breeding programmes.

Keywords: Groundnut, groundnut rosette disease, resistance, marker-trait association

5.1 Introduction

Cultivated groundnut (*Arachis hypogea* L., AABB, 2n = 4x = 40) is an annual crop belonging to the family *Fabaceae* and widely distributed throughout the tropical, sub-tropical, and warm temperate regions of the world, where it plays an important role as both food and cash crop (Maiti, 2002; Nautiyal *et al.*, 2002). In Malawi and other developing countries, apart from food security, groundnut contributes to poverty alleviation as a source of income and the nuts are eaten in various forms (Prasad *et al.*, 2010; Chala *et al.*, 2014). Moreover, Longwe-Ngwira *et al.* (2012) indicated that groundnut is the major legume crop in terms of value and quantity in Malawi, followed by pigeon pea, common bean, cowpea and soybean. Chikowo *et al.* (2015) reported that in Malawi the crop is predominantly grown by smallholder farmers under subsistence farming conditions and despite its importance, the yields are still low and suffer from fluctuations. Over the last three seasons the average yield was 759.77 kg ha^{-1}, which is less than half of the world average (1.64 t ha^{-1}) and one-third of the potential yield (3.0 t ha^{-1}) (Longwe-Ngwira *et al.*, 2012; FAOSTAT, 2018). Several biotic, abiotic and socio-economic factors have been indicated to constrain the groundnut production in Malawi and among them, groundnut rosette disease (GRD) is considered to be one of the major constraints (Simtowe *et al.*, 2010; Longwe-Ngwira *et al.*, 2012; Chikowo *et al.*, 2015).

Groundnut rosette disease is endemic to sub-Saharan Africa (SSA). It is caused by a complex of three agents (*Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and a *satellite*-RNA (satRNA) associated with GRV) and transmitted by an aphid (*Aphis craccivora* Koch) in a persistent manner (Brink and Belay, 2006; Waliyar *et al.*, 2007; Panguluri and Kumar, 2016). According to Olorunju and Ntare (2003), GRD is considered to be the most destructive groundnut disease in SSA and whenever it occurs, yield is reduced. Yield losses of up to 100% have been registered in susceptible cultivars in epidemic years (Naidu and Kimmins, 2007; Minde *et al.*, 2008). Efforts have been made to develop sustainable control methods to GRD and the use of resistant cultivars is known to be the most viable method to control the disease in groundnut production, especially for smallholder farmers (Naidu *et al.*, 1999; Waliyar *et al.*, 2007; Okello *et al.*, 2014). Although other methods are available and can be used, they are not economically practical and are difficult for smallholder farmers under subsistence farming conditions (Olorunju and Ntare, 2003; Brink and Belay, 2006). Moreover, chemical control has not proved to be effective, and improper use might cause environmental damages and development of insecticide-resistant biotypes (Naidu and Kimmins, 2007; Jackson, 2015).

Groundnut breeding programmes have been using phenotyping tools for selecting GRD resistant plants or progenies (Naidu *et al.*, 1999; Olorunju *et al.*, 2001; Pasupuleti *et al.*, 2013). However, conventional breeding has limitation when improving traits with quantitative inheritance, such as GRD resistance (Janila *et al.*, 2016). This is because there is a chance of selection bias due to failure of phenotypic screens and escapees (Cobb *et al.*, 2013). In contrast, genomic tools are robust, cost-effective, and reliable to enhance genetic gain for specific characters and the whole breeding efficiency (Pasupuleti *et al.*, 2013; Janila *et al.*, 2016). The development of genomic techniques in groundnut started recently and has slowly progressed due to the tetraploid nature of the crop, low marker polymorphism and lack of genome sequence resources (Janila *et al.*, 2016). Chu *et al.* (2011) indicated that the first variety developed using molecular techniques was registered in 2003 and since then, Japan, China, India and USA have been using marker-assisted breeding for groundnut improvement. Efforts have been made in identifying molecular markers linked to specific traits, such as rust and late leaf spot resistance (Hou *et al.*, 2007; Leal-Bertioli *et al.*, 2009; Khedikar *et al.*, 2010; Sujay *et al.*, 2012), aflatoxin contamination and *Aspergillus flavus* resistance (Lei *et al.*, 2006; Yanbin *et al.*, 2009), drought tolerance (Ravi *et al.*, 2011), protein content, pod and kernel traits (Gomez Selvaraj *et al.*, 2009), and high oleic acid content (Sarvamangala *et al.*, 2011; Wang *et al.*, 2011). However, few reports are available on DNA markers linked to GRD and the aphid resistance (Herselman *et al.*, 2004; Pandey *et al.*, 2014).

Research to develop high yielding and GRD resistant cultivars is needed and should be a priority. Therefore, the current study was designed to evaluate recombinant inbred lines (RILs) for resistance to GRD, which is very essential and will allow the identification of high yielding and resistant RILs for further advancement and release, contributing to groundnut production in Malawi. Assessment of association between DNA variants and GRD (marker-trait association) was also done to identify molecular markers that can be used for marker-assisted selection in future breeding programmes.

5.2 Materials and methods

5.2.1 Plant materials

The response to GRD was evaluated under field and glasshouse conditions. A total of 25 groundnut genotypes sourced from ICRISAT Malawi, which comprised 21 RILs derived from a bi-parental cross between Chalimbana (male and susceptible parent) and Nsinjiro (female and resistant parent), and two susceptible controls (CG7 and JL24) were used. JL 24 is highly susceptible to GRD, thus was also used as an infector-row.

5.2.2 Experimental sites

The materials were evaluated for resistance to GRD under artificial infestation at Chitedze Agricultural Research Station ($33^{0}38$'E and $13^{o}85$'S) during the rainy season, from 16th December 2017 to 5th May 2018. The station is located 16 km west of Lilongwe (Malawi) with an altitude of 1146 meters above sea level (masl). It is a hotspot area and experiences high GRD pressure during the growing season. Based on long-term climatic data, the station has an average minimum and maximum temperatures of 16^{o}C and 24^{o}C, respectively, with a mean annual rainfall of 892 mm. Weather data for the period of the trials are presented in Table 5.1. The soil used for the glasshouse experiment was collected from a forest field at Chitedze Agricultural Research Station, and soil samples from both field and glasshouse trials were collected and sent for analysis (Table 5.2 and Appendix 5.1).

