Breeding groundnut for resistance to rosette disease and its aphid vector, *Aphis craccivora* Koch in Malawi

by

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Thesis summary

Groundnut (*Arachis hypogaea* L.) is one of the most important legume crops in Malawi. However, production among smallholder farmers has declined in recent years. One of the constraints affecting groundnut production is groundnut rosette disease (GRD). Therefore, the main objective of this study was to develop appropriate groundnut cultivars that are resistant to GRD, combined with other traits preferred by farmers, in order to improve income and food security of smallholder farmers in Malawi and beyond. The specific aims were; (i) to assess groundnut cropping systems used by smallholder farmers in Malawi, their varietal preferences, and production challenges (ii) to assess the genetic diversity among groundnut germplasm collected from ICRISAT, the Chitedze gene bank and farmers (iii) to identify sources of resistance to GRD and to its aphid vector (iv) and to understand the type of gene action governing GRD resistance, and to identify groundnut genotypes suitable for use as parents in breeding for GRD resistance.

Assessment of groundnut cropping systems used by smallholder farmers, their varietal preferences, and production challenges was done by using a field survey and participatory rural appraisal (PRA) tools. The field survey was done in Lilongwe, Mchinji and Salima while the PRA was done in Kasungu, Lilongwe, and Salima. The assessment of genetic diversity among 106 groundnut genotypes collected from ICRISAT, Chitedze gene bank and farmers was done using 19 SSR markers. High throughput DNA extraction was done followed by polymerase chain reactions (PCR) after which the amplified products were analyzed. Evaluation of genotypes to identify new sources of resistance to GRD and its aphid vector was conducted under two test situations, one with high inoculum levels and one with low inoculum levels. Under high inoculum level, the infector row technique developed by Bock and Nigam (1990) which employs a susceptible variety as a disease spreader was used. While under low inoculum level, an aphid resistant variety instead of the infector row was used to control the aphids. Aphid resistance was studied under field and glasshouse conditions. Plants were planted in rows and at 14 DAS, 2 aphids were place on each plant. Aphid resistance was determined by observing the increase in number of the aphid population on the test plants. Gene action governing inheritance of resistance to GRD was studied under high disease pressure created by using viruliferous aphids. Parents and F_2 generations and their reciprocals were used in the study. The trials were laid out in a glasshouse and aphids were infested a week after germination and were killed after 7 days using Dimethoate. Disease data was collected at 7, 14, 21 and 28 days after aphid infestation.

The study on groundnut cropping systems, varietal preferences and production challenges revealed that most farmers grew groundnut alongside maize (*Zea mayis* L.) and beans (*Phaseolus vulgaris* L.) as food crops and tobacco (*Nicotiana tabacum* L.) and cotton (*Gossypium hirsutum* L.) as cash crops. The most preferred groundnut varieties grown by farmers were Chalimbana and CG 7. GRD was observed in half of the fields visited. However, 98% of the farmers interviwed had experienced it in their fields at some point, and 63.3% of the farmers believed that GRD was a major problem. Other challenges noted by farmers included lack of quality seed, poor extension support, lack of inputs, manipulation of the markets by buyers, and the failure of groundnut crops to meet the high standards required by the market. The examination of genetic diversity among 106 groundnut genotypes revealed a total number of 316 alleles with a mean of 17 alleles per locus. Polymorphic information content (PIC) and gene diversity values were high, which indicated that genetic diversity among the groundnut genotypes was high. The analysis of molecular variance indicated that 72.9% of the genetic variation observed in the genotypes was due to the variation between individuals within rather than between specific population groups. The evaluation of genotypes for resistance to GRD revealed five highly resistant genotypes namely ICG 9449, ICG 14705, ICGV-SM 05701, MW 2672 and MW 2694. Farmer preferred genotypes were rated as either moderately resistant or susceptible to GRD. Aphid resistance was only recorded in ICG 12991. Yield and GRD incidence were negatively and moderately correlated, which confirmed that GRD has the potential to reduce yield in groundnuts. The highly resistant genotypes were also high yielding except for genotype ICG 9449. Farmer preferred genotypes CG 7, Chalimbana and Tchayilosi, also gave above average yields, despite high disease incidence levels, which showed that these genotypes have tolerance to GRD. The study on gene action governing GRD resistance revealed information on combining ability effects of GRD resistance. The diallel analysis showed that GCA, SCA, reciprocal, maternal and non-maternal effects were all significant, which indicated that both additive and non-additive gene effects played a role in governing GRD resistance. The significance of SCA and reciprocal effects indicated that maternal parents played an important role in the expression of GRD resistance. However, the additive effects were predominant over non-additive gene effects. Four of the resistant genotypes, ICG 14705, MW 2694, ICGV-SM 05701, and MW 2672, were the best combiners for GRD resistance.

Generally, the study indicates that there is still a need to develop new varieties with resistance to GRD having traits preferred by farmers to enhance adoption. There is also a need for breeders to work with extension staff in promoting new varieties and also there is need for extension staff to actively provide information to farmers on production and marketing of groundnut. Groundnut is widely known to have a narrow genetic base which

has been a bottleneck to its improvement. However, the high genetic diversity observed in this study provides a basis for selection of appropriate parental genotypes for breeding programmes which can enhance further the broadening of the groundnut genetic base. Identification of the genotypes with high resistance to GRD in this study provides an opportunity to breed more GRD resistant materials. The observation that additive gene effects are predominant in governing GRD resistance means that GRD resistant materials can be improved by introgressing additive genes using recurrent selection breeding procedures. There is also a need to employ molecular techniques which can help in shortening the entire breeding process.

Declaration

I, **Justus Mtendere Martin Chintu**, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. The thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from another person.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from the person.

Signed …… Date……
Justus M.M. Chintu

This thesis has been submitted for examination with our approval as University of KwaZulu-Natal supervisors.

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Dedication

To my wife Angella, son Jeremy and my parents, Edmund and Angela Chintu

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Thesis introduction

1. Background

Groundnut (*Arachis hypogaea* L.) is one of the most important legume crop in the world, and it is grown in many countries in the tropical, sub tropical and warm temperate regions. It is mainly cultivated for its high quality edible oil and digestible protein. In 2010, groundnut was grown on a total area of 23.91 million ha worldwide with an estimated production of 37.95 million tonnes (unshelled) at an average yield of 1.58 tonnes ha^{-1} (FAO, 2012). About 90% of the global groundnut production comes from Asia and Africa, where it is mostly produced by smallholder farmers under rainfed conditions (ICRISAT, 2012). As such, groundnut has a great bearing on the nutrition and financial well-being of the smallholder farmers.

2. Importance of groundnut in Malawi

In Malawi, groundnut is an important crop in terms of area under cultivation and total production (Freeman *et al*., 2002). The main groundnut producing areas in the country are at medium altitudes about 600m above sea level (asl) in the Lilongwe, Mchinji, Kasungu, Mzimba and Rumphi plains, and on the lakeshore (about 200 m asl) in the Karonga and Salima flood plains (Figure 1) (Minde *et al*., 2008; Sangole *et al*., 2010). The crop is cultivated as a sole crop or in association with other crops. Groundnut kernels are commonly used in the homestead or sold in local markets. They are prepared in several ways for consumption including roasting, boiling, crushing into butter or adding to traditional vegetable dishes as a sauce and edible oil.

Groundnut is viewed as a cheap crop to produce by smallholder farmers in Malawi. It is grown during the rainy season, mostly with with no fertilizers or chemicals being applied (Sangole *et al*., 2010). In addition, farmers usually keep seed after each harvest for the next cropping season, hence, the only cost to producing groundnut is the land, and its preparation and management. As a legume crop, groundnut fixes nitrogen in the soil, therefore, improving fertility levels for the subsequent crops.

The main groundnut varieties grown in Malawi are Chalimbana, CG 7, and Manipintar (Simtowe *et al*., 2008). Despite the release of several new groundnut varieties, most farmers grow only landraces or old released varieties, which are susceptible to diseases and are low yielding (Minde *et al*., 2008). Some of the common landrace varieties include Tchayilosi, Kalisele, and Gambia. Recently lines include JL 24, ICGV-SM 90704, ICG 12991, and

Chalimbana 2005 were released to farmers. These lines have been renamed locally as Kakoma, Nsinjiro, Baka and Galum'bwako, respectively. Of these, ICGV-SM 90704 and ICG 12991 are resistant to groundnut rosette disease (GRD), which is the main disease affecting groundnut in Malawi.

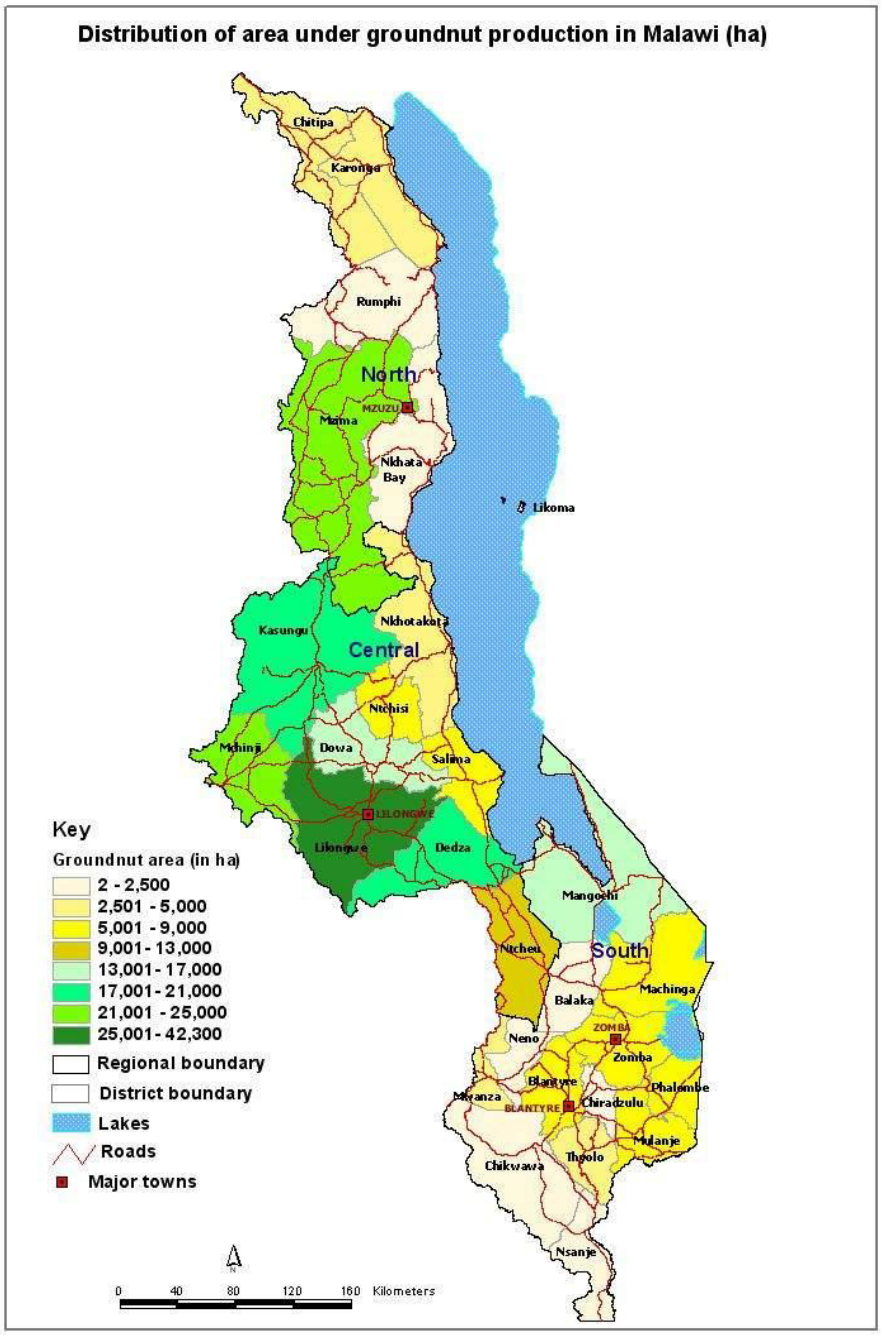

Figure 1.1 Map of Malawi showing groundnut producing areas (Simtowe *et al.*, 2008).

3. Groundnut production trends in Malawi

Groundnut has been important to smallholder production systems in Malawi for a long time (Nakagawa *et al*., 2009). Until the 1980s, Malawi was one of the biggest exporters of groundnut to Europe, and the crop ranked second in importance to maize in terms of land use and export earnings (ICRISAT, 2006; Siambi *et al*., 2007). Groundnut production and export were high from the 1960s up to early 1980s, when it declined to its lowest levels (Diop *et al*., 2004; Fekete *et al*., 2004). The decline in production and the stringent quality standards required by European markets caused Malawi to lose its groundnut market in Europe. As a result, groundnut was abandoned by male farmers for more profitable cash crops, and it has become a woman's crop (Minde *et al*., 2008).

In recent years, the government of Malawi, several private companies and organizations like ICRISAT have been working together to revive the production of groundnuts. The focus has mostly been on reviving the seed multiplication and delivery system, and increasing the awareness of farmers regarding agronomic practices that reduce plant diseases and quality specifications relating to aflatoxin contamination levels (Siambi *et al*., 2007). Consequently, production started to increase in the late 1990s from 23,933 tonnes in 1994/1995 to 190,112 tonnes in 2002/2003 (Minde *et al*., 2008). The total area of groundnuts cultivated in Malawi also rapidly expanded from 71,586 ha in 1996 to 200,000 ha in 2006 (Nakagawa *et al*., 2009). In 2010, it was estimated that groundnut was grown on 295,236 ha producing 297,487 tonnes at an average yield of 1007.6 kg ha^{-1} (FAO, 2012). As the world food prices are increasing, it is hoped that farmers will start producing groundnut on a large scale as an enterprise. Generally, there is considerable potential for the expansion of groundnut production for domestic consumption and export markets.

4. Challenges of groundnut production in Malawi

Groundnut production in Malawi is severely constrained by both biotic and abiotic stress factors. The most important biotic constraints of groundnut are diseases such as GRD, early leaf spot (*Cercospora arachidicola*), late leaf spot (*Phaseoisariopsis personata*), rust (*Puccinia arachidis*) and *Aspergillus* infestation resulting in aflatoxin contamination (Subrahmanyam *et al*., 1997; ICRISAT, 2006). Among the groundnut diseases, GRD is the most destructive, and can cause total yield loss in severe cases (Ntare *et al*., 2001). It is caused by a complex of three viruses, groundnut rosette virus (GRV), groundnut rosette assistor virus (GRAV) and satellite RNA (satRNA) and transmitted by a single species of aphid, *Aphis craccivora* Koch (Taliansky *et al*., 2000). Although, the disease occurs sporadically and at low levels in most growing seasons (Waliyar *et al*., 2007), the continuous

growing of susceptible varieties by smallholder farmers, coupled with poor farming practices and frequent droughts (Minde *et al*., 2008) create a more conducive environment for GRD to develop to epidemic levels on a regular basis. Such a scenario could be disastrous for the groundnut industry in Malawi.

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 in Malawi (Minde *et al*., 2008). Other factors such as the loss of key markets because of poor nut quality due to aflatoxins, and the absence of an organized system for seed production and delivery, have also limited the expansion of groundnut production (Siambi and Kapewa, 2004).

5. Research justification

Although GRD can be managed by the use of pesticides that control aphids, the insecticides are too expensive for the majority of smallholder farmers to purchase, and they are environmentally hazardous. Use of GRD resistant cultivars is a cost effective option to control the disease. There are several GRD resistant sources that have been identified among global groundnut germplasm (Subrahmanyam *et al*., 2000; Ntare *et al*., 2001). From these, several resistant varieties have been developed and released to farmers in Malawi, such as ICG 12991 and ICGV-SM 90704 (van der Merwe *et al*., 2001; Freeman *et al*., 2002; Deom *et al*., 2006; ICRISAT, 2006; Makkouk and Kumari, 2009). However, the adoption of these improved varieties in Malawi is low, because the new varieties do not carry key traits preferred by farmers. Therefore, new varieties are needed that combine high levels of GRD resistance with agronomic and quality traits that farmers want.

Information available indicates that most groundnut breeding programmes have only used elite breeding lines and cultivars to develop new varieties, causing the improved materials to have a narrow genetic base (Upadhyaya *et al*., 2002). This means that local varieties have been overlooked by modern breeders, despite their carrying of key traits desired by farmers. In order to widen the genetic base, and to capture traits preferred by farmers, there is need to involve a wide source of germplasm, including local varieties in breeding programmes. It is widely assumed that the narrow genetic base and the complex nature of the groundnut genome pose a serious bottleneck to groundnut's genetic improvement (Pandey *et al*., 2012). As such, there is also need to assess gene diversity among genotypes used for breeding. This information should helpful in making choices of parents for breeding with an aim to exploit the gene diversity to a maximum.

5.1 Goal of the research

The overall goal of this research was to contribute to improvement of income and food security levels of smallholder farmers in Malawi and beyond by developing appropriate groundnut cultivars that are resistant to GRD, combined with other traits preferred by farmers.

Therefore, the specific objectives to be achieved through this research were to;

i. Determine groundnut cropping systems, varietal preferences and production challenges of farmers in central region of Malawi

ii. Assess the genetic diversity available in the collection of groundnut germplasm to be used for developing new cultivars.

iii. Identify new sources of resistance to GRD and the aphid vector among the varieties collected from various sources, and evaluate the groundnut varieties for yield and yield related traits at the same time.

iv. Determine the genetic parameters governing inheritance of GRD resistance and identify the best combiners to be used as donor parents in developing GRD resistant groundnut varieties.

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CHAPTER 1

Literature review

1.1 Introduction

The chapter covers literature review on several aspects of groundnut (*Arachis hypogaea* L.) starting from the origin, distribution, taxonomy and botany of groundnut, its production, uses and economic importance and constraints to groundnut production. A second section on groundnut rosette disease covers disease distribution, symptoms, diagnosis, epidemiology, transmission, management and breeding for resistance. Screening techniques for groundnut rosette disease have also been discussed.

1.2 Origin, distribution and taxonomy of groundnut

Groundnut originated from South America in the coastal regions of Peru where evidence of its cultivation between 300 and 2500 BC is supported by archaeological reports (Stalker, 1997; Maiti, 2002). The crop is believed to have been distributed to other parts of the world by the Spanish and Portuguese explorers in the sixteenth and seventeenth century (Hammons, 1994). Today, groundnut is widely distributed and adapted in the tropical, sub-tropical and warm temperate regions of the world. The most important groundnut producing countries are India, China, USA, Brazil and parts of the western and southern Africa (Maiti, 2002).

Groundnut belongs to the *Leguminosae* family, tribe *Aeschymanomeneae*, subtribe *Stylosanthineae.* The genus and species names *Arachis hypogaea* are derived from greek words *arachos,* meaning weed, and *hypogea,* meaning underground chamber (Holbrook and Stalker, 2003). The genus *Arachis* encompasses a rich diversity of plant types containing both annuals and perennials (Knauft and Wynne, 1995). They are distinguished from most other plants by having geocarpic reproductive growth whereby the peg develops below the soil surface (Stalker and Simpson, 1995). 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) (Stalker, 1997; Burow *et al*., 2008;). There are 80 species in the genus *Arachis* divided into nine sections: *Arachis, Caulorrhizae, Erectoides, Extranervosae, Heteranthae, Procumbentes, Rhizomatosae, Trierectoides,* and *Triseminatae* (Valls and Simpson, 2005). Among the species, *A. hypogaea* is the only species that has been domesticated and is widely distributed for food and vegetable oil production around the world (Holbrook and Stalker, 2003).

The cultivated groundnut, *A. hypogaea*, is a tetraploid and is divided into two subspecies, *hypogaea* and *Fastigiata* Waldron. Each of the subspecies is further divided into botanical varieties; subsp. *hypogaea* into var. *hypogaea* and var. *hirsuta*, subsp. *fastigiata* Waldron into var. *fastigiata*, var. *vulgaris*, var. *peruviana* and var. *aequatoriana*. Only three botanical varieties, subsp. *hypogaea* var. *hypogaea*, subsp. *fastigiata* var. *fastigiata* and var. *vulgaris* are widely cultivated in the Americas, Africa, and Asia (Ferguson *et al.*, 2004). The subspecific and varietal classifications are based on morphological characteristics such as growth habit, branching patterns, pubescence, stem colour, and pod and seed size and shape (Krapovickas and Gregory, 1994). Intermediates between the subspecies are rare but do exist, which sometimes makes classification of the cultivated species difficult (Isleib and Wynne, 1983).

1.2.1 Botany

Groundnut is an annual plant with an indeterminate growth habit having a distinct main stem and a variable number of lateral branches (Shokes and Melouk, 1995). The stem is initially solid, upright or prostrate ranging from 120 to 650 mm in length, which then becomes hollow as the plant grows (Stalker, 1997). The branching pattern and distribution of vegetative and reproductive nodes along the main stem and lateral branches are the main traits which primarily distinguish the two subspecies, subsp. *hypogaea* and subsp. *fastigiata,* from each other (Holbrook and Stalker, 2003). The subsp. *hypogaea* has alternate branching to reproductive nodes and either a spreading or a bunching growth habit, while the subsp. *fastigiata* has sequential branching to reproductive nodes and an erect growth habit (Shokes and Melouk, 1995; Stalker, 1997). The groundnut leaves are mostly tetrafoliate and alternately arranged on the stems, however the subsp. *hypogaea* has dark green leaves while the subsp. *fastigiata* has light green leaves (Ramanatha Rao and Murty, 1994).

The groundnut plant produces flowers within four to six weeks after emergence continuing until late in the growing season, depending on the genotype and the environment (Shokes and Melouk, 1995; Stalker, 1997). Although flowering occurs above ground, seeds are produced below the soil surface. The flowers are variable in colour, ranging from light yellow to deep orange and sometimes white. Flowers are borne in the axils of leaves, usually with three flowers per inflorescence, but only one of these flowers opens at a given time (Stalker, 1997). The groundnut plant produces more flowers than the photosynthetic capacity to fill the pods and less than 20% produce mature pods even under ideal conditions (Donovan, 1963; Ramanatha Rao and Murty, 1994). The flowers are self pollinated. However, at locations

where bee activity is high, some cross-pollination can occur (Nigam *et al*., 1983). After fertilization of the ovule, an intercalary meristem becomes active and a pointed carpophore or gynophore, commonly known as a peg, is formed. The peg exhibits positive geotropism and grows downward into the soil where it becomes diageotropic and ceases to elongate and develops into a pod (Shokes and Melouk, 1995).

The pods are elongated spheres with various amounts of reticulation on the surface and/or constriction between seeds. Although pods usually develop below ground aerial pods can occur (Holbrook and Stalker, 2003). The pods may grow up to 80 mm x 27 mm and normally contain two to five seeds. Although the number of seeds per pod depends on the cultivar, it can also be influenced by season and other factors (Stalker, 1997). Seeds are either round or elliptical with pointed or flattened ends and range in their colours from off white to deep purple. Each seed consists of two large cotyledons, an epicotyl, and a primary root. The cotyledons comprise nearly 96 percent of the seed weight and are the major storage tissue for the developing seedling (Holbrook and Stalker, 2003).

1.3 Production, uses and economic importance of groundnut

Groundnut is a popular legume crop in the world, valued for its “nuts”, oil, meal, and vegetative residue (Bunting *et al*., 1985). It is mostly produced in areas where the mean rainfall is 600 - 1200 mm per annum and the mean daily temperatures in the range of 25-28^{o}C (CGIAR, 1994; Maiti, 2002). It is estimated that about 13.5 million ha are grown in Asia, 5.3 million ha in Africa, 1.2 million ha in the Americas, and 0.1 million ha in other parts of the world (Carley and Fletcher, 1995). In 2010, the total area under groundnut reached 23.91 million ha worldwide, with an estimated production of 37.95 million tonnes (unshelled) and mean yield of 1.58 tonnes ha^{-1} (FAO, 2012).

The groundnut crop offers many benefits to both commercial and subsistence farmers who produce it. As a food source, groundnut is highly nutritious, containing 20% carbohydrates (Ahmed and Young, 1982), 25-34% digestible protein (Naidu *et al*., 1999) and 36-54% oil (Knanft and Ozias-Akins, 1995). In many developing countries, groundnut is the principal source of digestible protein and vitamins such as thiamine, riboflavin, and niacin (Naidu *et al*., 1999). Groundnut seeds are consumed raw, roasted or boiled and can be processed for making soups and confectionary products, and can also be ground to produce peanut butter (Bunting *et al*., 1985). Generally, oil is the most important product of the crop and more than half of all groundnut grown in the world is used to produce oil (Stalker, 1997). Groundnut oil content and quality varies depending on the cultivar, geographical location, season and

growing conditions (Asibuo *et al*., 2008). The oil pressings, seeds and straw are also used in many countries as fuel and animal feed in the form of groundnut cakes and haulms (Stalker, 1997; Wesche-Ebeling *et al*., 2002).

Groundnut also provides cash to poor farmers in the developing countries of Asia and sub-Saharan Africa, and therefore, it contributes significantly to food security and poverty alleviation (Naidu *et al*., 1999). For instance, in Malawi groundnut is an important food and cash crop in smallholder agriculture providing approximately 25% of agricultural cash income (Minde *et al*., 2008). In many sub-Saharan African countries, women predominantly grow and manage the crop, hence its production has a direct bearing on the overall economic and nutritional status of women and children (Naidu *et al*., 1999).

Groundnut is a legume crop with root nodules, that can fix nitrogen in the soil, improving soil fertility, hence benefits the productivity of subsequent crops (Cox and Sholar, 1995). Studies have indicated values from 25% to 64% of plant N derived from fixation by groundnuts (Sprent, 1994). It is also a relatively drought tolerant crop (Stalker, 1997) and grows well despite minimal inputs making it suitable for low input agriculture practiced by smallholder farmers in the sub-Saharan Africa (Naidu *et al*., 1999).

Despite groundnut being an important crop among many smallholder households in Africa, there is a wide difference in yields from farms in Africa and those of other parts of the world. For instance, in 2010 the world mean yield for groundnut was 1580.7 kg ha^{-1}, while in Africa the production is pegged at 902.1 kg ha^{-1} compared to 3086.2 kg ha^{-1} realized in Americas (FAO, 2012) . Generally, yields of groundnut grown by smallholder farmers are consistently low (Stalker, 1997; Holbrook and Stalker, 2003).

1.4 Constraints to groundnut production

Groundnut production is constrained by several biotic and abiotic factors such as diseases, pests, aflatoxin contamination, nematodes and drought (Maiti, 2002). In the sub-Saharan region of Africa, diseases are generally regarded as a major constraint to groundnut production (Chiteka *et al*., 1992). The common diseases of groundnut are foliar and include rust, early leaf spot and late leaf spot. In addition to these, GRD which occurs only in Africa, is also a major production constraint (Nigam, 2008).

In Malawi, a large number of fungal, viral, and nematode diseases have been reported, but only a few are of economic importance (Babu *et al*., 1995). Diseases such as early leaf spot,

rust and GRD are widespread and reduce yields whenever they occur (Minde *et al*., 2008). It is estimated that early and late leaf spot diseases cause up to 70% yield loss (Monfort *et al*., 2004) while loses due to rust exceed 50% worldwide (Hagan *et al*., 2006). GRD also contributes significantly to the low productivity of the crop in Africa with epidemics costing an estimated US$156 million annually (Ntare *et al*., 2002; Monyo *et al*., 2008). Monyo *et al*. (2008) noted that Africa is the only place where GRD and leaf spot diseases regulary combine to cause devastating yield losses in groundnut crops.

Groundnut is also attacked by both pre- and post harvest insect pests that cause significant economic loses. Over 400 species of pests attack groundnut (Lynch, 1990). Knauft and Wyne (1995) indicated that foliar feeders of groundnut cause maximum yield loss when their feeding reduces photosynthetic area, especially during pod initiation and pod fill period. Apart from directly lowering yields, insects serve as vectors for viruses and also cause damage to pods and seeds making them undesirable for marketing (Stalker, 1997). In Africa and Asia, the most important insect pests are termites (*Microtermes* spp), white grubs (*Lachnosterna consanguinea* Blanchard), thrips (*Megalurothrips uitatus* Bagnall) as a vector of bud necrosis virus disease (tomato spotted wilt virus), leafhoppers (*Empoasca kerri* Pruthi and *E. fabae* Harris.), aphids (*A. craccivora*) as a vector of GRD, and lepidopterous defoliators (*Heliothis zea* Boddie) (Lynch, 1990). In the U.S.A., the lesser cornstalk borer and southern corn rootworm are the most important insects (Stalker, 1997). These pests can easily be controlled by application of pesticides an approach which is affordable in the developed world but is too expensive for the resource-limited farmers in Africa.

Erratic or insufficient rainfall is also a major constraint to groundnut production in rain-fed environments (Madhava *et al*., 2003). Groundnut is highly drought tolerant and can grow well in many areas of the world where most other food legumes fail to produce any yield (Holbrook and Stalker, 2003). However, low soil moisture regimes greatly limits groundnut production (Stalker, 1997). Minde *et al*., (2008) observed that groundnut production in Malawi is greatly affected by unreliable rainfall which is often followed by mid-season droughts. In addition, drought stress increases susceptibility of groundnut seeds to fungal infestation by *Aspergillus flavus* Link and *A. parasiticus* Speare which cause the kernels unhealthy for human consumption due to the aflatoxins that the fungi produce (Sanders *et al*., 1985; Waliyar *et al*., 2005). Aflatoxin contamination of groundnuts has been identified as a major constraint to trade of food crops in Africa (Lubulwa and Davis, 1994). In addition to frequent droughts, high aflatoxin contamination of groundnut kernels in Malawi has also been attributed to poor post-harvest handling techniques that enhance the growth of the fungi (Siambi *et al*., 2007). As a result, the market for Malawi's groundnut has been

adversely affected because the European Union banned imports of groundnuts from Malawi in the early 1990s when aflatoxin contamination exceeded acceptable levels (Nakhumwa *et al*., 1999). The lack of a market and dimishing yields caused many farmers to abandon groundnut for more lucrative cash crops such as tobacco. The result is that groundnut was reduced to being largely a subsistence crop grown mainly for household consumption, with only surpluses reaching the local and regional markets (Fekete *et al*., 2004). The government of Malawi, Private Companies and ICRISAT have made efforts in reviving the seed multiplication and delivery system, educating farmers on quality specifications, and promotion of agronomic practices that reduce the levels of plant diseases and aflatoxin contamination (Siambi *et al*., 2007).

Other constraints to groundnut production which are of minor importance in other regions range from production to economic factors. In Malawi, the constraints include use of low yielding groundnut varieties, declining soil fertility levels through poor crop management and low nutrient application, inadequate support services such as extension services and credit facilities, lack of seed, and a clash in labour demand (Minde *et al*., 2008). Lack of access to sufficient quantities of improved seed has been identified as the root cause of low groundnut productivity because it forces farmers to use low yielding varieties and recycled kernel as seed (Simtowe *et al*., 2009). There is also a lack of interest by commercial seed companies to breed and sell seed of self-polinated crops, which can be recycled by farmers hence making it uneconomic to breed them (Siambi and Kapewa, 2004). As a result, there is no established groundnut seed enterprise in Malawi which reliably produces and sells good quality groundnut seed.

1.4.1 Groundnut rosette disease (GRD)

GRD has been described as the most devastating disease of groundnut in the sub-Saharan Africa causing yield loses approaching 100% whenever an epidemic occurs (Ntare *et al*., 2002). It was first described in Tanganyika (now Tanzania) by Zimmerman in 1907 (Naidu *et al*., 1998) and since then recurrent epidemics have been reported in several countries. Epidemics of the disease are usually severe and highly unpredictable (Naidu *et al*., 1998; Naidu *et al*., 1999; Taliansky *et al*., 2000). For instance, an epidemic affected approximately 0.75 million hectares of groundnut in Nigeria in 1976, while in Zambia about 43,000 hectares of groundnut fields were affected in 1995 and in 1996 groundnut production in Malawi was reduced by 23% (Anonymous, 1996; Ntare *et al*., 2002). However, GRD usually occurs in low levels every growing season and its severity increases in crops grown late in the season (Waliyar *et al*., 2007).

