EVALUATION OF THE PERFORMANCE OF GROUNDNUT GENOTYPES AND THEIR RESISTANCE TO GROUNDNUT ROSETTE VIRUS

By

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THESIS ABSTRACT

The groundnut or peanut is one of the important legume crops of tropical and semi-arid tropical countries, where it provides a major source of edible oil and vegetable protein. The crop is mainly grown by smallholder farmers with little inputs, resulting in low yields of 700 kg/ha compared to Asia and south America which records 3500 kg/ha and 2500 kg/ha respectively. The low yields are due to a number of abiotic and biotic factors with diseases being a major constraint. Amongst the diseases, groundnut rosette disease can cause up to 100% yield loss when infection occurs. The objectives of this study were to; (i) evaluate the ICRISAT elite lines for rosette resistance using artificial inoculation, (ii) determine the effect of genotype by environment interaction of landraces and elite lines and select for stability and high yield, and (iii) determine the genotype by trait interaction for the landraces so as to select potential genotypes for use as parents in the breeding programme. To achieve objective one, glasshouse and field inoculation experiments were conducted using the infector row technique. In the glasshouse, the results revealed that ICGV SM 08503 and ICGV SM 01514 were resistant and showed 0% disease incidence while ICGV SM 01711, ICGV SM 09547, ICGV SM 09537, ICGV SM 08501 and ICGV SM 09545 showed moderate resistance with scores ranging from 1.1 to 1.7. ICGV SM 02724, ICGV SM 10005 and ICGV SM 08560 showed high susceptibility with scores as high as 4.6. However, the susceptible genotypes ICGM SM 10005, ICGV SM 02724 and ICGV SM 08560 showed low incidences of the disease in the field evaluation. At 60 days after sowing (DAS), the incidence ranged from 9.9% to 16.5% while at 80 DAS, it ranged from 18.6% to 23.8%. The highest score for disease incidence at 100 DAS was 27.3% for genotype ICGV SM 08560. The rest of the genotypes had 0% incidence. The yield per hectare ranged from as low as 0.32 ton/ha to as high as 1.03 ton/ha. ICGV SM 10005 recorded the lowest yield while ICGV SM 01711 was the highest yielding genotype with 1.03 ton/ha. For the genotype x environment study, a total of 11 groundnut genotypes from ICRISAT comprising of nine elite lines and two released cultivars as controls were evaluated over ten environments spread across the three agro-ecological zones of Zambia in the 2016/17 season. Additive main effect and multiplicative interaction (AMMI) and genotype and genotype by environment interaction (GGE) biplot models showed that ICGV SM 01711 and ICGV SM 02724 were high yielding recording 2.08 t/ha and 1.99 t/ha, respectively, compared to the average mean of 1.67 t/ha across all environments and showed relative stability. ICGV SM 10005 and ICGV SM 08560, which are Spanish genotypes, yielded 1.67 t/ha and 1.60 t/ha, respectively, compared to Luena (control) which yielded 1.23 ton/ha. ICGV SM 10005 had better relative stability over ICGV SM 08560 and Luena.

Genotype x trait analysis, correlation and path coefficient analysis on a total of eight landraces, two pre-released cultivars and five released cultivars showed a strong and highly significant correlation for grain yield with number of pods per plant, yield per plant, shelling percentage and 100-seed weight with r values of 0.86, 0.90, 0.94 and 0.23, respectively, at $P<0.001$ but 100-seed weight's correlation was not significant. The path coefficient analysis revealed that yield per plant, shelling percentage, number of pods per plant, 100-seed weight and days to maturity had a positive direct effect on grain yield while days to flowering had negative direct effect on grain yield. Genotype by trait (GT) biplot captured 83.00% of the variation due to genotype by trait interactions. Two land races, Kasele and Chalimbana performed relatively well in relation to MGV 4 and it was recommended that these could be hybridized with genotypes that have complementary features so that beneficial alleles are combined for improvement of the crop, while genotypes ICGV SM 01514, ICGV SM 01711 and Chishango can be used as sources of resistance genes.

DECLARATION

I, **Lutangu Jethrow Makweti**, declare that,

1. The research reported in this thesis, except where otherwise indicated is my original work.
2. This thesis has not been submitted for any degree or examination at any university.
3. This thesis does not contain other person' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing unless specifically acknowledged as being sourced from other researchers.
    a. Where other written sources have been quoted, then their words have been rewritten or rephrased, but the general information attributed them has been referenced.
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5. This thesis does not contain text, graphic or tables copied from the internet unless specifically acknowledged and the source being detailed in the thesis and in the reference sections

Signature…… Date…07/04/2018……

Lutangu Jethrow Makweti

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

Signature ……Date…08/04/2018……

Dr Julia Sibiya (Main Supervisor)

Signature……Date…….

Dr Augustine Gubba (Co-Supervisor)

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DEDICATION

The dissertation is dedicated to my parents, Mr Fredrick Makweti and Mrs Christine Makweti, my wife, Mayamiko Banda Makweti and our two lovely daughters Lilato and Liseli.

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LIST OF ACRONONYMS AND ABBREVIATIONS

AEA	Average Environmental Axis
AEC	Average Environmental Coordinate
AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of Variance
CSO	Central Statistics office
DAS	Days after Sowing
DTF	Days to Flowering
DTM	Days to Maturity
DIV	Disease Index Value
FAO	Food Agriculture Organisation
FAOSTAT	Food Agriculture Organisation Statistics
GEI	Genotype by Environment Interaction
GGE	Genotype and Genotype by Environment
GRAV	Groundnut Rosette Assistor Virus
GRD	Groundnut Rosette Disease
GRV	Groundnut Rosette Virus
GT	Genotype by Trait
GXE	Genotype by Environment
GYLD	Grain Yield
HSW	Hundred Seed Weight
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IPCA	Interaction Principal Component Analysis
MAL	Ministry of Agriculture and Livestock
METs	Multi Environment Trials
PCA	Principal Component Analysis
PDI	Percentage Disease Index
PPP	Pods Per Plant

RCBD	Randomised Complete Block Design
RT-PCR	Reverse Transcription Polymerase Chain Reaction
Sat RNA	Satellite Ribonucleic Acid
SSA	Sub-Saharan Africa
SH	Shelling percentage
TAS-ELISA	Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay
YPP	Yield Per Plant

CHAPTER ONE

THESIS INTRODUCTION

1.1 Background

Groundnut or peanut (*Arachis hypogaea* L.*)* is one of the important legume crops of tropical and semi-arid tropical countries, providing a major source of edible oil and vegetable protein. Groundnut seeds contain 47-53% oil and 25-36% protein. The crop is cultivated between 40ºN to 40ºS of the equator. The productivity of groundnuts varies from 3500 kg/ha in the United States of America to 2500 kg/ha in South America and Asia and less than 1000 kg/ha in Africa (Prasad et al., 2010). The crop is mainly grown in Asia and Africa and is characterised by low inputs, grown under rain-fed conditions by smallholder farmers with little or no mechanisation. It is mostly grown as a sole crop, mixed or intercropping and has low productivity (700 to 1000 kg/ha). The low yields are due mainly to various abiotic and biotic constraints. Abiotic stresses of prime importance include temperature extremes, drought stress, soil factors such as alkalinity, poor soil fertility and nutrient deficiencies while common biotic stresses include diseases like groundnut rosette disease (GRD), early and late leaf spot and rust (Nigam, 2014).

1.2 Importance of groundnuts in Zambia

Groundnut (*Arachis hypogaea* L.) is the second most important legume after beans in Zambia and second most grown crop after maize according to the 2015/16 crop forecast survey report (MAL and CSO, 2016). The local groundnut varieties grown are of the tan colour, early, medium to late maturing type and vary in seed size, e.g., Chalimbana which is big seeded and Solontoni which is small seeded. There are also some improved varieties like Chishango, MGV 4 and MGV 5 that are grown.

Groundnut seeds are rich in oil and protein and also a source of dietary fibre, minerals and vitamins. Groundnuts is now commonly been used as an affordable source of protein compared to animal protein (Savage and Keenan, 1994). The seeds are eaten raw, roasted, blanched, made into peanut butter or powdered and added to different vegetables or crops to form traditional dishes e.g. pumpkin leaves with groundnuts or porridge which is very nutritious. Groundnut straws are commonly used as animal feed. The crop is used in soil improvement as it fixes nitrogen into the soils and it is also used for cooking oil extraction. The crop requires less inputs in terms of fertilizers and it is therefore suitable for cultivation by

smallholder farmers (Smartt, 1994). It is a good cash crop that gives high returns on a small area and with all the listed uses, groundnut is a good crop for local and international trade (Okello et al., 2010).

In terms of exports, Zambia is still lagging behind as it exports very little compared to what is produced annually. According to FAO (2014), the highest export records were in 2003 with approximately 1, 800 metric tonnes while 2009 recorded a low, with less than 200 metric tonnes being exported (Figure 1.1). Aflatoxin, a toxin produced by the fungus called *Aspergillus flavus* and grows in soils and grains like groundnuts has been one of the major challenges to export trade in Zambia, as most of the groundnuts are harvested from small scale farmers recording aflatoxin levels beyond the acceptable international standard (Njoroge et al., 2016).

Figure 1.1 Zambia groundnut export trends over years from 2003 to 2013

Source: FAOSTAT (2017)

1.3 Production of groundnuts

In 2014, the crop was grown under a total area of 26.5 million hectares globally with an estimated production of 43.9 million tonnes (unshelled) and an average yield of 1.65 t/ha. Africa and Asia are the major producers of groundnuts worldwide with an estimated 90% share of the production. Most of the crop is produced under rain fed conditions by small scale farmers, with Asia having produced 25.6 million metric tonnes while Africa produced 13.9 million metric tonnes in 2014. Asia had a great average yield per hectare production of 2.4 t/ha while Africa recorded only 0.95 t/ha (FAO, 2014). Zambia is ranked 29th in the world in terms of groundnut production with a production of 143,591 metric tonnes (0.3% of world

production) and an average yield of 0.59 t ha^{-1}. China topped the world`s top ten producers of groundnuts in 2014, contributing 43% of the world`s total production (Figure 1.2)

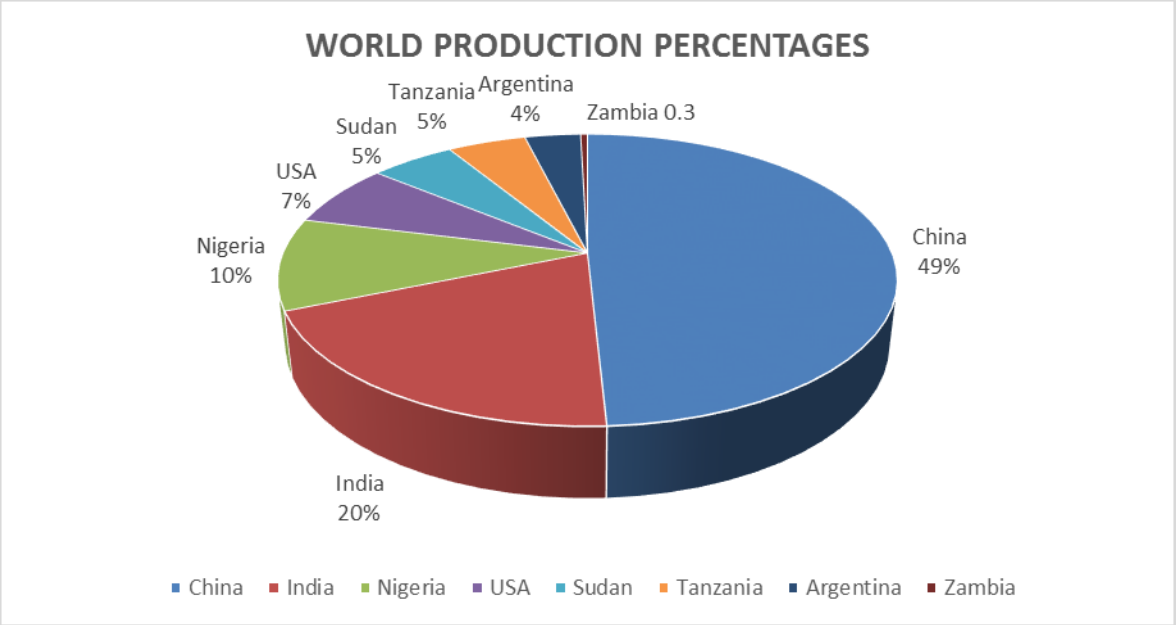

Figure 1.2 Chart showing the world's top 10 producers of groundnuts

Source; FAOSTAT (2017)

In 2015/16 season, Zambia produced 131,562 metric tonnes of groundnuts from 222,952 ha (Figure 1.3) with most of the production coming from eastern and northern provinces of the country (MAL and CSO, 2016).

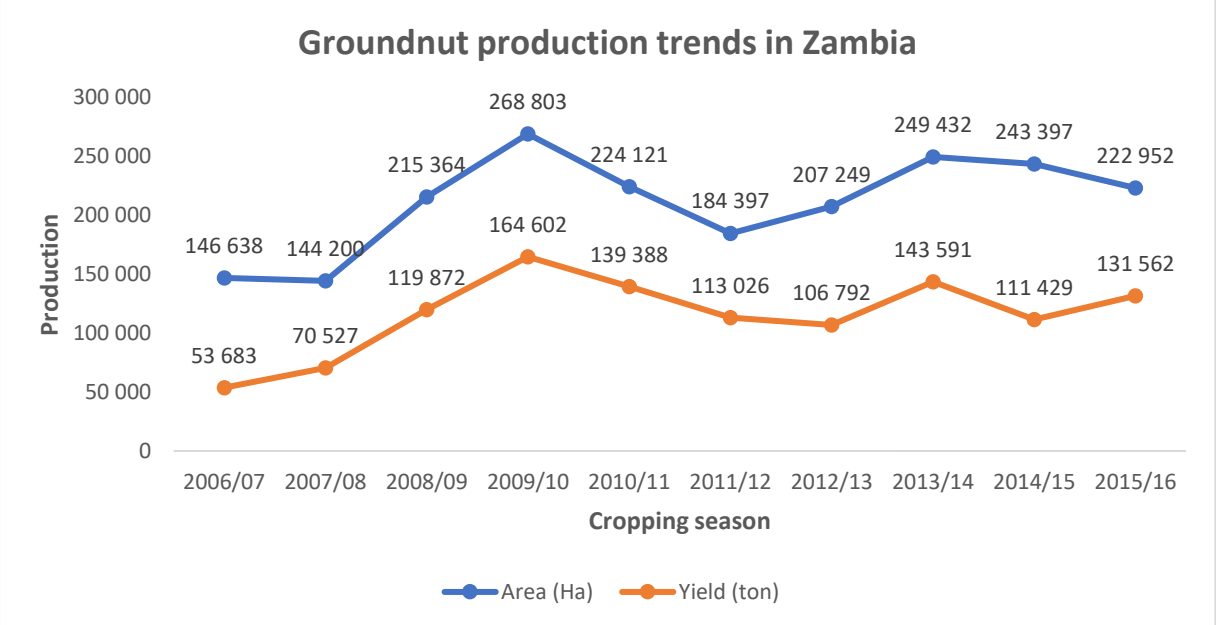

Figure 1.3 Area under production (ha) and production in metric tonnes in Zambia

Source: MAL/CSO (2017)

1.4 Production Constraints

With all these listed benefits of groundnuts to farmers and consumers, there are several factors that affect its production and these are grouped into biotic and abiotic factors. Reddy et al. (2003) reported that almost two thirds of the production areas worldwide do not receive enough rainfall leading to drought stress, thus affecting yield and quality of groundnuts. Declining soil fertility levels due to poor crop management practices and low levels of fertilizer application are also a major challenge for the groundnut industry (Minde et al., 2008). Important biotic factors include diseases and pests. Notable among them is early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Cercosporidium personatum*). According to Liu et al. (2013), these diseases affect groundnuts in all the growing regions. In addition, a combination of rust (*Puccinia arachidis*) and late leaf spot can cause yield losses ranging between 50-70% in Africa and India (Khedikar et al., 2010). The other major disease which is endemic to Sub-Saharan Africa (SSA) and can cause great destruction during an epidemic is groundnut rosette disease (GRD).

1.5 Groundnut rosette disease

This disease is rated as the most destructive viral disease affecting groundnuts and is known to cause about 30% yield loss in farmers' fields annually (Naidu et al., 1999). Three causal agents have been reported to be responsible for GRD development: *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and a satellite-RNA (Sat-RNA) which all have to be present for successful transmission of the virus by the aphid vector. In the event that they do not occur together, GRAV or GRV will only cause infection that will show no symptoms (Alhassan, 2013). Plants that are infected show short internodes and thick stems, while the leaflets will have small chlorotic, twisted and a distorted appearance (Bock et al., 1990). The disease is very destructive and can cause up to 100% yield losses (Adu-Dapaah et al., 2004). Huge economic losses were reported in Zambia and Malawi during 1994-1995 seasons where approximately 43,000 ha in eastern Zambia were affected leading to a loss of approximately $4.89 million and a production reduction of 23% in Malawi (Iwo and Olorunju, 2009). It reduces yield and increases the production cost. With its unpredictable nature, it has been known to cause almost US$156 million losses across Africa (Ntare et al., 2001). The level of yield loss depends on the stage at which the crop is infected, with seedling infection being the most devastating (Waliyar et al., 2007).

1.6 Problem statement and justification

The environment has a great effect on the performance of any cultivar resulting in different phenotypic expressions and performance of genotypes across different environments

(Crossa, 1990). The interaction between the performance of the cultivar as determined by its genetic composition and the environment can greatly influence its performance (Ding et al., 2007; Yan and Wu, 2008). Most cultivars in Zambia are adapted to Region II and partly Region III though there are problems with “pops” in region III due to highly leached soils caused by heavy rainfall. Region I is characterised by low rainfall and high temperatures making groundnut production a challenge. This situation requires cultivars that have broad adaptability and some that are specifically bred for certain environments to increase the groundnut production in Zambia. Many breeders have used the testing of genotypes across different locations over years and seasons to evaluate the stability of genotypes across the environments. This process helps breeders to develop breeding strategies that can help selection of superior cultivars for target environments (Kang, 2002).

Studies have shown that GRD can be controlled effectively through chemical applications and cultural practices, e.g., timely planting and recommended spacing (Davies, 1975). However, this is not practiced and many farmers cannot afford pesticides, leaving breeding for varieties with genetic resistance as a potentially more promising solution (Ntare et al., 2001). Control of the disease using host resistance has been used over the years with many varieties having been developed by different breeders, but in most cases, these varieties have turned out not to be resistant to all three causal agents. They are usually susceptible to GRAV indicating the lack of complete resistance and many cases have been cited where resistance has broken down Olorunju et al. (2001) and Bock et al. (1990).

Some landraces have been known to carry resistance to various foliar diseases. Olorunju et al. (2001) reported that breeding for GRD resistance was first initiated in the 1950s in West Africa, using landraces from Burkina Faso and Ivory Coast as their first sources of resistance. This resulted in development of long and early maturing resistant lines. Out of 31 lines that were evaluated for resistance to bacterial wilt by Jiang et al. (2007), 21 were landraces and only 2 out of 21 were susceptible to bacterial wilt.

In Zambia, there is high adoption and use of landraces that has contributed to low production of groundnuts and low average yields (Mofya-Mukuka and Shipekesa, 2013). These cultivars have low levels of disease resistance and low yields. An improvement of these cultivars can help boost production of groundnuts, as there will be high adoption since farmers are already familiar with the cultivars. However, before any objectives can be set in any breeding programme, the underlying causes must be understood and then a breeding strategy established for successful breeding. Therefore, assessment of suitable parents based on their differences is key to any breeding programme. Cultivated groundnuts have high levels of diversity agronomically, morphologically and physiologically and variability is available among the related wild diploid species. Even with these levels of diversity, groundnut breeders have

not fully exploited the genetic resource potential, which has led to the narrowing of the genetic base (Herselman, 2003). The crossing of elite by elite genotypes has led to reduced genetic gain and thus landraces can be a source of variation when they are included in the breeding programme (Khera et al., 2013).

1.7 Objective

It is against this background that this study was conducted. The main objective of the study was to evaluate elite and landrace lines and select those with resistance that are adapted to different environments, stable and with acceptable agronomics and yield traits preferred by farmers.

1.8 Specific objectives

The specific objectives were to:

- Evaluate elite lines for rosette resistance using artificial inoculation
- Study the genotype by environment interaction of landraces and elite lines and select for stability and high yield.
- Conduct a study on the genotype by trait association for the landraces to select potential genotypes for use as parents in the breeding programme.

1.9 Research Hypotheses

The following hypotheses were tested:

- There are significant differences in resistance to groundnut rosette disease among the ten test lines
- There are significant differences in yield across the 11 test lines across the ten test environments
- There is a significant relationship between grain yield and secondary traits

1.10 Dissertation outline

The dissertation is organised into five chapters with a journal paper design. With such a format, some unavoidable repetition in the references and some overlaps in introductory information between chapters will be observed. The referencing format used is based on the Crop Science journal style. The structure of the dissertation is as follows:

Chapter 1: Introduction

Chapter 2: Literature review

Chapter 3: Evaluation of elite groundnut lines for resistance to groundnut rosette disease

Chapter 4: Genotype by environment interaction analysis of elite groundnut genotypes for grain yield across diverse agro-ecological regions of Zambia

Chapter 5: Genotype by trait association analysis of Zambian groundnut landraces

Chapter 6: Overview of the study

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CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

In this chapter, a number of topics are covered that relate to the objectives of the study. The chapter covers the origin, spread and diversity of groundnuts and its biology. It looks at the production trends in the world and Zambia in particular and the factors that affect the production of groundnuts. A detailed account of groundnut rosette virus disease, with focus on the epidemiology, causal agents, management, diagnosis and the vector that is responsible for the disease are given. Progress that has been made in combating GRD is also highlighted. A review of the genotype by environment interaction, and genotype by trait and how effective they are in selecting the best genotypes across the different test environments will be presented.