Table 5.1: Weather data for the period of the experiments

Month	Minimum Temperature (^{o}C)	Maximum Temperature (^{o}C)	Rainfall (mm)	Relative Humidity (%)
December	19.63	28.73	170.40	75.33
January	18.08	29.13	52.00	68.82
February	18.57	27.74	160.60	76.57
March	18.09	28.02	209.10	76.13
April	16.03	26.72	4.13	74.00

May	14.36	26.30	0.00	58.60	
Average	17.46	27.77	-	71.57	
Total	-	-	596.23	-	

Table 5.2: Soil analytical data for the field and glasshouse trials

Soil sample	Soil texture	pH(H_2O)	OM (%)	TN (%)	P (ppm)	K (meq/100g)
Field	Sandy clay	5.42	3.07	0.7	15.08	0.45
Glasshouse	Sandy loam	6.02	4.83	0.6	10.57	0.49

pH-potential of hydrogen, OM-organic matter, TN-total nitrogen, P-phosphorous and K-potassium.

5.2.3 Experimental design and management

The trials were planted in a 5 x 5 square lattice design (Patterson *et al.*, 1978), with two replications due to seed limitations. In the field and in each replication, every genotype was planted in a two row plot of 3.0 m in length at a spacing of 0.6 m between rows and 0.1 m within a row. The plants were sown by hand at a rate of one seed per hill. In the glasshouse, a plot consisted of sixty plastic pots of 100 mm diameter and one seed per pot was sown. The field trial was conducted under rain-fed conditions and in the glasshouse the soil was kept moist throughout the experiment by daily manual irrigation as necessary. The trials were kept free of weeds and neither fertilizers nor pesticides were applied.

5.2.4 Disease inoculation

The test materials were infested with GRD using the infector-row technique described by Bock and Nigam (1988), which can result in 98% of incidence in susceptible cultivars. The infector rows consisted of the GRD susceptible genotype JL24 and were arranged systematically throughout the trials, where one row of the genotype JL24 was planted between two rows of the test materials as recommended by Bock and Nigam (1988). Prior to planting the trials, JL24 seedlings were raised and infected in the glasshouse. The heavily diseased seedlings were transplanted into each of the infector rows at 1.5 m spacing around 7 to 14 days after sowing (DAS). To increase the disease spread, viruliferous aphids (which act as vectors for the virus) were transferred from infected plants in the glasshouse to the infector rows and test materials using a camel's hair brush at a weekly basis up to 80 DAS. The number of viruliferous aphids, was increased by collecting non-viruliferous aphids from surrounding fields and placing them in a petri dish containing infected leaves. The aphids were allowed to feed for 30 minutes on the leaves to acquire the viruses and they were transferred to the infector rows and test materials (Bock and Nigam, 1988).

5.2.5 Data collection

Data were collected on percentage of disease incidence, days to flowering and maturity, plant height, number of branches, yield and its components, and shelling percentage. Disease data were recorded based on Waliyar *et al.* (2007) while yield and agronomic traits were recorded based on the groundnut descriptors (IBPGR and ICRISAT, 1992). Data, except for percentage of disease incidence and yield, were recorded on five randomly selected plants and 10 mature pods that were also randomly selected.

Percentage of disease incidence (PDI)

Observations on GRD development were recorded visually at 60, 80 and 100 DAS. The number of plants showing GRD symptoms in each plot was determined by counting and PDI was calculated as follows:

$$PDI\ (\%) = \frac{NIP}{TP} * 100$$

Where: PDI is the percentage of disease incidence, NIP is the number of plants showing GRD symptoms and TP is the total number of plants in a plot.

The final PDI was used to show GRD resistance (Iwo and Olorunju, 2009), as shown in Table 5.3. GRD is a viral disease and the method based on PDI, for assessment of genotypes for the disease resistance, is the widely used (Waliyar *et al.*, 2007). Severity was also recorded, using 1 to 5 rating scale, where: 1 = no symptoms, 2 = symptoms on 1 to 20% foliage but no stunting, 3 = symptoms on 21 to 50% foliage and stunting, 4 = severe symptoms on 51 to 70% foliage and stunting, and 5 = severe symptoms on 71 to 100% foliage, stunting and dead plants (Waliyar *et al.*, 2007). Severity scores were transformed by ln(x+1) before analysis in order to have residual terms following normal distribution (Gomez and Gomez, 1984).

Table 5.3: Scale of percentage of disease incidence for evaluation of groundnut genotypes for resistance to GRD

PDI (%)	Inference/ Host response
0-10	Highly resistant
11-30	Resistant
31-50	Moderately resistant
51 and above	Susceptible

Source: Waliyar *et al.* (2007)

Days to flowering (DTF) and days to maturity (DTM)

Days to flowering and maturity were determined as the number of days between sowing date and the date when 50% of plants in a plot had flowed and matured, respectively.

Plant height and number of branches

The number of both primary (NPB) and secondary branches (NSB), and plant height (PH) were measured at 85 DAS. Plant height was measured from the ground to the top of the main stem axis while the branch numbers were determined by counting.

Yield and yield components

Number of pods per plant (NPP) was recorded at harvest on the selected plants and a mean was determined for each plot. Pod length (PL) and pod width (PW) were measured at the lengthiest and widest points, respectively. The pods were sun dried to approximately 8-10% moisture content and then weighed to determine pod yield per plot. A pod sample of approximately 100 g, which was randomly drawn from each plot, was shelled then weighed and the shelling percentage (SP) was calculated as follows:

$$SP\ (\%) = \frac{SW}{PWT} * 100$$

Where: SP is the shelling percentage, SW is the seed weight and PWT is the pod weight before shelling.

Hundred seeds were counted and weighed from the shelled samples, and the hundred seed weight (HSW) was recorded and expressed in grams. Seed yield was estimated using the formula:

$$SYD = \frac{PY * 10000}{PS} * SP$$

Where: SYD is the seed yield (kg ha^{-1}), PY is the pod yield per plot (kg), PS is the plot area (m^2) and SP is the shelling percentage (expressed as a fraction).

5.2.6 DNA extraction and sequencing

Four seeds per genotype were planted in a 300 mm diameter plastic pot for leaf tissue sampling. The plastic pots were labelled accordingly and the planting was carried out in a glasshouse at Chitedze Agricultural Research Station. Seven days after emergence, young leaves from one plant of each genotype were sampled for genomic DNA extraction, which was done using the cetyltrimethyl ammonium bromide (CTAB) protocol with slight modification as described by Mace *et al.* (2003). The quality of the extracted genomic DNA was examined

using agarose (0.8%) gel electrophoresis and quantified by using spectrophotometric analysis. Each DNA sample was digested with restriction enzyme MsII and then sequenced on Illumina HiSeq 2000 at LGC Genomics, UK (Annicchiarico *et al.*, 2017). The raw Illumina data was aligned to the groundnut reference genome, cultivar Trifrunner (Dash *et al.*, 2016). SNP calling and filtering was implemented using GBS pipeline in Trait Analysis by Association, Evolution and Linkage (TASSEL 5) (Glaubitz *et al.*, 2014). A total of 6348 SNP markers with frequency above 10% and distributed in the whole groundnut genome were maintained for analysis.