GRD is caused by a complex of three agents, groundnut rosette virus (GRV), satellite RNA (satRNA), and groundnut rosette assistor virus (GRAV) (Taliansky *et al*., 2000). Waliyar *et al*. (2007) described the complexity of association of the GRD causing agents as unique, and whose origin and perpetuation in nature still remains a mystery. GRV belongs to the genus *Umbravirus* which is a group of imperfectly characterized plant viruses, each of which depends on unrelated helper luteovirus (or luteo-like virus) with transmission by aphids in a circulative, non-propagative manner (Taliansky *et al*., 1996). It has no structural (coat) protein therefore, it does not form conventional virus particles (Taliansky *et al*., 2000). In experiments, GRV can also be transmitted by grafting and mechanical inoculation (Waliyar *et al*., 2007). However, in nature GRV is transmitted by *A. craccivora* and it replicates autonomously in the cytoplasm of infected groundnut plants (Taliansky *et al*., 1996; Taliansky and Robinson, 2003). Taliansky and Robinson (1997) reported that isolates of GRV contain satRNA of about 900 nucleotide (nt) in length. Thus, GRV always occurs together with satRNA. The satRNA (subviral RNAs) belongs to the subgroup-2 (smaller linear) satellite RNAs which are single stranded, linear and non- segmented (Murant *et al*., 1988; Waliyar *et al*., 2007). It contains four large open reading frames (ORFs) (Taliansky *et al*., 1996) and several different variants of satRNA have been identified (Murant and Kumar, 1990; Blok *et al*., 1994). The satRNA is transmitted by aphids together with GRV and GRAV (Waliyar *et al*., 2007).

GRAV belongs to the family *Luteoviridae* and its biological properties are typical of a luteovirus (Taliansky *et al*., 2000). GRAV virions are non-enveloped, isometric shaped with 28nm diameter particles of polyhedral symmetry (Waliyar *et al*., 2007). Murant (1989) noted that the genome of GRAV is non-segmented, comprised of a single molecule of linear positive-sense, single stranded RNA of c.6900 nucleotides that encode for structural and non-structural proteins. Groundnut is the only known host of GRAV into which it is transmitted by aphids in a persistent manner and experimentally by grafting but not by mechanical sap inoculation (Waliyar *et al*., 2007).

The intimate interaction between GRAV, GRV and satRNA is crucial for the development of the disease. The GRAV acts as a helper virus in vector transmission of GRV and satRNA where they are packaged together in the coat protein of GRAV to form virus particles that are transmissible by the vector (Naidu *et al*., 1999). The satRNA also plays a key role in GRV transmission in that its presence in the source plant is essential for the GRAV-dependent transmission of GRV (Murant, 1990). It has also been observed that satRNA is largely responsible for GRD symptoms in groundnut (Murant *et al*., 1988). The different

variants of satRNA are responsible for the different GRD symptoms (Murant and Kumar, 1990). Thus, there are two predominant types of the disease, 'chlorotic rosette,' which is prevalent in the sub Saharan Africa (Naidu *et al*., 1999) and 'green rosette' which is found in the western, eastern and southern Africa (Wangai *et al*., 2001). Although GRAV on its own does not cause symptoms, a study by Naidu and Kimmins (2007) showed that GRAV infection, without GRV and satRNA affects plant growth and contributes to yield losses in groundnut. Therefore, any GRD control measures have to target all the three agents.

1.4.1.1 Disease distribution

GRD is limited to groundnut and only occurs in Africa despite the fact that its vector, *A. craccivora* occurs in almost all groundnut growing regions of the world (Waliyar *et al*., 2007). Since the time when the disease was first documented in Tanzania and South Africa, it has also been reported in Angola, Burkina Faso, Cote d'Ivoire, Gambia, Ghana, Kenya, Madagascar, Malawi, Niger, Nigeria, Senegal, Sudan, Swaziland, Uganda, and Zaire (now Democratic republic of Congo) (Naidu *et al*., 1999). It appears that the disease is spreading to most African countries and may reach other parts of the world outside Africa. As such, urgent attention is needed from both breeders and pathologists in order to limit its spread.

1.4.1.2 Disease symptoms

The two distinct forms of GRD 'chlorotic and green rosette' occur with variable symptoms within each type (Murant and Kumar, 1990; Naidu *et al*., 1999; Waliyar *et al*., 2007). The variations are said to be due to diversity among the causal agents (satRNA variants), differences in genotype response, variable climatic conditions, and mixed infections with other viruses (Naidu *et al*., 1999). GRD infected plants show stuntedness and appear bushy due to shortened internodes and reduced leaf sizes. Ansa *et al*. (1990) noted that stunting is more severe in groundnut infected by all three agents (GRAV, GRV and sat RNA) than in those containing GRV and satRNA only.

The leaves of plants affected by green rosette appear darker than the leaves of uninfected plant. Some leaves also show a light green and dark green mosaic. On the other hand, leaves of plants affected by chlorotic rosette appear curled with bright chlorosis and few green patches. In both forms, the disease symptoms appear either on the whole plant or in some branches or parts of the branches depending on the stage of infection. Naidu *et al*., (1998) indicated that early GRD infection especially before flowering results in severe or total yield loss. However, when GRD infection occurs between flowering and pod setting or

maturation the symptoms appear in some branches, only or part of the branches, and yield losses depend on the severity of the infections but in most cases, it is negligible (Waliyar *et al*., 2005). As such, losses to GRD incidences could be avoided or minimized if farmers were able to control aphids when the plants are young than later in the season.

1.4.1.3 Disease diagnosis

Detection of the causative agents of GRD is crucial in understanding the disease. GRD is diagnosed in the field based on the visual symptoms and through mechanical inoculation onto a suitable indicator host such as *Chenopodium amaranticolor* Coste and Reyn (Naidu *et al*., 1999). Naidu *et al*., (1998) indicated that detection and diagnosis of GRD based on symptoms and aphid transmission procedures are time consuming and labour intensive. There are improved methods which employ serological and nucleic acid based diagnostic techniques used to detect all the three agents of GRD (Waliyar *et al*., 2007). Triple antibody sandwich - enzyme linked immunosorbent assay (TAS-ELISA), which is used to detect GRAV, dot-blotch hybridization and reverse transcription-polymerase chain reaction (RT-PCR), are used to detect all three GRD agents in both the plants and aphids (Blok *et al*., 1995; Naidu et al., 1998; Waliyar *et al*., 2007). However, these techniques are expensive and require advanced technical skills to use; hence, diagnosis based on symptoms alone is used at most African research centres.

1.4.1.4 Disease epidemiology

Identifying sources of initial GRD infections early in the season can assist in devising ways to control the spread of the disease. The vector, *A. craccivora* is known to be present throughout the year and normally infest groundnut when the crop is young soon after emergence (Hildebrand *et al*., 1991). Infections of GRD when plants are young provide a great opportunity for rapid secondary infections (Naidu *et al*., 1998). The primary source of GRD infection is not known. However, it is believed that it survives in off-season infected crop plants or alternative host plants from which the aphids collect the inoculum before spreading the disease into the current crop (Naidu *et al*., 1999). Thresh (1983) noted that GRD is polycyclic because each infected plant serves as an inoculum source for increasing progressively the spread of the disease during the growing season. Although aphids occur in both winged and wingless forms, only the winged aphids are responsible for primary spread of the disease (Waliyar *et al*., 2007). Within the field, further spread of the disease is attained by apterae and nymphs of the *A. craccivora* (Naidu *et al*., 1998). Naidu *et al*. (1999) noted that knowledge of vector population dynamics, distribution and initial sources of inoculum

could greatly help in predicting GRD epidemics, and application of appropriate preventive and control measures beforehand.

1.4.1.5 Disease transmission

The aphid, *A. craccivora* is the only known vector of GRD and it is also a vector of several other plant viruses (Lynch, 1990). The GRD virus particles are transmitted in a persistent manner, but do not multiply inside the vector which is labelled as circulative transmission (Watson and Okusanya, 1967; Naidu *et al*., 1998; Waliyar *et al*., 2007). Aphids are polyphagous, brownish-grey in colour and feed on young shoots, leaves, inflorescences and fruits, and also on stems in herbaceous plants (Blackman and Eastop, 2007). The aphids do not necessarily cause serious damage to plants, although some damage has been observed in drought situations, especially in young plants (Singh and Oswalt, 1992). In the tropics, only females are found. These reproduce parthenogenetically, enabling rapid population increases, the speed of which is determined by prevailing climatic conditions and nutritional status of the host plant (Naidu *et al*., 1999; Blackman and Eastop, 2007). Aphids ingest phloem sap from their hosts through narrow piercing–sucking mouthparts called stylets (Goggin, 2007), which cause damage on plants especially the leaves (Knauft and Wynne, 1995). Misari *et al*. (1988) found that the aphid acquired the virus particles through sucking of the phloem sap and was able to transmit the particles throughout its entire life of 14 days. Strategies aiming at controlling the aphid population may reduce GRD incidences.

Dubern (1980) conducted a study on the transmission efficiency of both forms of GRD which showed that the minimum acquisition access and inoculation access periods by the aphid are 4.5 h and 3 min, respectively. The study also determined a latent period of 18 h in the aphid and a minimum time for transmission of 22.5 h. However, studies have indicated that aphids do not always transmit all GRD particles together. Naidu and Kimmins (2007) noted that spatial and temporal separation of GRAV from GRV and satRNA can occur under natural conditions in groundnut enabling the aphid to transmit either GRAV or GRV plus sat RNA separately. This occurs due to differences in inoculaton feeding behaviour of the aphid whereby if the aphid spent a short time of feeding, then only GRV and sat-RNA were transmitted but when the feeding time was longer such that the phloem cells were penetrated, then all the three agents including GRAV were transmitted (Waliyar *et al*., 2007). Studies have also shown that *A. craccivora* can transmit GRV and its sat RNA only from source plants that are also infected with GRAV which has a coat protein for encapsidation, meaning that transmission of GRD is not possible from diseased plants lacking GRAV (Okusanya and Watson, 1966; Murant, 1990; Naidu *et al*., 1999). This indicates that if found,

germplasm resources with resistance to GRAV can be of great use in breeding for resistance to the GRD.

1.4.1.6 Management of GRD

There are various methods that have been investigated and used to protect groundnut against GRD. These include the use of pesticides to control vector aphid population, the use of recommended cultural practices which delay onset and spread of both the vector and disease, and the use of resistant cultivars.

Earlier studies have shown that use of pesticides such as organophosphates can effectively control aphid populations hence reduce disease incidences (Naidu *et al*., 1999; Ntare *et al*., 2002). The timing of spray, dosage and type of pesticide used are crucial for efficient control of aphid populations (Waliyar *et al*., 2007). However, resource-limited farmers cannot afford to purchase these chemicals. The alternative is cultural practices. One of these is rouguing of infected volunteer plants and the plants infected early in crop life to prevent primary and secondary spread of the disease (Waliyar *et al*., 2007). Intercropping groundnuts with other crops such as maize, beans and sorghum has also been reported to decrease GRD incidences. This has been observed in countries like Malawi, Uganda and the Central African Republic (Naidu *et al*., 1999; Subrahmanyam *et al*., 2002). Studies have also shown that early sowing and dense planting are two practices that greatly reduce GRD incidences because early sowing ensures the establishment of the crop before aphid populations reach their peak and dense plantings discourage infestation since aphids prefer light airy conditions (Farrell, 1976). However, recommendations on sowing date and the use of dense plant stands have not been widely implemented by the smallholder farmers who give priority to other crops like maize and tobacco which are sown early and groundnut later. They also practice wide plant spacings to offset the risks of droughts (Ntare *et al*., 2002; Thresh, 2003).

Generally, host-plant resistance is considered to be the most cost-effective management measure against GRD because smallholder farmers seldom use the cultural or chemical control methods. Breeding work has led to the development of several GRD resistant cultivars that have been released in the sub-Saharan Africa (Ntare *et al*., 2001; van der Merwe et al., 2001; Deom *et al*., 2006). The earlier developed resistant varieties were seriously flawed in that they had a long growth period, making them unsuitable for areas where droughts are frequent, and therefore short duration cultivars would have been more appropriate (Naidu *et al*., 1998). However, early maturing sources of GRD resistance have been identified in the Spanish type of groundnut (*Arachis hypogaea* subsp. *fastigiata*, var.

vulgaris) (Naidu *et al*., 1999). Ntare *et al*., (2002) noted that most of the very few early maturing cultivars available also have some poor agronomic characteristics As a result, despite the fact that GRD resistant varieties have been available for the last 20 years, adoption of these varieties has been very low and as a result farmers continue to grow susceptible varieties whose yields are far below the world average (Edriss, 2003; Minde *et al*., 2008). This necessitates the need to search for more sources of resistance and to breed new varieties which combine GRD resistance with other agronomic traits.

1.4.1.7 Breeding for resistance to GRD and its vector

Breeding for GRD resistance involves making crosses between both resistant and susceptible varieties followed by selections in the segregating populations which are done through bulk and pedigree systems or their modifications (Olorunju and Ntare, 2002). The key in breeding is in selecting proper parents for making crosses. Breeding work started when GRD resistant varieties were disovered among late maturing landraces of Virginia type (*Arachis hypogaea* subsp. *hypogaea* var. *hypogaea*), during an epidemic of GRD that occurred in the 1950s in Senegal (Naidu *et al*., 1999; Olorunjua and Ntare, 2002). However, the earlier developed varieties were unsuitable for most areas in the sub-Saharan regions having short rain seasons. This meant that there remained a need to breed short duration, GRD resistant varieties (Naidu *et al*., 1998).

Research into breeding for host plant resistance by ICRISAT has contributed to the development of several groundnut genotypes and identification of germplasm lines with acceptable levels of field resistance to GRD (Olorunjua *et al*., 2001). The resistant lines rarely show GRD symptoms, indicating that they are highly resistant or tolerant to GRV and its satRNA which are responsible for symptoms (Subrahmanyam *et al*., 1998). Waliyar *et al*. (2007) noted that in spite of the availability of several sources of resistance, all the ICRISAT varieties seem to have the same resistance genes. Inheritance studies on their varieties have shown that resistance to both forms of GRD, green and chlorotic is controlled by two recessive genes (Olorunju *et al*., 1992; Ntare *et al*., 2002). The mechanism of resistance is reported to be to initial infection, restriction of virus movement, and restricted production of satRNA which induces symptoms (Ntare *et al*., 2002). It has been observed that all GRD resistant cultivars and germplasm lines contain resistance to GRV and satRNA only and not to GRAV (Naidu *et al*., 1999; Taliansky *et al*., 2000; Waliyar *et al*., 2007). Plants infected with GRAV show significant reduction in seed weight, meaning that GRAV infection without GRV and sat RNA affects plant growth and contribute to yield loss (Naidu and Kimmins, 2007). The complexity in the interaction of GRD viruses poses a challenge to breeders trying to

develop groundnut lines with durable resistance. However, there is still a need to search for additional sources which are also resistant to GRAV, or the use of other plant breeding strategies such as recurrent selection which can provide the basis for the development of durable resistance to GRD in groundnut.

Aphid resistance is another strategy that has been used in breeding programmes to control GRD. However, identification of sources of aphid resistance has not been very successful as there are very few genotypes available with resistance (Lynch, 1990). High levels of aphid resistance are mainly found in wild relatives of groundnut (Sharma *et al*., 2003). Aphid resistant varieties developed so far are susceptible to GRD but escape field infection (Thresh, 2003). In Malawi, an early maturing, drought tolerant Spanish-type groundnut germplasm line, ICG 12991, was released with resistance to the aphid (Deom *et al*., 2006). Knauft and Wynne (1995) noted that there is a strong relationship that exists between the amount of condensed tannin, procyanidin and the fecundity of aphids, which suggests that screening for procyanidin levels could help in identifying genotypes with resistance to the aphid. Chancellor (2002) indicated that antibiosis and non-preference are the mechanism of resistance in ICG 12991 groundnut variety where virus transmission is controlled through collapse and death of plant cells at the feeding site.

Studies have also been done to explore resistance to GRD in wild relatives of groundnut. High levels of resistance has been identified in some wild *Arachis* species, several of which show immunity to GRAV, GRV and satRNA, and the aphid vector (Subrahmanyam *et al*., 2001). This means that an opportunity exists for transferring the resistance genes from the wild relatives to the cultivated groundnut. Waliyar et al. (2007) indicated that a hybrid derivative that was developed from an interspecific cross of *A. hypogaea* x *A. chacoense* showed a high degree of GRD resistance. However, making of interspecific crosses between *A. hypogaea* and its wild relatives is difficult (Holbrook and Stalker, 2003), because interspecific hybrids have low fertility levels and offer limited genetic recombination hence preventing introgression of genes into cultivated species (Stalker, 1997). However, there is still need to exploit genes for resistance to GRD and its aphid vector sourced from wild *Arachis* species to broaden the genetic base of GRD resistance and to reinforce resistance in cultivated groundnut.

Adoption of “improved” groundnut varieties by smallholder farmers in Malawi is currently very low. In a study conducted in several districts in Malawi, Simtowe *et al*. (2009) found that 60% of sampled farmers were aware of at least one improved variety of groundnut but only 26% of them had attempted to grow at least one of these improved varieties. Adoption of new

technologies such as varieties is a complex issue among farmers in most parts of the world because it is determined by several factors. However, adoption can be enhanced by employing participatory methods in the development of the new varieties, whereby farmers are involved in the breeding process. There is also need to involve local germplasm in breeding programmes because they usually carry the key traits desired by farmers.

1.4.1.8 Sources of resistance and breeding methods

Background information of the parental materials helps in the selection process for an efficient breeding programme. The discovery of sources of resistance to GRD and the aphid vector could provide an opportunity for improving the groundnut crop in Africa. Evaluation of 12,500 lines from the ICRISAT gene bank collection of germplasm led to the identification of about 150 resistant sources of which 130 were long duration Virginia types and 20 were short duration Spanish types (Subrahmanyam *et al*., 1998). In addition, 65 new sources of resistance have also been identified in West Africa of which 55 are Virginia types and 10 are early maturing Spanish types, although the type of resistance acting in these gentoypes has not been determined (Waliyar *et al*., 2007).

Development of improved cultivars also requires an understanding of the nature of gene action governing key traits such as GRD resistance in the germplasm used for breeding. Use of a diallel mating scheme can identify the levels of general combining ability (GCA) and specific combined ability (SCA) in parental lines which reflects their ability to combine efficiently (Falconer and Mackay, 1996). GCA and SCA have been used in groundnut breeding to select the best parents for GRD resistance, early maturity and other agronomic traits using F_2 and F_3 generations (Adamu *et al*., 2008). Analysis of a diallel mating scheme provides estimates of genetic parameters such as gene action, number of genes, heritability, components of variation and linkage, and other genetic variance (Hill *et al*., 1998).

Molecular techniques have also been used to improve the groundnut crop. Technologies such as marker assisted selection and gene transformation offer a chance to improve breeding efficiency for traits of agronomic importance and increase the potential for introducing alien genes into the *A. hypogaea* genome (Stalker, 1997). Recently, a DNA marker for aphid resistance in groundnut was identified which provides a simple marker based method for screening aphid resistance (Herselman *et al*., 2004; Waliyar *et al*., 2007). Although molecular techniques have not been used directly in breeding for GRD resistance, marker assisted breeding offers an easier and faster approach for introgressing non-additive, recessive genes than conventional breeding methods (Pandey *et al*., 2012). Therefore,

combining molecular techniques with conventional methods can hasten further breeding work for GRD resistance.

1.4.1.9 Screening techniques for GRD resistance

Screening for resistance to GRD and the aphid vector is done under both glasshouse and field conditions. Breeders can use an effective screening technique developed by Bock and Nigam (1988) that permits the rapid field evaluation of large segregating populations, and inbred lines to identify resistance to GRD (Naidu *et al*., 1999). The technique involves planting a test row of uninfected plants flanked on either side by a row of a susceptible cultivar infested with aphids. This technique leads to a 99% success rate in spreading the disease to susceptible plants, hence, resistant cultivars are easily detected (Ntare *et al*., 2002). Mechanical sap inoculation can also be done to transmit and evaluate resistance but this only works for GRV and sat RNA (Waliyar *et al*., 2007). Grafting using scions from GRAV infected groundnut plants can be used to evaluate resistance to GRAV (Olorunjua *et al*., 1992). Naidu and Kimmins (2007) indicated that virus inoculation of groundnut seedlings before transplanting is an effective method to create high levels of synchronous infection for determining the effect of single infection of GRAV on the growth and yield of groundnuts and/or to compare different varieties or germplasm lines of groundnut for their reaction to GRAV under natural conditions. Confirmation of the presence of all disease agents during genotype evaluation can be done by using diagnostic assays such as TAS-ELISA or RT-PCR (Waliyar *et al*., 2007).

There are two rating methods that are being used to quantify GRD resistance in groundnut cultivars. Both methods use a rating of symptoms of infected plants, hence they evaluate resistance to GRV and satRNA which are responsible for producing symptoms (Waliyar *et al*., 2007). The first method employs a visual rating score using a 1-5 subjective scale where 1 = highly resistant and 5 = highly susceptible (Olorunjua *et al*., 1991).

The other method widely used is based on percent disease incidence (PDI) and plants are measured at an early stage of pod filling (Waliyar *et al*., 2007). The total number of plants in each row and the plants showing rosette symptoms (chlorosis with severe stunting) are counted once at 80 days and again at 100 days after germination. The PDI in each row and the mean percent incidence for each plot over the two counts are then computed to assess the resistance of the genotype to GRD.

It would be advantageous to exploit a combination of virus resistance genes and genes for vector-resistance to broaden the genetic base of resistance to GRD and to enhance their durability (Naidu *et al*., 1998). Screening for resistance to the aphid vector promises to be beneficial to GRD resistance breeding programs. Studies have shown that under field conditions, it is possible to identify vector resistance on cultivars by the comparative level of aphid colony establishment (Chancellor, 2002). Resistance is determined by the effect of the plant on the aphid physiological aspects such as instar development, reduced survival, lower bodyweight and reduced fecundity of adult aphids. The adverse effect of the plant on aphid survival, longevity and fecundity is termed as antibiosis, whereas the effect whereby the aphid is directed away from a plant is called antixenosis or non-preference (Thomas and Waage, 1996).

1.5 Genotype by Environment interaction

Studies conducted over the years have shown that genotype × environment interactions are widespread in groundnut (Knauft and Wynne, 1995). Significant GxE interaction, in particular, the crossover type tends to hinder genetic progress in breeding programs making it difficult to unambiguously select promising materials that perform consistently well across a wide range of environmental conditions (Nigam et al., 2003). Thus, the interpretation of genetic studies and predictions become complicated with GxE interaction. As such, multi-year and multi-location testing is necessary prior to cultivar release (Holbrook and Stalker, 2003; Knauft and Wynne, 1995). However, information on GxE interaction studies in groundnut is limited.

1.6 Summary

GRD has been a major focus of research for many years and advancement in understanding of the disease has helped in breeding several resistant cultivars. However, there are still many aspects which are not known about the disease such as: the origins of primary infection at the start of each season, the unpredictable fluctuations in the GRD incidences throughout the sub-Saharan Africa; why is GRD only endemic to the African continent, despite the aphid vector being present in other part of the world; how the virus survives out of season; and how far the aphid vector can travel and still transmit the GRD viruses.

Resistant cultivars are the most viable means to control GRD for the resource poor smallholder farmers. Surprisingly, most of the resistant cultivars developed so far have not yet gained popularity among the farmers, despite the threat posed by GRD. In this case, an

opportunity exists to develop GRD resistant cultivars that farmers want to grow. There is clear need to involve farmers in the breeding work in order to develop resistant and agronomically desirable varieties which can then stimulate adoption and diffusion of the developed materials. It is also important to involve the local landraces which farmers still grow in order to capture traits preferred by the farmers.

The varieties that have been developed so far are mainly resistant to GRV, and indirectly resistant to its sat RNA. There are a few cultivars that are resistant to the aphid vector. Resistance to GRV does not amount to immunity and can be overcome under high inoculum pressure or adverse environmental conditions. Another approach would be to breed for resistance to GRAV, which is essential for the multiplication and transmission of GRD. Such resistance has been found mainly in wild relatives. Introgressing GRAV resistance from wild species into cultivated groundnut might offer immunity to GRD. Another possible breeding strategy that can be exploited is to combine the resistance to GRD with resistance to the aphid vector.

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CHAPTER 2

Groundnut (*Arachis hypogaea* L.) varietal preferences and production challenges among smallholder farmers in the central region of Malawi

Abstract

Although groundnut (*Arachis hypogaea* L.)is an important crop among smallholder farmers in Malawi, production has diminished in recent years. Several constraints are believed to have affected productivity, including: use of low quality seed, weather changes, and diseases. This study was instituted, using a field survey and participatory rural appraisal (PRA) tools, to capture a solid understanding of the groundnut varietal preferences and production challenges faced by smallholder farmers in the central region of Malawi. A field survey was used to assess the occurrence of groundnut diseases in farmers fields and the PRA was conducted to determine the variety traits preferred by farmers and production and marketing challenges. A total of 30 fields were surveyed and over 120 farmers interviewed during the study in 4 districts of Malawi, namely Kasungu, Lilongwe, Mchinji and Salima. Most farmers (54%) had farms of between 1 to 2 ha. Most of the land (94%) was inherited from parents, while 6% purchased their farms. The majority of farmers grew groundnut alongside maize (*Zea mayis* L.) and beans (*Phaseolus vulgaris* L.), and tobacco (*Nicotiana tabacum* L.) and cotton (*Gossypium hirsutum* L.) as cash crops. Farmers sourced groundnut seed from a spectrum of sources, including local markets (84%), retention of their own seed (74%), agro-dealers (50%) and the government's subsidized seed programme (34%). The most widely grown groundnut varieties were Chalimbana (96%) and CG 7 (94%). Groundnut diseases observed in the fields during the survey included early leaf spot (*Cercospora arachidicala*) and late leaf spot (*Cercosporidium personatum*), rust (*Puccinia arachidis*) and groundnut rosette disease (GRD). Although GRD was observed in only 50% of the fields visited, 98% of the farmers had experienced it in their fields at some point, and 63.3% of the farmers believed that GRD was a major problem. Other challenges noted by farmers included the lack of quality seed, poor extension support resulting in a lack of technical advice, lack of inputs (fertilizers and agrochemicals), manipulation of the markets by buyers, and the failure of groundnut crops to meet the high standards required by the market. These findings suggest the need to develop new groundnut varieties with traits preferred by the farmers and their promotion to enhance adoption. There is also also the need for extension staff and researchers to work together in providing adequate information to farmers in terms of production and marketing of groundnut.

2.1 Introduction

Groundnut is an important crop for smallholder farmers in Malawi. The crop serves as a major source of protein, oil and income (Minde *et al*., 2008). Groundnut accounted for 27% of the land devoted to the cultivation of legumes (171, 000 ha) during the period 1991 - 2006 (Simtowe *et al*., 2010).

Generally, groundnut yields are low in Malawi. Several constraints reduce yields. These include the use of low yielding varieties, declining soil fertility, inadequate extension support services, limited access to agricultural credit facilities, and pests and diseases (Kumwenda and Madola, 2005; Siambi *et al*., 2007; Minde *et al*., 2008). Lack of access to sufficient quantities of improved seed, cause farmers to use low yielding varieties, and plant recycled grain as seed, hence lowers groundnut productivity (Simtowe *et al*., 2009). Commercial seed companies focus on the sale of hybrid seed of crop varieties that have to be re-purchased each season. They avoid self-pollinated crops, which can be recycled by farmers, making the breeding of these crops uneconomic (Siambi and Kapewa, 2004). Consequently, there is no established groundnut seed enterprise in Malawi.

In the past 10 years, ICRISAT and the Department of Agricultural Research have released several high yielding groundnut varieties with good levels of resistance to major biotic and abiotic stresses, such as GRD and drought (Freeman *et al*., 2002; ICRISAT, 2006; Makkouk and Kumari, 2009; Simtowe *et al*., 2009). However, adoption of these improved varieties has been very low. In a study conducted in several districts in Malawi, Simtowe *et al*., (2009) found that 60% of sampled farmers were aware of at least one improved variety of groundnut but only 26% of them had attempted growing at least one of these improved varieties.

Adoption of new technologies such as varieties is a complex issue among farmers in most parts of the world because it is determined by several factors. Akudugu *et al*. (2012) found that farm size, level of education, and access to funds were the main determinants of adoption of new technologies. On the other hand, Doss (2003) attributed the low adoption of improved technologies by farmers to, firstly, that the farmers are not aware of the novel technologies or their benefits; secondly, that the technologies are not readily available or are not available at the times needed; and lastly, that the adoption of the technologies seems to be unprofitable given the complex decisions that farmers make regarding land allocation and labor partitioning between agricultural and non-agricultural activities. In order to enhance adoption and diffusion of improved crop varieties, there is need for employment of participatory methods in the development of the new varieties, whereby farmers are involved

in the breeding process, especially in the development of selection criteria, and in the making of selection choices.

Generally, there is no literature published on previous groundnut breeding programmes in Malawi, that were targeted for small-scale farmers to show that breeders had efficiently analyzed farmers' varietal trait preferences and the market quality demands. The low rate of adoption of released varieties reflects the lack of involvement of farmers prior to developing them. According to Smolders (2006), participatory plant breeding (PPB) aims at developing locally adapted varieties that are adapted to the farmers' local environment and which consider the diverse traits that are valued by farmers. Participatory rural appraisal (PRA) techniques have been used in PPB to assess farmers' priorities, preferences in variety choice and to set breeding goals that meet famers' needs (Hall and Nahdy, 1999; Adu-Daapah *et al*., 2007). The use of well-applied PRA techniques in PPB results in a better client-oriented breeding programme and more efficient goal setting or product design, because the breeder gathers key information on the physical environment where the crop will be grown, the existing varietal diversity, the size of market, and the essential traits (Witcombe *et al*., 2005).

Several researchers have successfully used PRA in groundnut improvement programmes in other countries. For example, farmers in Ghana indicated that resistance to GRD was their most preferred trait in improved groundnut varieties (Adu-Daapah *et al*., 2007). Ntare *et al*., (2007) reported that in Mali, Niger, Nigeria and Senegal farmers were able to select 17 varieties based on the farmers' village level criteria which included high pod and fodder yield, resistance to diseases, taste, oil content, drought tolerance and marketability. PRA uses tools such as semi-structured interviewing, focus group discussions, preference ranking, mapping and modeling, seasonal and historical diagramming to identify and prioritize the production preferences and constraints (Theis and Grady, 1991).

In view of this, a study was implemented involving a survey and PRA in the central region of Malawi to find out farmers' varietal preferences and production challenges. The specific objectives were to;

i. Evaluate household characteristics, production practices and utilization of groundnut
ii. Determine varietal preference and essential traits in groundnut varieties
iii. Assess occurrence and severity of GRD
iv. Assess farmers' awareness and perceptions of GRD
v. Evaluate production and marketing challenges faced by groundnut farmers

2.2 Materials and Methods

2.2.1 Study areas

The study was conducted in the central region of Malawi which produces about 70% of the country's groundnut crop (Ngulube *et al*., 2001). The survey was conducted in three districts namely Mchinji, Lilongwe, and Salima while PRA was conducted in Kasungu, Salima and Lilongwe. Important characteristics of the study sites are given in Table 2.1 and Fig. 2.1.