2.2 Origin, spread, centre of diversity and taxonomy

Groundnut belongs to the *Leguminosae* family, tribe *Aeschymanomeneae*, subtribe *Stylosanthineae.* The genus and species names *Arachis hypogaea* are derived from a Greek word, *arachos,* meaning weed, and *hypogea,* meaning underground chamber. The most distinguishing feature from other plants is the ability of its peg to grow underground (Holbrook and Stalker, 2003; Stalker and Simpson, 1995).

Groundnut is known to have originated from South America, mostly likely the coastal areas of Peru where evidence of ancient cultivation has been chronicled by archaeologists (Stalker, 1997). The early Portuguese sailors are reported to have carried the two seeded groundnuts in the late 15th century to Africa, India and the Far East, while the Spaniards are believed to have carried the three seeded types in the early 16th century to Indonesia, China, and Madagascar. By the mid-16th century, groundnuts had managed to reach North America from Africa via slave trade as well as the Caribbean islands, Central America and Mexico. By the 19th century, groundnuts had become an important crop in West Africa, India, China and the USA (Hammons, 1994; Nigam, 2014).

The species of genus *Arachis* are perennial or annual legumes and made up of a large and diverse group of diploid (2n = 2x = 20 or 18) and allotetraploid (2n = 4x = 40) (Burow et al., 2008; Stalker, 1997). The genus *Arachis* comprises of 80 species which are further divided into nine sections: *Arachis, Caulorrhizae, Erectoides, Extranervosae, Heteranthae, Procumbentes, Rhizomatosae, Trierectoides,* and *Triseminatae* (Valls Jose and Simpson,

2005) and only *A. hypogaea* is domesticated and widely distributed around the world (Holbrook and Stalker, 2003). Cultivated groundnut has been botanically classified into two subspecies, which mainly differ in their branching pattern with subspecies *hypogaea* having alternate branching and subspecies *fastigiata* with sequential branching. Each subspecies is further divided into two botanical varieties; subsp. *hypogaea* into var. *hypogaea* (Virginia) and var. *hirsuta* and subsp. *fastigiata* into var. *fastigiata* (Valencia), var. *vulgaris* (Spanish), var. *peruviana* and var. *aequatoriana* (Kumar, 2004)

The lack or presence of flowers and regularly alternating vegetative and reproductive nodes on branches among the subspecies is used to morphologically differentiate the subspecies. In both botanical varieties, the *hypogaea* does not have the floral axes and branches on the main stem. There are different growth habits (spreading, intermediate and erect) while the size of the primary branches differs from the main stem. They have simple inflorescence and modest to prolific vegetative branches with interchanging pairs of vegetative and reproductive axes on branches. This subspecies typically has two seeds in each of the pods whose beak is not very noticeable, while the size of the seed is either intermediate or large. The testa colour generally is tan but other colours do exist (red, white, purple etc.). They have seed dormancy and are medium to late maturing (Ntare et al., 2008). On the other hand, the *fastigiata* has floral axes on the main stem with an uneven arrangement of vegetative and productive branches. The reproductive parts are predominantly on branches with simple inflorescence. They have an upright growth type with primary branches been shorter than the main stem. With a less prominent to no pod beak arrangement, this subspecies has two to four seeds per pod while the seed colour is same as the *hypogaea* with the only difference been the seed size which is medium to large. The treated seed dormancy is very little (Ntare et al., 2008).

2.3 Factors affecting production and yields

Groundnut production in Sub-Saharan Africa (SSA) is affected by several biotic and abiotic stresses like pests, diseases, drought, aflatoxin that can cause huge yield losses if not attended to. Among these constraints, diseases top the list on the major causes of yield losses in SSA (Chiteka et al., 1991; Maiti, 2002). Reddy et al. (2003) reported that almost two thirds of the production areas worldwide do not receive enough rainfall leading to drought stress. This leads to groundnuts being affected in terms of yield and quality. Declining soil fertility levels because of poor crop management practices and low levels of fertilizer application has also become a major challenge for the groundnut industry (Minde et al., 2008). Another factor which is biotic and spread worldwide is diseases and pests. Notable among them all is early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Cercosporidium personatum*). According to Liu et al. (2013), these diseases affect groundnuts in all groundnuts growing

regions. A combination of rust (*Puccinia arachidis*) and late leaf spot can cause yield losses ranging between 50-70% in Africa and India (Khedikar et al., 2010). The other disease which is endemic to Sub-Saharan Africa (SSA) and can cause great destruction when an epidemic strikes is Groundnut rosette disease (GRD), a viral disease of groundnuts.

2.4 Groundnut rosette virus disease

2.4.1 Distribution of ground rosette virus disease in Africa

Groundnut rosette disease (GRD) was first reported in 1907 in present day Tanzania and it is one of the most important viral diseases of groundnuts in Africa with epidemics reported in West Africa in 1975 (Gibbons et al., 1985). In the 1994-1995 seasons in central Malawi and eastern Zambia, an epidemic affected approximately 43,000 ha in eastern Zambia, causing an estimated loss of $4.89 million, while in Malawi, groundnut production was reduced by 23%. It is estimated that US$156 million is lost across Africa per year to GRD because of the unpredictability of the disease occurrence that in many case is sporadic (Ntare et al., 2001; Iwo and Olorunju, 2009). There have been reports of GRD in other parts of SSA with major occurrences reported in Burkina Faso, Ghana, Nigeria, Malawi, Mozambique and Uganda (Ntare et al., 2001).

2.4.2 Disease epidemiology and transmission

The aphid, *A. craccivora* is the only known vector of GRD and it is also a vector of several other plant viruses. It transmits the virus in a persistent and circulative manner (Naidu et al., 1998). There are three causal agents responsible for GRD development: *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and a satellite-RNA (Sat-RNA). In order for a successful transmission to occur, the three agents must operate in unison in the host plant (Alhassan, 2013). If they do not all occur at the same time, either GRAV or GRV only will cause infection with no symptoms or temporal minor mottle symptoms. Sat RNA is mainly responsible for disease symptoms, while the different forms of sat RNA are responsible for the different forms of the disease (Murant, 1990). The host range of *A. craccivora* has been studied widely in efforts to describe how the virus carrying aphids are able to survive during and off-season. It was shown that the aphid infects different plant species but has a great affinity for plants belonging to the *Leguminosae* family which accounts for 47% of the worlds` known hosts (Naidu et al., 1998).

The aphid itself does not cause damage to the host plant except under drought, whereby they affect young plants (Singh and Oswalt, 1992). They reproduce at a fast rate leading to increased population within a short time. This rapid increase is dependent on the prevailing climatic conditions and the health status of the host plant (Naidu et al., 1999). Misari et al.

(1988) reported that the aphid acquired the virus from the source plant by sucking in the phloem sap and then transferring the virus to other plants for the rest of its 14-day life cycle. The virus sources can be young shoots, leaves, fruits, stems and inflorescence (Blackman and Eastop, 2007; Rathore and Tiwari, 2017). However, the aphid does not always transmit all the three causal agents; GRV, GRAV and Sat-RNA during this transmission process as this is depended on the feeding behaviour and the duration of the feeding. If the aphid manages to penetrate the phloem cells during feeding, it can transmit all the three agents but if the feeding time is short and there is no phloem cell penetration, only GRV and Sat-RNA will be transmitted (Waliyar et al., 2007). The transmission of GRD is not possible if the source plant does not have GRAV because GRAV has a coat protein, which is for encapsulation (Murant, 1990; Naidu et al., 1999; Okusanya and Watson, 1966). Dubern (1980) in his study on the transmission efficiency of the two forms of GRD discovered that the acquisition access took 4.5 hours while the inoculation access took only 3 minutes. There was an 18 hours latent period and a minimum transmission time of 22.5 hours.

GRD is polycyclic in nature and once the plant is infected, it will act as a source of the inoculum, leading to rapid spread of the disease throughout the season. The wingless aphids are the ones known to be responsible for the initial spread of the disease (Waliyar et al., 2007). It is, therefore, imperative that the distribution, movement and source of inoculum is understood as this can help in making GRD epidemic predictions and appropriate measures for control and prevention put in place in time (Chintu, 2013).

2.4.3 Disease symptoms

Chlorotic and green rosette are the two common symptoms of rosette, with chlorotic type being common in SSA, while the green rosette distribution is unknown. Plants affected by the disease exhibit stuntedness in growth with bushy appearance (Naidu et al., 1998). However, field symptoms may vary depending on stage of infection, climatic conditions and presence of other viral infections (Naidu and Kimmins, 2007). Plants infected in the late growth stages show little symptoms in few of the branches. The symptoms may be other than the chlorotic and green symptoms (Naidu et al., 1999).

2.4.4 Disease diagnosis

The diagnosis of the disease maybe through visual assessment based on the symptoms exhibited by the infected plants or by mechanical inoculation onto a suitable indicator host such as *Chenopodium amaranticolor* which would show symptoms indicating the presence of GRV. However, this test is not always reliable because of the fluctuating temperatures in SSA (Naidu et al., 1999). With advancement in technology, improved diagnostic methods have been invented. A triple antibody sandwich enzyme-linked

immunosorbent assay (TAS-ELISA) can be used for GRAV diagnosis while for the diagnosis of each of the three GRD causal agents both in plants and aphids, a reverse transcription-polymerase chain reaction (RT-PCR) can be used (Waliyar et al., 2007).

Detection of GRV using visual assessment is not reliable as there have been cases reported where GRAV was detected in plants that did not show symptoms. Bock and Nigam (1988) reported observing GRAV antigens in 6 GRD resistant lines that had been exposed to aphid inoculation in Malawi. Olorunju et al. (1992) reported another case where GRAV was detected in 11 out of the 15 plants that showed no symptoms. Amoah et al. (2016) also concluded that symptoms alone are not reliable when screening for plants for resistance to the three causal agents of the disease after they discovered that all resistant lines tested positive for GRAV antigens.

2.4.5 Screening techniques

Over the years, different methods of screening for resistance have been employed. The most effective has been the infector row technique developed by Bock and Nigam (1987). This involves planting a test row of uninfected plants with rows of GRD infected plants on either side. Olorunju et al. (2001) screened different lines for resistance and by the end of three weeks after exposure to the inoculum, more than 95% of the susceptible lines were showing symptoms and the disease spread was very good both on the infector rows and the test lines indicating an even spread of the inoculum and effective screening. By the end of 20 days after exposure, the susceptible lines were showing 100% infection. Use of a good and efficient screening technique reduced the chances of any escapes. Others researchers including Amoah et al. (2016), Subrahmanyam et al. (1998), Subrahmanyam et al. (2001), Chintu (2013), Naidu et al. (1998) and many others have used this technique successfully.

Other methods for inoculation like grafting have been tested before (Bock and Nigam,1987), where both healthy and infected plants were used either as scions or as shoots and there were unexplained variations in the results. Olorunju et al. (1995) recommended the use of mechanical inoculation to screen for resistance. This involves grinding of infected leaves in a mortar mixed with 6.0 ml of buffer. The upper and lower part of the leaf is then dusted with carborundum and rubbed with inoculum using latex grooves and cheese cloth pads. Lower cases of escapes where recorded compared to earlier studies using infector row where some susceptible lines showed no symptoms (Olorunju et al., 1991). Olorunju et al. (1995) went on to recommend the use of mechanical inoculation but indicated that the field screening was time consuming and laborious as many infected plants had to be transplanted into the field. In as much as the mechanical procedure recorded less numbers of escapes, the procedure is time consuming and costly as chemicals have to be purchased. The number of lines that can

be evaluated using this procedure is also limited compared to the field screening using infector row technique.

2.4.6 Management and control

The use of pesticides in the control of GRD has been researched on and this method leads to reduced aphid population on plants. In delaying the spread of the disease and increase in aphid populations, different cropping practices have been found to be useful. Limited success has been achieved with each, and in recent years, efforts have focused on the use of different cropping practices and breeding for aphid and virus resistance for disease management (Davies, 1976; Naidu et al., 1999). Cultural practices are not practiced as many farmers cannot afford pesticides, leaving breeding for varieties with genetic resistance as a potentially more promising solution (Ntare et al., 2001).

2.5 Progress in managing rosette disease

Olorunju et al. (2001) reported that breeding for resistance was first initiated in the 1950s by the French Institut de Recherches pour les Huiles et Oléagineux (IRHO) in West Africa, using landraces from Burkina Faso and Ivory Coast as their first sources of resistance to GRD. These were used to breed for resistant cultivars and became the basis for resistance breeding in Africa. Through these efforts, long-duration varieties such as 69-101 (130 days to maturity), RMP 12, RMP 40 and RG 1 (140-150 days) and early maturing (90 days) Spanish (*A. hypogaea* L subsp. *fastigiata* var. *vulgaris*) were developed.

Through the years, a number of accessions have been screened for resistance to GRV and GRAV with several sources of resistance being reported (Subrahmanyam et al., 1998). Olorunju et al. (2001) conducted a study during the 1996 and 1997 growing season in which 2301 accessions from different sources and 252 advanced breeding lines derived the resistant crossing programme were evaluated. The infector row technique was used to screen these lines and 65 accessions were reported to have high levels of resistance, while 134 breeding lines were resistant. However, all disease resistant lines were susceptible to GRAV. According to Ntare et al. (2001), the major disadvantages of land race cultivars is that they take long to mature, usually 130-150 days thus making them prone to end of season drought. The available few early maturing cultivars that may be resistant are not preferred by farmers because of their poor agronomic traits leading to less adoption. The challenge then is to have short duration cultivars that are resistant with good agronomic traits like high yield that are adapted to SSA conditions.

ICRISAT launched a breeding programme in the early 1980s in Malawi for the development of resistant cultivars that are early maturing using the infector row technique for screening

genotypes. This technique leads to 99% infection of the susceptible plants (Ntare et al., 2003). Genotypes with resistance and yield higher than the susceptible genotypes by 19-93% under natural and high disease pressure have been developed and deployed to national breeding programmes in several SSA countries where they have been released while some are still being tested (Ntare and Olorunju, 2001; Ntare et al., 2002; Ntare et al., 2003). For example, in Zambia, ICGV SM 08503, ICGV SM 12991 and ICGV SM 90704 have been released as resistant lines and named as MGV 7, Katete and Chishango respectively while ICGV SM 01711 and ICGV SM 01514 are been tested by the variety release committee for possible release (SCCI, 2015).

All the released resistant cultivars and breeding lines that have been developed are not resistant to GRAV but only to GRV which leads to sat RNA resistance indirectly. Such genotypes do not develop symptoms (Bock et al., 1990; Naidu et al., 1999). GRV resistance does not offer immunity meaning under high disease pressure, the resistance breaks down (Bock et al., 1990). There have been reports of GRAV immunity present in wild species (Subrahmanyam et al., 2001; Subrahmanyam et al., 1998). This immunity can be transferred to cultivated groundnuts through conventional and molecular breeding approaches. Padgham et al. (1990) noted that aphid vector resistance is one area that can be exploited in the breeding programme as it has been identified in many existing breeding lines.

2.6 Genotype by environment interaction (GEI)

The efficiency of selection in any given breeding programme is dependent on the genetic variation that exists in a population (Falconer and Mackay, 1996). A lot of work has been done on yield improvement and one of the critical components of such research is adaptability and stability. The way the genotypes interact with the environments complicates the breeding and makes interpretation of experimental data and prediction very difficult, thereby reducing efficiency of selection. For quantitative traits like yield, the interaction can be due to changes in the ranking of the genotypes (Cooper and DeLacy, 1994). It is, therefore, imperative that a breeder knows the magnitude of the GEI in the development of cultivars, which have high yields and are stable across environments. The study of GEI in groundnuts can range from simple analysis of variance to more specific and complicated analysis (Amini et al., 2013). However, analysis of variance is not very informative when explaining GEI. It is for this reason than other statistical models such as regression and multivariate analysis have been developed and are more useful in understanding GEI (Ramagosa and Fox, 1993).

The additive main effects and multiplicative interaction (AMMI) and genotype, genotype by environment (GGE) method have been applied for analyzing multi-environment trials by many

researchers across different crops like maize (Kamut et al., 2013; Sibiya et al., 2012; 2013), in rice (Katsura et al., 2016), in soybean (Rao et al., 2002) and sorghum (Gasura et al., 2015) among many others. However, these models have not been fully exploited in groundnut breeding.

2.6.1 Additive main effects and multiplicative interaction (AMMI)

Plant breeders have to perform genotype by environment interaction (GEI) studies when testing cultivars across different environments. The biplot is a tool used to understand the GEI pattern graphically (Thillainathan and Fernandez, 2001). The AMMI is one of the most widely used statistical method and it is useful in understanding the pattern of interaction between genotypes and environments. The analysis provides information on the GEIs in the multi environment trials (METs) and is able to increase the accuracy of the yield estimates using the means. This will make recommendations more reliable and ensure repeatability thereby increasing the selection and genetic gain. The other output for the AMMI is that it helps in identifying the best genotypes across the different environments (Gauch, 2013; Hongyu et al., 2014; Kaya et al., 2002).

The breeder's goal is to choose genotypes that have good performance across different environments and poor analysis of inefficiency in the statistical model used to analyse GEI can give the breeder problems. The choice of the type of statistical method to use in the analysis of data depends on the type of data, the number of test environments and the accuracy of the data (Hongyu et al., 2014). Over the last 20 years, the AMMI models have been widely used in the analysis of yield trials and many researchers have published several reviews on AMMI and the GGE biplots (Dias and Krzanowski, 2006; Gauch, 2006; Gauch et al., 2008; Yan et al., 2007; Yang et al., 2009). The AMMI analysis is very useful in identification of high yielding genotypes that are stable and highly adaptable to different agro ecological zones, which can be useful in making recommendations for wide adaptation and the selection of useful testing sites (Gauch et al., 2011; Zobel et al., 1988).

The AMMI is able to separate the interaction components separately for each of the test environments (Bose et al., 2014). The principal components interaction (PCI) depend on the level of interactions that are regarded as significant (Kandus et al., 2010). The principal component analysis (PCA) is applied to evaluate the effect of the interaction from the additive ANOVA model. The plotting of the PCA scores in a biplot against each other provides the graphic examination and understanding of the GEI element. The combination of the biplot display and the stability analysis of the performance across different environments makes it easy to group the genotypes based on similarity of performance (Kaya et al., 2002; Thillainathan and Fernandez, 2001). However, the AMMI is not suitable for identifying

genotypes that are superior and to indicate which environments are suitable for the selected genotypes. This is where GGE biplot analysis becomes a very useful tool.

2.6.2 GGE biplot analysis

The GGE biplot is a data visualisation tool, which graphically displays GEI (Yan and Kang, 2002). It is an effective way of analysing mega-environment analysis e.g., “which-won-where” pattern, which makes it possible to recommend specific genotypes for specific mega-environments. This can be effectively used to evaluate environments and mean yield and stability of genotypes. This analysis tool is progressively being used in GEI data analysis in agriculture (Butron et al., 2004; Crossa et al., 2002; Dehghani et al., 2006; Kaya et al., 2006; Ma et al., 2004; Yan and Kang, 2003). GGE biplot analysis is a combination of tools from many other methods such as AMMI and regression (Ding et al., 2007). It enables the visualization of the row and column factors and the underlying factors in a simultaneously manner through its scatter plot arrangement. (Yan and Tinker, 2006). This makes it so useful in the evaluation of the genotype by environment and the identification of stable and adaptable genotypes (Ding et al., 2007). The genotypes regarded as being adaptable are those that perform relatively well across several environments. Ding et al. (2007) states that in the evaluation of environments, there is deduction of the discrimination ability of the environments and its representativeness of the ideal environment and this is what makes GGE such an important tool and lead to the increase in its application in agriculture (Yan and Tinker, 2006).

2.7 Genotype by trait association

In every breeding programme, objectives are set and these objectives will help decide which traits to focus on to achieve the set goals. One of the most complex traits is yield as it is highly affected by the environments and other undesirable linkages and associations with other traits (Yan, 2014). This makes selection based on yield only a very ineffective approach. Therefore, conducting trials across different diverse environments for different traits is an integral part of any breeding programme (Yan and Kang, 2002; Yan and Rajcan, 2002). This type of evaluation requires careful interpretation of the results and understanding of how it is useful to breeding and this is one of the major challenges breeders face (Yan and Kang, 2003). The different relationships that exits among the traits have a great impact on the decision as to which type of breeding selection method to use. It is common to find negative correlation among important traits in plant breeding which leads to selection challenges (Lewis, 2006). Xu-Xiao et al. (2008) stated that variation among the genotypes within and among populations is essential in breeding and these variations in terms of agronomic and plant structure traits should be carefully studied (Rubio et al., 2004).