5.2.7 Data analysis

5.2.7.1 Phenotypic data

Analysis of variance (ANOVA) was performed on percentage of disease incidence and the other recorded traits using the General Linear Model (GLM) in SAS version 9.4 (SAS Institute, 2015) and Genstat 18th Edition (Payne, 2014), following the tests of Shapiro-Wilk and Bartlett for residual normality and variance homogeneity, respectively. The model for the combined ANOVA was as follows:

$$Y_{ijkl} = \mu + G_i + E_j + GE_{ij} + R_{k(j)} + B_{l(kj)} + \varepsilon_{ijkl}$$

Where: Y is the observed genotype response, μ is the general mean, G is the effect of genotype, E is the effect of the environment, GE is the interaction effects of genotype and environment, R is the replication effect, B is the block effect and Ɛ is the error term.

Means were separated by least significant difference (LSD) at 5% level of significance. To determine the degree of relationship between disease and agronomic traits, correlation analysis was performed using Pearson's method and PROC CORR in SAS version 9.4 (SAS Institute, 2015). Since it is important to use traits with high heritability for marker-trait association (Laido *et al.*, 2014; Qin *et al.*, 2015), heritability for GRD incidence was estimated using the mean square values from the ANOVA table as follows (Singh *et al.*, 1993):

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2 + \sigma_e^2}$$

Where: H^2 is the broad-sense heritability; σ_g^2, σ_e^2, σ_p^2, σ_{ge}^2 are the variances due to genotype, environment, phenotype, and genotype and environment interaction, respectively.

5.2.7.2 Genotypic data

Marker-trait association between percentage of disease incidence and the 6348 SNP markers was performed in TASSEL 5, following the Mixed Linear Model (MLM) procedure and a

significant association was declared at *p value* < 0.001 (Bradbury *et al.*, 2007). Distribution of *p values* of associated SNP markers were generated using Manhattan plot with threshold of $\log_{10}$ (*p value*) (LOD) = 3 (Sindhu *et al.*, 2014).

5.3 Results

5.3.1 Percentage of disease incidence

The infector-row technique was effective in spreading the virus among the evaluated groundnut genotypes and the recorded response varied from symptomless plants up to stunting and bushy appearance due to shortened internodes. Analysis of variance (ANOVA) revealed highly significant differences ($p < 0.001$) for GRD incidence (Table 5.4 and Appendix 5.2). Highly significant ($p < 0.001$) differences between the environments were also observed, and interactions of genotype and environment were highly significant ($p < 0.001$). Generally, GRD incidence was higher under glasshouse than field conditions with final PDI mean values of 29.34% and 15.82%, respectively. PDI increased over time under both environments and genotypes with high PDI had high severity scores (Appendix 5.2). Under glasshouse, final GRD incidence varied from 12.69% (ICGV-SM 15604) to 77.69% (JL24) while at the field conditions it ranged between 0 and 72.28% (Chalimbana). RILs ICGV-SM 15607, ICGV-SM 15617, ICGV-SM 15618, ICGV-SM 15622 and ICGV-SM 15631 were symptomless under field conditions. Final PDI across environments ranged between 8.65% for ICGV-SM 15607 and 73.20% for JL24 with an average value of 22.58%. Out of the evaluated genotypes, two were highly resistant, twenty were resistant and three were susceptible, across the environments. The controls were susceptible with final PDI values of 59.23% (CG7) and 73.20% (JL24) while the male (Chalimbana) and female parents (Nsinjiro) were susceptible and resistant, with mean of 72.77% and 25.47%, respectively. Additionally, final GRD incidence had high broad-sense heritability estimate of 84.18%.

Table 5.4: Mean percentage of disease incidence (PDI) and overall disease response of RILs, parental genotypes and controls across field and glasshouse environments

	Field		Glasshouse		Across environments	
Genotype	PDI	Response	PDI	Response	PDI	Pooled Response
ICGV-SM 15604	8.84	HR	12.69	R	10.76	R
ICGV-SM 15605	6.94	HR	29.50	R	18.22	R
ICGV-SM 15606	2.32	HR	25.19	R	13.76	R
ICGV-SM 15607	0.00	HR	17.31	R	8.65	HR
ICGV-SM 15610	9.29	HR	20.29	R	14.79	R
ICGV-SM 15611	10.61	R	23.46	R	17.03	R
ICGV-SM 15612	4.17	HR	15.77	R	9.97	HR
ICGV-SM 15615	6.76	HR	28.46	R	17.61	R
ICGV-SM 15617	0.00	HR	26.27	R	13.13	R
ICGV-SM 15618	0.00	HR	21.92	R	10.96	R
ICGV-SM 15621	7.95	HR	15.69	R	11.82	R
ICGV-SM 15622	0.00	HR	31.15	MR	15.58	R
ICGV-SM 15623	13.29	R	29.88	R	21.59	R
ICGV-SM 15624	12.91	R	24.76	R	18.83	R
ICGV-SM 15627	18.47	R	29.00	R	23.73	R
ICGV-SM 15629	18.51	R	28.88	R	23.70	R
ICGV-SM 15630	20.69	R	17.79	R	19.24	R
ICGV-SM 15631	0.00	HR	22.92	R	11.46	R
ICGV-SM 15632	4.26	HR	23.72	R	13.99	R
ICGV-SM 15633	15.25	R	27.12	R	21.19	R
ICGV-SM 15635	8.70	HR	27.04	R	17.87	R
Parents						
Nsinjiro (female)	29.78	R	21.15	R	25.47	R
Chalimbana (male)	72.28	S	73.27	S	72.77	S
Controls						
CG7	55.77	S	62.69	S	59.23	S
JL24	68.72	S	77.69	S	73.20	S
Mean	15.82		29.34		22.58	
Genotype MS	623.89***		371.55***		931.70***	
Environment MS	-		-		4573.44***	
Gen X Env MS	-		-		63.75***	
LSD (5%)	5.79		11.25		8.60	
SED	2.73		5.31		4.20	
CV (%)	17.28		18.09		18.69	

Significant levels: *** significant differences at 0.1%, MS-mean square, Gen-genotype, Envenvironment, LSD-least significant difference, SED-standard error of difference, CV- coefficient of variation, HR-highly resistant, R-resistant, MR-moderately resistant and S-susceptible.