Table 2.1: Geographical and weather information of the four districts where study was conducted in Malawi

	Altitude (masl)	Latitude	Longitude	Area (Km^2)	Average rainfall (mm)	Average temperature (°C)	
						Maximum	Minimum
Kasungu	4403	13°02'S	33°29'E	7878	500 – 1200	28	9
Lilongwe	3440	13°59'S	33°47'E	6159	500 – 900	30	6
Mchinji	3877	13°49'S	32°54'E	3356	500 – 1100	36	20
Salima	525	13°45'S	34°30'E	2196	500 – 1000	32	16

* masl is metres above sea level

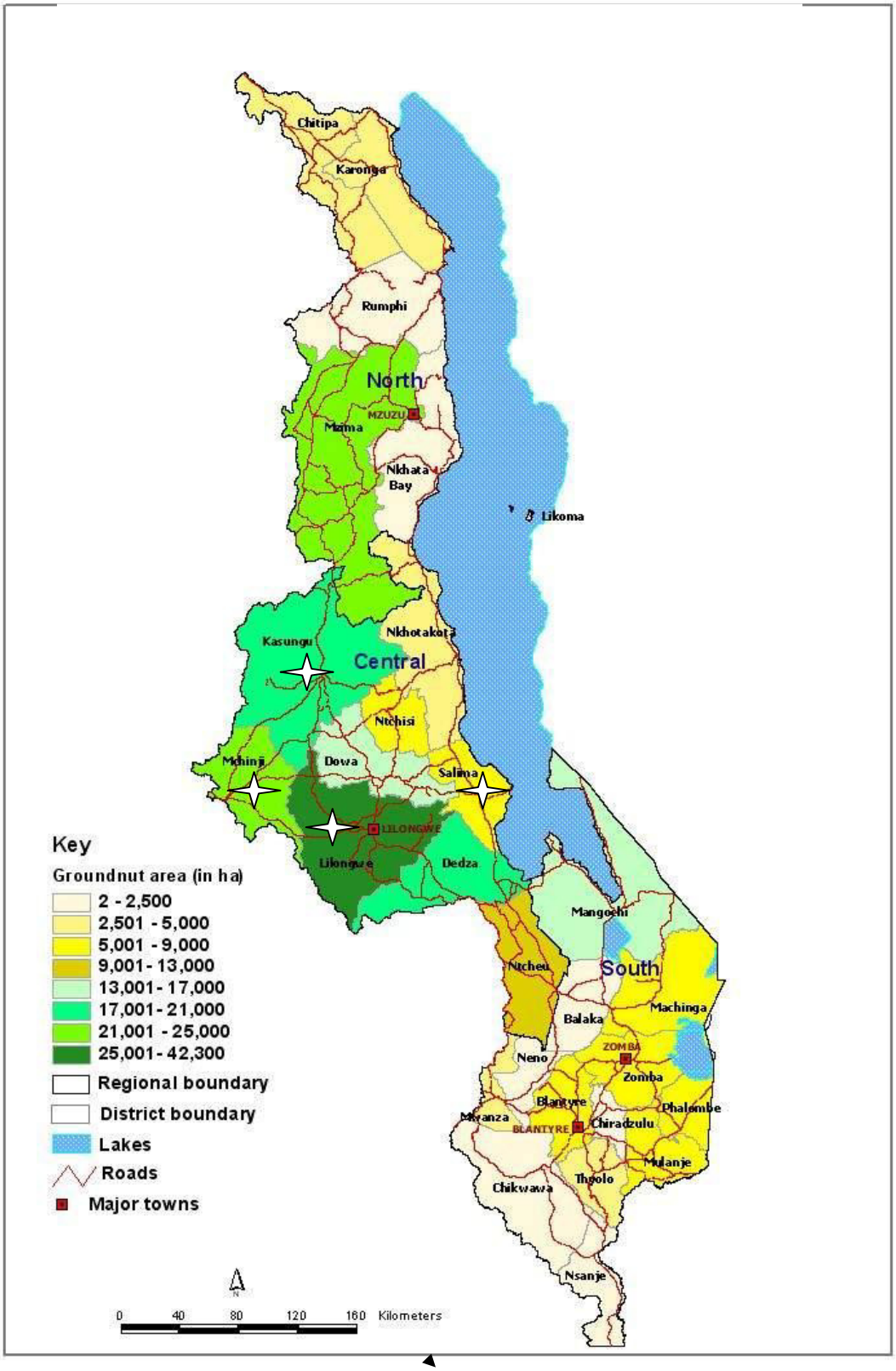

Figure 2.1: Map of Malawi showing area under groundnut production (Simtowe *et al*., 2010). Study areas are marked by stars.

2.2.2 The survey

The survey was conducted through field visits, observation and assessment. In each of the 3 districts, 10 farmers' fields were selected at random among groundnut growers with the help of extension officers who were familiar with the farmers and fields concerned. The information was captured on a pre-designed form under which the following were indicated; name of the farmer, location, date of planting, variety, and diseases. The location of each selected field was determined using a handheld GPS (GARMIN eTrex, personal navigator). In the field, the farmer gave information on the groundnut varieties planted and date of planting. Disease assessment involved selecting a total of 40 plants along 2 diagonals of each field (Figure 2.2). A walk around of the field was also done to assess the whole field for factors that may have contributed to disease incidence.

2.2.2.1 Assessment of GRD and other diseases

GRD was assessed based on percentage disease incidence (PDI) as described by Waliyar *et al*. (2007). A total number of 40 plants along 2 diagonals were randomly selected in each field and counted. Plants showing GRD symptoms as described by Waliyar *et al*., (2007) were selected from this sample and counted. Percentage disease incidence (PDI) was computed using the formula below;

$$PDI = \frac{\text{Number of plants infected along 2 diagonals}}{\text{Total number of plants along 2 diagonals}} \times 100$$

Leaf spot and rust diseases were scored based on a 1-9 scale (1 = no disease and 9 = plants severely affected and 50-100% leaves withered or defoliated) (Subrahmanyam *et al*., 1995).

2.2.3 Participatory Rural Appraisal

An extension planning area (EPA) in each district was chosen for farmer interviews and focus group discussions. The selection of an EPA in each district involved the principal investigator (breeder), an agricultural extension officer and a crop production officer under the Agricultural Development Divisions (ADD). The EPAs were selected based on their performance and history of groundnut production with the best being most likely to be chosen. In each EPA, farmers were initially organised for a focus group discussion (FGD), each group comprising of a total of ≥20 farmers (both men and women). A checklist with open ended questions was used for the FGDs. In total, there were 3 FGDs conducted in the 3 districts with >60 people involved. Later, farmers who were not involved in the FDGs were

randomly selected with the help of village heads and interviewed using a structured questionnaire (Figure 2.3). A total of 50 farmers, (27 men and 23 women), were interviewed. In total, the PRA involved ≥120 farmers. The location where each FGDs and farmer interviews were conducted was recorded using the handheld GPS.

a. b.

Figure 2.2. Assessment of groundnut diseases rust (a) and leaf spot (b) in Salima and Mchinji districts, in Malawi

Figure 2.3: Individual farmer interviews in progress in the field in Lilongwe district, Malawi

2.2.4 Data analysis

The data collected from the EPAs in the 3 districts were analysed using the Statistical Package for Social Scientists (SPSS).

2.3 Results

2.3.1 Household characteristics, landholding size and labour use

2.3.1.1 Household characteristics

The demographic characteristics of the households are presented in Table 2.2. About 92% of the households of farmers interviewed were male-headed with family sizes ranging from 2 to 10 members. It was also observed that 92% of the households were headed by men and women within the active age range (18-65 years) with only 8% headed by men or women over 65 years old. Up to 80% of the farmers had more than 10 years farming experience while 20% had less than 10 years experience. Of these, 52% depended solely on farming, while 44% conducted several other businesses (e.g. selling livestock, brewing beer, and fishing) and only 4% were employed.

Table 2.2. Demographic characteristic of farmers households in Malawi where survey was conducted

Characteristic		**District**			Total
		Kasungu	**Salima**	**Lilongwe**	
Sex of household head (%)	Male	94.0	88.0	93.0	91.6
	Female	6.0	12.0	7.0	8.3
Mean age of household head (%)	18 to 35 years	36.4	40.0	9.1	22.0
	36 to 65 years	54.5	25.7	34.3	70.0
	65years and above	9.1	34.3	50.0	8.0
Marital status (%)	Married	89.0	100.0	93.0	94.0
	Widow	11.0	0.0	7.0	6.0
	Divorced	0.0	0.0	0.0	0.0
	Single	0.0	0.0	0.0	0.0
Education of household head (%)	None	5.6	41.2	26.7	24.5
	Primary	83.3	47.1	46.7	59.0
	Secondary	11.1	11.7	26.7	16.7
	University	0.0	0.0	0.0	0.0
Occupation (%)	Farming only	55.6	35.3	66.7	52.5
	Business	38.9	64.7	26.7	43.4
	Working	5.6	0.0	6.7	4.1
Experience in farming (and in growing groundnut) (%)	1-5 years	5.5 (44.4)	11.8 (41.2)	6.7 (33.3)	8 (40)
	6-10 years	16.7 (22.2)	5.9 (17.6)	13.3 (0)	12 (14)
	11 - 25 years	50.0 (22.2)	52.9 (23.5)	33.3 (26.7)	46 (24)
	26 years and beyond	27.8 (11.1)	29.4 (17.6)	46.7 (40)	34.0 (22)

Note: (*) indicates farmers experience in years in growing groundnut

2.3.1.2 Land holding characteristics

The farm size per household ranged from less than 0.5 ha to over 2 ha (Table 2.3). About 54% of the households owned farms of between 1 to 2 hectares while 24% had farms of less than 1 hectare whereas 22% had farms bigger than 2 hectares. Most of the farms (94%) owned by the households was inherited with only 6% being rented or borrowed (Figure 2.4).

Table 2.3: Distribution of smallholder farms sizes in Kasungu, Lilongwe and Salima districts, Malawi

District	Distribution of farm sizes			
	≤ 0.5 ha.	0.6≥1 ha.	>1 to 2 ha.	>2 ha.
Kasungu	0.0	0.0	44.4	55.6
Salima	11.8	17.6	70.6	0.0
Lilongwe	6.7	40.0	46.7	6.7
Total	**6.0**	**18.0**	**54.0**	**22.0**

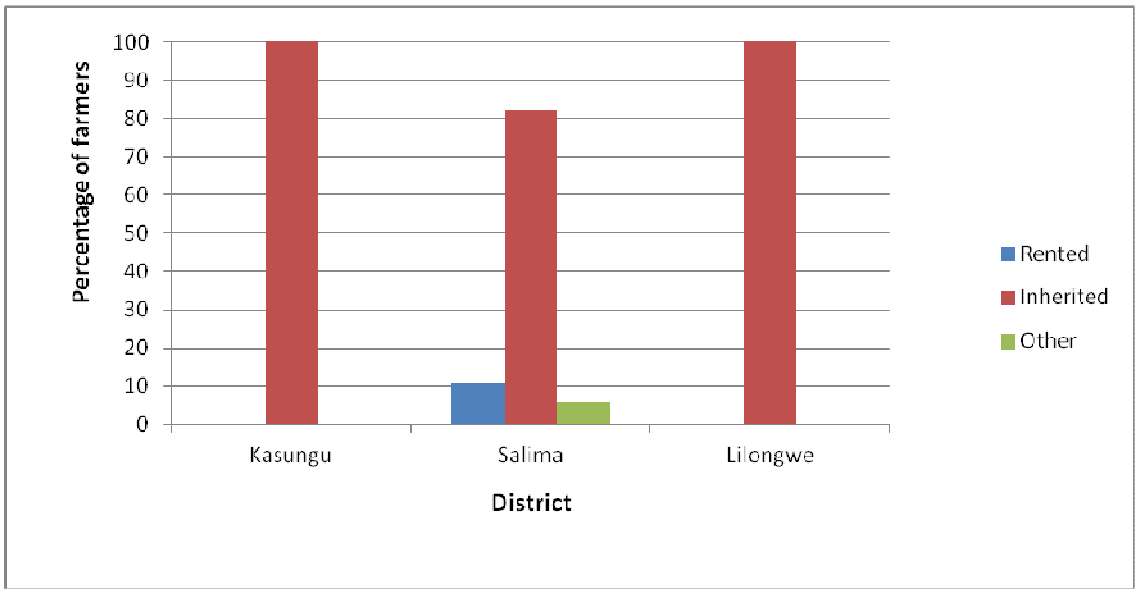

Figure 2.4: Sources of the farms (inherited versus rented or bought) owned by smallholder farmers in Kasungu, Lilongwe and Salima districts, Malawi

2.3.1.3 Labour use

All household members (from children to adults) provided labour for their fields (Figure 2.5). Forty six percent of households with large farms and other sources of income hired extra labour. The farm activities that most required the hiring of labour included land preparation, weeding, harvesting and processing. Although labour is hired for all crops, the focus group discussions revealed that most activities in groundnut fields were done by women and

children. Apart from family and hired labour, 16% of the farmers mainly in Kasungu and Salima also used village labour.

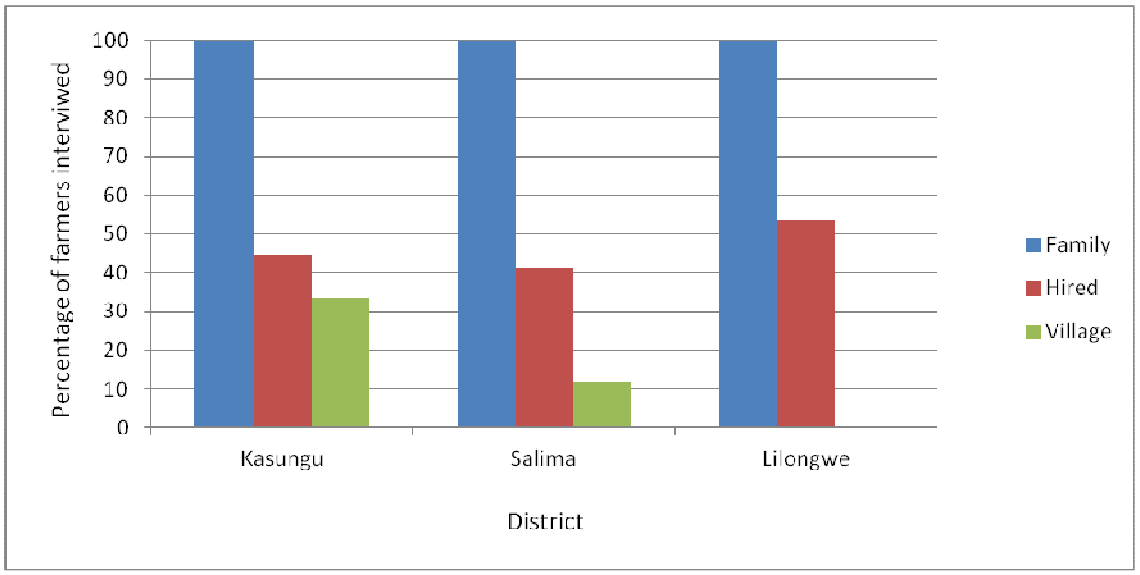

Figure 2.5: Source of labour used by surveyed households

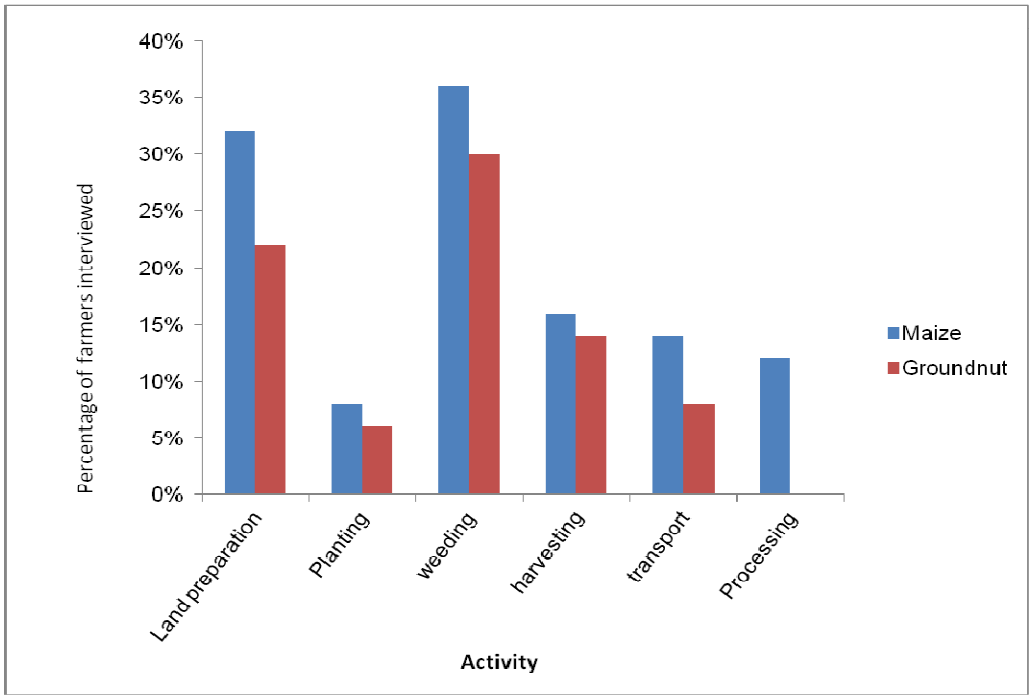

Figure 2.6: A comparison in labour hiring between maize and groundnut crop in Malawi

A comparison between labour hiring for maize and groundnut production showed that farmers hired more labour for maize than groundnut (Figure 2.6). For both crops weeding required most labour while planting required the least.

2.3.2 Cropping systems, crop production and seed sources

The main crops grown in the three districts are presented in Figure 2.7. All farmers interviewed grew both maize and groundnut, of whom 54% started growing groundnut within the last 5 to 10 years. About 65% other crops grown by farmers included: soybean, tobacco, cassava, sweet potatoes and cotton.

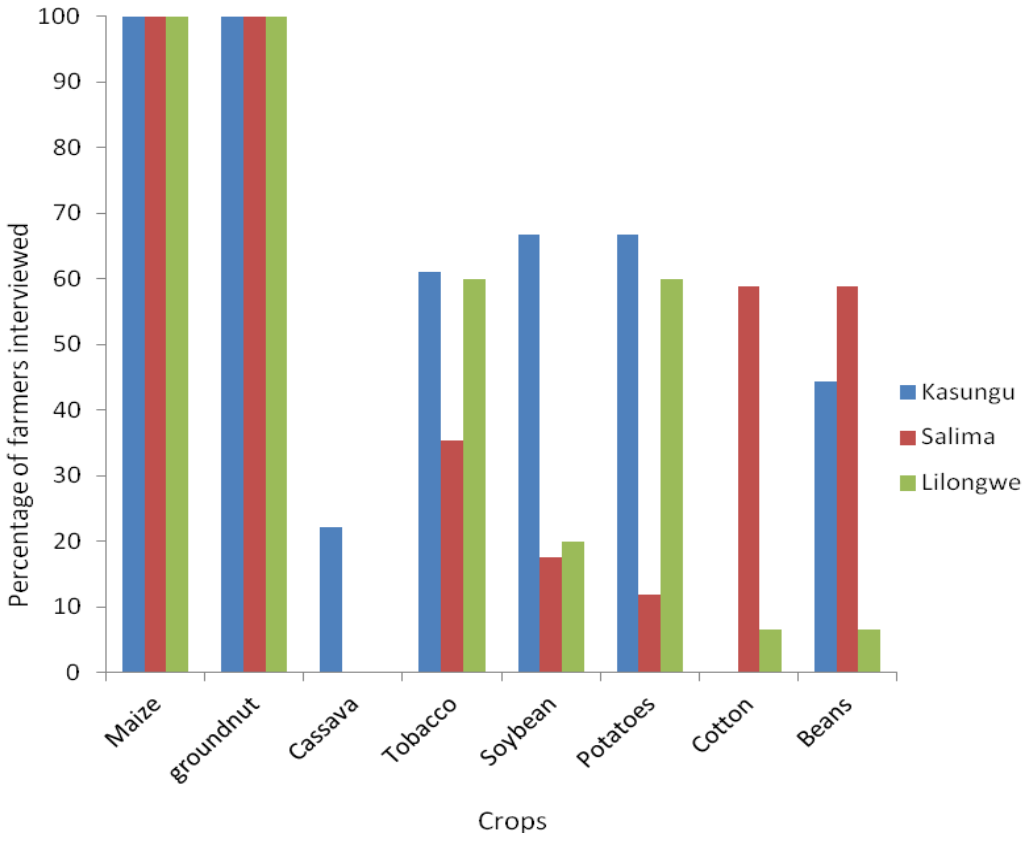

Figure 2.7: Main crops grown in Kasungu, Salima and Lilongwe districts, Malawi

The results presented in Figure 2.8 showed that farmers obtained seed from various sources including: local markets (84%), agro-dealers (50%), the government's farmers input subsidy programme (34%), and from NGOs (20%). However, most farmers (74%) also kept part of their own harvest for seed and depended on farmer to farmer seed exchanges.

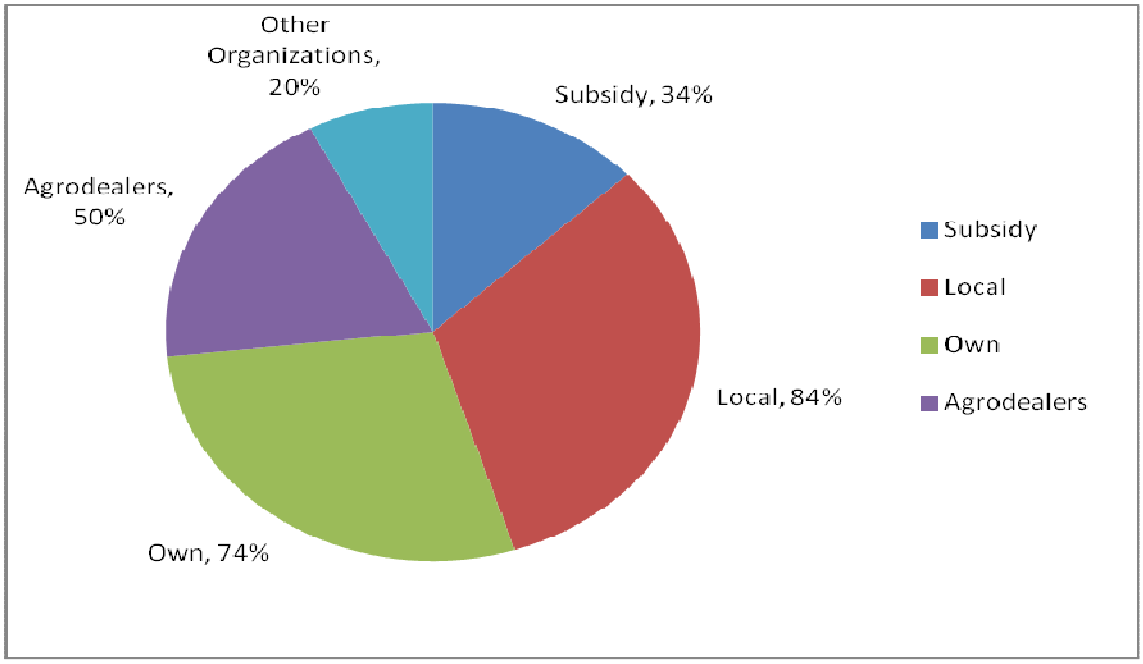

Figure 2.8: Main sources of groundnut seed grown by farmers in Kasungu, Lilongwe and Salima districts, Malawi

Sources of groundnut seed are presented in Figure 2.9. The majority of seed was obtained from farmers' previous harvests and local seed producers. Other sources of seed included agro-dealers, farmer to farmer seed exchanges, inheritance, farmer clubs, gifts and governments subsidy programme.

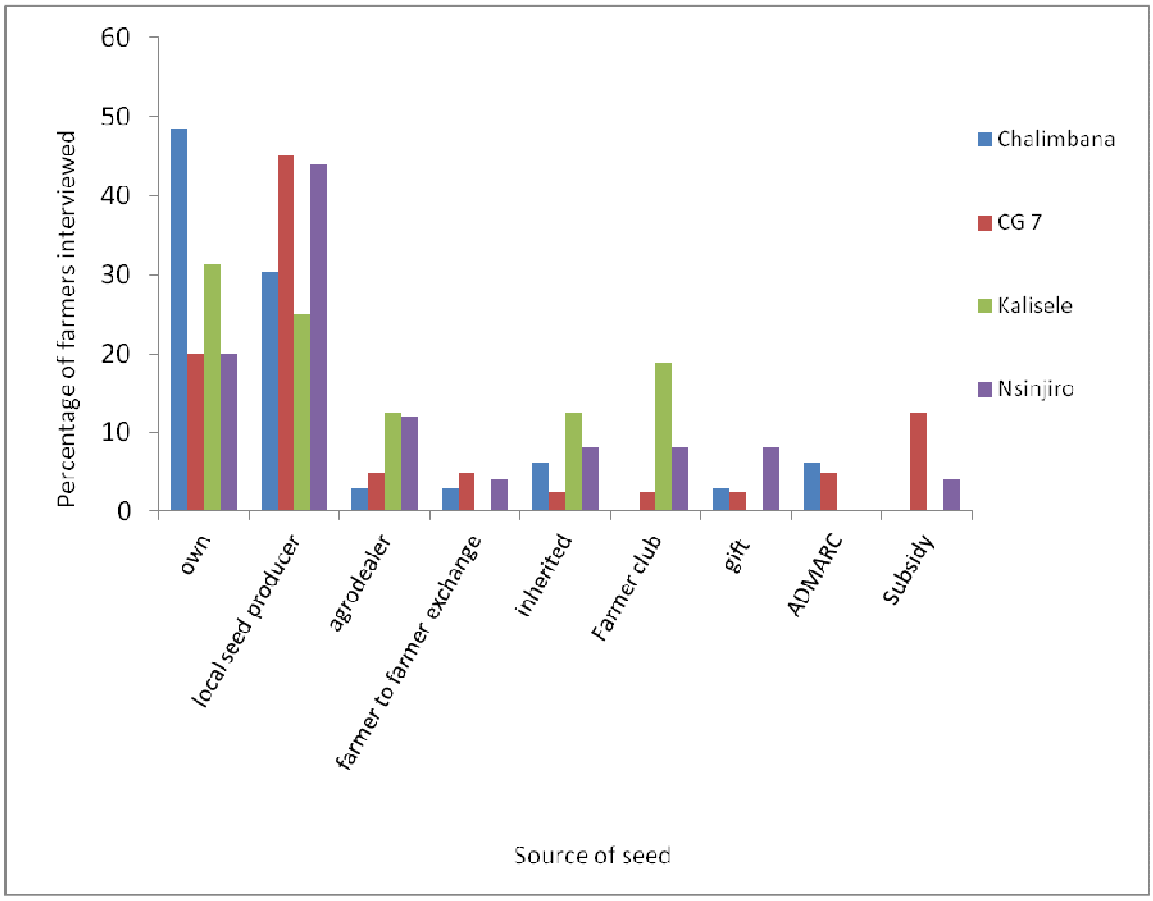

Figure 2.9: Sources of seed for four main groundnut varieties grown by farmers in Kasungu, Lilongwe and Salima districts of Malawi

2.3.2.1 Groundnut varieties

The groundnut varieties grown by farmers are Chalimbana, CG 7, Kalisele, Nsinjiro, Kakoma, Chalimbana 2005, Baka, and Manipintar (Table 2.4). Among the varieties, the most common in all districts were Chalimbana and CG 7 grown by 96% and 94% of the farmers, respectively. The least common varieties were Kakoma and Baka which have been recently released in Malawi.

Table 2.4 Groundnut varieties grown by smallholder farmers in three districts of Malawi (%)

	District			
Variety	Kasungu	Salima	Lilongwe	Total
Chalimbana	94.0	94.1	100.0	96.0
CG 7	88.9	100.0	93.3	94.0
Kalisele	83.3	35.3	80.0	66.2
Nsinjiro	11.1	70.6	26.7	36.1
Kakoma	0.0	17.6	0.0	5.9
Chalimbana 2005	5.6	29.4	46.7	27.2
Baka	0.0	5.9	0.0	2.0
Manipintar	27.8	70.6	53.3	50.6
Others (Gambia and Mawanga)	5.6	76.5	20.0	34.0

During focus group discussions, it was observed that the majority of farmers planted their groundnut later in the season after planting other major crops such as maize and tobacco. About 94% of farmers planted groundnut as a mono crop in rows with only 6% practicing mixed cropping.

2.3.2.2 Farmers' preferences for different groundnut varieties

Several attributes were given by the farmers as the basis for their preferences for particular varieties (Table 7). Yield and good taste were the most common positive attributes by farmers for most of the varieties grown. Other positive attributes included large grains, high oil content, ease of shelling, ease of pounding into groundnut paste, early maturity, and tolerance to diseases, pests and drought.

Negative attributes common for most of the varieties were late maturity, small grains, over branching, sprouting before harvest, oiliness, tolerance to drought, susceptibility to diseases and pests. Although, high oil content was given as a positive attribute, many who grew groundnut for home use viewed it as a negative attribute because when used to prepare paste for relish, the high oil content made the relish rancid more easily.

Farmers gave varying views on performance of groundnut varieties (Table 2.6). In general, CG 7 was viewed as the best variety with 79.5% of farmers ranking it from good to excellent while Chalimbana was ranked as fairly poor by 52% of farmers.

Table 2.5 Traits of popular groundnut varieties grown in Malawi

Variety	Trait	
	Positive	Negative
Chalimbana	Large grain, high yield, good taste, weighty,	Late maturity, susceptible to diseases, pests and drought, difficult to harvest (branchy)
CG 7	High yield, good taste, high oil content, easy to shell	Late maturity, susceptible to diseases, pests and drought, hard to pound, not good for paste
Kalisele	Good taste, tolerant to diseases and drought	Small grain, hard to shell, low yielding, hard to pound, susceptible to pests
Nsinjiro	Early maturity, high yield, good taste, easy to pound	Susceptible to diseases, sprouts in the field before harvesting
Kakoma	Early maturity, drought resistant, high yielding, marketable	Small and light (weight) grains, sprouts in the field before harvesting.
Chalimbana 2005	Large grain, good taste, high yield	

Table 2.6: Farmers rating of the four most popular groundnut varieties in Malawi

Variety	Score			
	Poor	Fair	Good	Excellent
Chalimbana	4.0	48.0	36.0	12.0
CG 7	0.0	20.5	48.7	30.8
Kalisele	9.1	27.3	45.5	18.2
Nsinjiro	20.0	10.0	40.0	30.0

2.3.2.3 Groundnut production and utilization

Most farmers grow their groundnut on about 0.5 ha of land (Table 2.7). On average most farmers produced 8-10 bags (50 kg) of unshelled groundnut and sold up to 50.5% on average of their produce (Figure 2.10). The remaining 49.5% was for home consumption and seed for the next season.

Table 2.7: Average groundnut field size

District	Area (ha)	Std. Dev.
Kasungu	0.51	0.46
Salima	0.50	0.44
Lilongwe	0.51	0.46

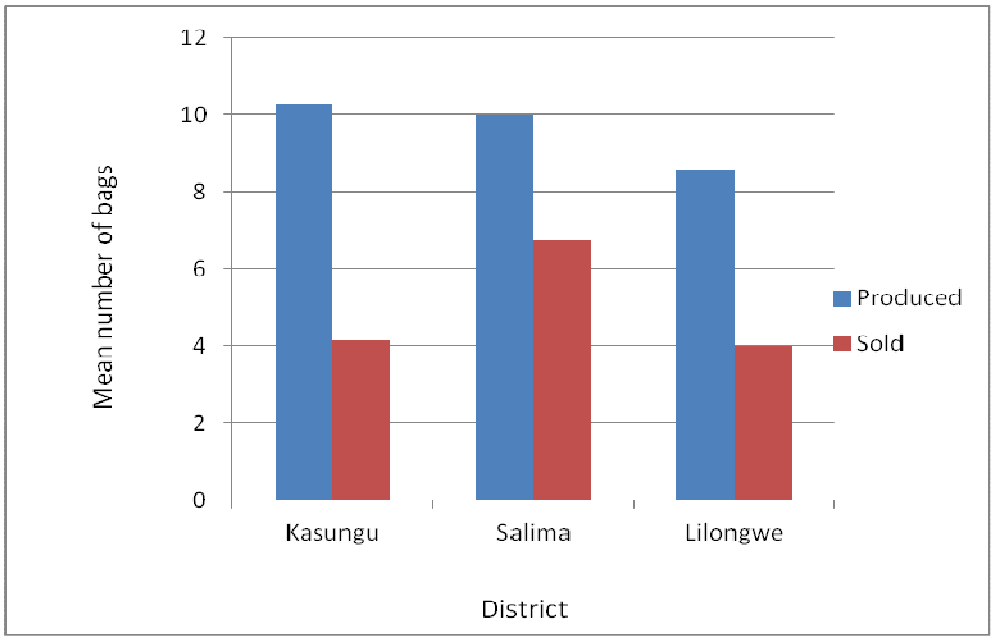

Figure 2.10: Average number of bags (50 kg) produced and sold in each of the districts surveyed in Malawi

2.3.2.4 Occurrence of diseases in farmers groundnut fields

There were several diseases observed in farmers fields which included early and late leaf spot, rust and GRD. Generally, GRD was observed in 50% of the farmers' fields visited (Figure 2.11). However, disease incidences were low (1-40%) in most of the fields. Other diseases observed included early leaf spot (ELS), late leaf spot (LLS) and rust.