Over the years, a number of methods have been applied in order to understand how traits are related across different crops by many researchers (Rao et al., 2014, Rubio et al., 2004; Sarwar et al., 2004; Shoba et al., 2012; Thakur et al., 2014; Tiwari et al., 2011) including use of genotype by trait (GT) biplot analysis for trait profiling. This tool uses the GGE technique to graphically display the GT interaction and allows for the establishment of the relationships that exist among the traits across the test genotypes (Yan and Frégeau-Reid, 2008; Yan and Rajcan, 2002). The GT biplot is able to provide information on the traits that are redundant and those that are useful and this information is helpful in identifying those traits that exhibit direct or indirect effect of the trait of interest. This informative way of collecting data on genotypes has been applied in other others legume crops like soybean (Yan and Rajcan, 2002), common bean (González et al., 2006), cowpea (Oladejo et al., 2011) and in cereals like wheat (Ali et al., 2008).

Correlation is another tool that is used to show the associations that exist among the traits and how they are related to the trait of interest. This is important because there is lack of consistence in how yield components relate to yield which makes breeding cultivars that are stable in terms of performance across different environments difficult (Shenkut and Brick, 2003). For any selection criteria to be useful, the traits under study must have high correlation with seed yield and display a low GE interaction coupled with high heritability (Rao et al., 2002; Yuan et al., 2002).

2.7.1 Correlation and path coefficient analysis

Knowing the associations that exist between or among traits is an important part of the breeding programme that can help in developing cultivars that are high yielding and suitable. Correlation studies among the traits of interest help in identifying these associations. However, knowing these associations is not enough, as it does not show which traits are directly affecting the trait of interest. Therefore, path coefficient analysis used in determining what type of effect each trait has on the trait of interest; direct or indirect. As the number of traits increase, the correlation becomes more complex and this is where path coefficient becomes useful as it provides information on the direct and indirect effects of the traits in relation to the trait of interest (Bhargavi et al., 2015, 2017). Understanding the relationship that exists among yield components is essential in increasing the yields and as such, a lot of attention must be given to the traits during selection of better cultivars in a breeding programme (Raghuwanshi et al., 2016). Many researchers have applied the concept of correlation and path coefficient analysis in groundnut breeding so has to understand the trait association that exist among the traits of interest (Ashutosh et al., 2017; Babariya and Dobariya, 2012; Bhargavi et al., 2015, 2017; Choudhary et al., 2013; Gomes and Lopes, 2005; Gupta et al., 2015; John et al., 2015).

2.8 Summary of the literature review

From the literature review that was conducted, the following gaps where identified,

- There has been no immunity to GRD that has been found in elite lines or released cultivars. Wild species have been known to carry immunity ot GRD but this immunity is yet to be introgressed into the cutivated groundnuts
- There has been no documented work done on the improvement of Zambian landraces for resistance and yield
- Production levels still remain low due to low yields as a result of use of unimproved cultivars by farmers, lack of access to improved seed and biotic and abiotic factors

This study will seek to address some of these gaps through the set objectives and hopefully offer a solution so some of these challenges faced by farmers.

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CHAPTER THREE

EVALUATION OF ELITE GROUNDNUT GENOTYPES FOR RESISTANCE TO GROUNDNUT ROSETTE DISEASE

Abstract

Groundnut rosette disease (GRD) is a destructive disease, which causes up to 100% yield loss. The objective of this study was to evaluate 10 ICRISAT advanced genotypes for rosette resistance. The study involved two glasshouse trials and one field trial. Infector row technique developed by ICRISAT was used to transmit the disease by using viruliferous aphids reared in a separate glasshouse on infected plants. JL 24, a susceptible variety was used as the spreader of the disease in both the glasshouse and the field. Disease severity was measured using a rating of 1-5 with a score of 1 representing resistant and 5 representing highly susceptible. The results in the glasshouse revealed that ICGV SM 08503 and ICGV SM 01514 were resistant while ICGV SM 01711, ICGV SM 09547, ICGV SM 09537, ICGV SM 08501 and ICGV SM 09545 showed moderate resistance. ICGV SM 02724, ICGV SM 10005 and ICGV SM 08560 showed high susceptibility. The field screening, however, revealed different results as genotypes that were regarded as resistant or moderately resistant showed no symptoms. The susceptible genotypes showed symptoms but the incidence and severity was lower compared to the glasshouse results. There were significant differences ($P<0.001$) among the genotypes for yield component traits like pod yield per plant, seed yield per plant, hundred seed mass, number of pods per plant and shelling percentage. The susceptible genotypes also recorded low mean performance in all these traits proving that the disease had an effect on the traits. Seed yield per hectare ranged from 314.93 kg/ha to 1033.58 kg/ha. Genotype ICGV SM 10005 recorded the lowest yield while ICGV SM 01711 was the highest yielding with 1033.58 kg/ha. Identified resistant genotypes are important donors for GRD resistance. High yielding and resistant genotypes are recommended for multi-locational yield testing. Based on the results, it was observed that the glasshouse screening was more reliable, had less external factor interference and produced high levels of infection when compared to the field screening.

3.1 Introduction

Groundnut rosette disease (GRD) is a very destructive disease that causes up to 100% yield losses. Huge economic losses due to GRD were reported in Zambia and Malawi during 1994-1995 outbreak. In this outbreak, approximately 43,000 ha in eastern Zambia were affected leading to a loss of approximately $4.89 million, and a production reduction of 23% in Malawi (Iwo and Olorunju, 2009). The disease reduces yield and quality and increases the cost of production. GRD is also unpredictable in nature, but has been reported to cause almost US$156 million in losses across Africa (Ntare et al., 2001). The level of yield loss depends on the stage at which the crop is infected, with seedling infection being the most devastating (Waliyar et al., 2007).

Three causal agents have been reported to be responsible for GRD development, namely, *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and a satellite-RNA (Sat-RNA). These three agents operate in unison for an effective transmission of the virus by the aphid. In the event that they do not occur together, GRAV or GRV will only cause infection that will not show any symptoms or temporal mild mottle symptoms (Alhassan, 2013).

Chlorotic and green rosette are the two common symptoms of the disease with the chlorotic type being the most common in the Sub Saharan Africa (SSA). Plants affected manifest severe stunting and have a bushy appearance. However, field symptoms may vary depending on the stage of infection, climatic conditions and presence of other viral infections (Naidu et al., 2007).

Visual assessment based on the symptoms exhibited by the infected plants has been employed in many case studies to indicate the presence of GRV. However, this test is not always reliable. Due to the fluctuating temperatures of SSA (Naidu et al., 1999) there have been cases reported where GRAV was detected in plants that did not show symptoms (Bock and Nigam, 1988, Olorunju et al., 1992). But with the progress made in technology, improved diagnostic methods have been invented, e.g., a triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) which can be used for the detection of GRAV while the reverse transcription-polymerase chain reaction (RT-PCR) can be used to detect each of the three disease causal agents (Waliyar et al., 2007). Amoah et al. (2016) also concluded that symptoms alone are not reliable when screening for plants for resistance to the three causal agents of the disease after he discovered that all resistant lines tested positive for GRAV antigens.

In a bid to manage the disease, many methods have been investigated. These methods include the use of pesticides to reduce the aphid populations, use of cropping practices like

early planting, high plant population, rouging all infected plants in the effort to delay onset and spread of both vector and disease and breeding for virus and vector resistance. But even with all these measures, limited success has been achieved with each, and in recent years, efforts have focused on the latter two tactics for disease management (Davies, 1976; Naidu et al., 1999). Cultural practices are not practiced as many farmers cannot afford pesticides, leaving breeding for varieties with genetic resistance as a potentially more promising solution (Chintu, 2013; Ntare et al., 2001). With the environment and food safety becoming of increasing concern all around the world, there is need to increase the efforts put into breeding for resistant varieties and ensure disease management strategies are put into practice by the farmers (Wynne et al., 1991). The hypothesis for this study was that there were significant differences in the resistance levels of the ten test lines. Therefore, the objective of the study was to screen elite lines for resistance and identify some lines that can be released as commercial cultivars or as sources of resistance in the breeding programme.

3.2 Materials and Methods

3.2.1 Plant Materials

Ten elite lines (Table 3.1) were used in this experiment. All the lines were sourced from the ICRISAT regional rosette resistant trial after they were identified as the best performers in terms of yield. ICGV-SM 08503 was used as a resistant control. The materials were a combination of medium maturing (Virginia) and the early maturing lines (Spanish).

Table 3.1 List of genotypes screened for rosette resistance

Genotype code	Genotype name	Source	Botanical group	Entry type
G1	ICGV SM 09537	ICRISAT-Malawi	Virginia	Elite Line
G2	MGV 7 (Control)	ICRISAT-Malawi	Virginia	Released Cultivar
G3	ICGV SM 10005	ICRISAT-Malawi	Spanish	Elite Line
G4	ICGV SM 02724	ICRISAT-Malawi	Virginia	Elite Line
G5	ICGV SM 08501	ICRISAT-Malawi	Virginia	Elite Line
G6	ICGV-SM 09545	ICRISAT-Malawi	Virginia	Elite Line
G7	ICGV SM 01711	ICRISAT-Malawi	Virginia	Elite Line
G8	ICGV SM 08560	ICRISAT-Malawi	Spanish	Elite Line
G9	ICGV SM 09547	ICRISAT-Malawi	Virginia	Elite Line
G10	ICGV SM 01514	ICRISAT-Malawi	Spanish	Elite Line

3.2.2 Experimental site

The trials were conducted in the season of 2016/17 at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) situated at Chitedze Research Station (33°38'E and 13°85'S). The site has an altitude of 1146 m above sea level, and receives approximately

1230 mm of rainfall, with moderate temperate ranging between 16–24°C. The rain season begins in December and ends in April/May.

3.2.3 Experimental design

3.2.3.1 Glasshouse experiment

The trial was laid out in a randomised complete block design (RCBD) with three replications and it was repeated to confirm the results (glasshouse A and B). Each test line was planted in six small pots, which had a radius of 250 mm and the spacing between the pots was 5 cm. Each pot had two seeds planted to make 12 seeds/plants per test line per replication. Fourteen days after sowing (DAS), JL24 cultivar (susceptible) was planted in polythene black plastic and infected with the virus, and placed in between each test line. Two days later, three viruliferous aphids were added to each of the plants in the experiment including the infector plants to ensure effective spread of the virus. Two weeks after the introduction of the viruliferous aphids, three more aphids where added per plant to increase the number of vectors.

Figure 3.1 Picture showing the arrangement of the trial in the glasshouse

Figure 3.2 Scoring for disease incidence and severity

3.2.3.2 Field experiment

In the field, a RCBD design was used with 3 replications. Each test line was planted on a plot size that was 3 m long with 4 rows. A spacing of 0.75 cm between rows and 15 cm between planting stations was used, and one seed was planted per station. Each plot was flanked by a row of the susceptible line JL 24. Two rows of the infector row were planted at the beginning of the trial, and then two more were planted across the blocks after the 5th plot in each replication. Finally, another two rows were planted after the last plot in each block. The infector rows were planted two weeks before the trial was planted.

After planting the trial, plants of JL 24 that were grown in the glasshouse (in pots) and infected with the virus prior to planting of the trial were then added to the infector rows in the field to start the infection process after germination of the test lines. Three infected plants were added in each infector row at set intervals. At weekly intervals up to 80 DAS, three viruliferous aphids, which had been reared in a glasshouse on infected plants were placed onto the infector rows and the test genotypes using a camel's hair brush.

Figure 3.3 A resistant line (Right) and a suscepatble JL 24 (Left)

Figure 3.4 Screening for resistance in the field

All the recommended management practices like weeding and re-ridging were employed. However, no herbicides or pesticides were used in this trial.

3.2.4 Data collection and statistical analysis

The test plants were monitored and evaluated for GRD symptoms at 20, 40 and 60 days after aphid infestation for the glasshouse experiment while in the field, the number of plants infected were counted and recorded at 60, 80 and 100 DAS. The number of plants showing GRD symptoms per test line were converted into Percent Disease Incidence (PDI) using the formula as described by Waliyar et al. (2007).

$$PDI = \frac{Number\ of\ \ plants\ \ showing\ \ GRD\ \ symptoms}{Total\ \ number\ of\ \ plants\ \ \ per\ test\ \ line}\ \ x\ 100$$

Severity was also recorded both in the glasshouse and field by scoring for each plant in the pots for glasshouse experiment and accessing the severity per row for the field experiment. The disease index value was determined using a method similar to Kuhn and Smith (1977) which is,

$$\frac{A+2B+3C+4D+5E}{Total\ number\ of\ plants\ treatment}$$

Where; A, B, C, D and E equals the number of plants with symptoms rated 1, 2, 3, 4 and 5 respectively. Yield and other selected traits were also collected and analysed using SAS version 9.4. The rating for disease severity that was used was 1 to 5 with 1 been highly resistant and 5 been highly susceptible; where 1= plants with no visible disease symptoms on foliage, 2 = plants with obvious symptoms and no stunting (1-20% foliage affected), 3 = plants with symptoms plus stunting (21-50 % foliage affected), 4 = plants with severe leaf symptoms and stunted (51- 70% foliage affected), and 5 = plants with severe leaf symptoms and stunting or dead plants (71-100% foliage affected) (Waliyar et al., 2007).

The conversion of yield into kg/ha was done using the formula

$$Gyld\left[=\left[\left(\frac{Pyld}{1000}\right)\times\left(\frac{10000}{netplot}\right)\times SH\%\right]\right.$$

Where:

Gyld = Grain yield in kg/ha

Pyld = Pod yield per net plot

SH% = Shelling percentage

The ANOVA model that was used was

$\gamma = \mu_i + \varepsilon$ where;

Y is the quantitative dependent variable

μ_i is the true mean value of the dependent variable for the i^{th} population, where there are ***k*** populations.

ε is the random error in the response not attributable to the independent variable.

3.3 Results

3.3.1 Combined analysis of variance for the field trial

The combined analysis of variance for pod yield per plant, seed yield per plant, hundred seed mass, pod number per plant and shelling % is presented in Table 3.2. There were significant differences among genotypes for the yield component traits, pod yield per plant, seed yield per plant, pod number per plant, hundred seed mass and shelling percentage.

Table 3.2 Analysis of variance for yield component traits under field conditions

Source	DF	Pod yield per plant	Seed yield per plant	Pod number per plant	Hundred seed mass	Shelling %
Block	2	3.24ns	3.46ns	1.36ns	0.07ns	26.22ns
Genotype	9	1570.66***	1134.979***	334.925***	5568.28***	7377.22***
Error	18	48.37	21.04	59.62	253.99	246.84
Total	29	1622.26	1159.48	395.91	5822.34	7650.28
Mean		18.31	11.48	12.99	42.33	57.42
LSD		2.80	1.85	3.12	6.44	6.35
CV%		9.0	9.4	14.0	8.9	6.5

*LSD - least significant difference at P<0.05, CV% - coefficient of variation

The entry means for the pod yield per plant, seed yield per plant, number of pods per plant, hundred seed mass and shelling percentage are displayed (Table 3.3).

Table 3.3 Means for yield component traits of ten genotypes under field conditions

Genotype code	Genotype	Pod yield per plant (g)	Seed yield per plant (g)	Pod number per plant	Hundred seed mass (g)	Shelling %
G1	ICGV SM 09537	22.98	14.35	16	55.67	63
G2	ICGV SM 08503	27.08	18.12	15	55.33	67
G3	ICGV SM 10005	4.50	1.54	7	24.00	34
G4	ICGV SM 02724	10.40	4.41	8	24.00	43
G5	ICGV SM 08501	22.49	15.44	15	53.00	69
G6	ICGV-SM 09545	19.08	12.60	14	44.33	66
G7	ICGV SM 01711	28.17	20.78	14	59.33	74
G8	ICGV SM 08560	11.48	3.11	9	22.25	27
G9	ICGV SM 09547	16.26	11.09	17	46.33	68
G10	ICGV SM 01514	20.68	13.33	14	39.00	64
Mean		18.31	11.48	13	42.33	57
LSD		2.80	1.85	3	6.44	6
CV%		8.95	9.42	14	8.90	6

*LSD - least significant difference at P<0.05, CV% - coefficient of variation

The analysis of variance for yield and disease incidence is shown in Table 3.4 while the means for grain yield, incidence at 60, 80 and 100 days after sowing are displayed in Table 3.5. There were highly significant differences among the genotypes for incidence, seed yield and days to maturity at P<0.001. In Table 3.5, the susceptible genotypes G3, G4 and G8 showed low incidences at 60, 80 and 100 DAS. At 60 DAS, the incidence ranged from 9.9% to 16.5% while at 80 DAS, it ranged from 18.6% to 23.8%. The highest score for incidence at 100 DAS was 27.3% for G8. The rest of the genotypes had 0% incidence. The yield per hectare ranged from as low as 314.93 kg/ha to as high as 1033.58 kg/ha. G3 recorded the lowest yield followed by G8 and then G4 among the susceptible genotypes. Their mean yield was below the trial mean of 601.5 kg/ha. G7 was the highest yielding genotype with a performance of about 71.8% above the mean (1033.58 kg/ha). No significant yield difference was observed between G7

and G5 but G7 was significantly different from the remaining genotypes. The susceptible genotypes showed no significant difference amongst themselves at all stages of scoring.

Table 3.4 Analysis of variance for yield and rosette incidence

Source	DF	Seed Yield (Kg/Ha)	Rosette 60DAS	Rosette 80DAS	Rosette 100DAS	Days to maturity
Block	2	19856.616	39.334	29.594	51.808	2.467
Genotype	9	1246160.277***	1114.336***	2903.226***	3563.632***	2894.967***
Error	18	260599.026	184.805	458.21	681.514	37.533
Total	29	1526615.919	1338.475	3391.03	4296.954	2934.967

DAS=days after sowing, DF=degrees of freedom

***, **. * = least significant difference at P<0.001, 0.01 and 0.05 respectively

Table 3.5 Genotypic means for yield, days to maturity and rosette incidence

Genotype Code	Genotype	Seed Yield(Kg/Ha)	Rosette 60DAS	Rosette 80DAS	Rosette 100DAS	Days to maturity
G1	ICGV SM 09537	501.9	0.0	0.0	0.0	130
G2	ICGV SM 08503	697.3	0.0	0.0	0.0	128
G3	ICGV SM 10005	314.9	12.2	21.5	24.2	106
G4	ICGV SM 02724	489.5	9.9	18.6	18.6	127
G5	ICGV SM 08501	888.3	0.0	0.0	0.0	129
G6	ICGV-SM 09545	592.6	0.0	0.0	0.0	125
G7	ICGV SM 01711	1033.6	0.0	0.0	0.0	128
G8	ICGV SM 08560	481.6	16.5	23.8	27.3	110
G9	ICGV SM 09547	490.6	0.0	0.0	0.0	127
G10	ICGV SM 01514	524.8	0.0	0.0	0.0	103
Mean		601.5	3.9	6.4	7.0	121
CV%		20.2				

CV% - coefficient of variation, DTM - days to maturity. DAS - days after sowing

3.3.2 Combined analysis of variance for the glasshouse trials

Table 3.6 shows the ANOVA for the glasshouse trials. There were significant differences among the genotypes and between the two trials which were taken as environments, at P<0.001. This showed how significant their effect was on disease incidence and genotype reaction to the disease.

Table 3.6 Combined analysis of variance for groundnut rosette disease infection

Source	DF	Sum of squares	Mean square
Environment	1	2.089	2.089***
Block(Environment)	4	0.965	0.241*
Genotype	9	72.057	8.006***
Genotype x Environment	9	8.750	0.972***
Error	36	3.377	0.094
Total	59	87.238	

*** = significant at P<0.001, ** = significant at P<0.01 and * = significant at P<0.05, DF=degrees of freedom

3.3.3 Disease reaction under controlled rosette environment

Table 3.7 and 3.8 shows the percentage infection and disease index values (DIV) for the two trials in glasshouse A and B. The trial was repeated to confirm the levels of resistance and also the disease severity levels for the test genotypes. The disease incidence progression in glasshouse A (Table 3.7 and 3.8) was more that in glasshouse B. It was observed that 30 days after inoculation, all the susceptible genotypes were showing symptoms on all plants ranging from 75% to 90%. However, in the resistant ones, the progression was very low and the few plants that showed mild symptoms were only recorded after 40 days after inoculation with less than 10% infection. Any plant that showed 0% disease incidence was rated 1 and considered resistant while those that showed mild symptoms on a few plants (<50%) were regarded as moderately resistant. Any genotype that scored more than 50% was regarded as susceptible. In rating these genotypes, the level of severity was also considered which was able to help conclude that the genotype was susceptible or resistant. G2 and G10 (control) recorded 0% incidence in both trials and were rated as resistant while G5, G6 and G7 had less than 10% disease incidence with plants been rated 1 for these genotypes recording 96%, 92% and 93% respectively. G3, G4 and G8 had 0%, 1% and 7% of the total plants rated 1 (no symptoms) respectively while the rest of the genotypes showed higher percentages of plants rated 1 there by ranking them as resistant to moderately resistant.