5.3.2 Yield and related traits

Yield and its traits were recorded under field environment and ANOVA revealed significant differences for these traits, except for shelling percentage (Table 5.5). The mean value of seed yield was 850.48 kg ha^{-1} with genotypes varying from 194.40 kg ha^{-1} for JL24 to 1122.20 kg ha^{-1} for ICGV-SM 15605. ICGV-SM 15621 (1116.70 kg ha^{-1}), ICGV-SM 15618 (1114.40 kg ha^{1}), ICGV-SM 15604 (1105.60 kg ha^{-1}) and ICGV-SM 15615 (1100.00 kg ha^{-1}) were also among the five top yielding RILs. These RILs were not significantly different in terms of seed yield at 5% significance level. Both parents yielded below the trial mean. Nsinjiro, the female parent produced 733.30 kg ha^{-1}, 34.66% lower than the highest yielding genotype and Chalimbana, the male parent yielded 261.10 kg ha^{-1}, 76.73% below the best yielder. Chalimbana yielded below all the RILs while Nsinjiro's yield was better than ICGV-SM 15631 (677.80 kg ha^{-1}) and
ICGV-SM 15627 (672.20 kg ha^{-1}) but lower than the others. The controls, CG7 (472.20 kg ha^{1}) and JL24 (194.40 kg ha^{-1}) also yielded lower than the trial mean and the RILs.

The number of pods per plant ranged from 12 to 35 with a mean of 22 and Chalimbana was the lowest producer. ICGV-SM 15606 produced the highest number of pods, followed by ICGV-SM 15605 (34), ICGV-SM 15618 (32), ICGV-SM 15615 (30) and ICGV-SM 15604 (30). These RILs, except ICGV-SM 15606, were amongst the five top yielding genotypes. The female parent Nsinjiro recorded pod number of 25, which was above the trial mean. The controls CG7 and JL24 produced 17 and 13 pods per plants, respectively. Hundred seed weight varied from 24.73 g (JL24) to 46.84 g (ICGV-SM 15629) with a mean of 38.99 g. Chalimbana (44.97 g) is large seeded and was among the genotypes with the highest HSW. The longest pods were produced by ICGV-SM 15606 (33.40 mm) while the shortest by Nsinjiro (23.10 mm). ICGV-SM 15629 (14.90 mm) and JL24 (9.25 mm) produced the widest and narrowest pods, respectively.

Days to flowering and maturity ranged from 32 and 108 for JL24 to 42 and 130 for Chalimbana, respectively. The mean plant height was 164.58 mm, the RILs ICGV-SM 15605, ICGV-SM 15629 and ICGV-SM 15621 were the tallest with average height of 202.50 mm, 201.70 mm and 195.00 mm, respectively, while the controls JL24, CG7 and the parent Chalimbana were the shortest with mean height of 110.00 mm, 116.70 mm and 131.70 mm, respectively. ICGVSM 15610 (3) and ICGV-SM 15615 (6) recorded the lowest and the highest number of

primary branches, respectively. The highest number of secondary branches were observed for Nsinjiro (14), ICGV-SM 15633 (14) and ICGV-SM 15629 (13) while the lowest were recorded for JL24
(2) and Chalimbana (6).

Table 5.5: Performance of RILs, parents and controls in respect of 12 agronomic traits under field conditions

Genotype	DTF	DTM	NPB	NSB	PH (mm)	NPP	PW (mm)	PL (mm)	SYDP SYD (kg ha^{-1}) (g)	SP (%)	HSW (g)
ICGV-SM 15604	41	123	5	12	171.70	30	12.20	30.00	1105.60	11.60	67.39	39.41
ICGV-SM 15605	39	120	5	13	202.50	34	10.00	30.90	1122.20	10.82	65.91	44.29
ICGV-SM 15606	40	119	5	12	182.50	35	14.50	33.40	955.60	10.70	63.72	40.61
ICGV-SM 15607	40	125	5	12	156.70	18	12.70	28.48	1072.20	9.85	65.20	38.37
ICGV-SM 15610	41	117	3	13	163.30	27	11.80	29.40	1072.20	11.35	66.50	39.22
ICGV-SM 15611	39	122	5	12	163.30	21	13.20	30.90	772.20	7.18	65.29	42.17
ICGV-SM 15612	40	120	5	10	155.00	14	12.50	30.30	861.10	7.91	68.56	41.65
ICGV-SM 15615	41	118	6	13	153.30	30	13.10	29.70	1100.00	10.34	66.10	36.97
ICGV-SM 15617	41	117	5	12	150.00	20	13.60	29.40	877.80	9.07	67.01	37.17
ICGV-SM 15618	40	120	5	12	186.70	32	12.80	31.63	1114.40	10.58	66.26	42.59
ICGV-SM 15621	39	117	5	13	195.00	22	12.20	31.70	1116.70	9.66	66.19	40.71
ICGV-SM 15622	41	120	5	12	180.00	23	11.90	28.30	738.90	9.05	61.04	30.75
ICGV-SM 15623	41	118	5	13	181.70	20	13.60	25.70	916.70	9.80	67.46	31.21
ICGV-SM 15624	39	119	4	11	156.70	24	12.90	28.70	1061.10	10.16	71.20	39.73
ICGV-SM 15627	39	115	5	12	145.00	12	13.90	28.50	672.20	9.06	64.57	34.43
ICGV-SM 15629	41	117	6	13	201.70	21	14.90	28.50	766.70	8.31	63.19	46.84
ICGV-SM 15630	41	117	5	12	164.20	16	12.90	30.20	927.80	10.50	64.83	31.61
ICGV-SM 15631	41	118	5	12	184.20	21	13.30	30.15	677.80	6.39	65.28	37.55
ICGV-SM 15632	40	123	4	11	140.00	25	12.30	26.60	994.40	10.63	71.04	42.38
ICGV-SM 15633	41	120	5	14	160.00	19	12.20	28.10	742.20	8.05	66.27	45.22
ICGV-SM 15635	40	122	5	10	176.70	26	11.80	29.80	933.30	10.03	68.68	44.10
Parents												
Nsinjiro (female)	40	121	5	14	185.80	25	12.30	23.10	733.30	6.38	65.02	39.87
Chalimbana (male)	42	130	4	6	131.70	12	13.80	30.30	261.10	5.00	71.20	44.97
Controls												

Genotype	DTF	DTM	NPB	NSB	PH (mm)	NPP	PW (mm)	PL (mm)	SYD (kg ha^{-1})	SYDP (g)	SP (%)	HSW (g)
CG7	38	123	5	8	116.70	17	13.00	30.10	472.20	5.39	69.23	38.28
JL24	32	108	4	2	110.00	13	9.25	23.30	194.40	1.80	77.38	24.73
Mean	40	119	5	11	164.58	22	12.67	29.09	850.48	8.78	66.98	38.99
Genotype MS	6.73**	19.05*	0.85*	10.94*	945.80*	63.37**	2.61*	12.57**	103472.87**	8.11**	21.33ns	45.57**
LSD (5%)	2.93	5.79	1.1	4.45	43.31	8.56	2.19	3.46	350.10	3.05		6.99
SED	1.38	2.73	0.52	2.10	20.43	4.04	1.03	1.63	165.10	1.44	3.78	3.30
CV (%)	3.49	2.29	10.72	18.44	12.41	18.11	8.15	5.61	19.42	16.40	5.65	8.46

Significant levels: ns, *, ** non-significant differences, significant differences at 5% and 1%, respectively; MS-mean square, LSD-least significant difference, SED-standard error of difference, CV- coefficient of variation; DTF-days to flowering, DTM-days to maturity, NPB-number of primary branches, NSB-number of secondary branches, PH-plant height, NPP-number of pods per plant, PW-pod width, PL-pod length, SYD-seed yield, SYDP-seed yield per plant, SP-shelling percentage and HSW-hundred seed weight.