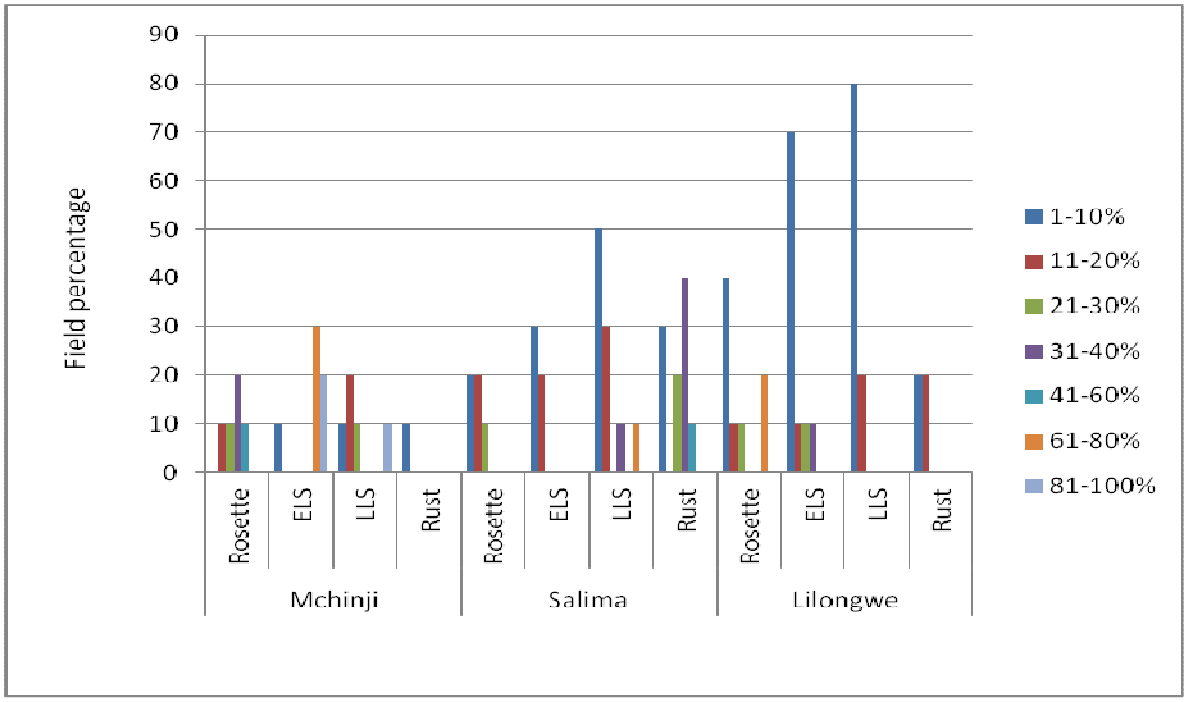

Figure 2.11: Groundnut fields infected by different diseases in Mchinji, Salima and Lilongwe districts

2.3.2.5 Farmers awareness and perception about GRD

Of the farmers interviewed, 98% had knowledge of and had experienced GRD in their fields. However, the disease was given different names in the different districts all of which were based on the symptoms expressed by diseased plants (Table 2.8). Generally, in Kasungu GRD was called Chakwinya (curled) and in Salima it was called Khate (leprosy) while in Lilongwe it was called Kadukutu (invisible pest burning plants).

Table 2.8: Local names given by farmers to the symptoms of GRD in Kasungu, Salima and Lilongwe

Local name	District		
	Kasungu	Salima	Lilongwe
Chakwinya	64.7	5.9	0.0
Chiwawu	0.0	11.8	6.7
Khate	0.0	64.7	26.7
Kadukutu	5.9	0.0	66.7
Chitukule	23.5	0.0	0.0
Chisaka	5.9	0.0	0.0
Kafumbata	0.0	11.8	0.0
Do not know	0.0	5.9	0.0

Table 2.9: Perceptions of farmers on the causes and modes of transmission of GRD in Salima, Kasungu and Lilongwe districts

		Doesn't know	Weather	Insects	Late planting	Aphids and mono cropping
Causes	Kasungu	47.1	17.6	17.6	11.8	5.9
	Salima	23.5	47.1	23.5	0.0	5.9
	Lilongwe	57.1	14.3	7.1	21.4	0.0
Transmission	Kasungu	47.1	35.3	11.8	0.0	5.9
	Salima	29.4	41.2	23.5	0.0	5.9
	Lilongwe	57.1	14.3	7.1	21.4	0.0
		Doesn't know	Chemicals	Early planting	Crop rotation	Field hygiene
Control	Kasungu	70.6	0.0	11.8	5.9	11.8
	Salima	88.2	5.9	0.0	5.9	0.0
	Lilongwe	78.6	7.1	14.3	0.0	0.0

Although symptoms of GRD were widely known, the majority of farmers did not know the real cause, mode of transmission or ways of controlling it (Table 2.9). Only 5.9% of the farmers believed that aphids were responsible for GRD incidence, while others attributed the disease to weather, mono-cropping, late planting and other insects.

Depending on their observation, 32.6% of the farmers indicated that CG 7 was the most susceptible variety to GRD followed by Chalimbana (23.9%), Kalisele (8.7%), Nsinjiro (4.3%) and Chalimbana (6.5%) However, 23.9% of the farmers believed that all varieties were equally susceptible to GRD. On the other hand, 50% of the farmers believed that

Chalimbana was the most resistant to GRD followed by CG 7 (22.2%), and Kalisele (22.2%) while 5.6% said that all varieties were resistant.

During the focus group discussions and interviews, 65.2% of the farmers indicated that they felt GRD incidences were increasing, while 26.1% indicated that GRD incidences were decreasing and 8.7% believed that there had been no change in GRD incidences over the years. Overall, 63.3% of the farmers acknowledged that GRD was an important problem that required an intervention.

2.3.2.6 Other production and marketing problems

The survey also revealed that apart from GRD, there were other production and marketing problems faced by groundnut farmers. The importance of problems varied from district to district (Figure 2.12). Overall, lack of inputs was the top most challenge farmers' face in production while diseases were the least. In marketing, farmers were faced with the question of high standards required in the market followed by inadequate markets.

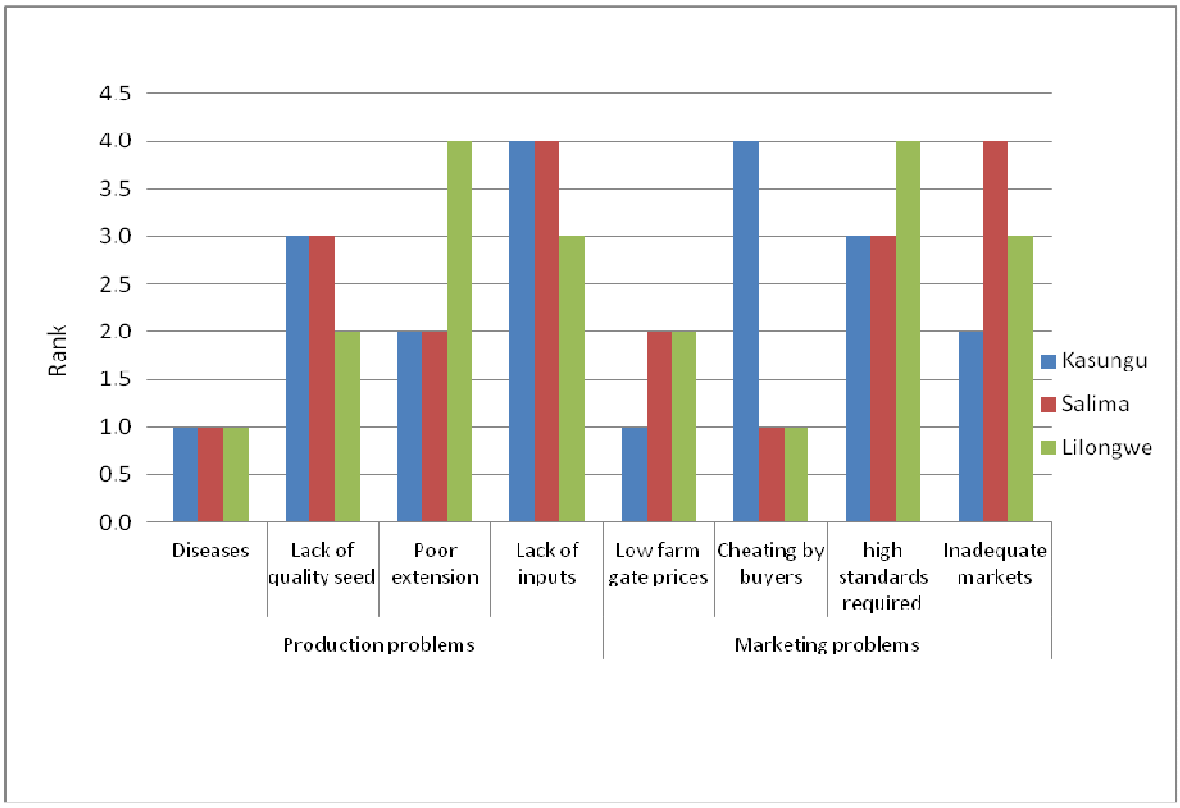

Figure 2.12: Ranking of problems faced by farmers in production and marketing of groundnut in Kasungu, Lilongwe and Salima

2.4 Discussion and conclusion

The results obtained in this study have elucidated the cropping systems, choices and constraints which farmers experience in producing groundnut. Farmers decisions' of what to produce and how to produce is determined by several factors including their age, household size, land holding size, education, occupation, and farming experience (Minde *et al*., 2008). In this study, the majority of the farmers interviewed had households headed by men within the active age group (18-65 years) and most were educated up to primary level. Most of the farmers did also not have formal jobs hence had more time to work on their farms. It is worthy noting that Malawi's economy is largely agro-based hence farming is very important for every household in the rural areas.

Land ownership is an important factor of food security in Malawi (Simtowe *et al*., 2009) as it determines how much a farmer can produce. Most of the land owned by farmers in the rural areas is inherited from parents as was confirmed in this study. The larger the land the more the crops the farmers grow in a season. However, it was observed that groundnut is often allocated to a small portion of the total farmers' field. Sintowe et al., 2008 observed that the total area planted to groundnut in Malawi from 1991 to 2006 was only 14% of the total area planted to maize. During focus group discussions, it was revealed that groundnut is usually left for women and children to produce while men focus more on major crops like maize and tobacco. This confirms the notion that groundnut is a woman's crop (Minde *et al*., 2008). Nationally, it is estimated that 20% of all farmers in Malawi grow groundnut and 85% of which are smallholder farmers (Sangole *et al*., 2010). Apart from maize, tobacco, and groundnut, farmers also grow several other crops which compete for space with groundnut. As a result, overall production of groundnut in most farmers' fields is still very low. However, as the tobacco market is decreasing and with efforts from the government to increase production of other crops apart from tobacco, there is an opportunity that the area for groundnut may increase.

The availability of several groundnut varieties in Malawi enables farmers to have a wide choice of what to plant. The study identified two most widely grown varieties namely Chalimbana and CG 7. Both varieties have been in Malawi for a period of more than 20 years and as such farmers are used to them. Chalimbana is large seeded and has good taste especially when eaten raw, fried, cooked or when used as paste in relish. The medium seeded CG7 has good taste and high oil content which is good for the market. Although, both varieties are marketed, Chalimbana is mostly grown for home consumption while CG 7 is mainly for the market. As such, CG 7's popularity is increasing and it is estimated that more than half of Malawi's groundnut produced is CG 7 (Minde *et al*., 2008). However,

Chalimbana and CG 7 are susceptible to GRD. But there are several other improved varieties that have been released having traits such as resistance to GRD, high yielding and early maturing which have not been adopted by most of the farmers probably because they lack key traits of interest to the farmers. It is worth noting that farmers still grow local groundnut varieties such as Kalisele and Gambia which are less yielding but probably have those unique traits which they prefer. Low adoption can also be attributed to the low level of education among most farmers which complicate their ability to understand new agricultural technologies (Akudugu *et al*., 2012). However, involvement of farmers in selection of traits and incorporation of the local varieties in a breeding programme can help in enhancing adoption of new varieties developed.

Agricultural production in Malawi is further hampered by the lack of inputs. Low income levels pose a major challenge to farmers to access agricultural inputs. Most of the farmers interviewed in this study did not have formal employment from which they can obtain cash. This could be the reason why most farmers use their own recycled seed and do not use chemicals to control pests and diseases. Lack of a structured groundnut seed enterprise provides the opportunity for cheap, low quality seed to circulate among the farmers through local traders and farmer to farmer seed exchanges. The Malawi government's input susbsidy programme which benefits farmers has a draw back that the majority of the farmers are not reached. The presence of local traders from where farmers obtain seed indicates existing opportunities for establishment of quality assured seed enterprises within farmers' reach.

A large proportion of farmers indicated that groundnut production was further hampered by lack of support and advice from extension staff and lack of inputs (herbicides, pesticides and fungicides). The inability of extension workers to reach out to farmers with information on varieties, inputs, markets and diseases is disastrous to increased agricultural production as a whole. For example, most of the farmers involved in this study were not aware of newly released varieties that can withstand some of the biotic and abiotic stresses related to groundnut production like drought and GRD. As such, farmers continued growing same old varieties which are susceptible to diseases and prone to droughts further reducing their overall production. In addition, lack of information on markets has led to exploitation of farmers by traders who usually dictate low prices. As a result, farmers become disinterested in producing more groundnuts for sale. However, these challenges can be solved by employing policies which can help to guide and protect farmers through the production cycle up to marketing.

The single most important disease affecting groundnut in Malawi was GRD. According to the farmers, the trend of GRD levels was increasing because of high frequency of the drought periods in Malawi. Although most farmers observed GRD in groundnuts, most of them did not know its cause, ways of transmission and how it could be controlled. In addition, the inadequacy of the extension system has failed to provide essential information to farmers. Of particular interest is that farmers in various areas had different names for GRD based on visual symptoms. However, as information about GRD is lacking among farmers, most still continue growing susceptible varieties, planting late in the season without applying any measures to control aphids. These actions combine to create conducive environments for GRD infection. Varieties which have been released recently with resistance to rosette include Chitala, Nsinjiro and Baka. As observed in this study, very few farmers had adopted them probably because of lack of certified seed and information about the varieties (Simtowe *et al.*, 2009). Therefore, it is imperative that in order to enhance adoption and use of improved varieties, breeding programmes should involve farmers from the initial stages where farmers can select traits according to their preference. There is also need for a governmental or NGO agencies to propagate and distribute adequate quantities of the certified seed of the improved varieties to smallholder farmers.

Apart from production problems, farmers also face marketing problems. Most farmers opt to produce less quantities of groundnut because of the unavailabilty of markets and exploitation by vendors. However, of recent, the government has put deliberate policies to promote production and marketing of groundnut and several other crops. It is hoped that the current situation will change and that farmers will start to produce more groundnut for sale.

This study has elucidated the cropping systems and the choices and constraints which smallholder farmers face when growing groundnut. The use of PRA tools has demonstrated its importance in obtaining information from farmers such as preferred traits to incorporate when breeding new crop varieties. Indeed, farmers face many challenges when producing groundnut. The use of agrochemicals to control aphids appears to be unaffordable to most smallholder farmers. The other recommended agronomic practices such as early and dense planting have not been adapted by farmers because they do not fit into their overall farming programmes, such as planting maize and tobacco first because they are more important crops. As such, it can be concluded that breeding and promotion of new varieties with traits preferred by farmers is the best approach to solving the problem of GRD in Malawi. The PRA identified the traits that farmers consider to be essential or important in groundnut cultivars. These priorities will be used in the subsequent breeding programme to breed novel cultivars that combine high levels of resistance with all other key traits that farmers prefer.

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CHAPTER 3

Genetic diversity of Malawian and other selected groundnut genotypes using SSR markers

Abstract

The existence of genetic diversity in germplasm collections is crucial for cultivar development. Twenty one SSR markers were used to assess the genetic diversity among 106 groundnut (*Arachis hypogaea* L.) genotypes collected from ICRISAT, the Chitedze genebank and farmers in Malawi, Tanzania and Zambia. DNA was extracted from leaf samples using the highthroughput DNA extraction method. DNA analysis was done following M13-tag polymerase chain reaction (PCR). The amplified PCR products were analyzed using a genetic analyzer. Data were analyzed using Powermarker V3.25, Arlequin v3.1 and DARwin 5.0 softwares. A total number of 316 alleles were revealed with a mean of 17 alleles per locus ranging from 7 (Ah1TC6G09, pPGPseq7H06, pPGPseq1B09) to 29 (pPGPseq2D12B). The high polymorphic information content and gene diversity values averaging 0.77 and 0.80 respectively indicated that genetic diversity among the groundnut germplasm was high. The analysis of molecular variance indicated that 72.9% of the genetic variation observed in the germplasm was due to the variation between individuals within rather than between specific population groups. Cluster analysis distinctly grouped the 106 accessions into four clusters with 2 major clusters comprising of genotypes from ICRISAT and the Chitedze genebank while the other 2 clusters comprised mainly accessions collected from farmers. Generally, the high genetic diversity observed in this study provides the basis for selection of appropriate parental genotypes for breeding programmes and mapping populations to further broaden the genetic base of groundnut cultivars in the East Africa.

3.1 Introduction

Groundnut (*Arachis hypogaea* L.) is a crop grown throughout the world and is adaptable to a wide range of environmental conditions. It has exceptional capacity to survive under the wide range of conditions under which it is grown. Substantial variations for morphological, physiological and agronomic traits have been observed in the crop. Upadhyaya *et al*. (2002a) studied a collection of groundnut germplasm accessions collected from various regions of the world and found significant phenotypic and agronomic diversity. Levels of morphological, physiological, and agronomic diversity are even higher in wild diploid species (Knauft and Wynne, 1995). However, very little polymorphism at molecular level has been detected in cultivated groundnut (Dwivedi *et al*., 2003).

Knowledge of the existence and extent of genetic diversity in crop species is of prime importance in plant breeding programmes for the development of improved cultivars. Traditionally, morphological traits coupled with reactions to pests, diseases and other stresses have long been used to determine the genetic relatedness or diversity existing within and between germplasm collections and characterizing them into varieties. However, such phenotypic associations tend to vary according to the environment (Knauft and Wynne, 1995) and are most useful for traits that are controlled by only a small number of genes (Brown-Guedira *et al*., 2000). As such, classifying germplasm collections based on phenotypic differences alone may not provide an accurate indication of genetic diversity (Menkir *et al*., 1997).

In groundnut, a large number of accessions have been evaluated and diverse sources for various traits such as early maturity, disease resistance, drought tolerance and others have been identified (Holbrook and Stalker, 2003; Upadhyaya *et al*., 2008). Utilization of these resources through conventional breeding has led to the development of a number of improved cultivars (Nigam *et al*., 2003; ICRISAT, 2004). However, most resource poor farmers in Malawi mostly still grow unimproved low yielding varieties. One of the reasons for low adoption of improved varieties, apart from limited access to seed, is that the new "improved" varieties are often flawed and do not perform well for key traits the farmers demand. As such, an important opportunity exists to develop high-yielding and resistant varieties, which also have traits required by farmers and their markets. Modern breeders have not fully utilized the local valuable germplasm, which farmers grow on their farms and of importance for breeding programmes. Upadhyaya *et al*., (2002b) noted that most groundnut breeding programmes that aim at rapid cultivar development have used elite

breeding lines and cultivars, resulting in the development of breeding materials with a narrow genetic base. Generally, the processes of plant breeding reduce genetic diversity within the improved crop species (Rauf *et al*., 2010). However, the narrow genetic base available to breeders and the complex nature of the groundnut genome pose a serious bottleneck to the genetic improvement of groundnut (Pandey *et al*., 2012b). Despite these challenges, the exploitation of the available diversity in groundnut germplasm and identification of appropriate characterization techniques still holds the key to its further improvement.

Recently, molecular (DNA based) technologies have become the favoured means of determining variation in large germplasm collections. Molecular markers provide useful information that enables conservationists to classify accessions reliably and for breeders to better estimate the genetic value of individuals subjected to selection, hence accelerating breeding progress (Hospital *et al*., 1997; Romera *et al*., 2009). Examples of molecular markers techniques include restriction fragment length polymorphism (RFLP) amplified fragment length polymorphism (AFLP), random amplified polymorphism DNA (RAPD), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) (Stafford, 2009). Generally, simple sequence repeats (SSRs) or microsatellites and single nucleotide polymorphism (SNP) markers are preferred for plant genetics and breeding applications (Pandey *et al*., 2012b).

Unlike all other DNA based technologies, SSRs have shown high levels of polymorphism enabling accession discrimination and assessment of genetic variation in cultivated groundnut (Ferguson *et al*., 2003; Varma *et al*., 2005; Gimene *et al*., 2007; Gautami *et al*., 2009; Liang *et al*., 2009). SSR markers consist of short, repeated sequences and are highly variable, co-dominant and easily detected from relatively small amounts of DNA after PCR amplification (Edwards and McCouch, 2007). Hundreds of SSR markers for cultivated groundnut have been developed (Pandey *et al*., 2012a). In their study, He *et al.* (2005) found eight useful markers to classify cultivated groundnut into botanical varieties where six markers were specific to botanical varieties *Arachis fastigiata* Waldron and *A. vulgaris*, C. Harz., one to *A. hypogaea* and *hirsute* Köhler, and one to *A. Peruviana* Krapov & W.C. Gregory and *A. Aequatoriana* Krapov & W.C. Gregory. Varshney *et al*., (2009) reported the development of the first genetic linkage map for cultivated groundnut based on SSR markers and its application for identification of QTLs for drought tolerance traits. Candidate genome regions controlling disease resistance such as late leaf spot (Chenault *et al*., 2009) and rust (Khedikar *et al*., 2010; Mondal *et al*., 2012) and a marker for *Sclerotinia* blight have also been identified using SSRs (Leal-Bertioli *et al*., 2009). Recently, a set of highly informative

polymorphic markers (199 SSRs with >0.50 polymorphic information content (PIC) have also been identified amongst 946 novel SSR markers, providing hope of accelerating further molecular genetics and breeding in groundnut (Pandey *et al*., 2012b). However, the number of molecular markers available for cultivated groundnut is still limiting (Wang *et al*., 2012). As such, identification of more markers for screening of resistance to several biotic and abiotic stresses that affect groundnut will be of great benefit to plant breeders.

This study used 21 SSR markers to determine the genetic diversity of a collection of groundnut germplasm assembled for use in a breeding programme for the development of cultivars resistant to groundnut rosette disease.

3.2 Materials and Methods

3.2.1 Plant Material

A total of 106 groundnut genotypes used in this study composed of local accessions, introductions and improved cultivars released through ICRISAT and the Malawi National Agriculture Research Services (NARS) (Appendix 3.1). Sixty seven genotypes were collected from ICRISAT, while 28 came from the Department of Agricultural Research Services (DARS) gene-bank at Chitedze Research Station, Lilongwe and 5 accessions were collected from farmers in the 3 districts Kasungu, Mzimba, and Rumphi of Malawi. Other genotypes also used in this study were sourced from Tanzania and Zambia. The genotypes from ICRISAT were randomly selected from a reference set of 288 genotypes while those from the gene bank comprised of the entire collection held by DARS. Genotypes from farmers were collected through visits to the districts and direct requesting farmers to provide cultivars that are local and referred to by their local names. The germplasm therefore constituted two populations, improved cultivars and germplasm accessions comprising of 21 and 85 genotypes, respectively.

3.2.2 DNA extraction

A total of 4 seeds per genotype were planted in seedling trays clearly marked as per genotype at the BecA-ILRI hub in Nairobi, Kenya and 7 days after emergence young leaves from each plant were sampled for DNA extraction. High throughput DNA extraction was done using the CTAB-based protocol described by Mace *et al*. (2003), omitting the phenol:chloroform step. The concentration of the extracted genomic DNA was determined electrophoretically using 0.8% (w/v) agarose gel by comparing DNA bands with a known λ DNA standard. DNA concentration was quantified using a Nanodrop ND-1000

spectrophotometer (Nano-Drop Technologies Inc., Rockland, DE, USA) and accordingly, was diluted to 10ng μl^{-1} for polymerace chain reaction (PCR).

3.2.3 SSR markers and Polymerase Chain Reaction

Twenty one SSR markers were used to assess the genetic diversity amongst the 106 groundnut genotypes (Table 1). These SSR markers were selected from previous studies (Ferguson *et al*., 2004; Moretzsohn *et al*., 2005) based on their informativeness and polymorphic information content. Analysis was done following the M13-tag polymerase chain reaction (PCR) described by Schuelke (2000). All forward primers contained an M13-tag (5'-CACGACGTTGTAAAACGAC - 3') on the 5' end that was fluorescently labelled to allow detection of amplification products. PCR amplification was performed in 10 µl in 384 well microtitre plates and each reaction comprised of 1 x PCR buffer (20 mM Tris-HCl, pH 7.6; 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5% (w/v) Triton X-100; 50% (v/v) glycerol), 2 mM $MgCl_2$, 0.16 mM dNTPs, 0.16 µM fluorescent labelled M13-forward primer, 0.04 µM forward primer, 0.2 µM reverse primer, 0.2 units of *Taq* DNA polymerase (SibEnzyme Ltd, Russia) and 30ng of template DNA. PCR reactions were performed on a GeneAmp 9700 thermocycler (Applied Biosystems) with initial denaturation of 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 59°C for 1 minute and 72°C for 2 minutes, followed by final elongation at 72°C for 20 minutes. Amplification was confirmed by running 4 µl of the products on a 2% (w/v) agarose gel stained with GelRed® (Biotium, USA) and visualized under UV light. Amplification products (1.5 µl – 3.5 µl of each) were co-loaded in sets of 3 to 4 markers together with the internal size standard, GeneScan™-500 LIZ® (Applied Biosystems) and Hi-Di™ Formamide (Applied Biosystems) and separated by capillary electrophoresis using an ABI Prism® 3730 Genetic analyzer (Applied Biosystems). Allele calling was performed with Gene Mapper 4.0 (Applied Biosystems).

3.2.4 Data analysis

Summary statistics on major allele frequency, allele number, availability, gene diversity, heterozygosity and PIC values (Botstein et al., 1980) were computed using PowerMarker V3.25 (Liu and Muse, 2005). Analysis of molecular variance (AMOVA) was performed using Arlequin v.3.1 (Excoffier *et al*., 2005). DARwin 5.0 (Perrier *et al*., 2003; Perrier and Jacquemoud-Collet, 2006) was used to calculate genetic dissimilarities between all possible pairs of varieties using simple matching coefficient. The dissimilarity coefficients were used to perform principal coordinates analyses (PCoA) and to construct a neighbour-joining tree (Saitou and Nei, 1987) with a bootstrapping value of 10 000 using DARwin v.5.0.

3.3 Results

3.3.1 SSR polymorphism, allelic richness and number of alleles

A total of 21 SSR markers were used in this study to provide information on the genetic diversity among the 106 genotypes of groundnut. Out of the 21 SSR markers, 3 markers (pPGPSeq19B01, pPGPSeq15C12, and Ah1TC6E01) were not considered in the analysis after results indicated that their use was affected by both high levels of missing data and pseudo-heterozygosity. Since groundnut is mostly a self-pollinated crop, it is expected to exhibit minimum levels of heterozygosity. The remaining 18 SSR markers comprised of 9 di-nucleotide repeat, 6 tri-nucleotide repeats and 3 compound microsatellites (Table 3.1). One marker, pPGPSeq1B09, seemed to amplify two different loci and was interpreted as having amplifying duplicate loci. Hence, allelic data were obtained for a total of 19 SSR loci amplified by the 18 SSR primer pairs. A total number of 316 alleles were observed. The number of alleles revealed per polymorphic locus ranged from 7 (pPGPseq7H06, pPGPseq1B09a, pPGPseq1B09b and Ah1TC6G09) to 29 (pPGPseq2D12B) with a mean of 17 alleles per locus. The amplicon sizes ranged from 127 to 362 base pairs across all loci and genotypes (Table 3.1).

Table 3.1: Estimates of genetic diversity of 106 germplasm collection screened using 19 SSR loci

	Marker	Repeat Unit	Allele size range (bp)	Major allele frequency	Genotype number	Allele number	Gene diversity[A]	Heterozygosity	PIC[B]
1	pPGPseq2D12B	$(TAA)_{16}$	237-330	0.18	46	29	0.9	0.06	0.9
2	pPGPseq7H06	$(CTT)_{12}$	300-321	0.50	9	7	0.68	0.01	0.64
3	pPGPseq1B09a	$(GA)_{19}$	284-298	0.46	7	7	0.68	0.00	0.63
4	pPGPseq1B09b		300-312	0.51	8	7	0.67	0.00	0.63
5	pPGPseq13E09	$(TAA)_{16}$	285-327	0.25	17	13	0.82	0.03	0.79
6	pPGPseq18C5	$(TAA)_{23}$	286-313	0.20	13	13	0.89	0.00	0.88
7	pPGPseq17E03	$(CTT)_{15}$	184-216	0.38	13	13	0.79	0.20	0.76
8	pPGPseq8E12	$(TTG)_6(TAA)_{15}$	201-228	0.47	22	17	0.72	0.09	0.70
9	Ah1TC1E01	$(GA)_{29}$	204-282	0.29	42	28	0.85	0.12	0.84
10	Ah1TC11A04	$(CT)_{16}(CT)_{33}$	170-218	0.44	25	20	0.74	0.13	0.71
11	Ah1TC4F12	$(CT)_{23}$	160-260	0.15	24	24	0.92	0.00	0.92
12	Ah1TC6H03	$(AG)_{21}$	227-254	0.17	18	14	0.87	0.13	0.86
13	Ah1TC302	$(CT)_{26}(CA)_7(CA)_5$	254-310	0.35	15	15	0.78	0.15	0.76
14	Ah1TC6G09	$(CT)_{18}$	127-157	0.54	6	7	0.51	0.00	0.40
15	Ah1TC7H11	$(AG)_{18}$	324-362	0.20	25	20	0.90	0.06	0.89
16	Ah1TC9F10	$(AG)_{31}$	257-300	0.16	35	23	0.91	0.14	0.90
17	Ah1TC11H06	$(AG)_{34}$	192-248	0.23	30	27	0.89	0.01	0.88
18	pPGPseq5D05	$(GA)_{32}$	261-295	0.22	15	15	0.87	0.00	0.85
19	Ah1TC1A02	$(TC)_{35}$	227-261	0.36	19	17	0.80	0.03	0.78
	Mean			**0.32**	**20**	**17**	**0.8**	**0.06**	**0.77**

[A]Gene diversity as explained by Weir and Hill (2002) , [B]Polymorphic information content as per Botstein et al. (1980)

The major allele frequency ranged from 0.15 (Ah1TC4F12) to 0.54 (Ah1TC6G09) with a mean of 0.32. The number of alleles analyzed as per population showed that out of the 19 polymorphic markers, 17 were polymorphic among the improved cultivars and 9 among the germplasm accessions. The mean number of alleles for the improved cultivars was 10.5 with a range of 5 to 17 whereas for the germplasm accessions, the mean was 11.8 with a range of 3 to 26 alleles per locus.

3.3.2 Gene diversity

Gene diversity shows the probability that two randomly chosen alleles from the population are different. In this study, it was noted that a marker detecting the least number of alleles also showed the lowest genetic diversity (Table 3.1). The gene diversity scores of the 19 polymorphic SSR loci ranged from 0.51 (Ah1TC6G09) to 0.92 (Ah1TC4F12) with a mean of 0.80. The mean gene diversities per population, over loci for the improved cultivars and the germplasm accessions were 0.61 and 0.67, respectively. Observed heterozygosity across all loci was very low ranging from 0 to 0.20 with a mean of 0.06. Six SSR markers were homozygous while seven showed heterozygosity ≤10% ranging from 1% to 9% and the other six markers showed levels of heterozygosity of ≤20% ranging from 12% to 20%. There was a large difference between the levels of gene diversity (0.80) and heterozygosity (0.06), indicating non-random mating structure according to Nei's unbiased estimate of gene diversity (Saitou and Nei, 1987). Among the improved cultivars and germplasm accessions, observed heterozygosity was also low at 0.13 and 0.03, respectively as compared to the mean expected heterozygoisty of 0.70 and 0.73, respectively. A total of 199 and 224 alleles associated with improved cultivars and germplasm accessions respectively were revealed by both the population-wise and locus-wise F-statistics (frequencies).

3.3.3 Polymorphic Information content

Generally, the SSRs used were highly polymorphic with PIC values between 0.40 (Ah1TC6G09) and 0.92 (Ah1TC4F12) with a mean of 0.77 (Table 3.1). Only one locus, Ah1TC6G09, showed a PIC value of <0.50.

3.3.4 AMOVA to partition the genetic variation

Analysis of molecular variance (AMOVA) partitioned the total genetic variation among and within the two populations namely the improved cultivars and germplasm accessions (Table 3.2). This revealed that the highest proportion of the total variation (72.9%) was among

individuals within the populations. The proportions of variation among and within the populations were lower at 17.4% and 9.69%, respectively.