Table 3.7 Percentage of disease incidence per plant from glasshouse A

		Percent Plant/Rating No						
Genotype Code	**Genotype**	**1**	**2**	**3**	**4**	**5**	**Disease Index Value**	**Disease Classification**
G1	ICGV SM 09537	79	17	4	0	0	1.4	Moderately Resistant
G2	ICGV SM 08503	100	0	0	0	0	1.0	Resistant
G3	ICGV SM 10005	0	32	20	28	20	4.6	Susceptible
G4	ICGV SM 02724	1	37	17	24	21	4.4	Susceptible
G5	ICGV SM 08501	96	2	2	0	0	1.1	Moderately Resistant
G6	ICGV-SM 09545	92	6	2	0	0	1.2	Moderately Resistant
G7	ICGV SM 01711	93	7	0	0	0	1.2	Moderately Resistant
G8	ICGV SM 08560	7	31	32	20	10	4.0	Susceptible
G9	ICGV SM 09547	72	26	2	0	0	1.6	Moderately Resistant
G10	ICGV SM 01514	100	0	0	0	0	1.0	Resistant
LSD							**0.63**	
Mean							**2.14**	
CV%							**17.2**	

*LSD-least significant difference at P<0.05, CV%-coefficient of variation

The disease severity was more in glasshouse A with a mean of 2.14. The rating for the susceptible lines ranged from 4 to 4.6 with genotypes G3 and G4 scoring the highest. While for the resistant category, the rating ranged from 1 to 1.6. Genotypes G2 and G10 were the only genotypes that scored a perfect 0% disease incidence and severity. For glasshouse B, the disease incidence and severity was not that much as the mean was 1.78 compared to 2.14 in glasshouse A. Genotypes G2, G5 and G10 showed good resistance with 0% infection.

Table 3.8 Percentage of disease incidence per plant from glasshouse B

		Percent Plant/Rating No					Disease Index Value	Disease Classification
Genotype code	Genotype name	1	2	3	4	5		
G1	ICGV SM 09537	67	32	1	0	0	1.7	Moderately Resistant
G2	ICGV SM 08503	100	0	0	0	0	1.0	Resistant
G3	ICGV SM 10005	33	37	24	4	1	2.5	Susceptible
G4	ICGV SM 02724	1	46	33	13	7	3.6	Susceptible
G5	ICGV SM 08501	100	0	0	0	0	1.0	Resistant
G6	ICGV-SM 09545	74	24	2	0	0	1.5	Moderately Resistant
G7	ICGV SM 01711	89	11	0	0	0	1.3	Moderately Resistant
G8	ICGV SM 08560	32	36	29	3	0	2.6	Susceptible
G9	ICGV SM 09547	67	30	3	0	0	1.6	Moderately Resistant
G10	ICGV SM 01514	100	0	0	0	0	1.0	Resistant
LSD							0.40	
CV%							13.0	
Mean							1.78	

*LSD - least significant difference at P<0.05, CV% - coefficient of variation

3.3.4 Differences among the test genotypes

The difference among the ten test genotypes' DVI means for glasshouse A and B are displayed in Table 3.9 and Table 3.10, respectively, show the differences among the DVI means for the ten test genotypes. The genotypes were significantly different in terms of their reaction to the disease. There was no significant difference between G3, G4 and G8, which had DVIs ratings of 4.6, 4.4 and 4.0, respectively, but all of them were significantly different from all the remaining genotypes. There were no significant differences among G1, G6, G7 and G5 in terms of disease severity but all these were different from G2 and G10, which showed complete resistance.

Table 3.9 Means for genotypes in respect of disease rating scores from glasshouse A

Genotype code	Genotype name	Mean
G3	ICGV SM 10005	4.6a
G4	ICGV SM 02724	4.4a
G8	ICGV SM 08560	4.0a
G9	ICGV SM 09547	1.6b
G1	ICGV SM 09537	1.4cb
G6	ICGV-SM 09545	1.2cb
G7	ICGV SM 01711	1.2cb
G5	ICGV SM 08501	1.1cb
G2	ICGV SM 08503	1.0c
G10	ICGV SM 01514	1.0c

*Means with the same letter are not significant

Glasshouse B trial also showed significant differences among the genotypes at $P<0.05$ with G4 scoring the highest in terms of disease severity while G2 and G10 again showed 0% disease symptoms. G4, G8 and G3 showed no significant difference among them but they were all susceptible though with less disease severity compared to glasshouse A. G1 and G7 were significantly different whilst G9, G6 and G7 were not significantly different.

Table 3.10 Means for genotypes in respect of disease rating from glasshouse B

Genotype code	Genotype name	Mean
G4	ICGV SM 02724	3.6a
G8	ICGV SM 08560	2.6b
G3	ICGV SM 10005	2.5b
G1	ICGV SM 09537	1.7c
G9	ICGV SM 09547	1.6dc
G6	ICGV-SM 09545	1.5dc
G7	ICGV SM 01711	1.3de
G10	ICGV SM 01514	1.0e
G2	ICGV SM 08503	1.0e
G5	ICGV SM 08501	1.0e

*Means with same letters are not significantly different

When a pairwise comparison was done for each pair of the genotypes (Table 3.11), it was revealed that there were highly significant differences (P<0.001) between the different genotype comparisons. G1, G2 and G10 were significantly different from G3, G4, and G8 while G3 was significantly different from G6, G7 and G9. G4, G3 and G8 were significantly different which ever genotype they were paired with.

Table 3.11 Pairwise genotype comparison for mean disease rating scores

Genotype comparison		Pr > |t|	Genotype comparison		Pr > |t|
G1	G10	0.003	G2	G9	0.0013
G1	G2	0.0024	G3	G4	0.0161
G1	G3	<.0001	G3	G5	<.0001
G1	G4	<.0001	G3	G6	<.0001
G1	G5	0.0056	G3	G7	<.0001
G1	G6	0.1727	G3	G8	0.117
G1	G7	0.0568	G3	G9	<.0001
G1	G8	<.0001	G4	G5	<.0001
G1	G9	0.8159	G4	G6	<.0001
G10	G2	0.9378	G4	G7	<.0001
G10	G3	<.0001	G4	G8	0.0002
G10	G4	<.0001	G4	G9	<.0001
G10	G5	0.8151	G5	G6	0.1287
G10	G6	0.0817	G5	G7	0.3345
G10	G7	0.2327	G5	G8	<.0001
G10	G8	<.0001	G5	G9	0.003
G10	G9	0.0016	G6	G7	0.5676
G2	G3	<.0001	G6	G8	<.0001
G2	G4	<.0001	G6	G9	0.1127
G2	G5	0.7552	G7	G8	<.0001
G2	G6	0.0697	G7	G9	0.0341
G2	G7	0.2045	G8	G9	<.0001
G2	G8	<.0001			
Mean		1.96			
LSD		0.36			
CV%		15.6			

*LSD - least significant difference at P<0.05, CV% - coefficient of variation

3.4 Discussion

Based on the results of this study, it was concluded that the level of resistance was higher in Virginia than in Spanish genotypes with Spanish recording only 1 out of the 3 Spanish lines (33%) in the study while Virginias only had 1 susceptible line (G4) out of the 7 Virginia lines that were tested. Subrahmanyam et al. (1998) and Olorunju et al. (2001) noticed similar observations in their study on the resistance levels of groundnuts across the three maturity groups. The severely infected plants were stunted with a bushy kind of appearance and the

leaves had reduced size, which affected pod yield. The susceptible lines G3, G4 and G8 showed that the disease affected the number of pod per plant, shelling percentage; seed yield per plant, pod yield per plant and hundred seed weight and caused massive reduction in each respective trait. The number of pods per plant were affected when compared to uninfected plants for the same genotype. This resulted in reduced seed yield and pod yield. The seed development was affected leading to less seed weight. All the genotypes that had rating of 5 died and did not produce any pods or seeds. Those that had a score of less than 4 produced few pods and most were single seeded. The rest of the pods were unfilled. The rest of the lines that showed resistance had higher pod number and seed yield. The plants that showed severe symptoms had a bushy appearance and were stunted. Similar finding was reported by Subrahmanyam et al. (2002), A'Brook (1964), Blackman and Eastop (1994), Appiah et al. (2016) and Olorunju et al. (2001). Genotypes that showed 0% incidence were regarded as been resistant while those that showed 100% incidence were regarded as highly susceptible. Those that showed a few plants with mild symptoms (<50%) were considered to be moderately resistant. This classification was used by other researchers and is in agreement with their findings (Blackman and Eastop, 1994; Hayatu et al., 2014).

The level of resistance in some of the ICRISAT advanced lines was demonstrated in this study. There were significant differences among the genotypes as displayed in Table 9 and 10. Genotypes G2 and G10 showed high level of resistance both in the field and in the glasshouse. Genotypes G7, G6, G1 and G5 showed moderate resistance to the virus as they had some plants that showed mild infections. However, they were not significant to cause any yield losses as there was no stunting of plants involved. When the genotypes were tested in the field, G1, G6, G5 and G7 showed no symptoms in any of the plots while G3, G4 and G8 that were susceptible in the glasshouse showed severe symptoms on some of the plants with some plants producing zero pods. A careful look at the genetic background of genotypes G2, G10 and G7 showed that they had inherited resistant genes from one of the parents used in the crosses (ICGV SM 90704) which was resistant. This could explain the reaction to rosette and the resistance levels they exhibited. Olorunju et al. (1991) in their study tested eight genotypes for disease resistance, and found that the resistant lines showed 86% mild symptoms in the field under high disease pressure though the symptoms were not significant to cause yield losses. This was also reported by Nutman et al. (1964) and Bock and Nigam (1988) who found that 1% of the resistant plants showed symptoms. This reaction can be attributed to several factors that include environmental conditions like rain, wind direction, which can affect the vectors ability to transmit the virus effectively and consequently lead to mortality, and aphid population (Meihls et al., 2010). During the 2016/17 season when the study was conducted, Chitedze research station received a lot of rain compared to the previous season and the level

of infection was so low in the field. In most cases, within a day or two after aphid application, the rains would come and wash away the aphids from the plants thereby affecting the transmission efficiency. This is in agreement with Olorunju et al. (1991) and Herselman et al. (2004) who made similar observations.

Based on this observation, it means that these moderately resistant genotypes (G7, G6, G5 and G1) can perform well in the field even with infected fields nearby as evidenced in this study where they recorded 0% infection, while the susceptible lines next to the plots showed symptoms. Herselman et al. (2004) stated that the number of plants that show symptoms in test genotypes indicate that they have all the three causal agents present and the efficiency of this transmission is as a result of vector population and frequency of the inoculation events.

Variations in the results from glasshouse and the field can also be an indication of the efficiency of the vector *A.craccivora* and its behaviour. This means that with high disease pressure, the resistance can break down (Naidu et al., 1999). Earlier reports have suggested that different mechanisms of resistance may work against GRV, GRAV and sRNA in resistant genotypes (Olorunju et al., 1991; 1992). Herselman et al. (2004) suggested that an understanding of these mechanisms would enable the designing of better strategies for breeding for resistance to all agents of the disease.

As for G3, G4 and G8, the results in the glasshouse and the field showed that plants that were infected exhibited severe symptoms, with some dying before harvest in the field while those that survived still produced no pods. It was, however, noted that the incidence levels in the field were lower when compared to the 100% disease incidence in the glasshouse screening trials. This difference in the results between the glasshouse and the field screening could have been attributed to the heavy rains that were experienced during the season which could have washed away the aphids thereby lowering their effectiveness in transmitting the virus. Research has shown that droughts or dry spells are good conditions for the spread of the virus (Naidu et al., 1999). Such kind of reaction indicates that in the event of a dry spell and a rosette epidemic, there would be huge yield losses.

3.5 Conclusion

The resistant lines identified in this study could contribute greatly to the national breeding programme in Zambia and this can help farmers have access to resistant varieties which will boost the production. Genotype G10 as much as it was a resistant variety showed lower yield in a study conducted across ten environments in Zambia (Chapter Four). It also lacked stability and as such, it is recommended that it be used as a source of resistant genes in breeding programmes. Genotype G7 however was high yielding when tested in ten environments in

Zambia and showed acceptable levels of stability. This genotype should be proposed for release once enough data is collected according to the release procedures of Zambia. The present study showed that only two lines had resistance to the disease with a score of one. It is also recommended that other sources of resistance like wild species where immunity to GRAV has been identified and some landraces should be explored and used in breeding programmes to expand the genetic base and improve on the resistance to GRAV which will reduce inoculum build up.

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CHAPTER FOUR

GENOTYPE BY ENVIRONMENT INTERACTION ANALYSIS OF ELITE GROUNDNUT GENOTYPES FOR GRAIN YIELD ACROSS DIVERSE AGRO-ECOLOGICAL REGIONS OF ZAMBIA

Abstract

Selection of disease resistant, adaptable, high yielding and stable varieties under different agro-ecological zones before release is an important part of any breeding programme as it impacts the adoption and productivity of the cultivars. The objective of this study was to evaluate and select genotypes that are both high yielding and stable across different environments for possible release and also identify environments that can be used for genotype selection depending on the objective. A total of 11 groundnut genotypes from ICRISAT comprising of nine elite lines and two released cultivars as controls were evaluated over ten environments spread across the three agro-ecological zones of Zambia in the 2016/17 season. The design used was a randomized complete block design (RCBD), replicated three times. Additive main effect and multiplicative interaction (AMMI), and genotype and genotype by environment interaction (GGE) biplot models were used to explore the G x E interaction. Results showed that environments E3, E5 and E10 were the best as they were both discriminating and representative, whilst environments E2 and E7 were non-representative. Environments E1 and E6 were non-representative and non-discriminating which rendered them useless. Based on mean yield ranking, genotypes G7 and G4 were high yielding resulting in 2.08 t/ha and 1.99 t/ha, respectively, compared to the average mean of 1.67 t/ha across all environments. Genotype G3, a Spanish type, yielded more than genotype G11 (control) also a Spanish type. Using IPCA1 and IPCA2, genotypes G7, G4, G5 and G2 (control) where identified as the best performing genotypes using AMMI though only G4 and G7 showed consistent performance, thus relative stability and adaptability across the ten testing environments. Genotypes G7 and G4 were identified as the ideal genotypes based on the GGE biplot analysis. Both AMMI and GGE classified the genotypes in a similar manner.

4.1 Introduction

The environment has a great effect on the performance of any genotype. This results in different phenotypic expressions and performance of genotypes across different environments (Crossa, 1990). The interaction between genotype performance as determined by its genetic composition, and the environment can greatly influence the performance of the genotype (Ding et al., 2007; Yan and Wu, 2008). The testing of genotypes across different locations over years and seasons has been used by many breeders to evaluate the stability of genotypes before release. This process helps breeders to develop breeding strategies that can identify superior cultivars for the target environments (Kang, 2002). Many terminologies have been used to refer to the genotype by environment interaction (GEI) analysis, e.g. specific adaptation, stability studies, mega environments (Yan and Hunt, 2002). For each specific environment, the mean yield data and the genotype`s yield stability has been used to evaluate the genotypes. With analysis of data from several years of testing over different environments, mega environments can be classified and identified (Casanoves et al., 2005).

Yusuf (2009), in his study of 49 maize varieties stated that the best varieties are those that have good stability in terms of performance across a wide range of different environments and such varieties exhibit small GEI effects as opposed to the unstable varieties that exhibit a large GEI effects. Varieties that exhibit large GEI effects complicate the breeder`s task of choosing the best varieties for the farmers.

The performance of any cultivar is a combination of the cultivar and the environment in which it is been tested or produced (Cooper and Byth, 1996). This is the reason why the potential cultivars must be tested in different locations and over years (Bernardo, 2002). The other component worth considering is the interaction that exists between the genotype and the environments (GEI) as it leads to genotypes responding differently to environmental changes (Crossa et al., 1991; Hallauer et al., 2010; Vargas et al., 1999). There are other factors that play a part in the GEI and these can be sources of the variation that is observed. Bänziger et al. (2006) cited that temperature, growing season duration, lack of enough water, sub-soil pH, rainfall and social economic factors are some of the factors that contribute to the presence of GEI. These can also include the biotic factors (Butron et al., 2004).

In this study, two types of analysis were used to identify the best genotypes and environments. Different methods have been used to evaluate genotypes across different environments for stability and performance. These methods include, among many others, coefficient of variation, linear regression, analysis of variance (ANOVA) (Zhang and Kong, 2002), additive main effects and multiplicative interaction (AMMI) model (Rad et al., 2013), and genotype plus

GEI (GGE) biplot analysis (Yan et al., 2000). Among these methods, the AMMI and GGE biplot analyses, were adopted in multi-environment trials (MET) two-way data matrices. The GEI has been the main focus in AMMI analysis, leaving out the effect of the genotype. With the focus on genotype effect, the GGE biplot model has been adopted widely as it is an effective method that can be useful in identifying differences among genotypes and the evaluation of test environment (Ding et al., 2007).

A combined analysis of variance can describe the main effects and be able to measure the interactions. However, ANOVA does not give enough information for explaining GEI. It is for this reason that AMMI model is used when understating GEI (Kaya et al., 2002). When the PCA scores are plotted against each other on the biplot, it becomes easy to visualise and interpret the components for GEI. A combination of the biplot display and the stability statistics for the genotypes will group the genotypes based on performance similarity across different environments (Thillainathan and Fernandez, 2001).

The GGE biplot is a data visualisation tool, which graphically displays GEI (Yan and Kang, 2002). It is an effective way of analysing mega-environment analysis e.g., “which-won-where” pattern, which makes it possible to recommend specific genotypes for specific mega-environments. This can be effectively used to evaluate environments, mean yield and stability of genotypes. This analysis tool is progressively being used in GEI data analysis in agriculture (Butron et al., 2004; Crossa et al., 2002; Dehghani et al., 2006; Kaya et al., 2006; Ma et al., 2004; Yan and Hunt, 2002; Sibiya et al., 2012; 2013).

The hypothesis being tested was that there were significant differences among the 11 test lines in yield across the ten test environment. Thus the objective of the study was to evaluate and select stable and high yielding genotypes for possible release and also identify environments that can be used for genotype selection depending on the objective.

4.2 Materials and Methods

4.2.1 Germplasm used

The genotypes were selected from the ICRISAT regional rosette resistant trial nursery. Nine best performing genotypes from the on-station nursery were selected. MGV 7 and Luena, which are released medium (Virginia) and early maturing (Spanish) cultivars, respectively in Zambia were used as controls because the trial consisted of four Spanish and five Virginia types (Table 4.1).

Table 4.1 List of the genotypes tested across ten environments

Genotype Code	Genotype Name	Source	Botanical Group	Entry Type
G1	ICGV SM 09537	ICRISAT-Malawi	Virginia	Elite Line
G2	MGV 7 (Control)	ICRISAT-Malawi	Virginia	Released Cultivar
G3	ICGV SM 10005	ICRISAT-Malawi	Spanish	Elite Line
G4	ICGV SM 02724	ICRISAT-Malawi	Virginia	Elite Line
G5	ICGV SM 08501	ICRISAT-Malawi	Virginia	Elite Line
G6	ICGV-SM 09545	ICRISAT-Malawi	Virginia	Elite Line
G7	ICGV SM 01711	ICRISAT-Malawi	Virginia	Elite Line
G8	ICGV SM 08560	ICRISAT-Malawi	Spanish	Elite Line
G9	ICGV SM 09547	ICRISAT-Malawi	Virginia	Elite Line
G10	ICGV SM 01514	ICRISAT-Malawi	Spanish	Elite Line
G11	Luena (Control)	ICRISAT-Malawi	Spanish	Released Cultivar

4.2.2 Site description

This study was carried out in Zambia across ten environments (locations). The environments were spread across the three agro-ecological zones that are classified based on soil type, rainfall received and temperatures. This study was carried out during the 2016/17 season. The sites and all the details about the sites are presented in Table 4.2.

Table 4.2 Description of the ten test environments

Site Code	Site Name	Agro-Region	Coordinates	Elevation (m)	Annual Rainfall (mm)	Average Temperature (°C)	pH	Soil Type
E1	Kalichero FTC	2	S13°30.184` E032°26.404`	943	1281	23.5	4.2	loamy sand
E2	Katete FTC	2	S14°04.960` E032°03.726`	1044	1125	22.8	4.5	sandy Loam
E3	Lundazi FTC	2	S12°17.323` E033°11.231`	1148	720	25.2	5.6	sandy clay loam
E4	Magoye	2	S15°59.612` E027°37.023`	1021	1046.8	24.4	5.5	sandy loam
E5	Mfuwe	1	S13°10.909` E031°56.338`	557	1051	25.1	5.0	loamy sand
E6	Mambwe FTC	1	S13°18.463` E032°02.079`	595	1160	26.5	4.2	loam
E7	Masumba	1	S13°13.297` E031°55.651`	550	1112.5	26.2	4.1	sandy clay loam
E8	Msekera	2	S13°39.007` E032°33.920`	1023	1242.2	24.8	5.5	clay loam
E9	Mufulira	3	S12°36.690` E028°08.789`	1217	1237.4	22.2	4.1	sandy clay loam
E10	Masaiti	3	S13°19.630` E028°24.912`	1209	1180	23.5	4.0	loamy sand

4.2.3 Experimental design and layout

The experimental layout used was a randomised complete block design (RCBD) with three replications, at each location. Planting was done by hand and each plot consisted of four ridges, 3 m long with an inter- row spacing of 0.6 m and intra-row spacing of 0.1 m. Fertiliser was applied at planting at the rate of 150 kg/ha for D-compound which has NPK ratios of 10:20:10, respectively. No pesticides or chemicals were applied during the growth period. The two middle ridges were harvested as net plot and the yield converted to yield per hectare.

4.2.4 Statistical analysis

4.2.4.1 Analysis of variance model

The data were analysed using GenStat version 18 (Payne et al., 2011). The combined analysis of variance (ANOVA) model used was:

$$\mathrm{Y}_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \mathrm{E}_{ij}$$

Where Yij is the yield of the genotype i in environment j, μ is overall yield mean, αi and βj are genotypic and environmental effect, (αβ)ij is the effect of interaction between the ith genotype and jth environment, $\in ij$ is the mean random error of the ith genotype and ej environment.