5.3.3 Relationship between disease incidence and agronomic traits

The summary of correlation coefficients (r) which describe the degree of association between final disease incidence and the other recorded traits is displayed in Table 5.6. Highly significant and strong negative correlations were observed between final GRD incidence with seed yield (r = -0.706, $p < 0.001$), plant height (r = -0.537, $p < 0.001$) and number of secondary branches (r = -0.681, $p < 0.001$). GRD incidence also showed negative correlations with number of pods per plant (r = -0.478, $p < 0.001$), hundred seed weight (r = -0.188, $p > 0.05$), pod width (r = 0.142, $p > 0.05$) and pod length (r = -0.291, $p < 0.05$), and a weak positive correlation with days to maturity (r = 0.004, $p > 0.05$). In addition, seed yield was strongly and positively correlated with number of pods per plant (r = 0.604, $p < 0.01$) and number of secondary branches (r = 0.566, $p < 0.001$). Weak positive correlations were also observed between seed yield and number of primary branches (r = 0.139, $p > 0.05$), days to maturity (r = 0.253, $p > 0.05$), plant height (r = 0.397, $p < 0.01$), pod width (r = 0.025, $p > 0.05$) and pod length (r =
0.280, $p < 0.05$).

Table 5.6: Person's correlation coefficients describing the association of GRD and agronomic traits of 25 groundnut genotypes tested under GRD infestation at field conditions

	NPB	NSB	DTF	DTM	PH	NPP	PW	PL	SYD	SYDP	SP	HSW
NSB	0.364**											
DTF	0.215	0.561***										
DTM	-0.042	0.124	0.491***									
PH	0.378**	0.576***	0.263	0.005								
NPP	0.203	0.353*	0.163	0.131	0.450**							
PW	0.192	0.207	0.409**	0.241	0.086	-0.052						
PL	0.053	0.073	0.268	0.227	0.257	0.256	0.286*					
SYD	0.139	0.566***	0.316*	0.253	0.397**	0.604***	0.025	0.280*				
SYDP	0.109	0.559***	0.466***	0.295*	0.314*	0.568***	0.142	0.375**	0.905***			
SP	-0.391**	-0.396**	-0.335*	0.092	-0.449**	-0.173	-0.318*	-0.263	-0.081	-0.144		
HSW	-0.009	0.294*	0.381**	0.528***	0.261	0.252	0.242	0.298*	0.309*	0.245	-0.074	
PDI	-0.268	-0.681***	-0.417**	0.004	-0.537***	-0.478***	-0.142	-0.291*	-0.706***	-0.653**	0.468***	-0.188

Significant levels: *, **, *** significant correlations at 5%, 1% and 0.1%, respectively; NPB-number of primary branches, NSB-number of secondary branches, DTF-days to flowering, DTM-days to maturity, PH-plant height, NPP-number of pods per plant, PW-pod width, PL-pod length, SYD-seed yield, SYDP-seed yield per plant, SP-shelling percentage, HSW-hundred seed weight and PDI-percentage of disease incidence.

5.3.4 Marker-trait association

The marker-trait association of percentage of disease incidence was tested against 6348 SNP markers. In total, 426 significant ($p < 0.05$) marker-trait associations were found and only those that had $p < 0.001$ were considered as significant (Table 5.7). These markers explained 36.58 to 82.64% of the total phenotypic variation and were located on eight chromosomes, namely A03, A07, B03, B05, B06, B08, B09 and B10. On chromosome B06, 10 marker-trait associations were found of which one of them explained the highest phenotypic variation (Marker R^2 = 82.64%). A marker on chromosome B05 explained the least proportion of phenotypic variation (Marker R^2 = 36.58%). The distribution of *p values* of associated SNPs with threshold of $-\log_{10}$ (*p value*) = 3 is shown in Manhattan plot (Figure 5.1).

Table 5.7: Summary of significanty associated SNP markers using Mixed Linear Model (MLM)

Marker	Chromosome	Position	*p value*	Marker R^2
SCM009803.1_46677195	A03	46677195	0.000455	75.48
SCM009807.1_16899504	A07	16899504	0.000493	79.34
SCM009807.1_25629160	A07	25629160	0.000386	78.10
SCM009807.1_37410692	A07	37410692	0.000348	78.10
SCM009807.1_69629827	A07	69629827	0.000348	81.35
SCM009813.1_144478824	B03	144478824	0.000348	36.58
SCM009813.1_76454497	B03	76454497	0.000348	73.09
SCM009815.1_49107917	B05	49107917	0.000348	36.58
SCM009816.1_103564954	B06	103564954	0.000348	78.10
SCM009816.1_12086279	B06	12086279	0.000364	79.08
SCM009816.1_15942502	B06	15942502	0.000348	78.10
SCM009816.1_19474194	B06	19474194	0.000982	82.64
SCM009816.1_38037328	B06	38037328	0.000386	80.70
SCM009816.1_48803162	B06	48803162	0.000464	79.34
SCM009816.1_52280418	B06	52280418	0.000448	78.10
SCM009816.1_73507048	B06	73507048	0.000414	62.50
SCM009816.1_80655911	B06	80655911	0.000348	63.10
SCM009816.1_99687994	B06	99687994	0.000561	78.10
SCM009818.1_68054497	B08	68054497	0.000348	78.10
SCM009819.1_15685908	B09	15685908	0.000378	77.84
SCM009819.1_27809758	B09	27809758	0.000348	76.50
SCM009820.1_142710697	B10	142710697	0.000348	77.63