A locus-by-locus AMOVA was also performed in order to obtain an estimate of how each locus contributes to the differentiation among and within the two population groups. The allelic variation revealed that each of the 19 SSR loci contributed significantly to the variation among individuals within the populations ranging from 54.3% to 94.25%. However, the contribution of each SSR locus to the variation between populations and among individuals was low with means of 20.22% and 6.68% respectively (Table 3.2).

3.3.5 Gene diversity among the groundnut genotypes

Using the genotyping data for 316 alleles obtained at 19 SSR loci, a principle coordinate analysis (PCoA) was carried out based on dissimilarity indices, which clearly distinguished the diversity pattern of the genotypes. A total of 20 Eigen values explained 50.45% of the total diversity. A PCoA plot of the first and second coordinates explained 10.42% and 7.52%, respectively of the total diversity clustering the test genotypes in 2 distinct groups (Figure 3.1). However, a plot of first and third axes, which explains 14.51% of the total diversity, clustered the genotypes into 3 distinct groups (Figure 3.2). The main cluster comprised of 84.4% of the genotypes, which included germplasm from local farmers (Tchayilosi and Kalisele), all the germplasm from the Chitedze gene bank and several varieties from ICRISAT. The second cluster comprised of 8.25% of genotypes, which included CG 7, Manipintar, ICGV-SM 01711, ICGV-SM 01721, ICGV-SM 90704, Chalimbana, MGV-5, RG 1 and Chitembana. The remaining cluster comprised of 7.34% of the germplasm, which include JL 24, Illanda, Pendo, Mwenje, ICG 12991, Malimba, ICGV-SM 99555, ICGV-SM 99557 and ICGV-SM 99568.

Table 3.2: Analysis of molecular variance (AMOVA) partitioning the genetic variation of the 106 groundnut genotypes the improved and unimproved germplasm populations

	Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage variation
	Among populations	1	257.53	0.77Va	17.41
	Among individuals within populations	426	2929.91	3.23Vb	72.90
	Within populations	427	183.50	0.43Vc	9.69
Locus by locus	Among populations		523.88	1.75	20.22
	Among individuals within populations		4842.15	6.32	73.10
	Within populations		222.00	0.577	6.68

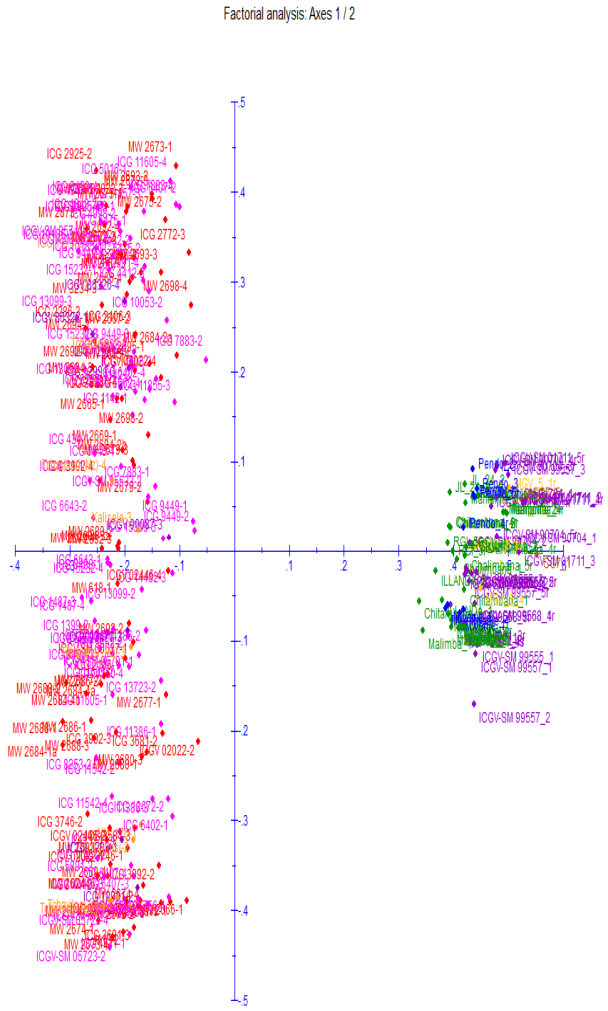

Figure 3.1: Scatter plot of axes 1 and 2 derived though PCoA based on the dissimilarity of 18 SSR markers across 19 loci for improved and unimproved germplasm.
Farmer preferred cultivars shown by yellow = MGV 5, blue = Pendo and Mwenje, green = Chalimbana, Chitembana, Malimba, Manipintar, Illanda, RG 1 and JL 24, orange = CG 7, black = Tchayilosi and Kalisele. ICRISAT and Chitedze gene bank cultivars shown by purple = Improved cultivars while red, pink and light purple = unimproved cultivars.

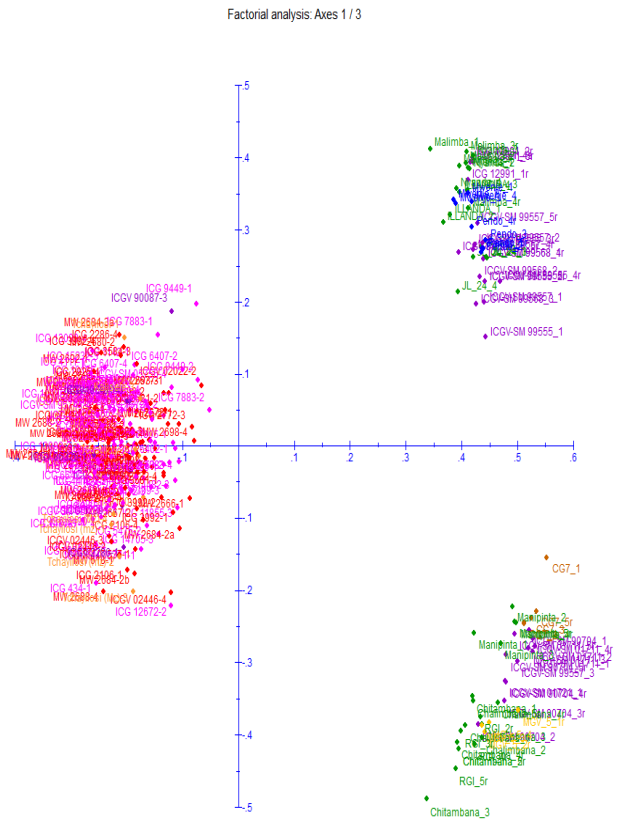

Figure 3.2: Scatter plot of axes 1 and 3 derived through PCoA based on the dissimilarity of 18 SSR markers across 19 loci for improved and unimproved germplasm.
Farmer preferred cultivars shown by yellow = MGV 5, blue = Pendo and Mwenje, green = Chalimbana, Chitembana, Malimba, Manipintar, Illanda, RG 1 and JL 24, orange = CG 7, black = Tchayilosi and Kalisele. ICRISAT and Chitedze gene bank cultivars shown by purple = Improved cultivars while red, pink and light purple = unimproved cultivars.

3.3.6 Genetic relationships among groundnut genotypes

The diversity among the test genotypes was further elucidated through an NJ tree constructed using DARwin5 (Figure 3.3) by following the Neighbor Joining (NJ) method (Saitou and Nei, 1987). The dendogram classified the germplasm into four major clusters (CL-1, CL-2, CL-3, CL-4) with the first two clusters (CL-1 and CL-2) further subdivided into sub-clusters. Clusters 1 and 2, were the largest and comprised mainly of genotypes obtained from ICRISAT and the Chitedze genebank. The local cultivars found in these clusters were Kalisele and Tchayilosi, however, Tchayilosi was found in both clusters while Kalisele was found only in CL-1. Genotypes ICG 9449, MW 2694, ICGV-SM 05701 and MW 2672, which are resistant to groundnut rosette disease (GRD) were grouped in CL-1 but each in a different sub-cluster. A clear distinction between the genotypes could further be observed in CL-3 and CL-4. Most of the groundnut varieties popular among farmers in Malawi (Chalimbana, CG 7, RG 1, Manipintar and ICGV-SM 90704) were grouped in CL-3 while

those popular in Tanzania (Illanda, Pendo, Mwenje) have been grouped in CL-4, as can be seen in the first and third axes of the PCoA plot. Interestingly, ICG 12991 and RG 1, which are also resistant to GRV disease were grouped in clusters CL-3 and CL-4, respectively, separate from the other resistant genotypes found in CL-1.

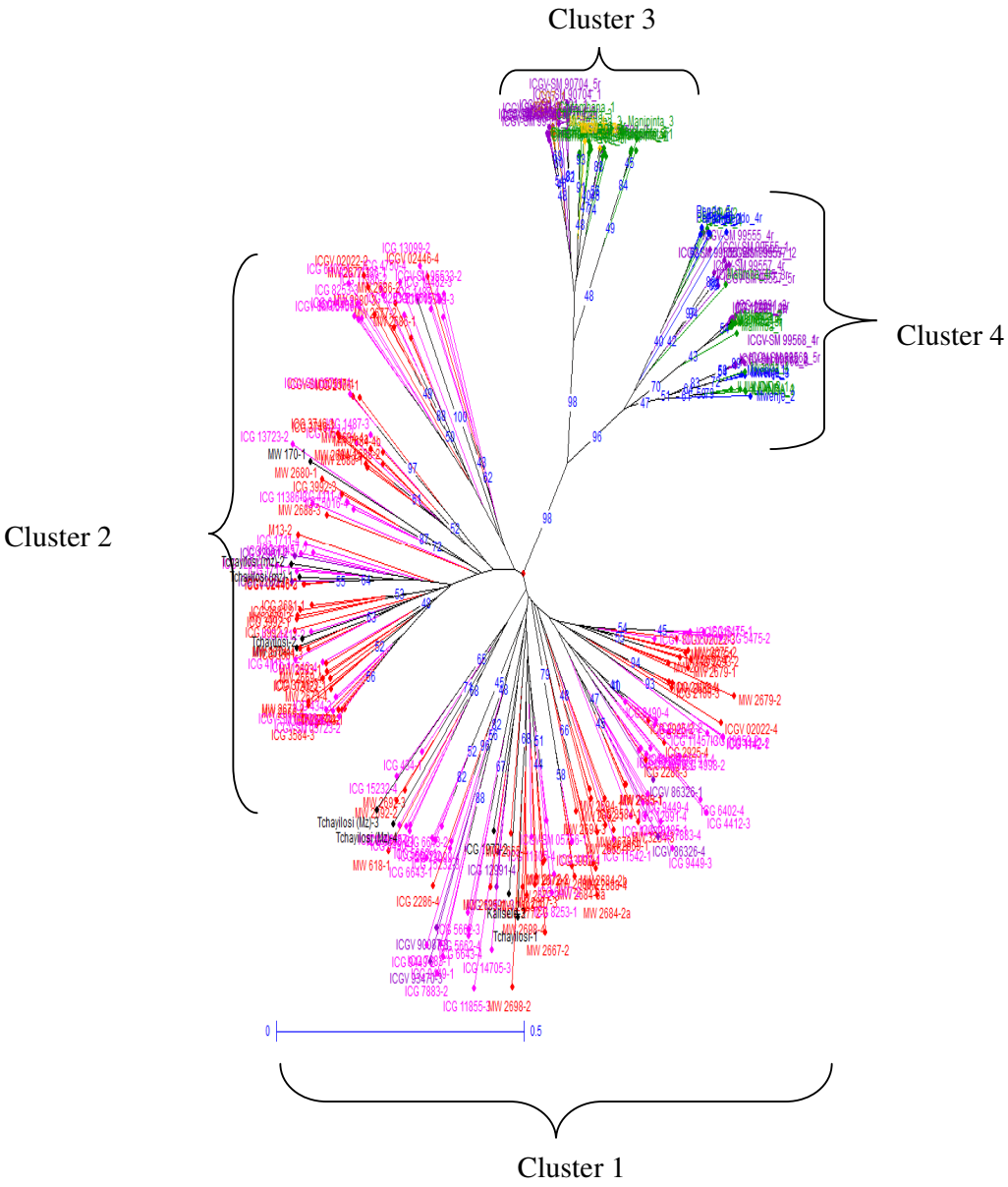

Figure 3.3: Genetic relationships among groundnut genotypes. Clusters 1 and 2: Improved and unimproved cultivars (shown in red, pink and light purple) from ICRISAT and the Chitedze genebank. Cluster 3: Farmer preferred cultivars shown in green = Manipintar, Chalimbana, RG 1, and in orange = CG 7, improved cultivars shown in purple = ICGV-SM 90704 and ICG 12991. Cluster 4: Farmer preferred cultivars shown in blue = Pendo and Mwenje, green = Malimba and Illanda. Improved cultivars shown in purple = ICGV-SM 99568, ICGV-SM 99555, and ICGV-SM 99557.

3.4 Discussion

Assessment of genetic diversity facilitates the identification of agronomically valuable and diverse germplasm for use in genetic enhancement of important traits such as disease resistance in groundnut. Furthermore, genetic characterization of genotypes can lead to utilization of wider gene pools which may result in identification of certain cross combinations with high recovery of genes for useful recombinants. There are several known sources of resistance to a number of biotic and abiotic stresses that have been identified and used to improve the groundnut crop through conventional breeding methods. However, conventional breeding methods have had limited success. As such, it is hoped that conventional breeding methods coupled with molecular techniques can help in dealing with complexity of genes governing the majority of desired traits (Pandey *et al*., 2012).

From this study, it is evident that genetic diversity exists among 106 genotypes of groundnut from Malawi, Tanzania and Zambia. In total, 19 SSR loci were analyzed using 18 SSR markers indicating amplification of one homoeolocus, possibly due to the tetraploid nature of groundnut or to primer sequence duplication within the genome (Varshney *et al*., 2009). All 19 SSR loci observed were highly polymorphic, as with other recent reports on diversity studies that have used SSR markers in groundnut (Mace *et al*., 2006; Tang *et al*., 2007). This shows that the number of SSR marker used in this stduy should be adequate to determine diversity among groundnut germplasm. Similar results have been reported (Kottapalli *et al*., 2007; Varshney *et al*., 2009). The high PIC and mean number of alleles observed per locus indicated that the markers were very informative. In this srudy, the PIC values were significantly higher than that reported in earlier studies (Mace *et al*., 2005; Gautami *et al*., 2009). Using AFLP, RAPD, DAF no polymorphic markers were detected on groundnut (Prakash and He, 1997; Herselman, 2003) and this could be attributed to a narrow genetic base of the groundnut varities grown (Dwivedi *et al*., 2003). The contradiction between earlier and recent studies shows how effective SSR markers are at detecting genetic diversity in germplasm. Cuc *et al*., (2008) indicated that the highly informative nature of newly developed markers is supported by the general theory that the degree of polymorphism of the SSR marker increases with the total length of the repeat. It is also important to note that the wide gene diversity discovered in this particular study may also have been due to the use of a large number of groundnut accessions obtained from different sources

Although, no specific trend of grouping was observed among the two primary population groups, improved and unimproved genotypes, the PCoA and neighbour joining tree broadly separated the germplasm into clusters. Of particular interest was the finding that the most

popular farmer cultivars were grouped together, while those from ICRISAT and the Chitedze genebank grouped in different clusters. This indicated that the genetic base of the germplasm in this study was broad and that the diversity came from different sources. Clusters CL-1 and CL-2 grouped together the majority of genotypes from ICRISAT and the Chitedze gene bank thereby indicating their relatedness and suggest that the source of these accessions could be the same. However, the presence of sub-clusters showed that the genotypes were also diverse since they derived from different botanical groups. This was elucidated further by AMOVA, which partitioned the total genetic variation among and within populations and this showed that the majority of genetic variation observed in the germplasm (72.9%) was due to the variation among individuals instead of being between specific population groups. Considering the F_{ST} value of 0.17, the degree of genetic differentiation between the populations was large. The genetic diversity observed in these germplasm could be important for groundnut breeding. These results can also be used to select the parental stocks for hybridization introgression of valuable genes into adapted groundnut varieties Varshney *et al*. (2009) proposed that classification of genotypes as per botanical types can be done by using a large number of SSR markers and fewer genotypes.

The relatedness observed between the germplasm from ICRISAT and the Chitedze gene bank is also worth noting, indicating that it may have been collected from similar sources or that the gene bank in Malawi holds the same genetic material as that of ICRISAT but under a different nomenclature. Two of the popular farmer varieties (Tchayilosi and Kalisele) were found dispersed among the ICRISAT and gene bank germplasm clusters, showing that they are different from the other farmer popular genotypes. Most of these are clustered together according to the country where they were adapted. For instance, varietyies Pendo, Nyanda, Mwenje and Illanda, which are found in Tanzania were grouped in one cluster, whereas Chalimbana and CG 7 as varieties found in Malawi were grouped in a separate cluster.

Genotypes with resistance to GRD such as RG 1 and ICG 12991 grouped separately within clusters containing popular farmer genotypes while the other resistant cultivars grouped in the main clusters. This possibly may indicate that the separated resistant genotypes in each of the clusters have different pedigrees or resources of resistance to GRD. Due to the complexity of the disease, the mechanism of resistance among these genotypes may indeed differ as observed in ICG 12991, in which the resistance to GRD is due to aphid resistance (Deom *et al*., 2006) compared to RG 1 which has resistance to the viral complex itself (van der Merwe *et al*., 2001). This dispersion of resistant genotypes in several clusters provides an opportunity for utilizing the diverse genotypes for developing mapping populations for

complex traits governing resistance of diseases like GRD and pyramiding different resistance genes.

As observed, molecular markers are useful tools in defining the genetic variation existing in populations and hence they can be useful in breeding programmes. For groundnut, which is a self-pollinated plant, selection of parents for crosses from related materials can indeed lead to narrowing of the genetic base. However, the information obtained in this study, shows that selection of parents from different clusters for crosses can lead to an increase in genetic variability and should lead to the future success of novel breeding programmes. In an ongoing research, the same genotypes were screened and parents were selected for GRD resistance and crossed with adapted varieties in Malawi (unpublished data). Although selection was based on phenotypic characteristics, the results of this study confirmed that the genetic variability that was created by crossing the germplasm from different clusters creates germplasm with a novel array of traits that may provide a basis for selection of new cultivars.

Conclusion

Plant breeding relies upon the genetic variability among the available germplasm. The results of this study highlight a reliable and efficient way of using molecular markers to identify gene diversity among genotypes. This provides key information needed for the choice of parents in a breeding programme aiming to exploit the gene diversity to a maximum looking for novel gene combinations as well as the use of established positive genes.

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Appendix 3.1: Groundnut genotypes employed in diversity study

S. No.	Cultivar name	Population	Property*	Source
1	ICGV 02446	Improved	Susceptible	ICRISAT
2	ICG 4343	Unimproved	Susceptible	ICRISAT
3	ICG 15232	Unimproved	Susceptible	ICRISAT
4	ICG 5016	Unimproved	Susceptible	ICRISAT
5	ICG 3746	Unimproved	Susceptible	ICRISAT
6	ICG 9449	Unimproved	Resistant	ICRISAT
7	ICG 13723	Unimproved	Susceptible	ICRISAT
8	ICGV-SM 05756	Improved	Susceptible	ICRISAT
9	ICG 11605	Unimproved	Susceptible	ICRISAT
10	ICG 434	Unimproved	Susceptible	ICRISAT
11	ICGV 90087	Improved	Susceptible	ICRISAT
12	ICG 11457	Unimproved	Susceptible	ICRISAT
13	ICG 5475	Unimproved	Susceptible	ICRISAT
14	ICG 4598	Unimproved	Susceptible	ICRISAT
15	ICG 1142	Unimproved	Susceptible	ICRISAT
16	ICG 6643	Unimproved	Susceptible	ICRISAT
17	ICG 4998	Unimproved	Susceptible	ICRISAT
18	ICG 12672	Unimproved	Susceptible	ICRISAT
19	ICG 2925	Unimproved	Susceptible	ICRISAT
20	ICG 14482	Unimproved	Susceptible	ICRISAT
21	M-13	Unimproved	Susceptible	ICRISAT
22	ICG 6402	Unimproved	Susceptible	ICRISAT
23	ICG 10185	Unimproved	Susceptible	ICRISAT
24	ICG 5662	Unimproved	Susceptible	ICRISAT
25	ICG 11386	Unimproved	Susceptible	ICRISAT
26	ICG 1487	Unimproved	Susceptible	ICRISAT
27	ICGV-SM 05723	Unimproved	Susceptible	ICRISAT
28	ICGV 02022	Improved	Susceptible	ICRISAT
29	ICG 14705	Unimproved	Resistant	ICRISAT
30	ICG 3584	Unimproved	Susceptible	ICRISAT
31	ICG 1399	Unimproved	Susceptible	ICRISAT
32	ICG 4111	Unimproved	Susceptible	ICRISAT
33	ICG 2772	Unimproved	Susceptible	ICRISAT
34	ICG 1711	Unimproved	Susceptible	ICRISAT
35	ICGV 02286	Improved	Susceptible	ICRISAT
36	ICGV-SM 05701	Unimproved	Resistant	ICRISAT
37	ICG 4412	Unimproved	Susceptible	ICRISAT
38	ICG 1415	Unimproved	Susceptible	ICRISAT
39	ICG 7883	Unimproved	Susceptible	ICRISAT

Appendix 3.1 (continued)

S. No.	Cultivar name	Population	Property*	Source
40	ICG 1274	Unimproved	Susceptible	ICRISAT
41	ICG 11855	Unimproved	Susceptible	ICRISAT
42	ICGV-SM 00537	Unimproved	Susceptible	ICRISAT
43	ICGV 93470	Improved	Susceptible	ICRISAT
44	ICGV-SM 95533	Improved	Susceptible	ICRISAT
45	ICG 8490	Unimproved	Susceptible	ICRISAT
46	ICG 15309	Unimproved	Susceptible	ICRISAT
47	ICGV 86326	Improved	Susceptible	ICRISAT
48	ICG 8253	Unimproved	Susceptible	ICRISAT
49	ICG 1973	Unimproved	Susceptible	ICRISAT
50	ICG 6407	Unimproved	Susceptible	ICRISAT
51	ICG 5891	Unimproved	Susceptible	ICRISAT
52	ICG 3681	Unimproved	Susceptible	ICRISAT
53	ICG 12921	Unimproved	Susceptible	ICRISAT
54	ICG 10053	Unimproved	Susceptible	ICRISAT
55	ICGV-SM 95741	Unimproved	Susceptible	ICRISAT
56	ICG 2286	Unimproved	Susceptible	ICRISAT
57	ICG 4750	Unimproved	Susceptible	ICRISAT
58	ICG 11542	Unimproved	Susceptible	ICRISAT
59	ICG 928	Unimproved	Susceptible	ICRISAT
60	ICG 13099	Unimproved	Susceptible	ICRISAT
61	ICG 12988	Unimproved	Susceptible	ICRISAT
62	ICGV 92234	Improved	Susceptible	ICRISAT
63	ICG 2106	Unimproved	Susceptible	ICRISAT
64	ICG 3992	Unimproved	Susceptible	ICRISAT
65	ICG 12991	Unimproved	Aphid resistant –Check	ICRISAT/Farmers
66	ICGV-SM 90704	Improved	Rosette Resistant- Check	ICRISAT/Farmers
67	JL 24	Improved	Susceptible check	ICRISAT/farmers
68	CG7	Improved	Susceptible	Farmers – Malawi
69	CHALIMBANA	Improved	Susceptible	Farmers – Malawi
70	MW 2698	Unimproved	Susceptible	DARS Gene Bank
71	MW 2684	Unimproved	Susceptible	DARS Gene Bank
72	MW 2665	Unimproved	Susceptible	DARS Gene Bank
73	MW 2673	Unimproved	Susceptible	DARS Gene Bank
74	MW 2680	Unimproved	Susceptible	DARS Gene Bank
75	MW 2693	Unimproved	Susceptible	DARS Gene Bank
76	MW/ 133	Unimproved	Susceptible	DARS Gene Bank
77	MW 2873	Unimproved	Susceptible	DARS Gene Bank
78	MW 2668	Unimproved	Susceptible	DARS Gene Bank
79	MW 2695	Unimproved	Susceptible	DARS Gene Bank
80	MW 2692	Unimproved	Susceptible	DARS Gene Bank

Appendix 3.1 (continued)

S. No.	Cultivar name	Population	Property*	Source
81	MW 2675	Unimproved	Susceptible	DARS Gene Bank
82	MW 618	Unimproved	Susceptible	DARS Gene Bank
83	MW 170	Unimproved	Susceptible	DARS Gene Bank
84	MW 146	Unimproved	Susceptible	DARS Gene Bank
85	MW 2667	Unimproved	Susceptible	DARS Gene Bank
86	MW 2679	Unimproved	Susceptible	DARS Gene Bank
87	RG 1	Improved	Resistant	DARS Gene Bank
88	MW 2685	Unimproved	Susceptible	DARS Gene Bank
89	MW 2666	Unimproved	Susceptible	DARS Gene Bank
90	MW 2677	Unimproved	Susceptible	DARS Gene Bank
91	MW 2688	Unimproved	Susceptible	DARS Gene Bank
92	MW 2684	Unimproved	Susceptible	DARS Gene Bank
93	MW 3234	Unimproved	Susceptible	DARS Gene Bank
94	MW 2669	Unimproved	Susceptible	DARS Gene Bank
95	MW 2672	Unimproved	Resistant	DARS Gene Bank
96	MW 2674	Unimproved	Susceptible	DARS Gene Bank
97	MW 2686	Unimproved	Susceptible	DARS Gene Bank
98	MGV 5	Improved	Susceptible	Zambia
99	Kalisele	Unimproved	Susceptible	Farmer –Malawi
100	Tchayilosi	Unimproved	Susceptible	Farmer – Malawi
101	Pendo	Improved	-	Tanzania
102	Illanda	Improved	-	Tanzania
103	Mwenye	Improved	-	Tanzania
104	Nyanda	Improved	-	Tanzania
105	Chitembana	Improved	-	Farmers – Malawi
106	Manipintar	Improved	-	Farmers – Malawi

***** Groundnut rosette disease results based on an experiment done at Chitedze Research Station, Malawi over 2 years from 2009/10 – 2010/11 growing seasons (Unpublished).

CHAPTER 4

Germplasm evaluation for selected agronomic traits and resistance to groundnut rosette disease and the aphid vector

Abstract

Evaluation of a germplasm collection for particular traits to find suitable parent material is important in a breeding programme. A total of 100 groundnut genotypes were evaluated to identify more sources of resistance to groundnut rosette disease (GRD) and its aphid vector at the Chitedze Research Station in Malawi over two seasons (summer 2009/10 and 2010/11). The study was done under high and low inoculum levels. Data collected included plant height, number of primary branches, number of pods per plant, pod length, number of seeds per pod, 100 seed weight, shelling percentage, pod yield, kernel yield and GRD scores. Disease incidence levels among genotypes averaged over two seasons were extremely high under high disease pressure (HDP) than under low disease pressure (LDP) environment. Five genotypes ICG 9449, ICG 14705, ICGV-SM 05701, MW 2672 and MW 2694 were highly resistant to GRD (0% disease incidence). Adapted genotypes were moderately resistant to susceptible to GRD. Aphid resistance was only in ICG 12991. Yield and GRD were significantly (r = -0.3, P<0.001; r = -0.5, P<0.001), negatively and moderately correlated in both low and high disease pressure environments, respectively. Because the yield of resistant varieties are high in both environments, GRD has the potential to reduce yield in groundnuts. In addition, the highly resistant genotypes yielded highly in both seasons except for genotype ICG 9449. Farmer preferred genotypes CG 7, Chalimbana and Tchayilosi gave above average yields despite high disease incidence levels, which showed that these genotypes have tolerance to GRD. The genotypes with high resistance or tolerance to GRD identified in this study can be recommended for release to farmers or for use in breeding for GRD resistant cultivars.

4.1 Introduction

Groundnut rosette disease (GRD) is one of the major diseases affecting groundnut in Malawi. The disease, endemic to the African continent, is caused by a complex of groundnut rosette virus (GRV), groundnut rosette assistor virus (GRAV) and satellite RNA (satRNA) (Taliansky *et al*. 2000). GRD usually appears in farmers fields in Malawi at low levels every growing season, however, the severity of the disease increases with late planting (Waliyar *et al*. 2007).

The GRD occurs in two predominant symptomatic forms, the “chlorotic” form and the “green” rosette form which are largely due to sat-RNA occurring in different forms (Murant and Kumar, 1990). The effects of the either forms of GRD on young plants include severe stunting due to shortened internodes and reduced leaf size leading to a bushy appearance (Naidu *et al*. 1999b). Yield loss due to GRD depends on the growth stage at which infection occurs whereby in seedlings infection leads to 100% yield loss while infection at the pod filling stage causes negligible effects (Naidu *et al*., 1999b; Waliyar *et al*., 2007).

The components of GRD are transmitted by a single aphid species, *Aphis craccivora* Koch. The virus particles are transmitted in a persistent manner, but do not multiply inside the insect host which is labelled as circulative transmission (Ntare *et al*., 2001). Naidu *et al*. (1999a) showed that the aphid does not always transmit all the three GRD agents together. Taliansky *et al*. (2000) explained that GRV and satRNA must be packaged within the GRAV coat protein to be aphid transmissible. As such, the success of transmitting all the three agents together is high when inoculation feeding period is longer or when the number of aphids per plant is high (Naidu *et al*., 1999a; Waliyar *et al*., 2007). The aphid acquires GRD particles from phloem sap after an acquisition feeding period of 4 h and 8 h followed by a latent period of 26 h 40 min and 38 h 40 min for chlorotic and green rosette, respectively (Misari *et al*., 1988). They also found that the aphids transmitted the virus particles for up to 2 weeks and beyond with transmission rates of 26-31%, when there were 1-2 aphids per plant, and 49% when there were five aphids per plant.

Several methods have been used to control GRD. Planting early in the season when the aphid population is low combined with a close plant spacing results in greatly reduced incidence of GRD (Naidu *et al*., 1999b; Taliansky *et al*., 2000; Waliyar *et al*., 2007). However, most smallholder farmers do not follow these recommendations. Chemical pesticides targeting aphids have been employed for GRD control. The timing, dosage, and type of insecticidal applications are critical for effectively diminishing the aphid vector

population especially where spray timing is based on an early forecast of vector migration into the crop (Naidu *et al*., 1999b). However, pesticide application and forecasting are too complicated and expensive for small scale farmers in Africa.

Planting resistant cultivars is the most effective and sustainable way of combating GRD. Several cultivars resistant to GRD and its aphid vector have been developed and released to farmers (Subrahmanyam *et al*., 1998; Ntare *et al*., 2001). In Malawi, several GRD resistant cultivars that have been released include ICGV-SM 90704, ICGV-SM 99568 and ICG 12991 (van der Merwe *et al*., 2001; Minde *et al*., 2008). However, these cultivars have not been adopted by small scale farmers probably because they lack key traits, hence GRD susceptible varieties are still commonly grown. Consequently, there is a great need for breeders to develop groundnut cultivars that combine resistance to GRD with good agronomic traits and grain quality.

Identification of good sources of resistance to GRD is crucial for groundnut breeding programmes. Subrahmanyam *et al*. (1998) reported that 116 groundnut lines with high levels of resistance to GRD were found among the global groundnut germplasm tested by ICRISAT. Aphid resistance has also been detected in a few cultivars, for example,ICG 12991, ICG 12988, and EC 36892 (Zeyong *et al*., 1995; Botternberg and Subrahmanyam, 1997; Minja *et al*., 1999). However, the need for resistant cultivars that meet farmers' demands necessitates the search for more sources of resistance. Therefore, this study was conducted to (i) identify GRD and aphid vector resistant materials among germplasm collected from various sources and (ii) to evaluate the groundnut germplasm for yield and yield related traits at the same time.

4.2 Materials and methods

4.2.1 Plant material

A total of one hundred groundnut genotypes were used in this study. The genotypes were sourced from ICRISAT Malawi (69), the Department of Agricultural Research Services (DARS) (27), and from farmers' fields (4) in the Kasungu, Lilongwe and Rumphi districts of Malawi. The genotypes from ICRISAT were randomly selected from a core collection of 288 genotypes while that from the DARS included all genotypes held at the gene bank. The genotypes sourced from farmers' collection were only used if they were named differently from the genotypes sourced from the ICRISAT and the DARS gene-banks. Three check genotypes (ICGV-SM 90704, ICG 12991 and JL 24) were included in the experiment.

4.2.2 Experimental site

The trials were conducted over 2 seasons, in 2009/2010 and 2010/11 at the Chitedze Research Station (33°38'E and 13°85'S), located 16 km west of Lilongwe, in the central region of Malawi along the Lilongwe-Mchinji road. The site lies at an altitude of 1146 m above sea level with a moderate temperature range (16 – 24°C). Malawi has a unimodal rainfall pattern which normally runs from October/November to April/May across the country and Chitedze receives a mean of 892 mm of rain annually.