4.2.4.2 Additive main effects and multiplicative interaction (AMMI) model

The AMMI model used was adopted from Gauch and Zobel (1989). Model diagnosis is essential in determining which member of the model family is best for a given dataset since AMMI constitutes a model family. The dataset presented in the current study is from a replicated yield trial which was analysed as a randomized complete block design. The contribution of each Interaction Principle Component Analysis (IPCA) to the total GEI sum of squares was determined. Biplots were plotted using the first two IPCAs to depict the relative performance of genotypes for yield and stability and therefore, the equation for the AMMI model used was as follows:

$$\mathrm{Y}_{ijk} = \mu + \alpha_i + \beta_j + \sum_n \lambda_n \delta_{in} \gamma_{jn} + \mathrm{P}_{ij} + \mathrm{E}_{ij}$$

Where Y_{ijk} is the mean in the *i*th genotype effect in *j*th environment and *k*th replication; and the additive components of the model which are μ is the grand mean, the *i*th genotype effect (αi), and the *j*th environment effect (βj). The terms λn δin γjn and p_{ij} constitute the multiplicative component, where λn, is the interaction principal component, αin, is the eigen vector for the genotypic principal component, γjn is the environmental principal component. Only first IPCAs are retained for analysis and the rest of the interaction variation is explained by the residual p_{ij}. The last component in the model ϵi, is the random error.

4.2.4.3 Genotype and genotype by environment interaction (GGE) model

Genotype and genotype by environment interaction biplot analysis was conducted in GenStat version 18 using the least squares means (ls) means (Payne et al., 2011). GGE biplots were constructed for grain yield from each environment. The comparison was between genotypes and their mean performance and stability across environments and the selection of the best environments and the highest yielding genotypes.

The GGE model used below was adopted from Yan and Kang (2002)

$$Y_{ij} - \mu - \beta_j = \lambda_1 \gamma_{i1} \delta_{j1} + \lambda_2 \gamma_{i2} \delta_{j2} + E_{ij}$$

Where Y_{ij} is the mean of *i*th genotype in the *j*th environment, μ is the grand mean, βj is environment main effect in the *j*th environment and $\mu + \beta_j$ is the mean of all genotypes in *j*th environment. The terms λ_1 and λ_2 are the singular values for the first and second principal components (PC1 and PC2), respectively; γ_{i1} and γ_{i2} are eigenvectors of the *i*th genotype for PC1 and PC2, respectively. The components δ_{j1} and δ_{j2} are eigenvectors of the *j*th environment for PC1 and PC2, respectively; and $\in_{ij}$ is the residual associated with the *i*th genotype in the *j*th environment.

4.3 Results

4.3.1 Combined analysis of variance

The combined analyses of variance (ANOVA) for the 11 genotypes tested across ten environments is presented in Table 4.3. The ANOVA indicated highly significant differences (P<0.001) for environments, genotypes and GEI. The mean yield across the ten environments was 1.67 t/ha with a coefficient of variation across all the environments of 17.9%.

Table 4.3 Combined analysis of variance for grain yield over ten environments

Source	DF	Sum of Squares	Mean Square
Environment	9	21.631	2.403***
Block(Environment)	20	1.738	0.0869ns
Genotype	10	17.775	1.778***
Genotype x Environment	90	54.231	0.603***
Error	200	17.885	0.089
Total	329	113.259	
Mean Yield	1670.05		
CV(%)	17.9		

*** = Significant at P<0.001, ns=non-significant, DF=Degrees of freedom, CV%= Coefficient of variation, DF=Degrees of Freedom

4.3.2 Additive main effect and multiplicative interaction analysis of variance for grain yield

The AMMI analysis of variance is presented in Table 4.4. The analysis revealed that the G, E and GXE multiplicative terms were significant at P<0.001. The model revealed that the interaction between genotypes and the environment accounted for approximately 58% of the treatment sum of squares (SS). The environments and genotypes however contributed significantly lower to the variations and accounted for 23% and 19% of the treatment SS, respectively. The mean squares for the first IPCA axis were larger than the residual indicating that the partitioning of the interaction sum of squares by AMMI was very effective. The G x E interaction (GEI) was further divided into seven interaction principal component analysis (IPCA) scores. All the interaction PCAs were highly significant (P<0.001 or <0.05). The first and second IPCAs captured 48.5% and 19.8% of the interaction sum of squares (SS) and degrees of freedom (DF), respectively. The second IPCA accounted for 19.8% of the interaction SS and 18% of the DF. The seven IPCA axes jointly accounted for 99% of the interaction SS, leaving 1% of the variation due to GEI in the residual. The residual accounted less than 1% of the total SS.

Table 4.4 Analysis of variance based on the AMMI model for grain yield (kg/ha) of 11 genotypes over ten environements

Source	D.F	SS	MS	Total Variation	GxE Explained (%)	Cumulative (%)
Treatments	109	93.64	0.8591***			
Block(Environments)	20	1.74	0.0869ns			
Genotypes (G)	10	17.78	1.7775***	18.98		
Environments (E)	9	21.63	2.4034***	23.10		
Genotype x Environment Interactions	90	54.23	0.6026***	57.92		
IPCA 1	18	26.31	1.4614***		48.51	48.51
IPCA 2	16	10.74	0.6711***		19.80	68.30
IPCA 3	14	6.02	0.4304***		11.11	79.41
IPCA 4	12	4.23	0.3524***		7.80	87.21
IPCA 5	10	2.71	0.2713***		5.00	92.21
IPCA 6	8	2.17	0.2711**		4.00	96.21
IPCA 7	6	1.61	0.2682**		2.97	99.18
Residuals	6	0.44	0.07		0.82	100.00
Error	200	17.88	0.09			
Total	329	113.26	0.34			

***, ** Significant at 0.0001 and 0.01 probability levels respectively. DF=Degrees of freedom, SS=Sum of squares, MS=Mean Square, IPCA=Interaction Principal Component Axis

4.3.2.1 Interaction principal components scores for genotypes and environments

Tables 4.5 shows the AMMI analysis data with IPCA1 and IPCA2 scores for the genotypes and the environments, respectively. The magnitude of deviation of any genotype from zero, which is the origin, either in the negative or positive direction is represented by the IPCA scores. The instability of the genotype is determined by the magnitude of its deviation from zero either in the positive or negative direction. The results also show the mean yields for each genotype and environment and their IPCA scores that were both positive and negative.

Mean yield for the 11 genotypes ranged between 1.23 t/ha and 2.08 t/ha. Genotype G11 had the lowest yield while genotype G7 had the highest. Genotypes G2, G4, G5 and G7 yielded above the overall mean of 1.67 t/ha while G9 and G3 had yields equal to the overall mean. The rest performed below the overall mean. Environment E6 recorded the highest environmental mean yield while E9 recorded the lowest mean. Genotypes with IPCA1 which had the same sign as IPCA1 for the environment meant that that specific genotype was adapted to that environment. Large IPCA scores, whether positive or negative meant high specific adaptation while low IPCA scores meant broad adaptability (Romagosa and Fox, 1993)

Table 4.5 Mean yield (ton/ha) of eleven genotypes tested in ten locations and their IPCA scores

Genotype Code	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	Mean GYLD	IPCAg1	IPCAg2
G1	1.09	2.31	1.49	1.78	1.21	1.49	1.12	1.67	1.28	1.53	1.50	-0.211	-0.577
G2	1.27	1.27	2.28	1.06	1.55	1.92	1.83	1.94	1.61	2.66	1.74	-0.610	0.585
G3	1.88	1.47	1.65	1.05	2.46	1.84	1.73	1.68	1.8	1.11	1.67	0.349	0.256
G4	2.07	2.23	2.28	1.87	1.88	2.04	1.71	2.03	1.26	2.6	2.00	-0.509	-0.157
G5	1.71	1.34	1.26	1.85	1.94	2.08	2.31	2.5	1.7	1.97	1.87	0.095	0.634
G6	1.44	1.11	2.23	1.83	1.27	1.25	1.6	1.8	1.23	1.39	1.52	-0.268	0.191
G7	1.51	2.25	2.88	2.49	1.99	1.56	1.93	1.64	1.49	3.05	2.08	-0.892	-0.293
G8	1.78	2.24	1.27	1.59	1.91	1.37	1.27	1.32	1.67	1.59	1.60	0.124	-0.539
G9	2.47	1.69	0.94	2.00	1.25	1.65	2.03	1.67	2.08	0.85	1.67	0.776	-0.095
G10	1.86	1.79	1.29	1.45	1.58	1.81	1.14	1.09	2.35	0.73	1.51	0.651	-0.392
G11	1.29	0.65	0.8	1.39	1.33	1.37	1.69	1.01	1.89	0.89	1.23	0.496	0.387
Mean	**1.66**	**1.78**	**1.90**	**1.68**	**1.40**	**2.16**	**1.42**	**1.56**	**1.27**	**1.88**	1.67		
IPCAe1	0.617	-0.057	-0.810	0.019	0.160	0.238	0.194	-0.068	0.790	-1.083			
IPCAe2	-0.125	-1.044	-0.021	-0.375	0.100	0.233	0.600	0.440	0.031	0.162			

GYLD= grain yield per hectare in tonnes

4.3.2.2 First best four AMMI selections per environment

The AMMI analysis revealed the best four genotypes in each environment (Table 4.6). Genotypes G7 and G5 appeared in 1st position in 3 environments each and in a total of 6 environments in the top four. Other genotypes which appeared in 1st position in a single environment where G10, G9, G3 and G4. The differences in the ranking of genotypes across the environments indicated the presence of GE crossover.

Table 4.6 First four AMMI selections per environment

Environment	Mean GYLD (ton/ha)	Score	Ranking per environment			
			1st	2nd	3rd	4th
E10	1.88	-1.083	G7	G2	G4	G5
E3	1.9	-0.81	G7	G4	G2	G6
E8	1.56	-0.068	G5	G4	G2	G6
E2	1.78	-0.057	G4	G1	G7	G8
E4	1.68	0.019	G7	G9	G6	G5
E5	1.4	0.16	G3	G7	G5	G8
E7	1.42	0.194	G5	G9	G7	G2
E6	2.16	0.238	G5	G2	G4	G3
E1	1.66	0.617	G9	G4	G3	G10
E9	1.27	0.79	G10	G9	G11	G3

GYLD=grain yield

4.3.2.3 Additive main effect and multiplicative interaction bi-plot

The IPCA1 and IPCA2 cumulatively contributed 68.31% to the GE interaction and when plotted against each other, the biplot in Figure 4.1 revealed that environments E2, E10, E3, E7 and E9 contributed greatly to the GE interaction effect, though environments E3 and E10 were similar as the angle between them was small. Genotype G2 performed better in high yielding environments like E6 and E10. Environments that are close to each other like E5, E6, E7 and E8 had similar response for the genotypes as depicted by the biplot. As for the genotypes, G4, G7, G6, G2 were adapted to E3 and E10 with G7 being the highest yielder while G1 and G8 were depicted as being adapted to E2 and E4. Genotypes G10 and G9 were adapted to E1 and E 9 while G3 was adapted to E6, E7 and E5. Similarly, G11 and G5 interacted well with E7 with E8 being another good environment for G6. Genotype G9 had the same yield as the average mean (1.67 t/ha) but was the most stable as it had APCA scores close to zero. The second most stable was G4 followed by G6, G3 and G7 which were also in close proximity to the stability axis.

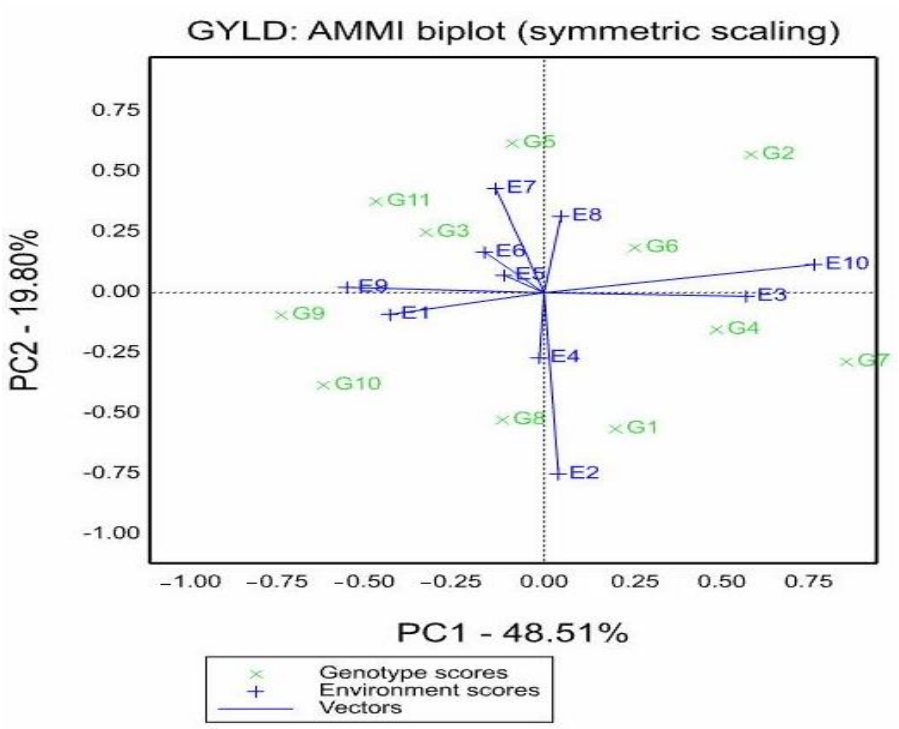

Figure 4.1 Biplot analysis of GE based on AMMI2 for the first two interaction principal component scores

4.3.3 Genotype and genotype by environments (GGE) biplot analysis

4.3.3.1 Mega environment classification and best genotypes

Figure 4.2 presents the polygon view of the GGE biplot. This biplot shows the best performing hybrid(s) for each environment and the groups of environments. The biplot rays divided the biplot into seven partitions and the environments appeared in four of them. Five environments (E2, E4, E5 E3 and E10) fell in one segment which was classified as one large mega environment, and the genotype at the corner of this segment was G7 meaning that it was the best performing genotype in these environments. Segment two contained three environments (E6, E7 and E8) and the best genotype was genotype G5. The remaining environments E1 and E9 were contained in smaller segments three and four, respectively. Genotype G1, G10 and G11 were the least performing genotypes in all environments with G11 only best in E9.

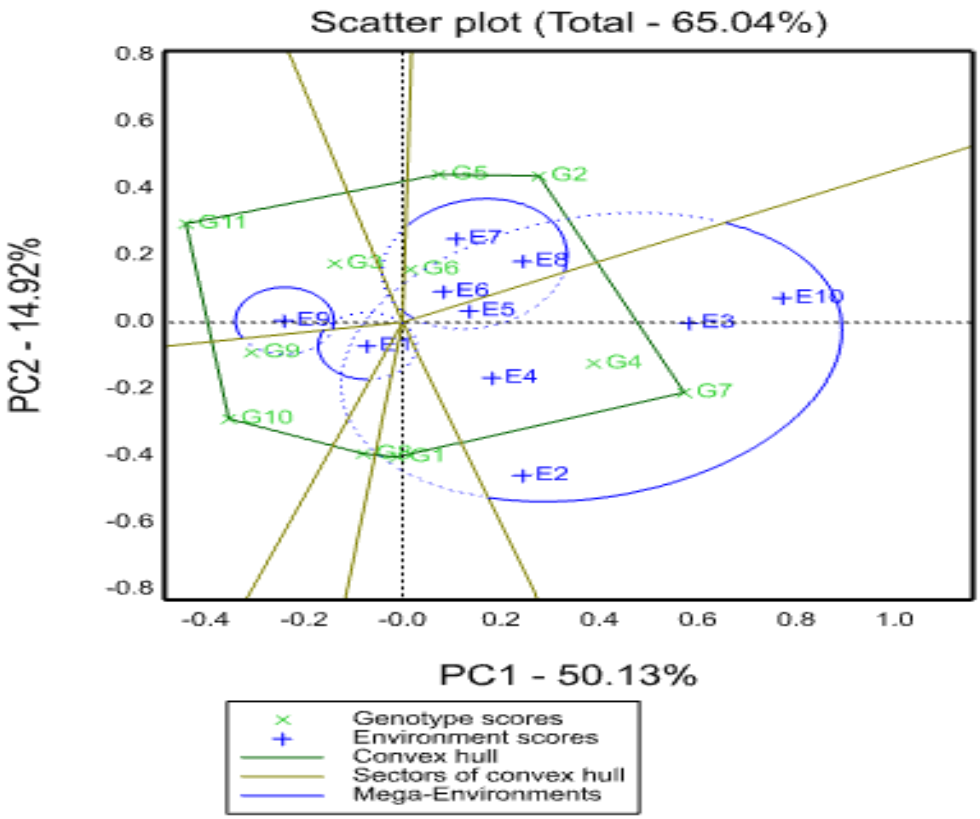

Figure 4.2 Biplot showing the best genotypes in each enviornment and mega environment classification

4.3.3.2 Ranking genotypes relative to the ideal genotype

The single arrow points towards the higher mean yield across all the environments (Figure 4.3). Genotypes with shorter perpendicular projections from the AEC axis are more stable whereas those with longer perpendicular projections are less stable. The other non-dotted line is the determinant of the variability (how unstable) in yield performance across the environments and it points in either directions and also indicates the mean yield point and any genotype on the left performed below the mean while those on the right side of the line performed above the mean. This therefore means that G7, G4, G2 and G5 performance was above the average across the environments with G7 being the highest yielder followed by G4 though in terms of stability, G4 was more stable than G7 as it was closer to the AEC axis. On the other hand, G3, G8, G9, G10 and G11 performed below the average with G3 and G9 being more stable across the environments compared to all the other genotypes. Genotype G4 and G7 were shown as the idea genotypes.

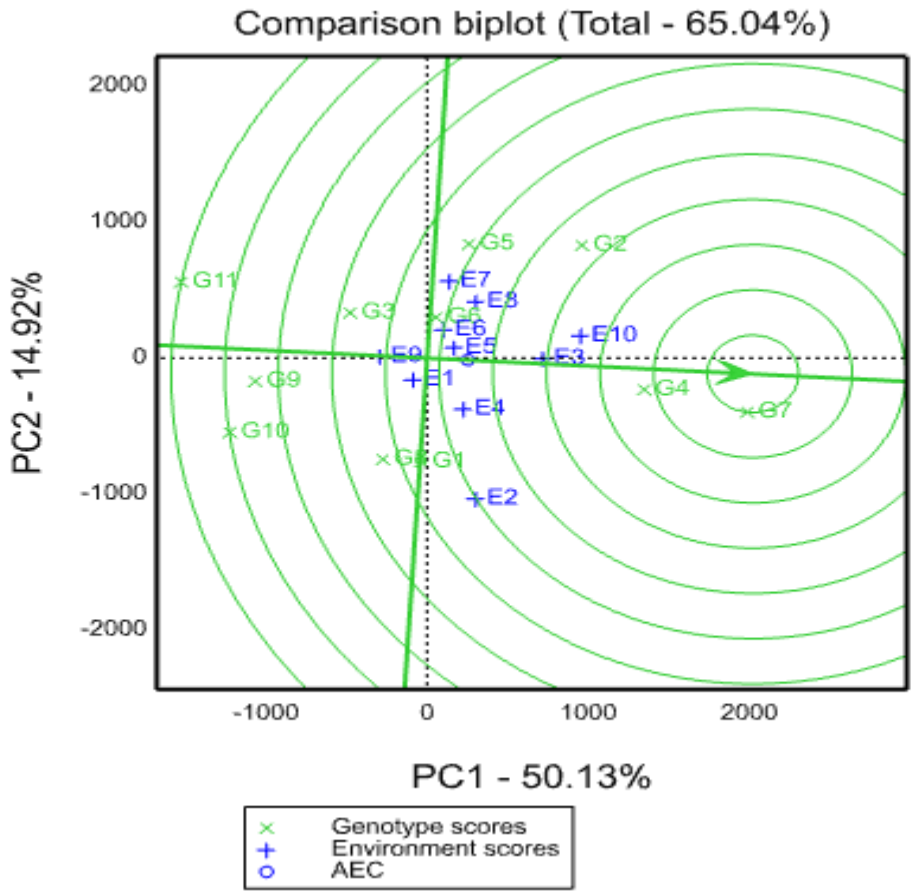

Figure 4.3 Biplot showing the ranking of genotypes relative to ideal genotype

4.3.3.3 Discriminating ability and representativeness of test environments

In order to identify environments that can be very effective in identifying best genotypes from a group of environments, test environment evaluation is necessary. An “ideal” test environment should have the ability to discriminating genotypes and be representative of the mega-environment (Yan, 2001). The discriminating ability of an environment is determined by the length of the vector and is related to the standard deviation of the means for the cultivars in the environment. A discriminating environment is more informative and gives information about the differences among the environments while a consistently non-discriminating environment are the ones that provides little information about the genotypes. A smaller angle between the environmental vector and the average environmental axis (AEA) means that the test environment is more representative of other test environments (Yan et al., 2007; Yan and Tinker, 2006). Therefore, based on Figure 4.4, environments E5, E6, E1 are non-representative and non-discriminating and are therefore not useful in testing genotypes’ performance. Environments E3, E7, E8, E9 and E10 are good for testing and selection of superior genotypes with E3 and E10 being the best ideal environments while E2, and E4 can be regarded as non-representative but discriminating and as such can be used for culling inferior genotypes.