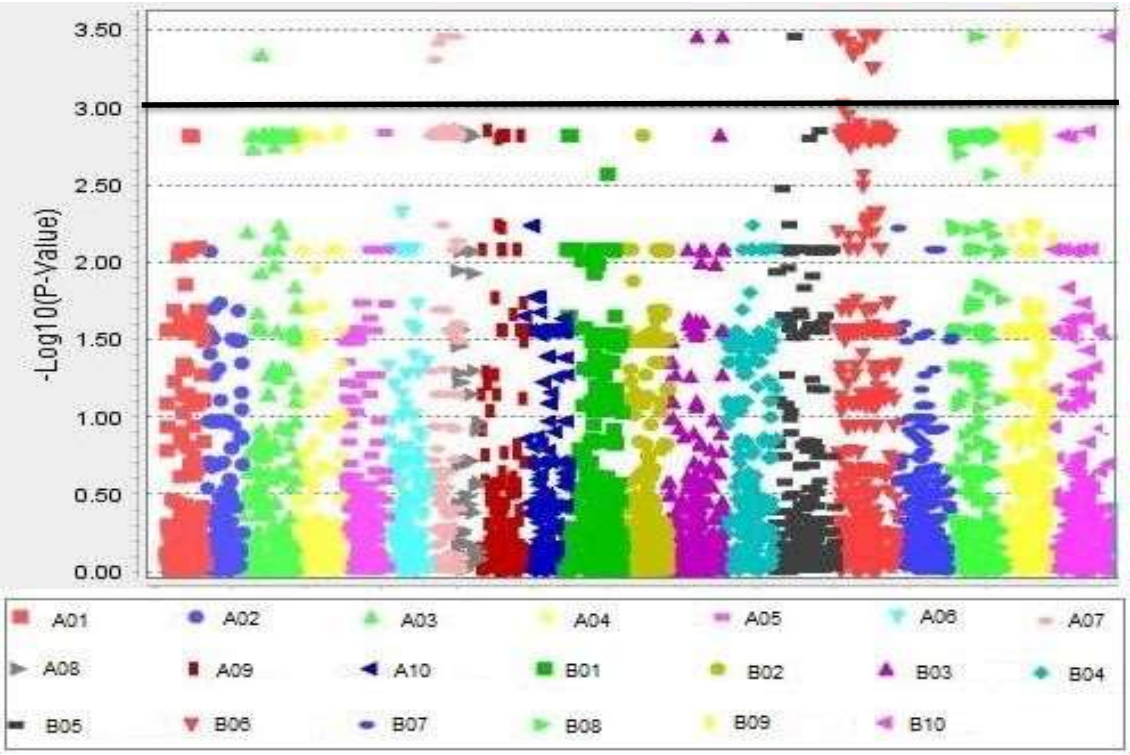


Figure 5.1: Manhattan plot of -$\log_{10}$ (*p values*) of the marker-trait association study using mixed linear model (MLM)

5.4 Discussion

Evaluating the GRD response of RILs and other genotypes is useful in determining their levels of resistance for further selection and advancement, or for selecting parents to start breeding programmes. The significant differences among the evaluated genotypes in all recorded traits suggest that there is genetic variability and selection may be effective. Mean PDI across the genotypes increased over time under both environments. Olorunju *et al*. (1991) and Mohammed *et al.* (2018) reported a similar pattern earlier, with the highest increase recorded on susceptible genotypes. Murant (1990) and Waliyar *et al*. (2007) also support this observation, as they considered GDR to be a polycyclic disease, whereby each infected plant serves as a source of inoculum for initiating a subsequent infestation by the movement of the aphid vector. Disease pressure was higher under glasshouse environment, indicating that this testing environment exhibited better conditions for GRD development and provides the best discrimination among the tested genotypes. These findings are consistent with Bock and

Nigam (1988) who used a glasshouse environment to identify susceptible “escapees”. The rains that occurred during the season may have disturbed the aphid population growth in the field while the glasshouse environment was protective and more conducive for aphid population growth, leading to higher GRD infection. Weather conditions, particularly rainfall, have been reported to influence the GRD development (Naidu *et al.*, 1999; Dwivedi *et al.*, 2003; Waliyar *et al.*, 2007).

There were genotypes that showed no symptoms under field conditions but mild symptoms on few young leaves in the glasshouse. The significant interaction between genotype and environment for GRD could explain these results, which are in agreement with earlier reports (Van der Merwe *et al.*, 1999; Iwo and Olorunju, 2009; Mohammed *et al.*, 2018). Moreover, Olorunju *et al.* (2001) and Waliyar *et al.* (2007) indicated that GRD resistance is not absolute since small portions of plants in resistant genotypes may show mild symptoms under high disease pressure, and Van der Merwe *et al.* (1999) reported that with high disease pressure the resistance can breakdown. GRD is a complex virus dependent on the interaction of three causal agents, GRAV, GRV and SatRNA. These genotypes, which showed no symptoms may have been infected by one of the agents (either GRAV or GRV), but not by SatRNA, which is responsible for GRD symptoms (Olorunju *et al.*, 1991; Waliyar *et al.*, 2007; Naidu and Kimmins, 2007).

The RILs showed good levels of resistance, indicating that they inherited genes for resistance from the female resistant parent Nsinjiro and the breeding objective which is to develop GRD resistant varieties may be achieved. Genotypes with high PDI mean were severely affected by the disease and an example of such genotypes were the controls JL24 and CG7, and the male parent Chalimbana. These susceptible genotypes showed severe symptoms that included reduced leaf size and bushy appearance due to shortened internodes. These symptoms have been reported to occur on susceptible genotypes, especially when the plants are infected at the early growth stage as it happened in this study (Nigam and Bock, 1990; Subrahmanyam *et al.*, 2002; Bua and Opio, 2014). Genotypes CG7, JL24, Chalimbana and Nsinjiro have been evaluated for GRD resistance under different environments in previous studies. Similarly, JL24, CG7 and Chalimbana were susceptible with PDI mean above 80% whereas Nsinjiro was resistant with PDI mean below 10% (Van der Merwe *et al.*, 2001; Muitia, 2011; Chintu, 2013). This indicates that Nsinjiro has stable resistance and can still be used as a source of GRD resistance for breeding programmes.

There was significant variation among genotypes on seed yield, suggesting that they had varied yield potential. The observed variation in seed yield was due to divergence of the

genotypes in terms of agronomic characteristics and GRD response. All the RILs yielded above the trial mean and the best five yielding RILs out yielded both parents, suggesting the existence of genetic gain for seed yield. Although these RILs showed no symptoms under field conditions, they may have been infected by either GRAV or GRV, or both and had their seed yield affected. This is in agreement with Naidu and Kimmins (2007), who reported that GRAV alone reduced seed yield in symptomless plants. Moreover, Van der Merwe *et al.* (1999) reported a yield reduction of up to 75% due to GRAV in symptomless genotypes grown under GRD environment. Hence, these genotypes should be evaluated under GRD freeenvironment to determine their yield potential. Most of the tested genotypes were medium maturing that require 800 to 1200 mm of rainfall and temperatures ranging from 24 to 30^{o}C for good growth and yield (Cillieres, 2011; Okello *et al.*, 2013; Engels, 2014). However, relatively lower temperatures (minimum and maximum of 17.46^{o}C and 27.77^{o}C, respectively) and rainfall (596.23 mm) occurred during the growing season, and may have negatively affected the seed yield. Furthermore, the seed yields recorded from the best five RILs were higher than the average yield in Africa (900 kg ha^{-1}), but lower than the yield obtained in the major groundnut-producing countries (2000-4000 kg ha^{-1}) (Singh and Nigam, 2016; FAOSTAT, 2018).