4.2.3 Experimental Design

4.2.3.1 High disease pressure

The trials were laid out in a 10 x 10 alpha lattice design with 2 replications. Each plot consisted of 2 rows, 3 m long and spaced at 0.75 m apart. Plants were sown by hand at 15 cm interval within rows at a rate of 1 seed per planting station. A high disease pressure environment was created using the infector row technique (Nigam and Bock, 1990) based on the susceptible genotype JL 24. Prior to planting of the trials JL 24 plants were raised in the glasshouse and infected with GRD. The GRD infected plants were transplanted into infector rows at 7 to 14 DAS. At weekly intervals up to 80 DAS, viruliferous aphids which had been reared in a glasshouse on rosetted plants were placed onto the infector rows and the test genotypes using a camel's hair brush. Non-viruliferous aphids were also collected from surrounding fields and later made to acquire the rosette virus complex. This was done by placing a rosetted leaf in a petri dish onto which non-virulifrerous aphids were placed and left for 30 minutes to feed on the leaf and acquire the viruses before placing them onto the test genotypes. No fertilizers, herbicides, or pesticides were applied in the trials. This was to simulate conditions under which groundnut is grown by farmers. Weeding and banking up of soil were also done as required. Harvesting was done after 150 DAS.

4.2.3.2 Low disease pressure environment

The trials were laid out in a similar arrangement to the high disease pressure environment (Section 4.2.3.1) except that the infector rows were planted with ICG 12991 which is resistant to the aphids in order to reduce GRD incidence. The trials were also not hand infested with viruliferous aphids.

4.2.3.3 Evaluation for aphid resistance

Aphid resistance trials were conducted both in the field and glasshouse at ICRISAT-Chitedze after the GRD resistance trials using a total of eleven genotypes of which five were GRD resistant, five were commercial varieties but susceptible to GRD in Malawi and one genotype that was known to be aphid resistant (ICG 12991). The experiment in the field was laid out in a Randomised Complete Block Design (RCBD) with 10 replications. The 11 genotypes were evaluated under a choice test where aphids placed on each plant were free to move and colonise genotypes they preferred. Each genotype was planted on a 2.0 x 0.15 m plot in a block spaced at 0.75 m apart. In the glasshouse, plants were planted in 250 mm wide pots spaced at 300 mm apart. The plants were covered with perforated plastic bags to stop the aphids from escaping after they had been placed on each plant. A total of 10 plants per genotype (100 plants in total) were arranged in a completely randomised design. In both the field and glasshouse experiments, 2 adult aphids collected from an aphid culture were placed on each plant at 14 DAS using a wetted brush. In both the glasshouse and field trials, each plant was then checked for the presence of aphids one day after infestation. When no live aphids could be found, fresh aphids were placed onto the plants.

4.2.4 Data collection

Disease incidence was scored at 80 and 100 DAS in all the environments as recommended by Waliyar *et al*. (2007). GRD incidence (%) (PDI) was determined by counting the number of infected plants in a plot and dividing them by total number of plants in the plot and multiplying by 100 (Table 4.1). The mean PDI over the two counts was then taken to reflect GRD resistance. To determine aphid vector resistance, aphid counts on each plant in both the field and glasshouse were taken at 7 and 14 days after infestation.

Table 4.1: An evaluation scale of percent disease incidence (PDI) for GRD in groundnut

PDI	Inference
Less than 10%	Highly resistant
11 - 30%	Resistant
31-50%	Moderately resistant
More than 50%	Susceptible

From the field experiments, selected agronomic traits were evaluated on 5 randomly selected plants of each plot and averaged. The data collected included; plant height (length (mm) of the main stem measured from the base to the tip of the plant, number of branches

(primary branches on the main stem), number of pods (total number of pods per plant) and number of kernels (average number of kernels contained in 10 pods per plot). Data on yield and related traits were recorded on per plot basis as follows; pods per plot which were harvested and sun dried to approximately 8-10% moisture content and then weighed to determine pod yield. Thereafter, the pods were shelled to determine kernel yield). Later, 100 seed mass weight which is the weight of 100 kernels from pods randomly drawn from each plot was weighed and shelling percentage was determined which is a 100g pod sample from each plot which was shelled and weighed. The formula for shelling % = (seed weight ÷ pod weight before shelling) x 100.

4.2.5 Data analysis

Disease incidence data were transformed by arcsine before analysis in order to stabilize the error variance (Gomez and Gomez, 1984). Data on GRD, yield and yield components were analysed in REML using Genstat 12th Edition statistical package (Payne *et al*., 2009). Genotypes were considered fixed effects, while year, genotype x year interaction, replication and blocks were fitted as random effects. The model for REML analysis was as follows;

$$Y_{ijkl} = \mu + G_i + S_j + GS_{ij} + R_k + B_l + \varepsilon_{ijkl}$$

Where: μ is the general mean, G are the genotype effects, S are the year effects, GS are the interaction effects of genotype and year, R are replication effects, B are the block effects and ε is the random term.

Correlations were done using Pearson's correlation procedure to determine the relationship between GRD incidence and agronomic performance. Analysis of variance was performed on aphid data counts following the standard procedure for analysing RCBD.

4.3. Results

4.3.1 Germplasm reaction to GRD under high disease pressure

Infector plants raised in the glasshouse developed up to 100% GRD in both seasons, 2009/10 and 2010/11. GRD symptoms on susceptible genotypes were observed starting from 7 days after infestation. In resistant to moderately resistant genotypes, disease development was slow and occurred much later after infestation. Disease development progressed symptomatically from leaf chlorosis, stunting and bushy appearance due to shortened internodes. Analyses for various traits over the two seasons are presented in

Table 4.2. There were highly significant (P≤0.001) differences between genotypes for all traits. Highly significant (P≤0.001) differences due to seasons were also observed for all traits except for plant height and number of primary branches.

Mean values of PDI, yield and other traits combined over the two seasons is presented in Table 4.3 and Appendix 4.1. PDI values ranged from 0% to 82.7% with a mean of 50%. Five genotypes were highly resistant, 6 resistant, 26 moderately resistant and 63 susceptible. The highly resistant genotypes included ICG 14705, ICG 9449, ICGV-SM 05701, MW 2694 and MW 2672. They showed no symptoms of GRD up to the end both the seasons. Farmer preferred genotypes namely Chalimbana, CG 7, and Tchayilosi were all moderately resistant having PDIs of 46.5%, 48.8% and 49.8% respectively while Kalisele was susceptible with a PDI of 64%. The resistant checks (ICGV-SM 90704 and ICG 12991) and the susceptible check (JL 24) were moderately resistant with PDIs of 32.7%, 35.8% and 46.2% respectively. Most of the susceptible materials dried before the end of the season especially in 2010/11 season due to the severity of the disease infection.

The mean values for pod yield over the seasons was 289.1 kg/ha with genotypes varying from 57.4 kg ha^{-1} (ICG 1142) to 969.4 kg ha^{-1} (ICG 14705). The susceptible check JL 24 had yields up to 33% and 73% higher than the resistant checks, ICGV-SM 90704 and ICG 12991, respectively. Three of the highly resistant genotypes, (ICG14705, ICGV-SM 05701 and MW 2672) were the top yielding genotypes producing 969.4 kg ha^{-1}, 736.1 kg ha^{-1} and 727.8 kg ha^{-1} respectively while ICG 9449 produced (166.7 kg ha^{-1}) 42% lower than the mean. Among the farmer preferred genotypes, Chalimbana produced (417.4 kg ha^{-1}) 44.3% higher than the overall mean compared to Kalisele which produced (174.1 kg ha^{-1}) 39.7% lower than the overall mean. The other traits with variations among genotypes included plant height (from 100 to 378 mm), number of primary branches (from 4 to 10 branches), number of pods per plant (from 2 to 18.8 pods), pod length (from 17.0 to 37.0 mm), number of kernels per pod (2 to 3 kernels), 100 seed mass weight (9.1 to 50.6g), shelling % (7.7 to 76.7%), and kernel yield (from 5.91 to 682.8 kg ha^{-1}).

Correlations among traits recorded for genotypes grown under high disease pressure are presented in Table 4.4. PDI was negatively correlated with pod yield ($r = -0.4$, $P<0.001$), kernel yield ($r = -0.3$, $P<0.001$), and number of pods per plant ($r = -0.4$, $P<0.001$). The correlation between PDI and number of seeds per pod was positive and significant ($r = 0.20$, $P<0.001$). However, no significant correlation between PDI and plant height was observed.

Table 4.2: Wald statistic for percent disease incidence (PDI), yield and other traits of 100 groundnut genotypes evaluated for 2 seasons under high disease pressure

		Wald statistic									
Source	df	PDI	Height (mm)	Number of primary branches	Number of pods per plant	Pod length (mm)	Number of seeds per pod	100 seed mass weight (g)	Shelling %	Yield (kg ha^{-1})	Kernel yield (kg ha^{-1})
Replication	1										
Genotype	99	402.8***	400.5***	527.3***	529.3***	527.4***	1009.7***	344.1***	395.9***	344.3***	289.5***
Year	1	930.1***	0	0	380.4***	67.5***	42.5***	11.3***	36.2***	10.0	3.1
Genotype x Year	99	163.2***	0.7	2.2	252.6***	205.9***	315.6***	107.8	117.1***	109.3	90.5
Error	198										

Note: *** Significant at P<0.001, df = degrees of freedom

Table 4.3: Genotypic means for percent disease incidence (PDI), yield and other traits over 2 seasons under high disease pressure

	Traits										
Genotype	PDI %	Inference	Height (mm)	Number of primary branches	Number of pods per plant	Pod length (mm)	Number of seeds per pod	100 seed mass weight (g)	Shelling %	Pod yield (kg ha^{-1})	Kernel yield (kg ha^{-1})
ICG 14705	1.7	HR	254	7.9	12.8	24.4	2.0	41.1	68.4	969.4	682.8
ICG 9449	0.0	HR	195	5.3	8.9	22.7	2.0	21.9	48.7	166.7	98.6
ICGV-SM 05701	0.0	HR	170	7.6	16.5	26.7	2.0	39.7	59.6	736.1	462.9
MW 2672	0.0	HR	178	5.8	11.6	27.0	2.0	40.1	55.9	727.8	414.1
MW 2694	0.0	HR	157	7.7	15.8	30.2	2.1	43.4	55.7	549.3	313.2
MW 2684	22.7	R	346	4.7	12.0	27.0	2.0	35.4	68.1	586.8	413.5
MW 618	24.2	R	238	5.0	13.2	23.1	2.0	27.5	68.4	500.7	343.4
CG7	48.8	MR	201	8.1	9.2	30.8	2.0	43.3	58.7	342.4	204.1
CHALIMBANA	46.5	MR	228	7.7	7.2	30.8	2.1	43.4	57.0	417.4	242.7
Tchayilosi	49.3	MR	196	5.8	8.8	28.3	2.2	31.9	56.8	409.0	248.6
Kalisele	64.7	S	162	7.1	6.3	23.4	2.0	32.9	33.0	174.1	56.0
MGV5	57.6	S	182	6.7	8.7	25.2	2.0	40.2	42.0	181.9	106.8
Checks											
Resistant											
ICG 12991	35.8	MR	300	5.9	11.6	22.0	2.0	26.1	67.2	348.6	236.4
ICGV-SM 90704	32.7	MR	209	6.7	13.9	27.2	2.0	32.9	49.9	409.7	246.9
Susceptible											
JL 24	46.2	MR	239	4.6	13.6	28.9	2.1	35.2	65.0	611.1	410.7
Mean	50.0		196	6.4	8.5	26.2	2.1	30.2	48.4	289.1	181.6
s.e.d. avg	11.5		41	1.0	2.1	2.5	0.1	5.4	9.0	138.2	102.5
s.e.d max.	11.8		50	1.2	2.8	3.2	0.2	6.5	10.8	176.9	122.0
s.e.d min	11.1		41	1.0	2.0	2.3	0.1	4.7	8.2	130.8	96.0

Note: HR = highly resistant, R= resistant, MR = moderately resistant and S = susceptible.

Genotypes presented in this table comprise of all those found to be highly resistant to GRD, all the farmer preferred varieties, the checks and other 3 randomly selected genotypes which were either resistant or susceptible.

Table 4.4: Correlations for percent disease incidence (PDI), yield and other traits among 100 groundnut genotypes evaluated over 2 seasons under high disease pressure

	100 seed mass	PDI	Height	Kernel yield	Number of pods per plant	Number of primary branches	Number of seeds per pod	Pod length	Pod yield	Shelling %
100 seed mass	-									
PDI	-0.08	-								
Height	0.06	0.03	-							
Kernel yield	0.44***	-0.33***	0.41***	-						
Number of pods per plant	0.14	-0.45***	0.11	0.36***	-					
Number of primary branches	0.19	-0.05	-0.28***	-0.16**	-0.01	-				
Number of seeds per pod	-0.11	0.21***	0.06	-0.04	-0.17**	-0.13	-			
Pod length	0.15	-0.04	-0.26***	-0.19**	-0.23***	0.17	0.23***	-		
Pod yield	0.47***	-0.40***	0.37***	0.98***	0.38***	-0.13	-0.04	-0.12	-	
Shelling %	0.52***	-0.09	0.34***	0.72***	0.25***	-0.19***	-0.00	-0.28***	0.65***	-

Note: **, *** Significant at P≤0.01 and P≤0.001, respectively

4.3.2 Germplasm reaction to GRD under low disease pressure

Under low disease pressure environment, a low PDI was observed on all genotypes over the two seasons (Table 4.5). However, significant differences among genotypes were detected for PDI, plant height, pod length, number of seeds per pod, 100 seed mass, shelling %, and yield. Highly significant differences due to season were also observed in all characters except PDI and number of seeds per pod. Furthermore, differences due to the genotype by season interaction were highly significant for height, pod length, 100 seed mass weight, shelling percentage, and yield. The mean values of yield and other traits are presented in Table 4.6 and Appendix 4.2. PDI for the genotypes ranged from 0 to 27.9% with a mean of 4.36%. Yield ranged from 191 kg ha^{-1} (ICG 2772) to 1017.3 kg ha^{-1} (ICGV-SM 00573) with a mean of 598.18 kg ha^{-1}. All the checks and farmer preferred genotypes had yields above average. However, Chalimbana, Tchayilosi and Kalisele produced 26.9%, 26.8% and 25.4% lower than the susceptible check JL 24, respectively, while CG 7 produced 2.79% higher than the check. The ranges for other traits were as follows; height (121.1 to 359.9 mm, mean = 226.2 mm), number of primary branches (3.5 to 11.1, mean = 6.55), number of pods per plant (5.87 to 20.49, mean = 13.05), pod length (21.19 to 39.1 mm, mean = 28.51), number of seeds per pod (1 to 4, mean = 2), 100 seed mass (23.22 to 54.87g, mean = 35.95), shelling % (37.36 to 74.97%, mean = 62.65%), and kernel yield (100.2 to 726.8 kg ha^{-1}).

Correlations among various traits for genotypes grown under low disease pressure are presented in Table 4.7. Significant, negative correlations were observed between PDI and pod yield ($r = -0.25$, $P<0.001$), kernel yield ($r = -0.23$, $P<0.001$), number of pods per plant ($r = -0.21$, $P<0.001$). Highly significant positive correlations were recorded between pod yield and 100 seed mass ($r = 0.24$, $P<0.001$), plant height ($r = 0.38$, $P<0.001$), kernel yield ($r = 0.97$, $P<0.001$). Non significant negative correlations were also recorded for all other traits.

Table 4.5: Wald statistic for percent disease incidence (PDI), yield and other characters of 100 groundnut genotypes evaluated for 2 seasons under low disease pressure

		Wald statistic									
Source	df	PDI	Height (mm)	Number of primary branches	Number of pods per plant	Pod length (mm)	Number of seeds per pod	100 seed mass weight (g)	Shelling %	Yield (kg ha^{-1})	Kernel yield (kg ha^{-1})
Replication	1										
Genotype	99	120.8	469.4***	345.5	284.1	1359.3***	672.9***	568.4***	446.2***	714.5***	751.1***
Year	1	4.3	154.2***	1.7***	1.3***	9.3**	1.3	38.9***	251.7***	252.3***	372.0***
Genotype by Year	99	74.4	179.7***	107.8	121.9	172.0***	125.9	193.1***	448.0***	187.7***	179.8***
Error	198										

Note : ** and *** significant at P≤0.01 and P≤0.001, respectively, df = degrees of freedom

Table 4.6: Means of 15 selected genotypes for percent disease incidence (PDI), yield and other traits evaluated for 2 seasons under low disease pressure

Genotype	PDI	Height (mm)	Number of primary branches	Number of pods per plant	Pod length (mm)	Number of seeds per pod	100 seed mass weight (g)	Shelling %	Yield (kg ha^{-1})	Kernel yield (kg ha^{-1})
ICG 14705	0.3	234	7.4	15.4	24.2	1.9	41.9	70.7	1013.8	726.8
ICG 9449	1.6	200	5.6	8.9	23.1	2.0	25.2	56.2	220.0	124.4
ICGV-SM 05701	0.0	173	9.5	16.9	29.6	2.0	46.4	68.8	823.3	570.0
MW 2672	0.0	173	5.0	15.6	29.8	2.1	45.6	59.4	851.7	508.8
MW 2694	0.0	200	8.1	18.4	32.4	2.0	46.5	61.1	762.1	477.4
MW 2684	2.1	360	3.5	12.9	29.1	2.0	38.3	72.4	884.5	641.9
MW 618	0.3	273	4.3	12.5	24.1	1.9	27.2	69.4	603.6	414.4
CG7	1.4	180	6.7	19.3	31.7	2.0	48.4	68.5	814.2	564.3
Chalimbana	1.9	237	7.1	14.2	34.4	2.1	45.5	58.6	580.8	351.4
Tchayilosi	7.0	241	5.2	11.6	29.7	2.3	39.3	63.8	581.4	399.7
Kalisele	6.8	203	7.9	13.3	29.3	2.4	40.9	58.9	592.7	393.0
MGV5	2.7	207	7.0	16.6	33.1	2.0	54.4	58.4	723.0	432.3
Checks										
Resistant										
ICG 12991	0.6	314	4.5	15.4	23.6	2.0	33.5	72.5	822.2	599.7
ICGV-SM 90704	0.6	192	7.3	17.1	30.1	2.0	41.2	65.6	927.8	611.7
Susceptible										
JL 24	3.7	271	5.2	16.2	28.8	2.1	37.5	71.3	795.0	577.1
Mean	4.4	226	6.6	13.0	28.5	2.2	36.0	62.7	598.2	391.9
s.e.d avg	6.1	35	1.3	2.4	1.5	0.2	4.2	5.2	108.7	78.8
s.e.d max	7.4	35	1.3	2.8	1.7	0.3	4.7	5.8	122.6	88.7
s.e.d min	5.9	34	1.2	2.4	1.5	0.2	4.1	5.2	105.7	76.6

Note: Same genotypes as in table 4.3

Table 4.7: Correlations among PDI, yield and other characters among 100 genotypes evaluated over 2 seasons under low disease pressure

	100 seed mass weight	Height	Kernel yield	Number of primary branches	Number of pods per plant	Number of seeds per pod	Pod length	Shelling %	PDI	Yield
100 seed mass weight	-									
Height	-0.13	-								
Kernel yield	0.24***	0.41***	-							
Number of primary branches	0.25***	-0.28***	-0.12	-						
Number of pods per plant	0.15	-0.02	0.41***	0.16	-					
Number of seeds per pod	-0.17***	0.12	-0.05	-0.15	-0.17***	-				
Pod length	0.29***	-0.12	-0.17***	0.11	-0.19***	0.46***	-			
Shelling %	0.28***	0.31***	0.63***	-0.09	0.18***	-0.08	-0.31***	-		
PDI	-0.02	-0.11	-0.23***	0.08	-0.21***	0.09	0.14	-0.08	-	
Yield	0.24***	0.38***	0.97***	-0.10	0.43***	-0.02	-0.09	0.48***	-0.26***	-

Note: *** Significant at P<0.001 respectively

4.3.3 Yield under conditions of high and low disease pressure environments

A comparison of yields of groundnut genotypes grown under high and low disease pressure in the same seasons is presented in Figure 4.1. For all genotypes, lower yields were observed under high disease pressure than under low disease pressure. The yields decreased by an average of 51.7%, with a range from 4.4% for ICG 14705 to 90.2% for ICG 1142. The decreases in yield averaged according to PDI classification for the highly resistant, resistant, moderately resistant and susceptible genotypes were 16.3%, 27.1%, 44.9% and 59.2% respectively.

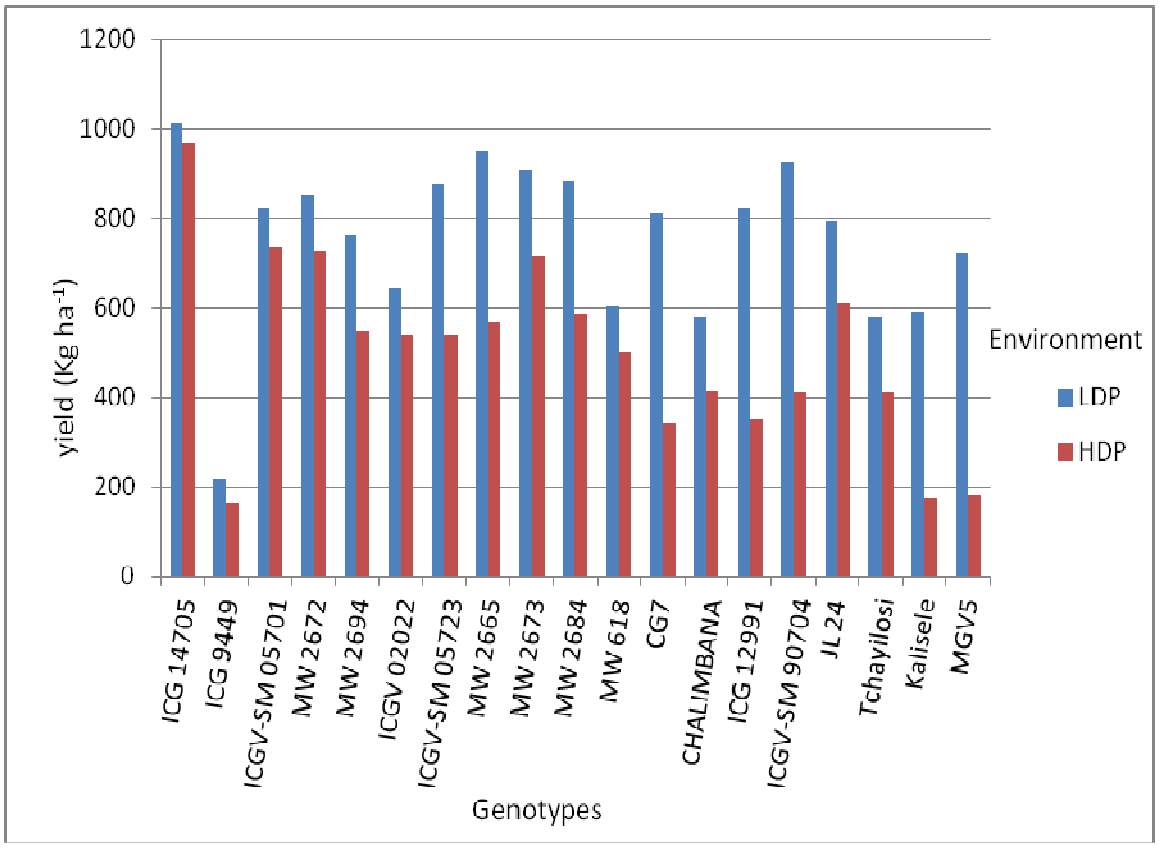

Figure 4.1: Mean yields of selected groundnut genotypes at low and high disease pressure

4.3.4 Resistance of 11 selected groundnut genotypes to aphid infestation

The results of the aphid resistance evaluation of 11 genotypes including controls under field and glasshouse conditions are presented in Table 4.8 and Table 4.9. In the field experiment, the aphid population mean decreased by 22.5% at 14 DAI to 8.06 aphids per plant from 10.4 aphids per plant at 7 DAI. This was probably due to heavy rainfall which may have washed

away the aphids from the plants before counting. However, significant (P<0.001) differences in aphid populations were observed among genotypes at 7 DAI. In the glasshouse experiment, aphid population increased by 306.7% from a mean of 26.9 aphids per plant at 7 DAI to 109.4 aphids per plant at 14 DAI. There were no differences in aphid population among genotypes at 7 DAI. However, highly significant (P<0.001) differences were observed at 14 DAI in aphid populations on the different genotypes. In both experiments, the resistant check genotype, ICG 12991 attracted very low aphid population growth at both 7 DAI and 14 DAI an indication of its resistance to aphids. Among the test genotypes and in comparison to the susceptible check, ICG 9449 attracted lower aphid populations at both 7 DAI and 14 DAI in both experiments confirming that it expresses a level of resistance to the aphids. None of the other test genotypes exhibited any level of resistance to the aphids in both the field and glasshouse experiments.

Table 4.8: Mean squares for aphid populations under choice (field) and no choice (glasshouse) tests

		Choice Test		No choice test	
Source	df	Count at 7 DAI	Count at 14 DAI	Count at 7 DAI	Count at 14 DAI
Genotype	10	260.6***	61.1	1290.5ns	17177***
Block	9	147.1	35.3	4988.4	3740
Error	90	77.7	35.6	791.4	4067

Note: *** and ns indicates significance at P≤0.001 and non significant respectively

Table 4.9: Mean aphid counts of 10 selected groundnut genotypes and the controls

	Choice test (Field)		No choice (Glasshouse)	
Genotype	Count at 7 DAI	Count at 14 DAI	Count at 7 DAI	Count at 14 DAI
CG 7	13.71	7.82	18.3	132.4
Chalimbana	21.15	8.79	33.2	130.5
ICG 14705	8.35	8.48	31	114.1
ICG 9449	6.75	4.16	21.3	58.3
ICGV-SM 05701	14.28	7.9	43.6	166.8
Kalisele	15.79	9.8	43.2	149
MW 26 72	7.48	8.67	21.1	102
MW 2694	8.97	11.49	26.6	139
Tchayilosi	6.88	10.23	21.4	91.6
Control				
Resistant				
ICG 12991	4.52	3.04	4.6	24.1
Susceptible				
JL 24	6.54	8.24	31.7	95.8
Mean	10.4	8.06	26.9	109.4
SE	5.09	5.09	23.2	52.7
LSD (P= 0.05)	7.482	2.66	25.06	56.81

4.4 Discussion

In this study, 100 groundnut genotypes of diverse origin were screened for GRD resistance and other selected agronomic traits. The use of infector row technique developed by Bock and Nigam (1988) in the high disease pressure environment was very effective in spreading GRD among the test genotypes in both seasons. The disease evaluation trials were conducted for two seasons in order to minimize disease escapees. Symptoms of GRD in susceptible test genotypes appeared as early as 7 days after initial aphid infestation. In contrast, under the low disease pressure environments the development of GRD was slow and low. In order to achieve maximum infection, artificial aphid infestation was done weekly up to 80 days after sowing. In spite of this, the highly resistant materials did not develop symptoms of GRD till the end of season, while those resistant to moderately resistant developed symptoms late in the season.

The highest levels of resistance characterized by 0% GRD incidence in both seasons, were detected on 5 genotypes ICG 9449, ICG 14705, ICGV-SM 05701, MW 2694 and MW 2672. However, for the rest of the genotypic differences were observed in susceptibility between the two seasons. Most genotypes were less susceptible to GRD in the 2009/10 season than in the 2010/11 season. This could be attributed to differences in environmental conditions existing immediately after aphid placement on test plants. In 2009/10 seasons, it was observed that on several occasions aphid infestation was disturbed by heavy rains, while in 2010/11 dry spells were experienced which provided coducive environment for aphid population growth, consequently, leading to high GRD infection among the test genotypes. High rainfall normally increases aphid mortality (Meihls *et al*., 2012).

Inclusion of the farmer preferred genotypes CG 7, Chalimbana, Kalisele, and Tchayilosi in this study helped to understand why farmers choose these genotypes. None of these genotypes were resistant to GRD confirming the need for farmers to have access to cultivars with appropriate agronomic traits, which also carry an adequate level of resistance to GRD to guard against yield losses in those seasons when GRD is prevalent. It is also worth noting that the resistant checks (ICGV-SM 90704 and ICG 12991) were all moderately resistant /susceptible to GRD indicating that their resistance level did not work well under conditions of high disease inoculum pressure. Waliyar *et al*. (2007) reported similar observations of the ineffectiveness of resistance in genotypes with known levels of resistance when inoculums levels were extremely high. ICG 12991 was resistant to the aphid vector of GRD and not to the viral complex causing the disease (Deom *et al*., 2006). As such, ICG 12991 can easily be susceptible in conditions where aphids have access on the genotype. Comparatively, ICG 12991 and ICGV-SM 90704 were as badly affected by GRD as the susceptible check JL 24. These results contradict previous studies where ICG 12991 and ICGV-SM 90704 were more resistant to GRD than JL 24 (van der Merwe *at al*., 2001; Deom *et al*., 2006). It appears that the five highly resistant genotypes identified in this study have a different mechanism of resistance which is more durable than that of the previously released varieties (ICGV-SM 90704 and ICG 12991) which may be worthy exploiting in subsequent breeding projects.

The genotypes with high levels of resistance to GRD and the farmer preferred genotypes were also screened for aphid resistance under field and glasshouse conditions in order to understand further the mechanism of GRD resistance. Aphid counts on two count dates were used as indicator of resistance and susceptibility of the genotypes. The resistant check (ICG 12991) had the lowest aphid population on all count dates and in all conditions, confirming that it is highly resistant to the aphid. ICG 9449 also expressed a higher level of resistance to the aphid than the other GRD resistant genotypes and maintained low aphid

populations. However, all other genotypes were susceptible to the aphid vector. This result confirmed that the mechanism of resistance in the highly GRD resistant genotypes identified in this study were not due to high levels of aphid resistance as per ICG 12991 but to the physiological resistance to the virus complex itself. This confirms the observation that genotypes resistant to the aphid vector are rare (Lynch, 1990). The genotype, ICG 12991 has been the most widely used to develop cultivars with resistance to the aphids (Herselman *et al*., 2004; Waliyar *et al*., 2007; Pandey *et al*., 2012).

Across Africa where GRD is prevalent, it greatly affects groundnut yields (Ntare *et al*., 2001). Similarly, in this study yield was highly affected by GRD especially under high disease pressure. The relationship between yield and GRD carried significant negative correlations under both LDP and HDP environments, meaning that yield was always reduced in the presence of GRD, irrespective of the inoculum load. One of the major effects of GRD is that affected plants develop fewer pods and many of these do not contain kernels. Consequently, most of the susceptible genotypes had very low yields. On the other hand, yield was not greatly affected in all the resistant genotypes except for ICG 9449 that was consistently low yielding. Among the most resistant genotypes, ICG 14705 was the best performing, with consistent high yields across the two environments. High yielding is a crucial trait for farmers, hence the highly resistant genotypes identified in these trials could be good parents to include in a breeding program with exception of ICG 9449. It would be helpful to elucidate the mechanism of resistance acting in each of these genotypes. Taliansky *et al*. (2000) noted that the resistant materials that were screened previously were not resistant to GRAV but to GRV and satRNA. The presence of GRAV alone affects yield even in plants which virtually show no symptoms of GRD infection (Naidu and Kimmins, 2007). It is also worth noting that among the susceptible farmer preferred cultivars, Tchayilosi, CG 7 and Chalimbana still gave considerable yield even in the presence of high GRD pressure. This shows that these genotypes are tolerant to GRD which could be one of the reasons why farmers choose to grow them. The susceptible check, JL 24 also yielded better than most other susceptible genotypes, and the resistant checks in both seasons indicating that it carries a high level of GRD tolerance. This is a contradiction of previous studies where JL 24 yielded far less than the resistant genotypes (van der Merwe *et al*., 2001).

Screening of groundnut genotypes for GRD resistance is complex. However, using visual symptoms and traits such as yield, resistant and tolerant cultivars can be identified. The primary objective of this research was achieved with several genotypes performing well for GRD resistance or tolerance and for their high yields. These characteristics make them good

varieties for release to farmers or to use as parents in a groundnut breeding project to deliver new cultivars with resistance to GRD and which farmers would choose to grow.