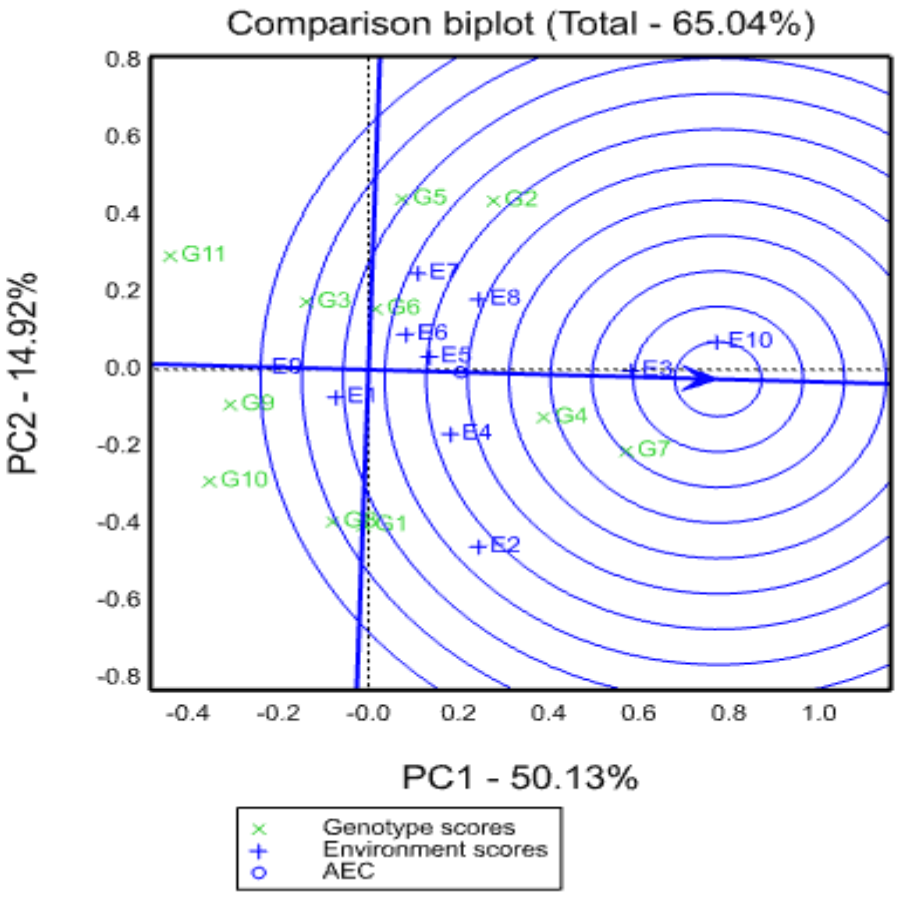

Figure 4.4 Biplot showing the ranking of the test environments based on ideal environment

4.3.3.4 Relationships among environments

The relationship that exists between two environments is determined by the cosine angle between the two environmental vectors. Environments will acute angles between them are positively correlated while those that have obtuse angles are negatively correlated. Those with right angles between them are not related. The angle size between the two environments indicates the similarity between the environments in discriminating the genotypes (Yan and Tinker, 2006). In Figure 4.5 the bi-plot shows the relationships among the test environments. There was a strong positive correlation among environment E3, E10, E5, E6, E8 E7 (acute angle) while E2 showed a negative correlation with E6, E7 and E9. E8 and E2 showed no correlation. E3 and E10 showed the highest positive correlation. E6 and E8 were also highly correlated. Based on the size of the angle between the environments, the environments were grouped in to three with E3 and E10 forming the first group, E4 and E2 formed the second group while the rest fell in the third group.

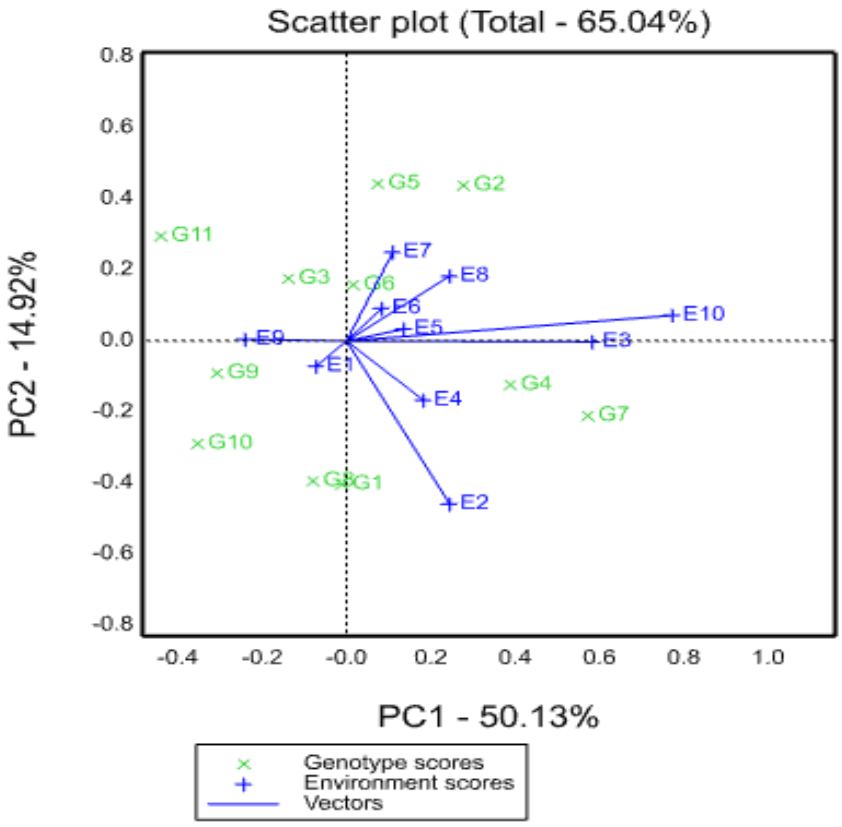

Figure 4.5 Biplot showing the relationship among the environments

4.4 Discussion

4.4.1 Genotype X environment interaction using AMMI analysis

In this study, the G+E+GEI variation was explained in three proportions which are genotype (G), the environment (E) and the genotype by environment interactions (GEI). GEI had the biggest contribution to the variation with 57.92% of the total treatment sum of squares while genotype contributed 18.98% with environment only contributing 23.92%. This is in agreement with other researchers (Makinde and Ariyo, 2011; Negash et al., 2013; Sewagegne et al., 2013) who also reported lower than 50% environmental contribution to the variation and lower than the GEI contribution. The high GEI percentage was as a result of genotypes performing differently in different environments due to crossover interaction However, most researchers have recorded huge contributions on variations due to environment which were above 75% (Kaya et al., 2002; Mengesha, 2013; Ndhlela et al., 2014; Nkhata, 2016) attributing it to the diverse nature of the environments.

After an AMMI analysis, it was shown that the contributions of GEI, E and G to the variation were all highly significant ($P<0.001$). This meant that the environments were different and led to differences in grain yield. The GEI sum of squares was about 3.5 times larger than that for genotypes and 2.5 times greater than that for environments, which explained the differences

in the response of genotypes across environments. The variation amongst environments is an indication of the need for multi-environment yield trials. The large yield differences due to change in environment is applicable to genotype evaluation and mega environment classification (Fox and Rosielle, 1982; Gauch and Zobel, 1996)**.** When GEI is larger than the genotype contribution, it suggests that there is a possibility of having different mega environments (Ndhlela et al., 2014).

Genotype G4 and G7 were the most stable genotypes as they had the highest IPCA1 scores and low IPCA2 scores (close to zero). This agrees with Kaya et al. (2002) who noted that a biplot created using the IPCA scores for the environment and genotype of the first two AMMI components showed that genotypes with larger IPCA1 and lower IPCA2 scores gave high yields and were stable while genotypes with lower IPCA1 and larger IPCA2 scores had low yields and were unstable. These genotypes, even in poor performing environments were able to perform above average. It was also noted that genotypes G10, G11 (controls) consistently had low yields in most sites and as such could not be regarded as superior genotypes. Genotype G9 performed below the average mean performance but it was stable as it had IPCA2 scores close to zero. This agrees with other researcher who identified low yielding stable varieties that may need to be tested in specific environments to realise their full potential. Makinde and Ariyo (2011) reported eight groundnut genotypes that consistently performed below average mean and among these, three were stable while Kaya et al. (2002) reported two bread wheat genotypes that performed below average but where very stable across the environments. The reason for the low yields for G11, G10, G3 and G8 when compared to the rest was due to the fact that they are early maturing varieties (Spanish) characterised by small seed size while the rest were medium maturing varieties (Virginia) characterised by big seed size. This study and the method used to identify genotypes with the best performance and relatively stable is in agreement with other researchers in sesame, finger millet, rice, wheat, groundnuts, proso-millet and maize (Lule et al., 2014; Makinde and Ariyo, 2011; Ndhlela et al., 2014; Negash et al., 2013; Tariku et al., 2013; Yan and Tinker, 2006; Zhang et al., 2016) who used AMMI and GGE biplots to identify the best performing stable genotypes across diverse environments. It should, however, be noted that they all recommended a minimum of two season's data to confidently say a genotype was stable and high yielding.

To evaluate the environments, an AMMI analysis was done that focused on environment and genotypes. The IPCA scores were both negative and positive for genotypes and environments which is similar to other researchers' results where they reported both negative and positive scores (Kaya et al., 2002; Mengesha, 2013; Silveira et al., 2013). Based on the IPCA scores and the AMMI biplot, it was noted that genotype G2 had specific adaptation to environments

E3, E8 and E10 where it performed better. The IPCA1 scores for the environment and the genotypes were in agreement with the biplot results. Genotypes G4, G7, G6, G2 were adapted to E3 and E10 with G7 being the highest yielder while G1 and G8 were depicted as being adapted to E2 and E4. Genotypes G10 and G9 were adapted to E1 and E 9 while G3 was adapted to E6, E7 and E5. Similarly, G11 and G5 interacted well with E7 with E8 being another good environment for G6. In terms of high yields and stability, genotype G9, G4, G6, G3 and G7 were depicted as the most stable with G4 and G7 having yielded higher that overall mean while G9 and G3 had the same yield levels as the overall mean. Other researchers have found similar results in different crops like groundnuts (Alhassan, 2013), maize (Ndhlela et al., 2014), wheat (Kaya et al., 2002), rice (Katsura et al., 2016), finger millet (Lule et al., 2014) and beans (Wilson, 2016). These results show that G4 and G7 are good for wide adaptation as they showed both stability and high yields while other genotypes like G2 and G5 showed less stability but had high yields which implies they are adapted to specific environments.

4.4.2 Genotype by environment interaction based on GGE biplot analysis

As breeders, the desire is to breed and release cultivars that are both stable and high yielding. To ascertain which genotype performed best and was stable, a GGE bi-plot analysis was used. Roostaei et al. (2014) states that the ideal genotype must have a high PC1 value (high mean productivity) and a PC2 value near zero (high stability). The selection of the best genotypes was based on the performance of each genotype across the different environments, its stability and adaptability. Using PC1 and PC2, it was possible to identify genotype G7, G4, G5 and G2 (control) as the best performing genotypes though only G4 and G7 showed consistent performance, relative stability and adaptability across the ten testing environments. Yan and Tinker (2006) stated that the best candidate genotypes are expected to be stable and have high mean yield performance across all test environments. In reality, such genotypes are rarely found. Therefore, high yielding stable genotypes can be considered as standards for the evaluation of genotype. The AEC ordinate approximates the genotypes' contributions to GEI, which is a measure of their stability or instability.

Ndola (E10) was the most discriminating environment as it had the largest PC1 score and the longest environment vector. For the environments that showed a high correlation like Lundazi FTC (E3) and Ndola (E10), if the same trait is being measured among the same genotypes, selection can be done indirectly and applied across the environments. The existence of such unique correlation among test environments shows that there is a possibility of reducing the number of sites used for testing as this would not significantly affect the results and will lead to a reduction in cost of testing the genotypes (Gauch et al., 2008). For an environment to be regarded as been representative of other test environments, it has to have a smaller angle with the AEA. The correlation coefficient between the genotypic values in a certain

environment and across the environments is reflected by the cosine angle between the environments. This helps determine the environment which is more representative of the other environment in a mega environment and can be used to test genotypes that can also perform well in the other environments (Yan and Tinker, 2006; Yan et al., 2007). Lundazi FTC (E3) was the most representative, followed by Ndola (E10) and Mfuwe (E5), whilst Katete FTC (E2) and Masumba (E7) were the least representative. Lundazi FTC (E3) and Ndola (E10), been representative and discriminating can be used for selecting generally adapted genotypes, and the discriminating and non-representative environments, such as Katete FTC (E2) can be used for culling inferior genotypes and also for selection of specifically adapted genotypes. Kalichero FTC (E1) and Mambwe FTC (E6) had the shortest vectors and hence qualified as non- representative and non-discriminating which rendered them useless. Alam et al. (2015) and Ndhlela et al. (2014) made similar conclusions on environments.

The environments were classified into four mega environments. Yan et al. (2007) stated that if the mega environment classification is repeated over years, it would be advisable to evaluate test genotypes in each of the mega environments. The season in which the study was carried out was characterised by heavy rains such that the low rainfall environments like E5, E6 and E7 which are in the valleys and usually receive less than 800 mm of rainfall annually received more than a 1000 mm. Hence this evaluation must be repeated before a conclusion is made on the classification of the mega environments. As this was a one season evaluation, one must be cautious not to make a conclusion until another year or two of testing so as to rule out any unfounded underlying causes to the variations observed. This is in agreement with Ndhlela et al. (2014) who also found some disparities in environment correlations after his study of the maize multi environment trial and attributed it to seasonal weather and climatic conditions. Bänziger et al. (2006) cited that temperature, growing season duration, lack of enough water, sub-soil pH, rainfall and social economic and biotic factors (Butron et al., 2004) are some of the factors that contribute to the presence of GEI.

4.4.3 Comparison between AMMI and GGE biplot analyses

In this study, AMMI biplot had a high percentage for the sum of PC1 and PC2 score compared to GGE (68.31% and 65.04% respectively). Yan et al. (2007) stated that the greater the percentage the more confidence the research has in explaining the variations but that did not mean that biplots with smaller percentages are useless. Both biplots had high percentages that were sufficient in explaining the G + GE. However, GGE biplots were able to give more information and were easy to interpret as they could visually explain the interactions between environments and genotypes compared to the AMMI biplot. GGE biplots were able to classify the environments into mega environments and show the “which won where” genotypes clearly and visually unlike the AMMI biplot where the best performing genotypes are not clearly shown

especially when there are so many genotypes (Yan et al., 2007). This is in agreement with other researchers who made similar observations (Casanoves et al., 2005; Samonte et al., 2005; Yan and Rajcan, 2002; Yan and Tinker, 2006). In terms of means and stability, the GGE biplot was superior as it was clear and easy to construct and interpret compared to the AMMI biplot. It clearly displayed the ideal genotypes and their stability. Malvar et al. (2005) and Voltas et al. (2005) explained similar observations in their study of AMMI and GGE biplot analysis of maize and the use of biplots for the identification of best genotypes in multi environments. Discriminating and representative environments were easily identified using the GGE biplot and were visually presented in manner that was easy to identify and interpret. Many researchers have used GGE biplots in this regard to identify discrimination and representative environments which is an essential part in plant breeding (Thomason and Phillips, 2006; Yan and Hunt, 2002).

Even though GGE gave more visual information which was easy to interpret, both AMMI and GGE classified the genotypes similarly and identified G7 and G4 and the highest and stable genotypes in the study.

4.5 Conclusion

The study revealed that environments, E3 an E10 were the best environments that were both discriminative and representative. Lundazi FTC (E3) was the most representative, followed by Ndola (E10) and Mfuwe (E5), whilst Katete FTC (E2) and Masumba (E7 were the least representative. Lundazi FTC (E3) and Ndola (E10) were both discriminating and representative and as such can be used for selecting generally adapted genotypes, and the discriminating and non-representative environments, such as Katete FTC (E2) can be used for culling inferior genotypes and also for selection of specifically adapted genotypes. Kalichero FTC (E1) and Mambwe FTC (E6) were non- representative and non-discriminating which rendered them useless. The AMMI and GGE bi-plots revealed that G7, G4 were high yielding recording 2.08 t/ha and 1.97 t/ha, respectively, compared to the average mean of 1.67 t/ha across all environments. G7 had a yield advantage of 19.6% over the control G2 (1.74 t/ha) while G4 yield advantage was 4.8% over G2 which was highly significant at $P<0.001$. Genotype G3, a Spanish type of variety yielded more than G11 (control) which was a Spanish type as well with a yield advantage of 26%.

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CHAPTER FIVE

GENOTYPE BY TRAIT ASSOCIATION ANALYSIS OF ZAMBIAN GROUNDNUT (*Arachis hypogaea* L.) GENOTYPES

Abstract

There is very little information available on the trait profiling of groundnut genotypes in Zambia. The objectives of this study were to evaluate the performance of 15 groundnut genotypes that consisted of eight landraces, two pre-released cultivars and five released varieties as controls based on multiple traits. This was done to identify genotypes that are superior in desired traits which can be candidates for release and commercial production or can be used as parents in the breeding programmes for further improvement of landraces and other genotypes. The experiment was laid out in a randomised complete block design (RCBD) with three replications across three environments. The traits under study included; days to 50% flowering (DTF), days to maturity (DTM), shelling percentage (SH%), weight of 100 seeds (HSW), pods per plant (PPP), yield per plant (YPP), pod yield (PYLD) and grain yield per hectare (GYLD). Grain yield showed highly significant positive correlation with PPP, SH% and YPP with r values of 0.86, 0.94 and 0.90, respectively at $P<0.01$. Path coefficient analysis revealed that YPP, SH%, HSW, PPP and DTM had a positive direct effect on GYLD while the DTF had a negative direct effect on GYLD with DTF showing a positive indirect effect on GYLD via YPP. Genotype by trait (GT) biplot captured 83.00% of the variations due to genotype by trait interactions. The GT analysis revealed that genotype G12 had high values for PPP, SH%, HSW, YPP and GYLD while genotype G8 ranked lowest for PPP, SH%, YPP and GYLD. Two landraces, genotypes G3 and G4 performed relatively well in comparison to genotype G12 and can be crossed with genotypes with complementary features so that beneficial alleles are combined for improvement of the crop. Genotypes G9, G10 and G15, can be used as sources of resistance genes to introgress into the landraces, which lack rosette resistance.

5.1 Introduction

Yield is a complex trait that has low heritability and is highly affected by the environment and other undesirable linkages and associations with other traits (Yan, 2014). This makes selection based on yield only an ineffective approach. Therefore, conducting trials across different diverse environments for different traits is an integral part of any breeding programme (Yan and Kang, 2002; Yan and Rajcan, 2002). This type of evaluation requires careful interpretation of the results as there are many factors influencing yield. Different relationships that exist among the traits have a great impact on the decision as to which type of selection method to use. It is common to find negative correlation among important traits in plant breeding which leads to selection challenges (Lewis, 2006; Xu-Xiao et al. 2008). Rubio et al. (2004) stated that variation among the genotypes within and among populations is essential in breeding and these variations in terms of agronomic and plant structure traits should be carefully studied.

Different methods have been used to understand how traits are related across different crops by many researchers and the traits have been profiled (Rao et al., 2014; Rubio et al., 2004; Sarwar et al., 2004; Shoba et al., 2012; Thakur et al., 2014; Tiwari et al., 2011). One method used for profiling traits is the genotype by trait (GT) biplot analysis and it is fast becoming an effective tool. This tool uses the GGE technique to graphically display the genotype by trait associations and allows for the visualization of the relationships that exist among the traits across the test genotypes (Yan and Frégeau-Reid, 2008; Yan and Rajcan, 2002). The GT biplot is able to provide information on the traits that are redundant and those that are useful and this information is helpful in identifying traits that exhibit direct or indirect effects on the trait of interest. This informative way of collecting data on genotypes has been applied in other legume crops like soybean (Yan and Rajcan, 2002), common bean (González et al., 2006), cowpea (Oladejo et al., 2011) and also in cereals like wheat (Ali et al., 2008).

Correlation analysis is also used to show the associations that exist among the traits and how they are related to the trait of interest. This is important because there is lack of consistence in how yield components relate to yield which makes breeding cultivars that are stable in terms of performance across different environments difficult (Shenkut and Brick, 2003). For any selection criteria to be useful, the traits under study must have high correlation with seed yield and also display a low GE interaction coupled with high heritability (Rao et al., 2002; Yuan et al., 2002).

Knowing the associations that exist between or among traits is an important part of the breeding programme that can help in developing cultivars that are high yielding and suitable. Correlation studies among the traits of interest help in identifying these associations. However, knowing these associations is not enough as it does not show which traits directly affect the

trait of interest. Therefore, path coefficient analysis is useful in determining what type of effect each trait has on a trait of interest; direct or indirect. As the number of traits increases, correlation becomes more complex and this is where path coefficient becomes important as it provides information on the direct and indirect effects of the traits in relation to the trait of interest (Bhargavi et al., 2015; 2017)

The hypothesis for this study was that there is a significant relationship between yield and secondary traits. Therefore, the objectives were to evaluate the performance of 15 groundnut genotypes that consisted eight landraces, two pre-released cultivars and five released varieties as controls based on multiple traits so as to identify genotypes that are superior in desired traits which can be candidates for release and commercial production or can be used as parents in the breeding programmes for further improvement.