The negative correlations between GRD incidence with seed yield, number of pods per plant, hundred seed weight, plant height and number of secondary branches indicate that plant growth and seed yield were negatively affected by GRD. Similar findings have been reported (Muitia, 2011; Chintu, 2013; Mohammed *et al.*, 2018). Moreover, these findings are consistent with Subrahmanyam *et al.* (1997) and Waliyar *et al.* (2007) who indicated that GRD affects plant growth leading to stunting, reduced number of pods per plant which many of them do not produce seed, reduced seed weight and number of branches. This was mainly observed on the susceptible genotypes JL24 and Chalimbana, which produced the lowest yields. Whenever GRD occurs, the yields are greatly affected (Ntarea *et al.*, 2003; Minde *et al.*, 2008). Moreover, yield reduction on JL24, Chalimbana and other susceptible genotypes due to GRD infestation was reported earlier (Olorunju *et al.*, 1991; Hayatu *et al.*, 2014; Appiah *et al.*, 2016). On the other hand, seed yield was not greatly affected on the resistant genotypes. Politowski and Browning (1978) and Råberg *et al.* (2007) indicated that tolerant genotypes have susceptible response and support the same amount of pathogens as other susceptible genotypes, but still yield considerably well. An example of such genotypes is CG7, which was susceptible and had a final PDI of 55.77% and yield of 472.20 kg ha^{-1}. The significant positive correlations between seed yield with number of pods per plant, hundred seed weight and number of secondary branches, indicate the direct contribution of these traits to seed yield. Hence, selection criteria should consider these traits for improvement of seed yield, as indicated by Patil *et al.* (2006)

and Yusuf *et al.* (2017). The positive correlations between seed yield with plant height and days to maturity suggest that tall and late maturing genotypes have enough time and capacity to accumulate photo-assimilates resulting in higher seed yields.
Zaman *et al.* (2011) reported similar observations.

The observed high broad-sense heritability for GRD incidence confirms the value of the phenotypic data in the present marker-trait association analysis, as supported by Laido *et al.* (2014) and Qin *et al.* (2015) who reported the relevance of traits with high heritability for marker-trait association. The current study identified 22 highly significant ($p < 0.001$) markertrait associations (MTAs), which will add to previously reported genomic regions influencing GRD and the aphid vector resistance. To check repeatability, association analysis was implemented based on severity and the reported MTAs were found significant but with lower R^2 (data not shown).

Several research efforts have been directed at identifying regions controlling various agronomic traits to facilitate marker-assisted selection in groundnut improvement (Lei *et al.*, 2006; Hou *et al.*, 2007; Yanbin *et al.*, 2009; Sujay *et al.*, 2012). The traits include diseases such as rust, early and late leaf spot, but few efforts have been directed towards GRD and its aphid vector. Most of the highly significant MTAs were mapped on the B sub-genome, suggesting that this sub-genome carries more genes of GRD resistance than the A subgenome. In contrast, Pandey *et al.* (2017) identified 42 QTLs linked to resistance to other diseases, where most of them were mapped on the A sub-genome. Markers linked to the aphid vector were identified by Herselman *et al.* (2004), which explained up to 79.06% of the total phenotypic variation and were located on chromosomes A01, A02, A03 and A04, and the current study also identified one MTA located at A03. Pandey *et al.* (2014) reported two markers linked to GRD resistance which explained up to 39.29% of the total phenotypic variation and were located on chromosome B04 while in the current study, no MTA was mapped on this chromosome. Divergence on type of markers and populations used could be the cause of these differences, since Pandey *et al.* (2014) used SSRs while SNPs were used in the current study.

5.5 Conclusions

Out of the evaluated genotypes, two were highly resistant, twenty were resistant and three were susceptible, across the environments. Yield varied and ICGV-SM 15605, ICGV-SM 15621, ICGV-SM 15618, ICGV-SM 15604 and ICGV-SM 15615 were among the resistant and high yielding lines. Strong negative correlations between GRD incidence with seed yield and number of pods per plants were observed, indicating the negative effect of GRD on seed yield.

Twenty-two highly significant marker-trait associations were identified, which will add to previously reported genomic regions influencing GRD and the aphid vector resistance, to be used for marker-assisted selection in groundnut breeding programmes.

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Appendix 5.1: Soil analytical data for the field and glasshouse trials

	Environment	
Parameter	Field	Glasshouse
Soil texture class	Sandy clay	Sandy loam
pH (CaCl2)	4.66	5.26
pH (H2O)	5.42	6.02
Organic carbon (%)	1.51	2.38
Organic matter (%)	3.07	4.83
Estimated N (%)	0.15	0.24
Total N (%)	0.70	0.60
Phosphorous (ppm)	15.08	10.57
Potassium (meq/100g)	0.45	0.49
Calcium (meq/100g)	9.62	10.42
Magnesium (meq/100g)	2.44	3.32
Sodium (meq/100 g)	0.23	0.23
Copper (ppm)	0.35	0.28
Zinc (ppm)	0.21	0.43
Manganese (ppm)	7.34	7.02
Iron (ppm)	9.63	54.74
Sulphur (ppm)	53.07	126.27

Appendix 5.2: Mean percentage of disease incidence and transformed disease severity index of RILs, parental genotypes and controls across field and glasshouse environments

	Percentage of disease incidence			Transformed severity		
Genotype	60 DAS	80 DAS	100 DAS	60 DAS	80 DAS	100 DAS
ICGV-SM 15604	5.75	6.83	10.76	0.89	0.86	1.11
ICGV-SM 15605	6.44	11.14	18.22	0.85	0.88	0.91
ICGV-SM 15606	7.25	8.59	13.76	0.83	0.82	1.01
ICGV-SM 15607	3.85	5.50	8.65	0.81	0.81	0.80
ICGV-SM 15610	5.13	9.36	14.79	0.87	0.95	1.00
ICGV-SM 15611	10.41	11.10	17.03	1.06	1.04	1.26
ICGV-SM 15612	4.76	4.85	9.97	0.82	0.87	0.98
ICGV-SM 15615	6.81	8.23	17.61	0.88	0.92	1.04
ICGV-SM 15617	7.69	8.65	13.13	0.78	0.84	0.88
ICGV-SM 15618	6.05	10.58	10.96	0.89	0.90	0.93
ICGV-SM 15621	5.40	9.34	11.82	0.83	0.94	1.18
ICGV-SM 15622	4.42	9.50	15.58	0.91	0.80	0.88
ICGV-SM 15623	8.30	10.61	21.59	0.95	1.07	1.20