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Appendices

Appendix 4.1: Mean values of percent disease incidence (PDI), yield and other related traits evaluated over two seasons under high disease pressure

Genotype	Transformed PDI	PDI	Height (mm)	Number of primary branches	Number of pods per plant	Pod length (mm)	Number of seeds per pod	100 seed mass weight (g)	Shelling %	Pod yield (Kg/ha)	Kernel yield (kg/ha)
CG7	0.1	48.8	201	8.1	9.2	30.8	2.0	43.3	58.7	342.4	204.1
CHALIMBANA	0.1	46.5	228	7.7	7.2	30.8	2.1	43.4	57.0	417.4	242.7
ICG 10053	0.1	54.7	188	3.9	6.7	34.4	2.5	22.8	43.5	218.7	114.3
ICG 10185	0.1	58.2	160	7.2	8.4	31.1	2.0	35.3	37.4	157.4	78.6
ICG 11386	0.1	41.0	198	7.0	7.4	29.9	1.9	33.4	44.1	252.8	112.4
ICG 1142	0.1	82.7	159	5.9	8.9	28.6	3.4	9.1	9.1	57.4	6.8
ICG 11457	0.1	59.8	110	8.7	4.2	33.6	2.0	24.1	33.0	215.3	75.8
ICG 11542	0.1	65.1	250	5.5	8.6	20.0	2.0	21.9	42.7	116.0	56.2
ICG 11605	0.1	62.2	129	5.9	6.5	29.8	2.7	22.5	44.1	188.0	119.5
ICG 11855	0.1	68.4	134	9.0	8.4	29.0	2.0	29.0	35.0	110.4	34.6
ICG 12672	0.1	57.1	185	7.0	3.1	26.5	1.7	22.0	27.8	108.3	83.9
ICG 1274	0.1	59.2	223	5.5	5.9	28.2	2.3	30.5	40.4	129.9	96.9
ICG 12921	0.1	44.9	224	6.0	5.8	24.6	2.0	28.2	50.9	161.1	77.9
ICG 12988	0.0	35.3	214	5.6	11.3	19.7	2.0	25.6	74.6	379.2	279.6
ICG 13099	0.1	57.3	178	5.4	5.6	19.8	2.4	19.8	22.5	104.2	39.0
ICG 13723	0.1	60.9	106	10.0	8.5	22.7	2.0	26.6	43.6	168.8	89.5
ICG 1399	0.1	51.8	194	5.1	5.7	24.8	2.5	21.3	31.3	156.2	70.6
ICG 1415	0.1	66.9	200	5.5	9.4	22.5	2.1	23.6	34.4	134.7	49.9
ICG 14482	0.1	57.3	180	8.5	5.0	25.8	2.0	29.3	31.9	111.1	68.3
ICG 14705	0.0	1.7	254	7.9	12.8	24.4	2.0	41.1	68.4	969.4	682.8
ICG 1487	0.1	60.1	133	6.5	9.2	21.2	2.0	25.0	59.6	159.0	171.3

ICG 15232	0.1	48.5	173	4.2	9.1	26.3	2.0	35.0	65.1	347.2	224.8
ICG 15309	0.1	61.3	201	4.7	8.5	30.1	3.1	25.2	37.0	136.8	49.9
ICG 1711	0.1	55.4	143	4.7	11.4	22.8	2.0	25.0	47.4	148.6	73.8
ICG 1973	0.1	68.2	217	6.3	8.5	19.5	2.0	24.2	44.1	229.2	130.9
ICG 2106	0.1	57.1	172	6.7	8.3	17.0	2.0	25.6	56.8	326.9	194.3
ICG 2286	0.1	60.7	272	5.7	9.8	27.1	2.0	34.3	66.3	281.2	298.8
ICG 2772	0.1	51.3	163	3.8	2.0	20.2	2.0	19.3	19.3	158.3	58.9
ICG 2925	0.1	58.3	103	6.8	5.2	32.4	2.3	30.9	40.8	212.0	103.6
ICG 3584	0.1	39.8	253	4.5	11.6	19.0	2.0	25.1	61.1	452.1	318.6
ICG 3681	0.1	47.2	193	4.0	7.9	31.0	2.8	28.6	60.6	295.8	176.1
ICG 3746	0.1	61.6	269	6.1	11.0	22.7	2.0	24.2	59.0	276.4	181.5
ICG 3992	0.1	56.7	146	6.5	2.4	19.6	2.0	25.3	34.9	161.1	111.2
ICG 4111	0.1	74.1	180	7.5	4.9	23.2	2.0	29.9	49.8	128.5	111.5
ICG 434	0.1	47.9	275	4.2	10.1	26.4	2.2	26.9	57.9	384.7	327.7
ICG 4343	0.1	54.6	114	9.9	7.6	30.5	2.0	36.4	45.8	138.2	110.8
ICG 4412	0.1	52.4	107	8.8	2.9	29.5	2.2	27.0	31.3	138.0	65.9
ICG 4598	0.1	39.9	199	10.1	4.7	25.5	3.1	23.6	35.0	116.7	62.9
ICG 4750	0.1	55.6	135	5.9	6.4	22.9	2.0	23.8	35.7	84.0	55.6
ICG 4998	0.1	70.5	116	8.8	6.5	22.7	1.9	37.7	41.6	97.9	74.5
ICG 5016	0.1	63.1	100	8.3	6.8	35.5	2.0	34.2	38.0	229.2	90.5
ICG 5475	0.1	65.7	142	4.5	4.7	26.8	2.4	17.2	34.2	109.0	88.5
ICG 5662	0.1	44.5	173	6.7	7.0	25.3	2.0	44.3	48.2	205.6	164.6
ICG 5891	0.1	56.7	153	8.5	6.7	28.1	2.5	23.0	34.0	131.9	114.9
ICG 6402	0.1	57.3	144	8.9	6.8	23.0	2.0	22.4	41.7	165.7	74.1
ICG 6407	0.1	46.3	228	5.6	9.8	27.2	2.2	32.0	55.7	450.0	273.9
ICG 6643	0.1	60.1	218	4.1	3.4	35.8	3.4	22.0	40.7	256.5	106.0
ICG 7883	0.1	57.8	140	6.4	2.3	35.9	2.0	17.3	7.7	99.1	5.9
ICG 8253	0.1	55.6	248	4.5	11.3	22.4	2.2	26.1	46.1	242.4	139.0
ICG 8490	0.1	55.4	258	5.6	5.2	27.0	2.0	22.6	44.6	149.3	104.4
ICG 928	0.1	52.2	129	7.7	5.9	23.3	2.0	33.7	44.0	125.0	107.0

ICG 9449	0.0	0.0	195	5.3	8.9	22.7	2.0	21.9	48.7	166.7	98.6
ICGV 02022	0.0	26.1	232	4.7	10.3	25.4	2.3	28.5	63.6	539.6	334.9
ICGV 02286	0.1	60.3	247	4.8	7.4	24.5	2.0	35.5	47.4	525.7	189.2
ICGV 02446	0.1	59.3	163	6.7	9.5	22.0	2.0	31.8	47.2	272.9	173.1
ICGV 86326	0.1	56.2	164	6.1	2.9	33.8	2.1	50.6	58.1	187.5	103.4
ICGV 90087	0.1	62.0	141	6.2	7.3	23.1	1.9	30.8	60.8	359.3	301.8
ICGV 92234	0.1	71.5	164	7.0	4.5	25.3	2.0	21.2	21.2	91.7	34.6
ICGV 93470	0.1	60.5	141	4.5	8.6	27.5	2.0	32.5	55.6	404.9	256.3
ICGV-SM 00537	0.1	68.2	239	6.7	10.0	26.7	2.2	33.6	56.3	333.3	202.9
ICGV-SM 05701	0.0	0.0	170	7.6	16.5	26.7	2.0	39.7	59.6	736.1	462.9
ICGV-SM 05723	0.0	18.6	284	4.4	18.8	20.9	2.0	35.3	62.1	538.2	347.2
ICGV-SM 05756	0.1	45.7	219	5.1	14.5	22.2	2.0	32.1	63.7	429.9	291.5
ICGV-SM 95533	0.1	36.7	378	5.1	8.9	31.6	2.9	27.4	53.3	516.7	301.7
ICGV-SM 95741	0.1	57.4	107	4.8	6.0	23.7	3.0	29.9	56.1	300.0	248.7
ICGV-SM 99573	0.1	35.5	286	5.3	12.1	26.1	2.0	33.1	64.2	438.9	307.8
Kalisele	0.1	64.7	162	7.1	6.3	23.4	2.0	32.9	33.0	174.1	56.0
M-13	0.1	58.6	131	10.2	5.1	29.3	2.0	36.2	54.5	259.3	180.9
MGV5	0.1	57.6	182	6.7	8.7	25.2	2.0	40.2	42.0	181.9	106.8
MW 146	0.1	59.9	109	8.2	8.8	25.1	2.2	35.1	48.0	138.2	118.2
MW 170	0.1	55.3	247	4.8	10.7	25.9	2.0	25.7	56.9	267.4	173.9
MW 2665	0.0	23.9	299	5.5	12.4	20.1	2.0	27.3	74.2	568.1	421.5
MW 2666	0.1	37.9	245	7.3	7.9	24.1	2.0	21.7	21.7	174.3	39.7
MW 2667	0.1	50.4	113	6.7	8.5	23.7	2.0	38.6	59.6	275.0	231.9
MW 2668	0.1	54.8	163	5.5	4.9	27.5	2.0	35.7	63.6	331.2	355.8
MW 2669	0.1	64.8	183	8.0	5.7	28.5	1.9	27.9	25.6	131.3	45.0

MW 2672	0.0	0.0	178	5.8	11.6	27.0	2.0	40.1	55.9	727.8	414.1
MW 2673	0.0	20.1	295	5.4	13.7	20.7	2.0	30.2	76.7	715.3	550.6
MW 2674	0.1	35.1	249	5.2	18.4	20.3	2.0	26.3	73.6	567.4	414.3
MW 2675	0.1	50.5	160	8.4	12.1	29.3	2.0	43.1	64.0	350.7	222.6
MW 2677	0.1	41.6	241	10.1	14.7	29.5	2.0	38.9	51.9	376.4	202.3
MW 2679	0.1	58.0	244	7.5	7.9	30.8	2.6	35.0	44.6	325.0	151.7
MW 2680	0.1	64.0	236	7.2	5.1	27.6	3.1	29.3	52.9	288.2	159.6
MW 2684	0.0	22.7	346	4.7	12.0	27.0	2.0	35.4	68.1	586.8	413.5
MW 2685	0.1	64.2	166	8.5	11.7	25.4	2.0	33.4	46.1	181.2	87.1
MW 2686	0.1	63.0	272	5.1	10.0	28.7	1.9	42.8	62.5	466.7	311.9
MW 2688	0.1	35.0	282	5.6	12.4	24.5	1.9	35.7	60.6	561.1	345.8
MW 2692	0.1	59.8	139	8.5	7.6	25.3	2.0	35.2	61.0	207.6	217.8
MW 2693	0.1	62.5	151	6.2	5.5	28.1	2.0	21.9	17.0	91.7	20.4
MW 2694	0.0	0.0	157	7.7	15.8	30.2	2.1	43.4	55.7	549.3	313.2
MW 2695	0.1	54.9	267	6.4	6.8	24.7	1.7	24.5	40.2	266.0	137.3
MW 2698	0.1	42.6	267	8.5	9.2	30.0	1.9	42.8	53.9	375.0	211.3
MW 2873	0.1	37.5	216	8.1	9.0	27.8	1.9	47.4	59.6	390.3	230.4
MW 3234	0.1	49.1	151	5.0	3.9	37.0	2.1	23.7	23.7	177.8	62.8
MW 618	0.0	24.2	238	5.0	13.2	23.1	2.0	27.5	68.4	500.7	343.4
MW/ 133	0.1	50.4	226	6.4	11.6	26.9	2.1	32.0	49.0	153.5	70.9
Tchayilosi	0.1	49.3	196	5.8	8.8	28.3	2.2	31.9	56.8	409.0	248.6
Checks											
Resistant											
ICGV-SM 90704	0.0	32.7	209	6.7	13.9	27.2	2.0	32.9	49.9	409.7	246.9
ICG 12991	0.1	35.8	300	5.9	11.6	22.0	2.0	26.1	67.2	348.6	236.4
Susceptible											
JL 24	0.1	46.2	239	4.6	13.6	28.9	2.1	35.2	65.0	611.1	410.7
Mean	0.1	50.4	194	6.5	8.4	26.2	2.1	30.2	48.0	284.0	178.0
s.e.d avg	0.00938	11.48	41.1	1.02	2.131	2.541	0.1384	5.381	8.957	138.2	102.5

s.e.d max	0.009586	11.76	50.2	1.226	2.763	3.224	0.1689	6.505	10.79	176.9	122
s.e.d min	0.009128	11.13	40.	0.9917	2.027	2.346	0.1367	4.706	8.18	130.8	96.02

Appendix 4.2: Mean values of percent disease incidence, yield and other related traits evaluated over two seasons under low disease pressure

Genotype	Transformed PDI	PDI	Height (mm)	Number of primary branches	Number of pods per plant	Pod length (mm)	Number of seeds per pod	100 seed mass weight (g)	Shelling %	Yield (kg ha^{-1})	Kernel yield (kg ha^{-1})
CG7	0.1	1.4	180	6.7	19.3	31.7	2.0	48.4	68.5	814.2	564.3
CHALIMBANA	0.1	1.9	237	7.1	14.2	34.4	2.1	45.5	58.6	580.8	351.4
ICG 10053	0.3	12.0	209	4.3	8.8	34.5	3.3	28.3	55.2	398.7	220.2
ICG 10185	0.1	4.0	177	7.7	8.6	30.9	2.0	39.7	49.7	202.8	100.2
ICG 11386	0.1	1.7	206	5.9	13.6	30.8	2.0	37.9	53.5	548.5	304.2
ICG 1142	0.1	2.8	247	5.5	10.2	31.1	2.9	33.3	65.3	587.3	390.0
ICG 11457	0.2	7.9	141	8.3	10.9	31.0	2.5	34.2	54.0	284.1	155.3
ICG 11542	0.2	6.1	263	5.7	11.5	22.3	2.1	28.9	64.9	446.0	303.5
ICG 11605	0.2	6.8	241	6.3	11.2	32.0	3.4	30.9	69.2	684.6	490.1
ICG 11855	0.6	28.0	145	9.1	11.1	31.7	2.0	39.8	48.2	295.5	156.9
ICG 12672	0.0	0.7	254	6.7	15.2	31.5	2.0	38.4	47.1	574.8	314.2
ICG 1274	0.2	6.8	329	5.3	10.5	29.1	2.6	35.1	56.7	439.5	280.4
ICG 12921	0.2	3.6	278	5.6	9.3	26.3	2.1	37.4	65.7	420.8	284.5
ICG 12988	0.1	2.4	264	5.6	19.8	21.2	2.0	32.3	75.0	901.8	675.1
ICG 13099	0.2	5.2	272	5.5	9.8	30.1	2.5	29.8	64.4	542.5	354.5
ICG 13723	0.2	4.4	138	10.5	15.7	23.7	1.5	27.7	70.7	613.7	434.4
ICG 1399	0.2	5.9	27.2	4.4	11.2	24.9	2.4	27.9	64.4	501.2	327.4
ICG 1415	0.2	7.0	277	5.2	13.8	23.5	2.4	28.0	65.7	609.5	409.8

ICG 14482	0.2	4.4	202	7.9	14.5	28.6	2.0	44.2	61.9	529.6	352.4
ICG 14705	0.0	0.3	234	7.4	15.4	24.2	1.9	41.9	70.7	1013.8	726.8
ICG 1487	0.1	4.3	208	5.5	11.8	23.3	2.0	25.6	72.6	457.6	339.0
ICG 15232	0.2	4.0	195	5.1	10.7	28.1	2.0	42.9	65.6	605.1	389.8
ICG 15309	0.2	4.3	310	5.0	9.9	34.9	3.7	25.5	63.2	455.8	298.6
ICG 1711	0.1	2.8	182	5.4	14.6	24.3	2.0	26.7	65.6	564.9	386.0
ICG 1973	0.1	3.5	228	6.1	15.2	22.1	2.0	23.2	69.1	460.5	343.2
ICG 2106	0.1	1.1	225	6.4	17.3	23.8	2.0	31.5	72.7	698.5	512.9
ICG 2286	0.1	1.6	222	6.6	8.2	30.5	2.0	32.8	49.2	248.1	147.2
ICG 2772	0.1	2.4	186	8.5	13.6	30.0	2.1	41.0	56.7	191.3	115.2
ICG 2925	0.2	4.6	121	8.3	13.7	34.0	2.9	38.2	60.5	381.8	233.4
ICG 3584	0.1	2.2	291	5.1	14.6	21.4	2.0	27.5	70.0	564.5	392.9
ICG 3681	0.2	9.4	238	5.5	9.7	34.9	3.1	33.6	64.1	545.5	352.7
ICG 3746	0.0	0.6	276	5.3	14.2	22.6	2.0	25.2	74.2	493.4	360.4
ICG 3992	0.3	8.3	165	7.4	10.9	29.4	2.1	43.9	54.1	305.4	167.2
ICG 4111	0.2	4.6	250	8.6	13.0	23.3	1.5	34.6	63.5	586.8	367.0
ICG 434	0.1	3.1	263	4.4	12.4	26.8	2.6	28.2	67.9	430.7	293.5
ICG 4343	0.2	4.1	178	9.0	14.4	28.3	2.3	36.7	64.4	726.5	481.2
ICG 4412	0.2	4.0	140	11.2	14.3	32.6	2.6	39.2	61.7	494.2	306.7
ICG 4598	0.1	2.7	259	7.4	13.0	29.5	2.6	32.7	59.2	798.5	498.3
ICG 4750	0.3	10.4	209	5.9	11.3	27.8	2.2	37.1	70.0	388.8	272.0
ICG 4998	0.2	4.3	157	7.7	10.7	27.2	2.0	38.8	51.6	433.0	255.1
ICG 5016	0.4	16.1	158	7.0	8.4	33.9	2.0	41.9	56.5	396.8	231.6
ICG 5475	0.3	6.7	174	4.8	12.4	29.4	2.7	31.5	60.5	555.0	361.4
ICG 5662	0.2	5.3	195	7.1	9.6	30.8	2.0	54.9	64.1	528.6	339.5
ICG 5891	0.1	2.6	167	9.8	14.1	29.4	2.5	33.8	57.6	288.3	164.5
ICG 6402	0.2	5.7	187	9.5	13.6	23.2	2.0	25.6	60.0	379.2	240.1
ICG 6407	0.1	4.0	236	5.4	10.9	29.6	2.5	34.9	69.1	543.3	382.1
ICG 6643	0.3	13.0	299	4.1	7.7	36.4	3.7	26.0	50.3	324.7	166.4
ICG 7883	0.3	14.7	174	7.1	5.9	36.0	2.0	39.0	44.5	221.7	107.0

ICG 8253	0.2	5.9	245	4.8	9.4	25.4	2.2	26.7	66.3	424.5	288.4
ICG 8490	0.1	1.6	284	6.4	14.5	26.6	2.1	32.2	63.1	478.6	308.3
ICG 928	0.3	11.9	166	9.7	15.4	28.0	2.0	38.7	61.6	420.7	261.3
ICG 9449	0.0	1.6	200	5.6	8.9	23.1	2.0	25.2	56.2	220.0	124.4
ICGV 02022	0.1	1.0	229	4.5	11.8	28.5	2.3	31.2	67.0	644.6	428.9
ICGV 02286	0.1	2.5	248	4.8	11.9	24.1	2.0	32.2	69.2	560.8	390.4
ICGV 02446	0.3	7.1	208	7.4	17.7	26.3	2.0	28.2	60.7	714.4	439.8
ICGV 86326	0.1	1.9	202	7.7	10.6	32.3	2.1	44.6	66.0	429.1	293.7
ICGV 90087	0.1	1.1	214	5.4	11.9	28.5	2.1	40.2	71.9	862.4	626.8
ICGV 92234	0.1	1.4	198	6.4	8.2	25.8	2.6	31.9	49.9	299.1	160.4
ICGV 93470	0.1	1.8	156	4.7	11.2	27.2	2.0	35.2	69.0	624.9	434.1
ICGV-SM 00537	0.0	0.0	296	6.9	17.6	33.1	2.5	40.7	61.8	1017.3	612.4
ICGV-SM 05701	0.0	0.0	173	9.5	16.9	29.6	2.0	46.4	68.8	823.3	570.0
ICGV-SM 05723	0.0	0.0	275	4.3	15.3	23.1	2.0	36.8	71.4	878.3	616.1
ICGV-SM 05756	0.0	0.0	244	4.7	20.5	23.0	2.0	39.0	72.1	1014.9	726.2
ICGV-SM 95533	0.1	1.0	323	5.2	11.4	31.3	3.1	29.3	62.3	920.8	590.9
ICGV-SM 95741	0.1	1.4	235	6.1	13.9	33.5	3.1	34.7	59.0	782.7	480.8
ICGV-SM 99573	0.1	0.9	333	4.6	12.8	29.0	2.0	35.3	71.0	732.8	520.0
Kalisele	0.2	6.8	203	7.9	13.3	29.3	2.4	40.9	58.9	592.7	393.0
M-13	0.3	13.3	136	10.0	14.2	32.0	2.0	38.8	54.2	546.5	290.3
MGV5	0.1	2.7	207	7.0	16.6	33.1	2.0	54.4	58.4	723.0	432.3
MW 146	0.1	3.8	138	8.5	13.4	27.9	2.4	33.3	61.3	558.4	350.7
MW 170	0.1	2.8	271	5.3	12.5	25.9	2.0	29.4	70.4	644.6	460.4
MW 2665	0.0	0.0	327	4.9	17.8	22.1	2.0	27.7	71.0	953.1	655.4
MW 2666	0.1	2.3	256	6.7	9.1	28.4	2.0	26.4	37.4	470.8	264.7
MW 2667	0.4	21.3	146	6.9	16.4	26.4	1.5	40.2	67.1	586.7	390.4
MW 2668	0.1	1.2	177	6.7	12.9	28.6	2.1	39.5	63.7	660.9	416.9
MW 2669	0.2	7.7	191	7.4	16.7	29.7	2.0	35.4	54.7	395.0	228.9
MW 2672	0.0	0.0	173	5.0	15.6	29.8	2.1	45.6	59.4	851.7	508.8
MW 2673	0.0	0.0	319	5.3	13.3	22.7	2.0	29.1	73.7	909.8	668.9

MW 2674	0.0	0.5	279	5.0	13.5	21.2	2.0	27.2	71.8	746.1	541.6
MW 2675	0.2	5.5	179	9.2	15.9	30.8	2.1	50.7	71.1	786.2	564.9
MW 2677	0.1	1.6	239	8.2	11.9	30.4	2.1	45.1	60.0	671.3	404.6
MW 2679	0.0	0.3	289	7.1	12.1	35.7	2.3	33.2	59.4	925.4	572.9
MW 2680	0.2	5.5	297	7.3	13.4	29.3	3.1	32.4	67.9	895.5	610.2
MW 2684	0.1	2.1	360	3.5	12.9	29.1	2.0	38.3	72.4	884.5	641.9
MW 2685	0.3	12.1	172	7.6	11.5	26.6	2.0	44.8	67.4	606.1	411.6
MW 2686	0.1	4.0	238	5.2	12.8	30.9	2.2	40.3	65.3	555.7	361.2
MW 2688	0.1	0.5	251	5.6	16.6	26.7	1.9	42.7	69.2	1009.6	695.7
MW 2692	0.1	3.6	168	9.4	14.2	26.3	2.0	45.1	71.7	622.7	444.8
MW 2693	0.1	1.4	190	6.4	15.6	31.8	1.9	44.9	56.6	722.7	420.1
MW 2694	0.0	0.0	200	8.1	18.4	32.4	2.0	46.5	61.1	762.1	477.4
MW 2695	0.1	2.2	280	6.3	13.6	27.4	2.0	34.2	52.4	605.5	353.0
MW 2698	0.0	1.0	266	7.5	13.1	31.2	2.0	48.1	56.6	827.9	524.8
MW 2873	0.2	12.4	237	8.7	12.8	26.3	2.0	45.5	66.1	542.5	346.2
MW 3234	0.2	4.3	211	6.8	7.4	39.1	2.5	33.0	37.4	436.5	228.0
MW 618	0.0	0.3	273	4.3	12.5	24.1	1.9	27.2	69.4	603.6	414.4
MW/ 133	0.1	1.3	242	7.1	14.8	29.4	2.2	34.7	51.3	687.9	357.0
Tchayilosi	0.2	7.0	241	5.2	11.6	29.7	2.3	39.3	63.8	581.4	399.7
Checks											
Resistant											
ICGV-SM 90704	0.0	0.6	192	7.3	17.1	30.1	2.0	41.2	65.6	927.8	611.7
ICG 12991	0.1	0.6	314	4.5	15.4	23.6	2.0	33.5	72.5	822.2	599.7
Susceptible											
JL 24	0.2	3.7	271	5.2	16.2	28.8	2.1	37.5	71.3	795.0	577.1
Mean	0.2	4.5	225	6.6	12.9	28.5	2.2	35.9	62.4	590.5	385.6
s.e.d avg	0.1	6.1	35	1.3	2.4	1.5	0.2	4.2	5.2	108.7	78.8
s.e.d max	0.1	7.4	35	1.3	2.8	1.7	0.3	4.7	5.8	122.6	88.7
s.e.d min	0.1	5.9	34	1.2	2.4	1.5	0.2	4.1	5.2	105.7	76.6

CHAPTER 5

Gene action governing inheritance of resistance to groundnut rosette disease in groundnut (*Arachis hypogaea* L.)

Abstract

Groundnut rosette disease (GRD) is an important disease affecting groundnut in Africa hence, development of new resistant varieties is necessary. This study was instituted to examine combining ability effects in order to understand the type of gene action governing resistance to GRD and to identify groundnut genotypes suitable for use as parents in breeding for GRD resistance. A total of 90 family were generated from a 10 x 10 diallel cross. The F_1 plants were selfed to produce the F_2 generation. Thereafter, the parents and F_2 populations including reciprocals were evaluated under high disease pressure created by infesting the groundnut plants with viruliferous aphids in an experiment that was laid out in a randomised complete block design. Disease incidence data based on GRD symptoms were recorded at 7, 14, 21 and 28 days after aphid infestation. Genetic variability was observed among the populations in terms of GRD infection. The diallel analysis showed that GCA, SCA, reciprocal, maternal and non-maternal effects were all significant ($P \leq 0.001$), which indicated that both additive and non additive gene effects play a role in governing GRD resistance. The significance of SCA and reciprocal effects indicated the important role of maternal parents in the expression of GRD resistance and the importance of parental selection for groundnut improvement. However, the additive effects were predominant over non-additive gene effects as indicated by the general predictor ratio which was close to unity (0.95). Among the resistant parents, 4 genotypes ICG 14705, MW 2694 ICGV-SM 05701, and MW 2672 were the best combiners for GRD resistance. Overall, the cross between ICG 14705 and Chalimbana had the highest specific combining ability in the desirable direction. The predominance of additive effects means that groundnut resistance to GRD can be improved by introgressing additive genes using recurrent selection breeding procedures.

5.1 Introduction

Groundnut rosette disease (GRD) is caused by a complex of groundnut rosette virus (GRV), groundnut rosette assistor virus (GRAV) and its satellite RNA (satRNA) and they are transmitted by an aphid (*Aphis craccivora* Koch). The disease mainly affects groundnut in Africa and has the potential to cause total yield loss in severe cases (Naidu *et al*., 1999). Although, the disease occurs sporadically and at low levels in most years, its severity increases in crops that are sown late (Waliyar *et al*., 2007). As such, GRD poses a great threat to groundnut production in Malawi where most farmers grow their groundnut crop late in the season after sowing their primary crops especially maize (*Zea mayis* L,) and tobacco (*Nicotiana tabacum* L.).

Efforts to combat GRD have led to the development of several improved cultivars with acceptable levels of resistance (Naidu *et al*., 1999; Ntare *et al*., 2001; Ntare *et al*., 2007). In spite of the availability of the resistant cultivars, most farmers in Malawi still grow unimproved groundnut cultivars probably due to lack of preferable traits (Simtowe *et al*., 2010; Unpublished data, 2012). However, in order to increase groundnut production, breeding is needed of more resistant cultivars that carry a number of traits preferred by farmers.

Development of improved cultivars requires an understanding of the genetic background and nature of gene action governing key traits such as GRD resistance in the germplasm used for breeding. This information is necessary for planning appropriate breeding and selection strategies (Zhang *et al*., 2005). The diallel mating design has been extensively used to obtain such information. Analysis of a diallel design partitions the total variation of population data into general specific combining ability of parents (GCA) and specific combining ability (SCA) of the crosses (Griffing, 1956; Hill *et al*., 1998). Thus, GCA provides estimates of additive gene effects while SCA estimates non-additive gene action (Falconer and Mackay, 1996).

In groundnut breeding, diallel mating designs have been used to study the nature of the genetic control governing traits such as disease resistance, yield and yield related traits. Additive gene action controls the majority of yield quality traits while seed size is governed by non additive gene action (Hariprasanna *et al*., 2008). In groundnuts, leaf spot and rust resistance are controlled by both additive and non-additive gene action while bud necrosis virus is governed by additive gene action (Buiel, 1996; Pensuk *et al*., 2002; Vishnuvardhan *et al*., 2011). Combining ability studies have also been done on GRD and Adamu *et al*.,

(2008) reported that additive effects were predominant over non-additive effects in governing GRD resistance and other yield related traits. However, information on the combining abilities of resistance to GRD is limited for Malawian groundnut genotypes.

Resistance to GRD has been reported to be governed by two independent recessive genes (Nigam and Bock, 1990; Olorunju *et al*., 1992). In all cultivars tested to date, the resistance is only to the GRV and satRNA and not to GRAV which interact together to cause GRD (Waliyar *et al*., 2007). satRNA is responsible for the symptoms seen, however, it relies on GRV for packaging and both require GRAV for packaging and transmission by the aphid vector (Taliansky *et al*., 2000). This complex interaction of GRD viruses poses a challenge to breeders trying to develop groundnut lines with durable resistance. Evaluation of resistance to GRD has been rated on a 1-9 scale or as percentage disease incidence or both (Waliyar *et al*., 2007). However, the drawback to this approach is that symptoms only indicate the presence of satRNA and GRV, and not GRAV. Recently, assays based on reverse transcription-polymerase chain reaction (RT-PCR) have been used to detect all the components of GRD in groundnut samples (Kumar and Waliyar, 2007). However, the use of RT-PCR is expensive and needs advanced biotechnological equipment and skills. As such, symptom based methods are widely used. Therefore, the objective of this study was to determine the genetic parameters governing the inheritance of GRD resistance in groundnuts and to identify the best combiners to be used as donor parents in developing GRD resistant groundnut varieties.

5.2 Materials and methods

5.2.1 Genotypes used for hybridization

Ten groundnut lines selected from germplasm previously screened for GRD resistance were used in this study (Table 5.1). The resistant lines were ICG 9449, ICG 1405, ICGV-SM 05701, MW 2694 and MW 2672 while the susceptible lines were farmer preferred cultivars CG 7, Chalimbana, Kalisele and Tchayilosi and a recently released susceptible genotype JL 24.

Table 5.1: Characteristics and type of reaction to GRD of the different groundnut parental lines used for the diallel crossing

Parent	Source	Seed size	Seed colour	Reaction to GRD
ICG 9449	ICRISAT	small	Red	Resistant
ICG 14705	ICRISAT	medium	Tan	Resistant
ICGV-SM 05701	ICRISAT	medium	Tan	Resistant
MW 2694	DARS	medium	Tan	Resistant
MW 2672	DARS	medium	Tan	Resistant
JL 24	ICRISAT/FARMERS	medium	Tan	Susceptible
CG 7	FARMERS	Large	Red	Susceptible
Chalimbana	FARMERS	Large	Tan	Susceptible
Kalisele	FARMERS	medium	Tan	Susceptible
Tchayilosi	FARMERS	Large	Tan	Susceptible

5.2.2 Hybridization using a 10 x 10 full diallel mating scheme

The ten groundnut lines were used as parents in a 10 x 10 full diallel mating design according to the method formulated by Griffing (1956). The parental lines were crossed in all combinations, with reciprocals, but ignoring selfs to generate 90 families. Crosses were made in both the field and glasshouse following the method of Norden (1980) in 2009/10 and these were repeated in the 2010/11 seasons. Emasculation was done in the afternoons between 2:00 - 4:00 p.m. followed by pollination the next day between 6:00 - 8:00 a.m. Each emasculated flower was marked by a single thread and once pollinated another thread was added such that each pollinated flower had double threads. F_1 plants were then planted in the off-season under irrigation in 2010/11. Backcrosses and selfs were also done to obtain BCF_1 and F_2 seeds. However, hand pollinations generated only small numbers of F_1 seeds, and the seed obtained from backcrosses were not enough for genetic evaluation. Hence, these studies were conducted using the parents and the F_2 progenies.