5.2 Materials and Methods

5.2.1 Plant materials

The genotypes used in this experiment were landraces collected from the eastern province of Zambia. These were collected from different farmers in Katete, Chipata, Lundazi and Petauke. Five controls were included in the trial of which three were released varieties MGV4, MGV5 (medium duration) and Luena (short duration) while two were pre-released varieties ICGV SM 01711 (medium duration and rosette resistant) and ICGV SM 01514 (short duration and rosette resistant). The remaining ten were landraces from the Spanish and Virginia botanical groups. Genotype details are presented in Table 5.1.

Table 5.1 List of goundnut geneotypes evaluated

Genotype Code	Genotype Name	Source	Botanical Group	Entry Type	Rosette Response
G1	Kayoba	Zambia	Spanish	Landrace	Susceptible
G2	Kadonokho	Zambia	Virginia	Landrace	Susceptible
G3	Kasele	Zambia	Virginia	Landrace	Susceptible
G4	Chalimbana	Zambia	Virginia	Landrace	Susceptible
G5	Solontoni	Zambia	Spanish	Landrace	Susceptible
G6	Kayoba 1	Zambia	Virginia	Landrace	Susceptible
G7	Kayoba Runner	Zambia	Virginia	Landrace	Susceptible
G8	Kanjuthe Kamun`gono	Zambia	Spanish	Landrace	Susceptible
G9	ICGV SM 01514	Zambia	Spanish	Pre-released	Resistant
G10	ICGV SM 01711	Zambia	Virginia	Pre-released	Resistant
G11	MGV 5	Zambia	Virginia	Released Variety	Moderate
G12	MGV 4	Zambia	Virginia	Released Variety	Susceptible
G13	Luena	Zambia	Spanish	Released Variety	Susceptible
G14	Katete	Zambia	Spanish	Released Variety	Resistant
G15	Chishango	Zambia	Virginia	Released Variety	Resistant

5.2.2 Site description

This study was carried out in Zambia across three environments during the 2016/17 season. The experiment was set up at three locations namely, Msekera Research Station, Masumba Technical Research Site and Copperbelt Research Station, each representing an agro-ecological zone (Region I, II and III). These sites are in Chipata, Mambwe and Mufulira districts, respectively. The site description details are presented in Table 5.2 and Table 5.3. Generally, Msekera is in a zone that receives rains between 800 and 1000 mm annually while Masumba is in the valley, a place where less than 800 mm of rain is expected. Mufulira falls in zone II where the rains can go above 1000 mm annually. This was one of the criteria used to select these sites.

Table 5.2 Site description

Site Code	Site Name	Agro-Region	Coordinates	Elevation	Annual Rainfall	Average Temperature
E1	Masumba	1	S13°13.297` E031°55.651`	550m	1112.5	26.2
E2	Msekera	2	S13°39.007` E032°33.920`	1023m	1242.2	24.8
E3	Mufulira	3	S12°36.690` E028°08.789`	1217m	1237.4	22.2

Table 5.3 Soil properties

Site Name	pH CaCl2	Org C%	N %	P Ppm	K ppm	Ca ppm	Mg ppm	Na ppm	CEC me%	Soil type
Mufulira	4.1	0.54	0.03	13	49	445	364	16	5.4	sandy clay loam
Masumba	4.1	0.28	0.02	11	178	440	115	58	3.76	sandy clay loam
Msekera	5.5	0.4	0.02	10.1	102	2840	268	13.85	5	clay loam

5.2.3 Experimental design and layout

The experiment was laid out in a Randomised Complete Block Design (RCBD) with three replications. Hand planting was done and each plot consisted of four ridges, 3 m long with inter row spacing of 0.6 m and intra row spacing of 0.1 m. Fertiliser was applied during planting at 150 kg/ha of D-compound fertilizer which has NPK ratios of 10:20:10, respectively. No pesticides or chemicals were added during the growth period. The two middle ridges were harvested as net plot and the plot yield was converted to yield per hectare. Plots were hand weeded throughout the growing seasons. Only, the middle two rows in each experimental plot were used for data collection.

5.2.4 Data collection and analysis

Five randomly selected plants from each plot across the three replications were sampled and selected at the time of harvesting. The traits that were observed were pod yield and its components including pods per plant, shelling percentage, weight of hundred seeds, days to flowering, days to maturity, kernel weight. Number of days from emergence to 50% flowering and 75% physiological maturity were recorded as days to flowering and days to maturity, respectively. Days to 50% flowering, grain yield and days to maturity were recorded on plot basis, data on the rest of the traits were recorded on five randomly selected plants and an average value was used for the statistical analysis.

Days to 50% flowering, days to maturity, grain yield and hundred seed weight were recorded based on measurements and observations made on the entire two middle rows of each plot.

Flowering date was recorded by visual observations, and days to 50% flowering was recorded when 50% of the plants in the centre two rows had one open flower. Grain yield was calculated from the entire middle two rows, which were hand harvested and threshed. Harvested seeds were dried at room temperature to 7% moisture content prior to weighing. Five 100-seed samples from each plot were weighed and mean 100-seed weight was recorded.

To compute G x E interactions for seed yield and plant traits, a combined analysis of variance across environments (sites) was performed. Analysis of variance (ANOVA) and genotype by trait analysis were performed using PC-SAS version 9.4 while genotype by trait analysis was performed using GGE biplot analysis (Yan, 2001; Yan and Kang, 2003) so as to determine which genotype was best and for what trait. The trait means were standardised before using them in the biplot analysis. Biplot analysis was based on Model 2 which requires standardisation, centering and transformation of data sets (transformation=0, centering=2 and scale=1). The GGE biplot model equation for genotype by trait interaction biplot analysis is presented as follows:

$$\frac{\mathrm{T}_{ij}-\beta_{ij}}{s_j} = \sum_{n=1}^{2} \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij} = \sum_{n=1}^{2} \xi^{*}_{in} \eta^{*}_{jn} + \varepsilon_{ij}$$

Where: T_{ij} is the mean value of genotype i for trait; $j\beta_j$ is the average value of all genotypes for trait j while S_j is the standard deviation of trait j among genotype means. λ_n is the singular value for Principal Component (PCn); ξ_{in} is the PCn score for genotype I; η_{jn} is the PCn score for trait j and ε_{ij} is the residual associated with genotype i in trait j.

The Pearson correlation coefficient was computed using the formula in SAS 9.4 software

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

Where; r is the Pearson coefficient correlation, x is the dependent variable, y is the independent variable while n is the sample size

The path coefficient analysis was done using Microsoft excel 2013 through the use the Pearson`s correlation results from SAS 9.4. These were then transformed into path coefficient analysis to show the contribution of each trait to the dependent variable. This analysis revealed direct and indirect effects on the primary trait which was grain yield. The model used as suggested by Akintunde (2012) is as follows:

$$y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + U$$

Where y is the dependable variable (GYLD) while $a + b_1X_1 + b_2X_2 + b_3X_3 + U$ are the correlation variables with the assumption that each is independently contributing to the dependent variable y

5.3 Results

5.3.1 Genotype by trait association

The combined analysis of variance for each agronomic trait is presented in Table 5.4. All studied traits were significantly affected by environment at P<0.001 and P<0.05 levels except PPP and YPP. Significant differences (P<0.001) was found among the genotypes for all the traits under study. Among interaction effects, GEI was significant for all evaluated traits at P<0.001 and P<0.01 levels, signifying that the trait genotypic values were influenced by the environment. All traits showed no significant variations within the replication across the environments except for PYLD which showed significant variation at P<0.01. Table 5.5 contains a contrast of means for evaluated genotypes on the basis of each studied trait across environments using Tukey comparison test at 5% level of probability. No single genotype showed superiority in all traits and as such, each genotype should be characterised by its trait profile. Based on YLD, genotype G12, followed by G14 and G11, performed well across environments, whereas G8 and G5 showed low yield performance.

Table 5.4 Combined analysis of variance for the studied traits

		Mean Square							
Source	DF	DTF	DTM	SH%	HSW	PPP	YPP	PYLD	GYLD
Environment	2	312.067***	184.030***	93.791**	153.489***	39.800ns	10.195ns	3181200.59***	858569.130***
Block(environment)	6	0.467ns	3.207ns	25.957ns	9.348ns	15.267ns	5.473ns	294554.03**	113077.416ns
Genotype	14	101.016***	222.792***	239.222***	483.479***	41.559***	55.705***	764366.89***	370805.980***
G x E	28	11.654***	13.696***	89.172***	126.235***	30.292**	31.000**	431680.760***	229677.004***
Error	84	0.379	2.453	19.015	10.031	11.314	10.787	69442.18	40833.280
Total	134								

GYLD= grain yield (kg/ha), DTM=days to maturity, SH%=shelling percentage, HSW= weight of 100 seeds (g), DTF= days to 50% flowering, YPP= yield per plant (g), PPP=number of pods per plant (g), PYLD=pod yield (kg/ha), ***, ** = Significant at P<0.001 and P<0.01 respectively, ns = significant at P>0.05

Table 5.5 Comparison of the 15 genotypes across three environments

Genotype Code	Genotype Name	DTM	DTF	HSW	PPP	YPP	SH%	PYLD	GYLD
G1	Kayoba	129.7.a	35.8dc	52.9bac	12.6b	14.8c	61.8bdec	1540.4bdc	963.0bc
G2	Kadonokho	124.7b	35.1e	52.8bac	14.3ba	16.5bac	65.6bdac	1631.4bdac	1065.7bac
G3	Kasele	124.7b	36.9a	56.8ba	19.2a	21.9a	61.8bdec	1835.3bdac	1148.3bac
G4	Chalimbana	129.7a	35.3de	52.6bac	14.8ba	21.5ba	53.5e	2204.6ba	1189.4bac
G5	Solontoni	126.3ba	33.3f	52.3bdac	14.4ba	14.3c	64.9bdac	1292.6d	837.0c
G6	Kayoba 1	124.7b	36.1bc	47.8bdc	15.6ba	15.3bc	65.9bac	1617.3bdac	1073.9bac
G7	Kayoba Runner	126.3ba	35.8dc	45.1dec	17.4ba	16.5bac	61.7bdec	1714.7bdac	1065.2bac
G8	Kanjuthe Kamun`gono	129.7a	36.6ba	56ba	15.2ba	16.8bac	55.9de	1441.7dc	816.3c
G9	ICGV SM 01514	115.1c	26.9h	45.4dec	18.7ba	15.8bac	68.7bac	1533.0bdc	1048.8bac
G10	ICGV SM 01711	126.3ba	33.6f	59.1a	13.7ba	16.8bac	71.8a	1750.9bdac	1260.7bac
G11	MGV 5	128ba	36.7ba	62.4a	13.6ba	18.9bac	66.6bac	2086.0bac	1398.6ba
G12	MGV 4	124.7b	35.7dce	60.6a	19.56a	21.6ba	64.1bdac	2322.5a	1506.5a
G13	Luena	115.7c	26i	42.1de	16.6ba	16.7bac	68.1bac	1904bdac	1267.1bac
G14	Katete	115.7c	32.7g	35.6e	16.7ba	18.2bac	71.6ba	2054bac	1441.7ba
G15	Chishango	124.7b	35.3de	53.9bac	14.8ba	15.5bc	60.8dec	1838.6bdac	1119.8bac

GYLD= grain yield (kg/ha), DTM=days to maturity, SH%=shelling percentage, HSW= weight of 100 seeds (g), DTF= days to 50% flowering, YPP= yield per plant (g), PPP=number of pods per plant (g), PYLD=pod yield (kg/ha). Means followed by the same letter in a column are not significantly difference

5.3.2 Correlation coefficients

The computed Pearson's correlation coefficients (Table 5.6) showed the relationships among the traits. PPP, SH% and YPP showed positive significant correlation with GYLD while DTF showed a significant negative correlation with GYLD. The correlation between GYLD and HSW, though positive was not significant.

Table 5.6 Pearson's correlation coefficients between the traits analysed for 15 genotypes across three environments

	Trait					
	DTF	DTM	PPP	SH%	HSW	YPP
DTF						
DTM	0.602ns					
PPP	-0.756*	-0.397ns				
SH	-0.585ns	-0.420ns	0.775**			
HSW	-0.545ns	-0.299ns	0.356ns	0.055ns		
YPP	-0.778**	-0.617ns	0.917**	0.851**	0.455ns	
GYLD	-0.682*	-0.422ns	0.856**	0.936**	0.226ns	0.901**

GYLD= grain yield (kg/ha), DTM=days to maturity, SH%=shelling percentage, HSW= weight of 100 seeds (g), DTF= days to 50% flowering, YPP= yield per plant (g), PPP=number of pods per plant (g),

**, * = Significant at P<0.01 and P<0.05 respectively, ns = non-significant

5.3.3 Path coefficient analysis

Results of path coefficient analysis (Table 5.7) showed that SH% had the largest direct effect on GYLD with r = 0.61. YPP had the second largest direct effect on GYLD with an r value of 0.31 while HSW, DTM and PPP recorded low direct effects of 0.01, 0.11 and 0.07 respectively. DTF showed a negative direct effect on GYLD but showed positive indirect effect on GYLD via YPP.

Table 5.7 Path coefficient analysis showing direct (diagonal and bold) and indirect effects (off diagonal) of different characters on pod yield in groundnuts

	Trait						
	DTF	DTM	PPP	SH	HSW	YPP	GYLD
DTF	**-0.0922**	0.0667	-0.0550	-0.3568	-0.0049	-0.2393	-0.6815
DTM	-0.0555	**0.1109**	-0.0289	-0.2559	-0.0027	-0.1898	-0.4220
PPP	0.0697	-0.0440	**0.0728**	0.4721	0.0032	0.2822	0.8560
SH	0.0540	-0.0466	0.0564	**0.6095**	0.0005	0.2618	0.9356
HSW	0.0502	-0.0332	0.0259	0.0337	**0.0090**	0.1399	0.2255
YPP	0.0718	-0.0684	0.0668	0.5187	0.0041	**0.3076**	0.9006

DTM=days to maturity, SH%=shelling percentage, HSW= weight of 100 seeds (g), DTF= days to 50% flowering, YPP= yield per plant (g), PPP=number of pods per plant

5.3.4 Genotype by trait biplot analysis

The interaction between the genotypes and the traits is shown in Figure 5.1. The biplot in Figure 5.1 shows data for 15 genotypes that were tested in three environments. The genotypes expressed differently and those closest to certain traits indicated the traits that were closely related to the genotype. The PC1 and PC2 explained 59.95% and 23.05% of the interaction, respectively, giving a total of 83.00%. Significant differences were observed among the traits in relation to genotypes and their performance. The significance level ranged from significant ($P<0.01$) to highly significant ($P<0.001$).

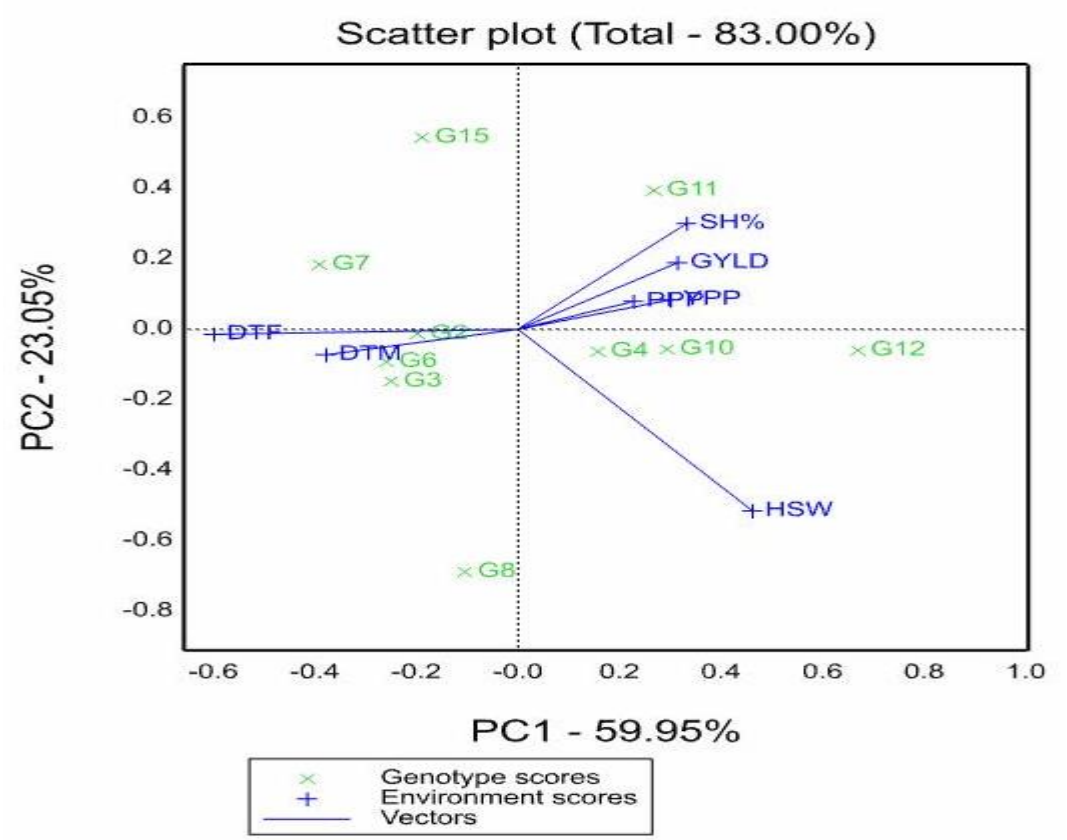

Figure 5.1 The genotype by trait biplot showing the interaction between traits and genotypes

5.3.5 Comparison of trait profiles of two specific genotypes

5.3.5.1 High yielding vs low yielding

The profiles for the traits of two genotypes can be easily compared on the GT biplot. The line that is perpendicular to the line joining the two genotypes divides traits into two groups which shows that each of the two genotypes had larger values for a number of the traits of interest. Figure 5.2 shows the comparison between the best yielding and the lowest yielding genotype and the traits they are associated with which made them be ranked the highest and the lowest. Genotype G12 was the highest yielding while genotype G8 was the lowest yielding genotype. Genotype G12 showed good performance in terms of GYLD, YPP, SH% and PPP. Genotype G8 had high values in DTF and DTM. HSW was intermediate as both genotypes showed high values. These traits showed high significance levels at $P<0.001$ and $P<0.01$.

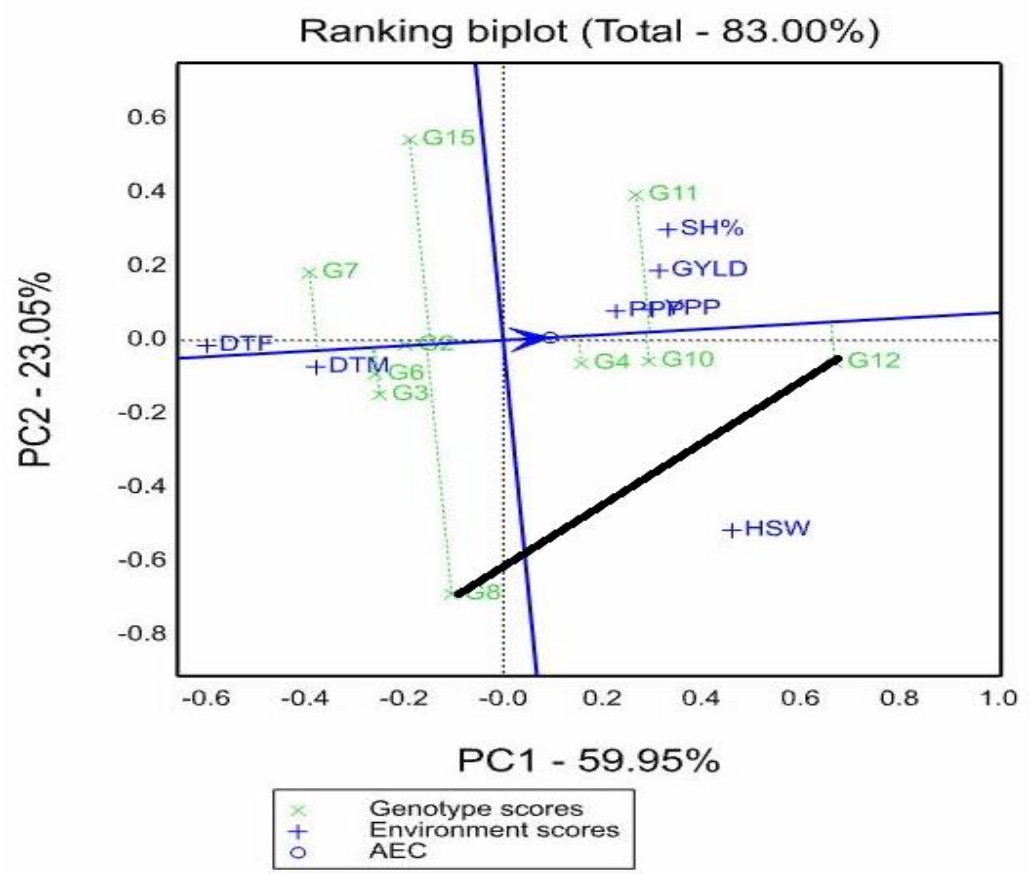

Figure 5.2 Genotype by trait biplot showing the comparison between the highest and lowest yielding genotypes

5.3.5.2 Comparison between landrace and released cultivar

Figures 5.3 and 5.4 show the comparison between the released cultivar G12 and two landraces G4 and G3 respectively. Genotype G12 recorded high values for GYLD, PPP and YPP compared to genotype G3 and G4. Genotype G4, however, had high values in HWS but lower values for GYLD, PPP and YPP as evidenced by the region in which they are falling on the biplot. Genotype G3 only recorded high values in DTM and DTM when compared to G12.