ICGV-SM 15624	12.51	14.60	18.83	0.95	0.99	1.17
ICGV-SM 15627	6.09	12.41	23.73	0.81	0.91	1.19
ICGV-SM 15629	13.16	18.19	23.70	1.03	1.09	1.25
	Percentage of disease incidence			Transformed severity		
Genotype	60 DAS	80 DAS	100 DAS	60 DAS	80 DAS	100 DAS
ICGV-SM 15630	13.36	15.86	19.24	1.08	1.18	1.26
ICGV-SM 15631	0.00	0.00	11.46	0.69	0.69	0.74
ICGV-SM 15632	12.35	14.31	13.99	0.97	0.98	1.14
ICGV-SM 15633	8.95	7.89	21.19	0.94	1.14	1.26
ICGV-SM 15635	7.33	12.90	17.87	1.00	1.04	1.20
Parents						
Nsinjiro (female)	18.76	23.64	25.47	1.21	1.38	1.43
Chalimbana (male)	58.59	62.42	72.77	1.50	1.55	1.62
Controls						
CG7	39.83	53.22	59.23	1.33	1.49	1.54
JL24	55.84	62.87	73.20	1.64	1.70	1.74
Mean	13.16	16.50	22.58	0.98	1.03	1.15
Genotype MS	737.3***	909.28***	931.70***	0.18***	0.23***	0.19***
Environment MS	1774.25***	2828.59** *	4573.44***	0.22***	0.1**	0.06ns
Gen X Env MS	38.58***	30.07***	63.75***	0.02ns	0.02ns	0.03ns
LSD (5%)	3.85	4.80	8.60	0.17	0.17	0.18
SED	1.89	2.36	4.20	0.08	0.08	0.09
CV (%)	20.32	20.18	18.69	11.72	11.19	10.78

Significant levels: ns, **, ***-non-significant differences, significant differences at 1% and 0.1%, respectively; MS-mean square, LSD-least significant difference, SED-standard error of differences and CV-coefficient of variation, DAS-days after sowing.

CHAPTER 6 GENERAL RESEARCH OVERVIEW

6.1 Introduction

Groundnut (*Arachis hypogea* L.) is a popular oilseed crop worldwide with an important role as both food and cash crop. In Malawi and other developing countries, it is mainly grown by smallholder farmers under low-inputs with average yield ranging between 700 and 1000 kg ha^{-1}, which is about 67-77% below the yield registered in the major groundnut-producing countries (3000 kg ha^{-1}). Several abiotic, biotic and socio-economic aspects constrain the crop production, and groundnut rosette disease (GRD) which can cause up to 100% yield losses in

susceptible cultivars, is among the major constraints. The use of resistant cultivars is the most viable method to control the disease, therefore, breeding for high yielding and GRD resistant cultivars is needed and should be a priority. This chapter provides a summary of the study findings and their implications in developing high yielding and GRD resistant cultivars which will contribute to the improvement of groundnut production in Malawi and other developing countries.

The objectives of the study were to:

a) Determine genetic variability for GRD response and yield traits in selected groundnut accessions under natural infestation
b) Assess the relationship between seed yield and its related traits, and analyse agromorphological diversity in selected groundnut accessions under natural GRD infestation
c) Evaluate groundnut recombinant inbred lines for resistance to GRD and perform SNP marker-trait association analysis.

6.2 Summary and implication of the findings

6.2.1 Assessment of groundnut accessions for genetic variability under natural rosette infestation

There were highly significant differences among the accessions for yield and GRD related traits. The environmental conditions were conducive for GRD development with disease incidence ranging between 4.09 and 69.18%, and seed yield varying from 53.60 to 1046.40 kg ha^{-1}. Out of the evaluated genotypes, two were highly resistant, 12 were resistant, 11 were moderately resistant and three were susceptible. ICG 12988 was highly resistant and the highest yielding accession. The accessions with resistant responses can be used as parents for GRD resistance breeding programmes. Seed yield, number of pods per plant, plant height, GRD incidence and number of secondary branches showed high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV). Moderate variation (PCV and GCV) were observed for days to flowering and pod width while shelling percentage and days to maturity showed low variability. High heritability estimates coupled with high genetic advance were recorded for number of secondary branches, plant height, seed yield and final GRD incidence, indicating that phenotypic selection based on the mean would improve these traits. Improvement for number of primary branches and shelling percentage based on the evaluated

accessions would be limited since they have low genetic potential due to lack of variability, low heritability and genetic advance.

6.2.2 Correlation, path coefficient and genetic diversity analysis in selected groundnut accessions under natural rosette infestation

Correlation analysis revealed positive association between seed yield with number of pods per plant, plant height, shelling percentage, hundred seed weight and number of primary branches. GRD incidence showed negative correlation with seed yield and yield related traits. Sequential path analysis indicated that high seed yield was directly associated with taller plant types, higher number of pods per plant and hundred seed weight, which were a result of higher pod width, lower GRD incidence and number of secondary branches. Thus, more weight should be given to these traits when improving seed yield in groundnut, particularly under GRD infestation. Cluster analysis revealed existence of diversity among the evaluated groundnut accessions and geographical origin did not have any influence on the clustering pattern. Principal component analysis generated three components which cumulatively explained 77.44% of the total variation among the accessions. PCA biplot was effective in showing the genetic distance among the accessions with results consistent to those of the cluster analysis. The estimated Shannon-Weaver diversity indices for qualitative traits were high, indicating the existence of high diversity among the selected accessions and agreeing with results from cluster and principal component analysis.

6.2.3 Evaluation of groundnut recombinant inbred lines and SNP based marker-trait association analysis for resistance to rosette disease

Analysis of variance revealed significant differences among the lines in all recorded traits indicating the existence of genetic variability and possibility of effective selection. There was a significant interaction between genotype and environment for disease incidence, and the higher incidence values were recorded under glasshouse conditions. ICGV-SM 15605, ICGVSM 15621, ICGV-SM 15618, ICGV-SM 15604 and ICGV-SM 15615 were among the resistant and high yielding lines. The study identified 22 highly significant marker-trait associations, which will add to previously reported genomic regions influencing GRD and the aphid vector resistance. These markers will be useful in classifying groundnut germplasm based on the GRD response and for their use in marker-assisted selection, once validated.

6.3 Conclusions and recommendations

The overall objective of the research was to assess variability, and generate new genetic resources and information relevant for GRD resistance breeding in Malawi. The study revealed existence of genetic variability for the recorded traits and presence of genetic diversity in the groundnut accessions, providing opportunity for parent selection that can be used for breeding high yielding and GRD resistant cultivars. It is recommended to select for maximum number of pods per plant, taller plants, higher seed weight, larger pods and minimum GRD or any other disease incidence when improving yield in groundnut. GRD resistant and high yielding lines were identified and it is recommended that further evaluations be conducted on these lines, particularly under GRD free-environment, to determine their yield potential. SNP markers linked to GRD were identified and their validation is recommended before large-scale application.