5.2.3 Disease evaluation of the parental and F_2 populations

Evaluation of GRD resistance was done under high disease pressure in a glasshouse at ICRISAT Lilongwe, Malawi in 2012. The evaluations were run in batches concurrently because of limited space and pots. A total of 60 seeds of each parental and 60 seeds of each of the 90 F_2 populations were planted in 100 mm diameter plastic tubes (one seed per tube) laid out in a randomised complete block design with three replications.

The high disease pressure environment was created through aphid infestation. Aphid colonies were reared on the susceptible genotype JL 24 in the glasshouse prior to planting of the experiments. A week after germination of the experimental plants, each plant was infested with 10 viruliferous aphids using camel's hair brush following the method by Naidu and Kimmins (2007). Thereafter, the aphids were killed 7 days after infestation by spraying with dimethoate at 6.5g/10 litres water.

Each of the test plant was routinely checked and evaluated for GRD symptoms at 7, 14, 21 and 28 days after aphid infestation. The number of plants showing GRD symptoms per population were computed into percent disease incidence (PDI) by using the following formula; PDI = (Number of plants showing GRD symptoms ÷ total number of plants per plot) x 100 as described by Waliyar *et al*., (2007). Disease severity was assessed at 28 days after infestation (DAI) by using a 1-5 rating scale (Olorunju et al., 2001) where 1 = no symptoms, 2 = GRD symptoms on 1-20% foliage but no obvious stunting, 3 = GRD symptoms on 21-50% foliage and stunting, 4 = severe GRD symptoms on 51-70% foliage and stunting, and 5 = severe GRD symptoms on 71-100% foliage, stunted or dead plants. A disease severity index was calculated according to Olorunju *et al*., (1991) as follows; (A + 2B + 3C + 4D + 5E)/total number of plants assessed per plot where A, B, C, D, and E equal to the number of plants assessed per plot, respectively.

5.2.4 Data analysis

The percent disease incidence data of the parental and F_2 populations were transformed by arcsine before analysis in order to stabilize the error variance (Gomez and Gomez, 1984). Thereafter, the data were analysed using the Diallel- SAS procedure by Zhang *et al*., (2005) according to method 1 and model 1 suggested by Griffing (1956). The procedure partitions the variance into 3 components; (i) due to general combining ability (GCA), (ii) due to specific combining ability (SCA) and (iii) due to reciprocal effects. The model used was as follows:

$$Y_{ij} = \mu + g_i + g_j + S_{ij} + r_{ij} + \varepsilon_{ijk}$$

where,

Yij = mean phenotypic value of (i x j)th genotype over replication k (k=1, 2, 3,...b)

μ = general population mean

g_i and g_j = GCA effects of the ith and jth parents, respectively

S_{ij} = SCA effects of the ijth cross

rij = reciprocal effect associated with the ijth cross

ε_{ijlk} = residual effect

The estimates of genetic components were obtained based on the expectations of the mean squares as under;

Component due to GCA, σ^2 GCA = ($MS_{GCA} - MS_{error}$) / 2n

Component due to SCA, σ^2 SCA = (MS_{SCA} - MS_{error})

Where,

MS_{GCA} = variance due to GCA

MS_{SCA} = variance due to SCA

MS_{error} = mean error

n = number of replications

Reciprocal effect r_{ij} = ½($Y_{ij} - Y_{ji}$) where i < j.

The significance of estimates of variance due to GCA, SCA and reciprocals was tested using F- values at P<0.01, and P<0.05 levels while significance of estimates of GCA, SCA and reciprocals was tested using their respective standard errors.

5.3 Results

5.3.1 Reaction of parent lines to GRD infection

GRD infection differed greatly among the parental genotypes (Table 5.2). Percent disease incidence (PDI) ranged from 0 to 100% with the highest incidence observed on CG 7 and JL 24, while the lowest incidence was observed MW 2694. In general, the susceptible genotypes had above 50% PDIs while the resistant genotypes had less than 10% PDIs.

Table 5.2: Percent disease incidence means of 10 groundnut genotypes used as parents in a 10 x 10 diallel

Genotype	Number of plants tested	PDI mean	GRD Classification
CG 7	60	100.00	S
CHALIMBANA	60	80.00	S
ICG 14705	60	1.75	R
ICG 9449	60	7.22	R
ICGV-SM 05701	60	3.33	R
JL 24	60	100.00	S
KALISELE	60	69.55	S
MW 2672	60	10.00	R
MW 2694	60	0.00	R
TCHAYILOSI	60	54.21	S

5.3.2 Reaction of F_2 progenies to GRD infection

The F_2 progenies differed in their reaction to GRD infection and symptoms appeared as early as 7 days after aphid infestation (Table 5.3, Figure 5.1). A total of 35% of progenies developed from crosses between resistant parents (R x R) did not develop GRD symptoms while, 65% developed symptoms with PDIs ranging from 10 to 75%. However, the symptoms were moderate, mostly appearing on a single leaf with a severity index of 2. Among the F_2 progenies of resistant and susceptible (R x S) crosses, 20% of the progenies did not develop GRD symptoms while the other 80% developed GRD with PDI ranging from 10 to 87% and severity index ranging from 2 to 4. All cross progenies between susceptible and resistant (S x R) parents and between susceptible parents (S x S) developed GRD symptoms with severity indices ranging from 2 to 4. The PDIs for S x R and S x S progenies ranged from 14 to 97% and 37 to 100%, respectively. In general, GRD disease progression was highest in S x S cross progenies and was lowest in R x R cross progenies (Figure 5.1). The average PDI for S x S progenies was almost 50% at 7 days after infestation while in R x R progenies the average PDI remained low between 10 to 20% until 28 days after infestation (Figure 5.1).

Table 5.3: Percent disease incidence means and severity indices of F_2 progenies arising from 10 parental lines (above diagonal) and reciprocals (below diagonal) in a 10 x 10 diallel cross

Parents	1	2	3	4	5	6	7	8	9	10
1. ICG 9449		10 (2)	33 (2)	0 (1)	0 (1)	97 (4)	66 (2)	87 (3)	93 (4)	50 (2)
2. ICG 14705	45 (2)		0 (1)	14 (2)	25 (2)	75 (3)	20 (2)	95 (3)	72 (2)	80 (3)
3. ICGV-SM 05701	33 (2)	0 (1)		0 (1)	75 (2)	35 (2)	29 (2)	74 (2)	43 (2)	67 (2)
4. MW 2694	63 (2)	14 (2)	20 (2)		0 (1)	43 (2)	72 (2)	89 (2)	39 (2)	68 (3)
5. MW 2672	14 (2)	0 (1)	59 (2)	11 (2)		44 (2)	67 (2)	27 (2)	43 (2)	14 (2)
6. JL 24	50 (2)	10 (2)	30 (2)	30 (2)	60 (2)		77 (3)	83 (4)	71 (2)	74 (2)
7. CG 7	82 (3)	87 (3)	23 (2)	73 (2)	72 (2)	80 (3)		100 (4)	56 (2)	67 (2)
8. CHALIMBANA	74 (4)	0 (1)	50 (2)	42 (2)	20 (2)	94 (3)	95 (4)		90 (4)	37 (2)
9. KALISELE	63 (3)	0 (1)	62 (2)	0 (1)	46 (2)	46 (2)	67 (3)	77 (3)		86 (3)
10. TCHAYILOSI	27 (2)	75 (2)	83 (2)	0 (1)	24 (2)	94 (3)	30 (2)	53 (2)	50 (2)	

(*) Disease index values calculated based on GRD symptoms using the following rating scale; 1 = no symptoms, 2 = leaf symptoms and no stunting, 3 = leaf symptoms plus stunting (general plant size) ranging from slightly to discernible to about 30%, 4 = symptoms plus stunting about 30 to 70%; and 5 = symptoms plus stunting greater than 50%. Numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 denote ICG 9449, ICG 14705, ICGV-SM 05701, MW 2672, MW 2694, JL 24, CG 7, Chalimbana, Kalisele and Tchayilosi respectively

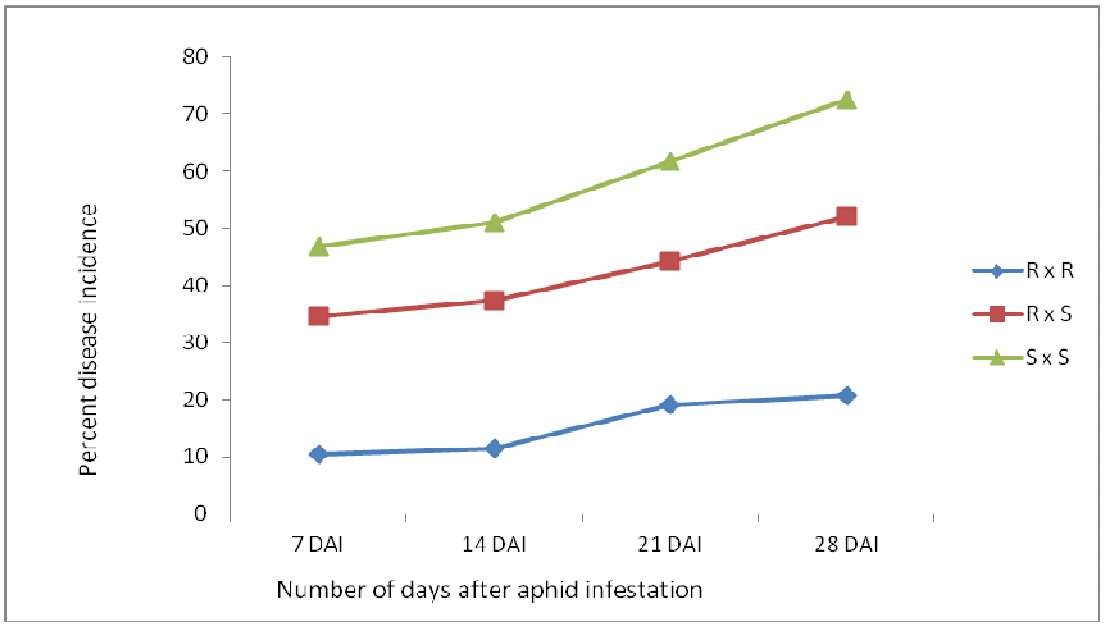

Figure 5.1; Disease progress curve of GRD in F_2 progenies derived from crosses between GRD resistant and susceptible genotypes.

* R x R = resistant by resistant cross, R x S = resistant by susceptible cross and also susceptible by resistant cross, S x S = susceptible by susceptible cross. Data points represent average PDIs averaged over 3 replications at 7, 14, 21 and 28 days after aphid infestation.

5.3.3 Gene action governing GRD resistance

Mean squares due to general combining ability (GCA), specific combining ability (SCA), reciprocal effects, maternal and non maternal effects are presented in Table 5.4. All mean squares were significant (P=0.001) for GRD resistance. The general predictor ratio (Baker, 1978) was calculated to be 0.95.

Table 5.4: Combining ability mean squares for GRD percent disease incidence under artificial aphid infestation

Source	df	MS
GCA	9	3.22***
SCA	45	0.32***
Reciprocals	45	0.34***
Maternal	9	0.67***
Non maternal	36	0.26***
GPR[a]		0.95

***Significant at P<0.001

[a]General predictability ratio (GPR) = 2MSGCA/ (2MSGCA + MSSCA)

The GCA estimates for GRD resistance among the 10 parental lines are presented in Table 5.5. Based on the rating scale used in this study where low values represented low disease and high values represented high disease, significant negative values indicate contribution of a parental line towards resistance while positive values indicate parental contribution towards susceptibility. A total of 5 lines (ICG 9449, ICG 14705, ICGV-SM 05701, MW 2672 and MW 2694) exhibited significant negative GCAs while the other 4 parents (JL 24, CG 7, Chalimbana, and Kalisele) exhibited significant positive GCA values. The GCA for Tchayilosi was positive but not significant.

Table 5.5: General combining ability (GCA) effects for groundnut rosette percent incidence under high disease pressure environment

Parents	Estimate
ICG 9449	- 0.086*
ICG 14705	- 0.279***
ICGV-SM 05701	- 0.193***
MW 2694	- 0.252***
MW 2672	- 0.212***
JL 24	0.238***
CG 7	0.298***
Chalimbana	0.289***
Kalisele	0.136***
Tchayilosi	0.059ns

Note: *, *** and ns indicate significance at P=0.05, P=0.001 and non-significant, respectively.

The estimates for SCA, reciprocal effects and least square means are presented in Table 5.6 (See also appendix 5.1). Significant negative SCA values indicate desirable crossing combinations. The crosses and reciprocals exhibited varying degrees of compatibility for GRD resistance. In general, 17 of the 90 F_2 progenies and reciprocals showed significant negative SCA effects for GRD resistance. Among the Rx R crosses, ICG 9449 x MW 2672 and among S x S crosses CG 7 x Tchayilosi with its reciprocal and Kalisele x Tchayilosi also had significant negative SCA effects for GRD resistance. The remaining 13 crosses and reciprocals which showed significant SCA effects were between resistant and susceptible parents (Table 5.6).

Table 5.6: Specific combining ability (SCA) effects for groundnut rosette disease resistance of selected crosses that had significant negative arising from a 10 x 10 diallel crosses

Cross	Cross type	SCA
ICG 9449 X MW 2672	R x R	-0.32**
ICGV 14705 X JL 24	R x S	-0.24*
ICGV-SM 05701 X JL 24	R x S	-0.23*
ICGV-SM 05701 X CG 7	R x S	-0.28**
MW 2694 X Kalisele	R x S	-0.24*
MW 2672 X Chalimbana	R x S	-0.28**
CG 7 X Tchayilosi	S x S	-0.60**
Reciprocals		
ICG 9449 X JL 24	R x S	-0.57***
ICG 14705 X JL 24	R x S	-0.29*
ICG 14705 X Chalimbana	R x S	-0.68***
ICG 14705 X Kalisele	R x S	-0.34**
ICGV-SM 05701 X Chalimbana	R x S	-0.26*
MW 2694 X Chalimbana	R x S	-0.26*
MW 2694 X Kalisele	R x S	-0.33**
MW 2694 X Tchayilosi	R x S	-0.48***
CG 7 X Tchayilosi	S x S	-0.29*
Kalisele X Tchayilosi	S x S	-0.36**

Note: *, **, *** indicates significance at P<0.05, P<0.01 and P<0.001, respectively.

5.4 Discussion

Artificial infestation of test plants with viruliferous aphids was effective in transmitting GRD as observed on the susceptible groundnut lines. As such, resistant lines for inclusion in a GRD breeding programme could be identified. Parental lines differed significantly in their reaction to GRD resistance based on percent disease incidence (PDI). Two groups were identified among the parents based on their resistance levels to GRD. These were: highly resistant with 5 genotypes namely ICG 9449, ICG 14705, ICGV-SM 05701, MW 2694 and MW 2672; and highly susceptible consisting of 5 genotypes including JL 24, CG 7, Chalimbana, Kalisele and Tchayilosi. However, there is still a need to determine whether the resistance was to all components of the viral complex (GRAV, GRV and satRNA) or only to GRV and satRNA.

Differences among the F_2 progenies in reaction to GRD infection were also observed. Disease progressed much slower in progenies from R x R crosses and more rapidly in progenies from S x S crosses. Symptoms of GRD observed on 13 progenies arising from crosses between resistant parents were moderate. The development of symptoms in these lines was probably due to the high disease pressure and the stage at which the plants were infected. However, the mildness in the symptoms and the low disease progression indicates a high level of quantitative resistance to GRD. Similar observations have been reported by Misari *et al*., (1988) where some F_2 progenies arising from R x R crosses showed mild symptoms of GRD. On the other hand, disease progress in progenies from R x S crosses was intermediate between that of R x R and S x S crosses. The disease reaction in progenies from R x S crosses showed segregation because some remained resistant while others showed mild to severe symptoms. It would be important to verify the resistance observed in these segregating materials to determine the number of genes involved.

From the diallel analysis, mean squares due to GCA, SCA, reciprocals, maternal and non-maternal effects were all significant. The significance in the GCA and SCA effects indicate that both additive and non-additive gene action are important in the inheritance of GRD resistance. In addition, the significance in reciprocal effects showed that cytoplasmic factors also played a role in GRD resistance. This was further shown by the partitioning of reciprocal effects into maternal and non maternal effects. The significance of maternal effects indicate that variation in GRD resistance was also influenced by cytoplasmic genetic factors or environmental preconditioning of the maternal parents while the significant non-maternal effects indicated the influence of factors due to interaction of cytoplasmic and nuclear genes (Wu and Matheson, 2001). However, the influences underlying non-maternal effects are difficult to explain (Lopez *et al*., 2003). In general, considering the mean squares and the general predictor ratio which was close to unity, the contribution of GCA effects to variation among the crosses was much higher than the contribution of SCA, reciprocals, maternal and non-maternal effects. This means that additive gene action is predominant in governing GRD resistance in the groundnut lines tested. Similar observations were made by Adamu *et al*., (2008) who reported that GRD was controlled mainly by additive gene action.

The estimates of GCA effects can be used to identify suitable sources of GRD resistance as indicated by significant negative values of the individual parents. In this study, all resistant genotypes expressed significant negative GCA values meaning that they are all potential sources of resistance to GRD. However, the best combiners with comparatively high negative CGA values were ICG 14705, ICGV-SM 14705, MW 2694 and MW 2672. The

genotype ICG 9449 had the lowest negative GCA value indicating that it may not be as a good combiner as the other resistant genotypes. On the other hand, in this study, positive GCA effects showed the contribution of parents towards susceptibility as was observed for parents JL 24, CG 7, Chalimbana and Kalisele. Thus, crosses with these susceptible parents will reduce GRD resistance. However, the GCA value for the susceptible parent Tchayilosi was not significant although positive indicating that it does not contribute substantially to enhancing susceptibility to GRD.

Considering the SCA and reciprocal effects, 17 cross combinations had significant negative effects indicating their suitability for GRD resistance. However, the highly significant SCA effects suggests that selection for GRD resistance would be more appropriate in later generations than F2s when non-additive gene effects have been reduced through selfing. The results also indicate that the significant SCA effects did not follow the expected performance based on the GCA values of two parents involved which could mean that the inheritance of GRD resistance is complex. Although, the majority of the crosses with desirable SCA effects were between resistant x susceptible parents, some were between two susceptible parents and others between two resistant parents. A reciprocal cross between resistant and susceptible parents ICG 14705 x Chalimbana and the susceptible parents CG 7 x Tchayilosi showed the highest two SCA values. There were also two reciprocal crosses involving susceptible parents with significant negative SCA values, all which had Tchayilosi as one of the parents. This indicates that apart from crosses between resistant and susceptible or between resistant parents, GRD resistance could also be produced among crosses involving susceptible genotypes. Hakizimana *et al*., (2004) indicated that resistance between susceptible parents is possible due to transgressive segregation or inter- and intra locus gene interactions.

The large number of reciprocal crosses with significant SCA effects confirms that maternally inherited effects were also important in GRD resistance. These results contradicted a study by Misari *et al*., (1988) where cytoplasmic and/or maternal effects were not observed in the inheritance of GRD resistance. However, although the maternal effects were small, these results suggest that the resistant parents could be used as female parents in breeding for GRD resistance.

In conclusion, this study has revealed that additive gene effects were predominant over non-additive gene effects for GRD resistance. The study also showed that all the resistant parental lines can be used in developing breeding populations for GRD resistance. The best line identified with good GRD resistance was ICG 14705 and had the best combining ability

when crossed with Chalimbana. As such, these parental lines are worth exploiting in developing GRD resistant materials. In addition, maternal effects also played a significant role in the inheritance to GRD resistance. This implies that when developing breeding populations, the female parent must be GRD resistant. For future studies, it will be necessary to focus on the performance of the crosses with regards to duration to maturity, yield and other related traits which are equally important in developing GRD resistant materials. The information on these traits will enable the breeder to form proper breeding strategies for developing high yielding, GRD resistant cultivars carrying farmer preferred traits which are needed by farmers in Malawi.

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Appendix 5.1: SCA for groundnut rosette disease arising from a 10 x 10 diallel crosses

Crosses	Cross type	SCA
ICG 9449 X TCHAYILOSI	R x S	0.23
ICG 9449 X ICG 14705	R x R	-0.025
ICG 9449 X ICGV-SM 05701	R x R	-0.003
ICG 9449 X MW 2694	R x R	-0.043
ICG 9449 X MW 2672	R x R	-0.321**
ICG 9449 X JL 24	R x S	0.003
ICG 9449 X CG 7	R x S	0.002
ICG 9449 X CHALIMBANA	R x S	0.283
ICG 9449 X KALISELE	R x S	0.384
TCHAYILOSI X ICG 9449	R x S	-0.135
TCHAYILOSI X ICG 14705	R x S	-0.058
TCHAYILOSI X ICGV-SM 05701	R x S	0.057
Tchayilosi X MW 2694	R x S	-0.482***
Tchayilosi X MW 2672	R x S	0.183
Tchayilosi X JL 24	S x S	0.150
Tchayilosi X CG 7	S x S	-0.292*
Tchayilosi X CHALIMBANA	S x S	-0.024
Tchayilosi X KALISELE	S x S	-0.356**
ICG 14705 X ICG 9449	R x R	0.189
ICG 14705 X TCHAYILOSI	R x S	0.683***
ICG 14705 X ICGV-SM 05701	R x R	-0.208
ICG 14705 X MW 2694	R x R	0.036
ICG 14705 X MW 2672	R x R	-0.020
ICG 14705 X JL 24	R x S	-0.244*
ICG 14705 X CG 7	R x S	0.092
ICG 14705 X CHALIMBANA	R x S	-0.013
ICG 14705 X KALISELE	R x S	-0.200
ICGV-SM 05701 X ICG 9449	R x R	-0.199
ICGV-SM 05701 X TCHAYILOSI	R x S	0.403**
ICGV-SM 05701 X ICG 14705	R x R	-0.000
ICGV-SM 05701 X MW 2694	R x R	-0.019
ICGV-SM 05701 X MW 2672	R x R	0.533***
ICGV-SM 05701 X JL 24	R x S	-0.238*
ICGV-SM 05701 X CG 7	R x S	-0.280**
ICGV-SM 05701 X CHALIMBANA	R x S	-0.009
ICGV-SM 05701 X KALISELE	R x S	0.207
MW 2694 X ICG 9449	R x R	0.300
MW 2694 X TCHAYILOSI	R x S	0.171
MW 2694 X ICG 14705	R x R	0.062
MW 2694 X ICGV-SM 05701	R x R	0.217
MW 2694 X MW 2672	R x R	-0.055
MW 2694 X JL 24	R x S	-0.046
MW 2694 X CG 7	R x S	0.315**
MW 2694 X CHALIMBANA	R x S	0.232*
MW 2694 X KALISELE	R x S	-0.235*
MW 2672 X ICG 9449	R x R	0.062
MW 2672 X TCHAYILOSI	R x S	-0.271
MW 2672 X ICG 14705	R x R	-0.169
MW 2672 X ICGV-SM 05701	R x R	0.051

MW 2672 X MW 2694	R x R	0.162
MW 2672 X JL 24	R x S	0.087
MW 2672 X CG 7	R x S	0.119
MW 2672 X CHALIMBANA	R x S	-0.278**
MW 2672 X KALISELE	R x S	0.106
JL 24 X ICG 9449	R x S	-0.579***
JL 24 X TCHAYILOSI	S x S	-0.142
JL 24 X ICG 14705	R x S	-0.293*
JL 24 X ICGV-SM 05701	R x S	-0.116
JL 24 X MW 2694	R x S	0.074
JL 24 X MW 2672	R x S	0.081
JL 24 X CG7	S x S	-0.069
JL 24 X CHALIMBANA	S x S	0.113
JL 24 X KALISELE	S x S	-0.162
CG 7 X ICG 9449	R x S	0.275
CG7 X TCHAYILOSI	S x S	-0.602**
CG 7 X ICG 14705	R x S	0.502***
CG 7 X ICGV-SM 05701	R x S	-0.045
CG7 X MW 2672	R x S	0.184
CG7 X MW 2694	R x S	-0.029
CG7 X JL 24	S x S	0.078
CG7 X CHALIMBANA	S x S	0.177
CG7 X KALISELE	S x S	-0.209
CHALIMBANA X ICG 9449	R x S	-0.127
CHALIMBANA X TCHAYILOSI	S x S	-0.258
CHALIMBANA X ICG 14705	R x S	-0.678
CHALIMBANA X ICGV-SM 05701	R x S	-0.255
CHALIMBANA X MW 2694	R x S	-0.264*
CHALIMBANA X MW 2672	R x S	-0.055
CHALIMBANA X JL 24	S x S	0.061
CHALIMBANA X CG 7	S x S	-0.060
CHALIMBANA X KALISELE	S x S	-0.087
KALISELE X ICG 9449	R x S	-0.209
Kalisele X TCHAYILOSI	S x S	-0.038
KALISELE X ICG 14705	R x S	-0.338**
KALISELE X ICGV-SM 05701	R x S	0.136
Kalisele X MW 2694	R x S	-0.330**
Kalisele X MW 2672	R x S	0.016
Kalisele X JL 24	S x S	-0.107
Kalisele X CG 7	S x S	0.039
Kalisele X CHALIMBANA	S x S	-0.077

Thesis overview

Groundnut (*Arachis hypogaea* L.) is an important crop in Malawi. However, several challenges continue to limit its production. Groundnut rosette disease (GRD) which is one of the major constraints was the main focus of this study. The main aim of this study was to develop appropriate groundnut cultivars that are resistant to GRD, combined with other traits preferred by farmers in order to improve food security of smallholder farmers in Malawi and beyond. The specific objectives of this study were therefore to (i) determine the groundnut production systems of smallholder farmers, their varietal preferences and production constraints; (ii) to assess the genetic diversity among groundnut germplasm collected from various sources (iii) to evaluate the groundnut germplam with the aim of identifying the best available sources of resistance to GRD and the aphid vector and evaluate the materials for yield and yield related traits; and (iv) to determine the genetics of resistance to GRD and identify the best combiners to be used as donor parents in developing GRD resistant cultivars, with good ancillary traits.

In order to accomplish these, several materials and methods were used. A field survey and participatory rural appraisal (PRA) tools were used to assess groundnut cropping systems used by smallholder farmers, their varietal preferences, and production challenges. SSR markers were used to assess the genetic diversity among 106 groundnut genotypes collected from ICRISAT, Chitedze gene bank and farmers. Field and glasshouse trials were performed to evaluate genotypes to identify new sources of resistance to GRD and its aphid vector. The identified resistant materials and farmer preferred genotypes were crossed in a 10 x 10 diallel mating scheme from which 90 families were generated. The F_1s were selfed to produce F_2s. Glasshouse trials were then run to determine the type of gene action governing inheritance of resistance to GRD using parental materials, F_2 generations and their reciprocals.

Significant findings of the study:

a. Groundnut production is allocated to small portions of land, and left for women and children to produce. Men are more concerned with other major crops like tobacco and maize.

b. Farmers obtain seed from various sources; own recycled seed, local traders, farmer to farmer seed exchange. It was perceived that most of this seed was of low quality and not true to type. These have a huge impact on groundnut productivity.

c. Groundnut rosette disease is a widely known disease; however, the majority of farmers do not know its cause and ways to control it. It was observed that the most widely grown groundnut varieties were Chalimbana and CG 7 which are susceptible to GRD. The released groundnut varieties were less familiar with farmers.

d. Farmers face other production and marketing challenges such as poor markets, lack of inputs and lack of technical support from extension.

e. Genetic diversity among the germplasm collected for use for evaluation of GRD and aphid vector evaluation was high. Germplasm from ICRISAT and the department of research in Malawi were clustered together meaning there were from same source. However, farmer preferred cultivars were clustered separately.

f. Five genotypes were identified as highly resistant to GRD, which included ICG 9449, ICG 14705, ICGV-SM 05701, MW 2672 and MW 2694. None of the farmer-preferred genotypes were resistant to GRD. Vector resistance was confirmed in ICG 12991. The highly resistant materials were high yielding except for ICG 9449.

g. Mean squares due to general combining ability (GCA), specific combining ability (SCA), and maternal and non-maternal effects were all significant, indicating that both additive and non-additive gene effects are important in the inheritance of GRD resistance. However, additive effects were predominant.

h. The best line identified with good GRD resistance was ICG 14705. It expressed its best combining ability when crossed with Chalimbana.

Breeding implications and future research needs

The survey on groundnut production systems in Malawi showed that although GRD is an important disease, adoption of newly released GRD resistant varieties by farmers is very low. This indicates the need for breeders to involve farmers in developing new varieties and incorporate local varieties in their breeding programmes in order to capture farmer preferred traits which could help in enhancing adoption among farmers. However, incorporating local varieties in developing new varieties could be a challenge in that some traits may be negatively correlated with yield and GRD resistance. It was also observed in this study that farmers did not know of any improved GRD resistant varieties released in Malawi. This reflects the clear need for breeders to work with extension staff or NGOs involved in seed distribution to promote the use of the released improved GRD resistant varieties. Lack of good quality seed which was revealed during PRA shows that an opportunity exists for the marketing of groundnut seed in Malawi. Lack of knowledge among farmers on the cause,

transmission and control measures of GRD also shows the need for extension agents in corroboration with scientists to educate the farmers on ways of reducing GRD, which could help in increasing groundnut productivity in Malawi.

Previous studies characterized groundnut as having a narrow genetic base (Upadhyaya *et al*., 2002). The narrow genetic base and the complex nature of the groundnut genome combine to pose a serious bottleneck to the genetic improvement of groundnut (Pandey *et al*., 2012). However, genetic analysis of groundnut germplasm used in this study revealed a relatively high level of genetic diversity. This provides key information needed for the choice of parents in breeding programmes aiming at exploiting the gene diversity to a maximum. It was also observed that GRD resistant genotypes were placed in several clusters. This provides an opportunity to use the genotypes for constructing mapping populations for GRD resistance and to pyramid different resistance mechanisms. Future molecular work could focus on identifying markers linked to GRD resistance.

In this study, use of the infector row technique developed by Bock and Nigam (1988) was effective in spreading GRD among all test genotypes. This proved to be the best method for evaluating GRD resistance. New sources of GRD resistance identified in this study could be released to farmers or exploited further in breeding programmes to develop new GRD resistant cultivars. All GRD resistant genotypes identified previously were resistant to groundnut rosette virus (GRV) and its satellite RNA (satRNA) and not to the groundnut rosette assistor virus (GRAV). All three components interact to cause GRD. In this study, screening was based on symptoms such that genotypes with no GRD symptoms were characterized as highly resistant. As such, there is still a need to determine if the resistance was against all three virus components (GRV, GRAV and satRNA). Breeding for resistance to all three viruses causing GRD could also be complex because of the nature of their interaction.

Resistance to the aphid vector has been found in few cultivars. In this study it was confirmed in one cultivar, ICG 12991. The identification of markers closely linked to aphid resistance by Herselman *et al*., (2004) could be used to develop more varieties with aphid resistance. Breeders could also look at possibilities of combining resistance to the aphid and GRD resistance or tolerance.

The genetic studies showed that the GRD resistance was largely governed by additive gene effects. The predominance of additive effects means that GRD resistance in groundnut can be improved by the accumulation of more positive, additive genes using recurrent selection

breeding procedures. Non-additive gene effects also played a role in governing resistance. This showed that when developing GRD resistant materials, the female parent should be resistant. Although, significant GCA effects indicated that all the resistant genotypes could be used for developing breeding populations for GRD resistance, ICG 9449 consistently gave low yields, making it an undesirable parent. For future research, it will also be necessary to focus on the performance of the crosses with regards to duration to maturity, yield and other related traits that are equally important to farmers. Identification of crosses among susceptible parents that showed high SCA effects indicated that GRD resistance could also be developed by crosses involving susceptible parents. As these susceptible parents are already popular among farmers, developing them could probably enhance adoption, hence, reduce GRD incidences in farmers' fields.

In general, molecular techniques could be used together with conventional breeding, in order to enhance the screening and the breeding for GRD resistance. Marker assisted selection has proved effective in crops where recessive genes are involved as in resistance for GRD (Nigam and Bock, 1990) and where there is a need for gene pyramiding (Pandey *et al*., 2012). Moreover, the detection of all the three viral agents of GRD needs molecular techniques such as reverse-transcription polymerase chain reaction (RT-PCR).

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