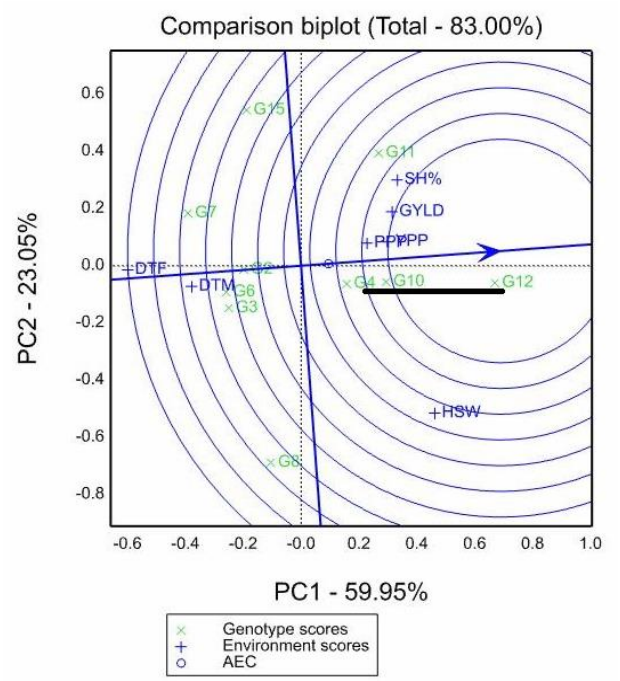

Figure 5.3 Geotype bt trait biplot showing comparison between G12 and G4

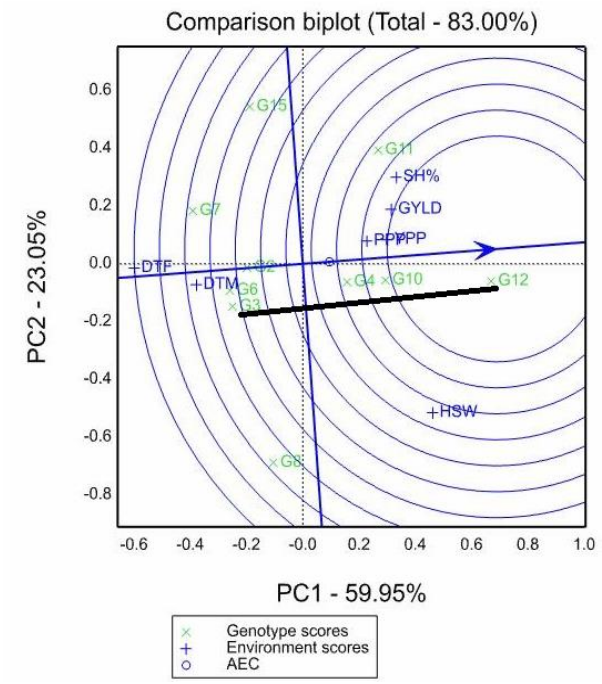

Figure 5.4 Genotype by trait biplot showing comparison between G12 and G3

5.3.6 Relationships among traits

The cosine angle between trait vectors for any two traits can be used to determine the correlation coefficient between the two traits (Yan and Kang, 2002). In the GT biplot, when a vector is drawn from the origin to the point where the trait is located on the biplot, one is able to visualise the relationship that exists between any two traits. This will give information on the association and the trait profiles across the 15 genotypes (Yan and Frégeau-Reid, 2008). Figures 5.5 shows the traits that were prominent and the relationship among the traits. YPP, GYLD, SH% and PPP showed positive correlation and association as evidenced by the acute angles between them. They showed significant differences among the genotypes and across the different environments. GYLD showed strong positive correlation with PPP, SH%, YPP and HSW and a weak negative correlation with DTF and DTM.

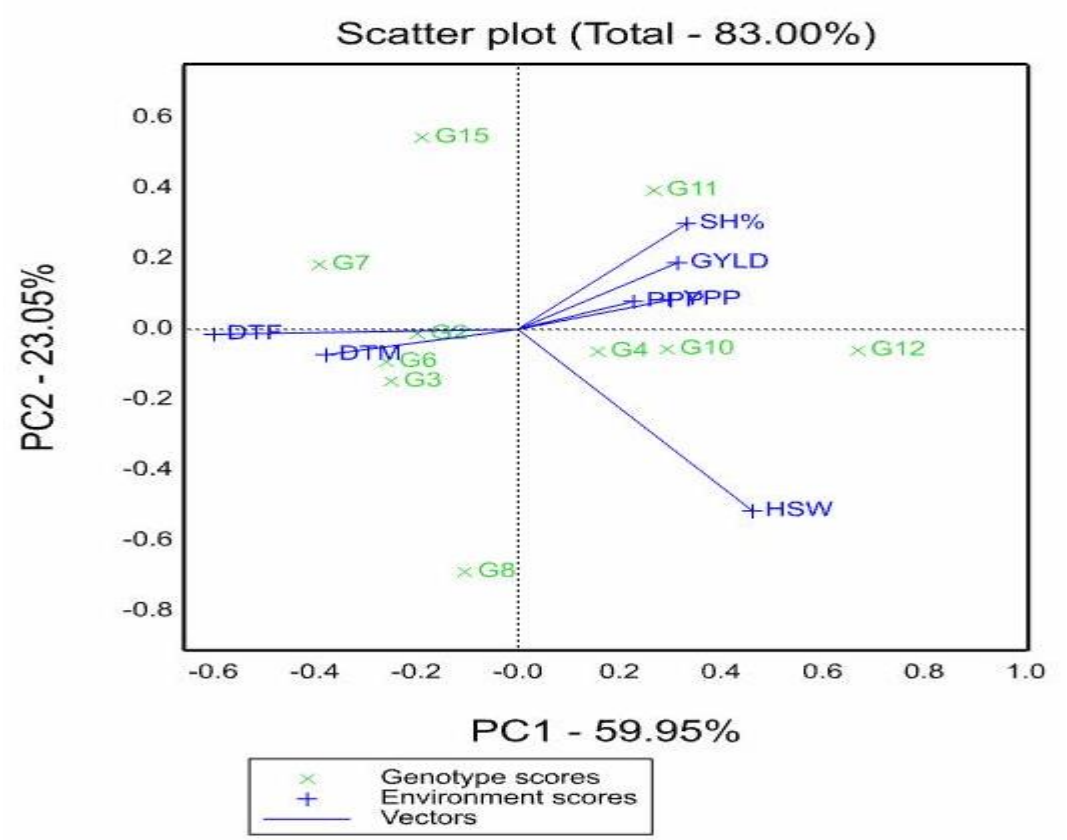

Figure 5.5 Genotype by trait biplot showing the relationship among the traits

5.3.7 Visual identification of the best genotypes based on multiple traits

The best way to view the interaction between the traits and the genotypes is by the use of the polygon view under the GT biplot. Figure 5.6 represents the polygon view of a GT biplot generated from data on eight agronomic traits of 15 genotypes across three environments and shows the “which is good for what biplot analysis. The GT biplot explained 83.00% of the total variation of the standardized data. The polygon was divided into five sectors and in each sector, there was a genotype identified as the best performer or poorest performer in relation to traits of interest. Genotype G12 showed dominion for performance under HSW, YPP, PPP and GYLD while G11 had good SH%. Genotype G7 had good high number of days under DTM and DTF. The rest of the genotypes performed poorly in relation to traits under study. Figure 5.7 illustrates the mean and stability ranking for the genotypes based on the traits under study. Most genotypes showed good stability values across the three test environment as evidenced by the length of their vectors except for G8 and G15 which were unstable and performed poorly. Genotype G2 was the most stable as it was right on the stability axis.

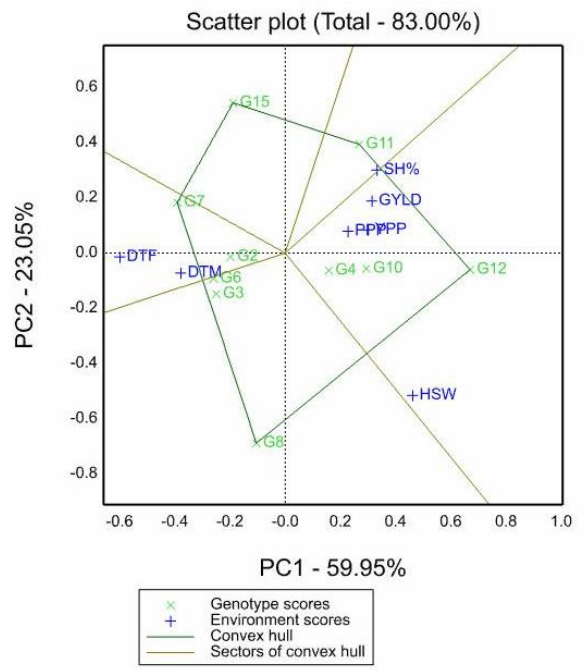

Figure 5.6 Genotype by trait biplot showing the "Which trait was good for what genotype"

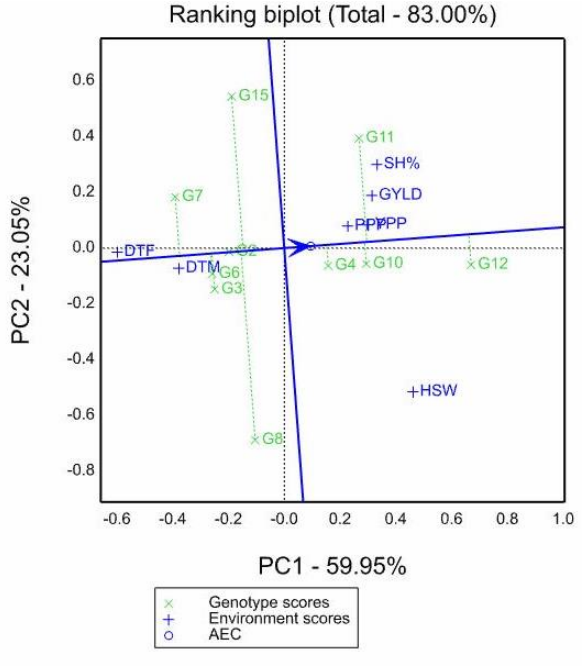

Figure 5.7 Genotype by trait biplot showing the mean vs stability

5.4 Discussion

Raghuwanshi et al. (2016) stated that knowing the associations that exist between or among traits is an important part of the breeding programme that can help in developing cultivars that are high yielding and suitable. But knowing these associations is not enough as it does not show which traits are directly affecting the trait of interest. In this study, GYLD was found to be highly significantly and positively correlated with PPP, YPP, SH% and HSW while there was a negative correlation with DTM and DTF. Similar results were reported by other researchers including Khanpara et al. (2010), Meta and Monpara (2010), and Choudhary et al. (2013) who observed a positive interrelationship between grain yield per plant and the number of pods per plant and 100 seed weight, while Vekariya et al. (2011) and Babariya and Dobariya (2012) reported positive correlation between grain yield and shelling percentage. If the angle between two traits is less than 90°, then the traits are positively correlated while if the angle is greater than 90°, then there is a negative correlation. The traits are not correlated if the angle is a right angle (Yan and Kang, 2002). These associations were confirmed by the Pearson correlation coefficients between any two traits. However, some discrepancies might

be expected as the PC1 *vs* PC2 biplot explained only 83.00% of the variations attributed to the genotype and genotype by trait interactions.

Thus, on the basis of correlations PPP, YPP, SH% and HSW proved to be the important traits influencing GYLD in groundnuts and they can serve as marker indicator traits for improvement in grain yield and need to be given importance in selection to achieve higher grain yields. The interrelationship among yield components would help in increasing the yield levels and therefore, more emphasis should be given to these components while selecting better types in groundnut. DTF and DTM had negative association with the rest of the traits under study. This relationship was expected as landraces used in this study have long DTM with low yields while the improved lines used as controls had shorter DTM compared to land races but had higher yields. Most groundnut researchers found positive association between DTM and GYLD which was expected as they were dealing with improved materials with the same botanical group which shows high correlation between the DTM and GYLD especially in Spanish and Virginia genotypes (Chishti et al., 2000; Gupta et al., 2015; Pavithradevi et al., 2014; Raghuwanshi et al., 2016).

When a path coefficient analysis was done, it revealed that HSW, PPP, YPP and SH% had positive effect on GYLD with YPP and SH% having the highest (r=0.3 and 0.6 respectively). DTF showed a low negative effect on GYLD. These results are similar to Raghuwanshi et al. (2016) and Kumar et al. (2012) who reported high positive effect of yield per plant on GYLD. This suggests that these traits are critical in ensuring high yield in groundnuts and a selection of traits that show a direct effect on yield and positive correlation with GYLD individually or in combination would ease the selection process (Nigan et al., 1984). Traits like DTM, HSW and PPP showed low to moderate positive effect on GYLD which is in agreement with reports by other researchers who have done similar studies in groundnuts (John et al., 2015; Makinde and Ariyo, 2012). Landraces are known to be very variable and record low PPP compared to improved genotypes.

The GT biplot is very useful in the identification of redundant traits and this can lead to reduced cost in measuring traits in experiments while still maintaining precision and accuracy. It is therefore suggested that either GYLD, PPP, SH% or YPP can be used as selection criteria instead of having to collect data on all four because they recorded high positive correlation. Similarly, high correlations between PPP and YPP suggests that one of the two can suffice as a selection criterion. Mohammadi and Amri (2011) suggested that such analysis can help reduce costs of data collection significantly. These traits would be sufficient in the groundnut breeding programme as they made a big difference between the best performing genotype G12 and the worst performing genotype G8.

Information on the performance of some of the genotypes and the traits they are associated with is very important in identifying potential parents that can be used in breeding programme. For example, genotype G3 and G4 are landraces that have potential and can be crossed to genotype G12 to improve on their yield performance and also be crossed to G9 which has high rosette resistance since all landraces tested in this study were rosette susceptible. Yan and Frégeau-Reid (2008) reported that genotypes with long genotype vectors indicate that they have high levels for one or more traits and such genotypes, superior or not can be very useful in a breeding programme for some of their useful traits.

5.5 Conclusion

The study concluded that G12 had the best collection of traits (YPP, PPP, GYLD, HSW and SH%) that are necessary for high yields and this explains why it has been one of the most grown groundnut variety in Zambia over the years having been released in 1991 (SCCI, 2015). The two landraces performed well on average and one of them is genotype G4 popularly known as Chalimbana among the local farmers. Another one is genotype G3. These two genotypes have potential of being released as cultivars but with a few improvements in traits like disease resistance, days to maturity and grain yield. It is, therefore, recommended that these be crossed to lines that have rosette resistance and are high yielding while G14 (released variety) can be used to improve on earliness in these two landraces that mature above 135 days.. It is recommended that backcross breeding method be used so as to maintain the genotype phenotypically while introgressing an important trait. This will help in quick uptake and adoption by farmers who are already familiar with these genotypes. It is also recommended that this study be repeated to check for the repeatability of the results and the number of sites be increased so as to get a clear picture of the performance of these genotypes in line with the traits of interest.

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CHAPTER SIX

OVERVIEW OF THE STUDY

6.1 Introduction

Groundnut is an important legume crop of tropical and semi-arid tropical countries, where it provides a major source of edible oil and vegetable protein. In Africa, the crop is mainly grown by smallholder farmers with little inputs resulting in low yields ranging between 700 and 1000 kg/ha on average when compared to Asia and other continents. Various abiotic and biotic constraints contribute to the low yield with diseases being the major constraint, particularly groundnut rosette disease; a viral disease that can cause up to 100% yield loss when infection occurs. In addition, lack of access to improved seed leaves many farmers relying on local varieties that are low yielding and susceptible to diseases.

The objectives of this study where to;

- Evaluate the ICRISAT elite lines for rosette disease resistance using artificial inoculation.
- Ascertain the genotype by environment interaction of landraces and elite lines and select for stability and high yield.
- Conduct a study on the genotype by trait interaction for the landraces so as to select potential genotypes for use as parents in the breeding programme.

The following hypotheses were tested in this study

- There are significant differences in resistance to groundnut rosette disease among the ten test lines
- There are significant differences in yield across the 11 test lines across the ten test environments
- There is a significant relationship between grain yield and secondary traits

The aim of this chapter is to summarize the research done, state the findings and make recommendations for such findings.

6.2 Summary of main findings

6.2.1 Evaluation of the ICRISAT elite lines for rosette disease resistance using artificial inoculation

The following were the findings;

- About 70% of the test genotypes showed resistance to rosette disease.

- In the glasshouse, genotypes G2 and G10 were resistant and showed 0% disease incidence while G7, G9, G1, G5 and G6 showed moderate resistance with scores ranging from 1.1 to 1.7. The rest were all susceptible with ratings as high as 4.6.
- In the field, G4, G3 and G8 showed moderate susceptibility to rosette while the rest had 0% incidence.
- Rosette incidence in the field had an effect on pod yield, hundred seed weight, pods count, yield per plant, shelling percentage and yield per plot.
- Genotypes G7, G10, G9, G1 and G6 showed resistance to rosette and this could have been attributed to one of the parents which is rosette resistant.

6.2.2 Genotype by environment interaction of elite lines and selection for stability, adaptability and high yield

The following were the major findings in this study;

- The 2016/17 season was a good season with rains starting early and ending on time which led to zero incidences of rosette in the GxE field study.
- Environments, Lundazi (E3), Mfuwe (E5) and Ndola (E10) were identified as the best environments that were both discriminative and representative and best for selecting generally adapted genotypes, whilst Katete FTC (E2) and Masumba (E7) were non representative but discriminative and could be useful for culling inferior genotypes and selection of specifically adapted genotypes. Kalichero FTC (E1) and Mambwe FTC (E6) were non-representative and non-discriminating which rendered them useless. This grouping and testing for environments can help save resources for the breeding programme as other sites can be dropped and others used to represent them.
- Genotypes G7 and G4 were high yielding recording 2.08 t/ha and 1.99 t/ha, respectively, compared to the average mean of 1.67 t/ha across all environments.
- G7 had a yield advantage of 19.6% over the control G2 (1.74 t/ha) while G4 yield advantage was 4.8% over G2. G3, a Spanish yielded more than G11 (control) which was a Spanish as well with a yield advantage of 26%.
- Genotypes G7, G4, G5 and G2 (control cultivar) were the best performing genotypes though only G4 and G7 showed consistent performance, relative stability and adaptability across the ten testing environments.
- G7 and G4 were identified as the ideal genotypes based on the GGE biplot analysis and would be recommended for release.

6.2.3 Genotype by trait interaction for the landraces to select potential genotypes for use as parents in the breeding programme

The following were the findings;

- Grain yield showed strong highly significant correlation with yield per plant, number of pods per plant, 100-seed weight and shelling percentage with r values of 0.90, 0.86, 0.23 and 0.94, respectively, at P<0.001 though correlation with 100-seed weight was not significant
- Path coefficient analysis revealed that yield per plant, shelling percentage, days to maturity, 100-seed weight and number of pods per plant had a positive direct effect on grain yield while days to 50% flowering had a negative direct effect on grain yield.
- Genotype by trait biplot (GT) captured 83.00% of the variations due to genotype by trait interactions.
- The control cultivar, G12 was the highest yielding with high values for pods per plant, 100-seed weight, yield per plant and shelling percentage while G8 ranked lowest.
- G3 and G4 which are landraces performed relatively well in comparison to G12 (control cultivar) and had yields above the mean in all the sites.

6.3 Recommendations

Based on the findings of the study, the following were the recommendations;

- Genotypes G7 (ICGV SM 01711) and G4 (ICGV SM 02724) should be recommended for commercial release. But it should be noted that genotype G7 (ICGV SM 01711) should be recommended for wide adapatation due to its combination of high yield, stability and rosetted resistance while genotype G4 (ICGV SM 02724) was high yielding but should be recommended for specific adapattion due to it susceptibility to rosette.
- There is need to repeat this study to ensure repeatability of the findings before a conclusion can be drawn.
- There is need to make use of the molecular tools which are now available so as to confirm if the type of resistance observed in the genotypes is due to all the causal agents. Most GRD resistant lines are only resistant to GRV and Sat-RNA and not to GRAV.
- Genotypes G9 (ICGV SM 01514) in the landrace trial had low yields but showed good resistance to rosette. This and genotype G15 (Chishango) should be recommended as a source of rosette resistance genes and G9 (ICGV SM 01514) can be crossed to landraces to reduce the days to maturity and introgress rosette resistance genes while genotype G12 (MGV 4) in the same trial can be used to improve yield of the landraces.
- A deliberate landrace improvement programme should be established using back cross breeding so as to improve the local varieties which are preferred by farmers.

This will help in the quick adoption and uptake of the newly improved local varieties since they would have maintained most of the traits prefered by farmers.

6.4 Conclusion

The study was a success as it helped identify high yielding, stable and rosette resistant lines that can be recommended for commercial cultivation and can also be used as sources of rosette resistance. This is useful in the improvement of landraces and other lines. The traits of focus when breeding for yield were highlighted in the analysis.