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GENETIC ANALYSIS OF RESISTANCE TO ROSETTE DISEASE OF GROUNDNUT (*Arachis hypogaea* L.)

By

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Groundnut rosette disease (GRD), transmitted naturally by aphids, *Aphis craccivora*, is the most destructive viral disease of groundnut (*Arachis hypogaea* L.) in Nigeria and causes serious yield losses to farmers. The narrow genetic base among groundnuts has impeded efficient utilization for development of host resistance to GRD. Studies were undertaken in Nigeria to: (i) ascertain farmers' knowledge of and preferences for rosette resistant genotypes; (ii) assess the genetic diversity among aphid and rosette resistant genotypes using microsatellite markers; (iii) exploit genotype x environment interaction towards improved selection efficiency to obtain high-yielding varieties; and, (iv) determine the mode of inheritance of resistance to groundnut rosette disease. A participatory rural appraisal (PRA) involving 90 farmers was conducted in two groundnut producing communities in Northern Nigeria. Early maturing genotypes and GRD resistance were the most important farmer preferred traits. Farmers ranked insect pests and inadequate rainfall as the most important causes of groundnut rosette disease. Majority of farmers across the study areas were doing nothing to avert the disease. Some farmers however rogue infected plants and use GRD resistant varieties when available. Genetic diversity and association of simple sequence repeat (SSR) markers with GRD resistance were detected in a set of 50 cultivated groundnut genotypes with different levels of resistance to GRD. Out of 170 bands amplified from 36 primers, 166 were polymorphic (97.65%). Each amplified 2 to 12 microsatellite loci, with an average of 4.74 loci per primer. The Polymorphic Information Content value of each marker ranged from 0.19 to 0.82. Average pairwise genetic distance among the 50 genotypes was 0.31. The largest distance was 0.51 (between ICGV – IS – 07812 and RS006F4B1 – 31) and the shortest distance was 0.05 between ICGV – IS – 07865 and ICGV – IS – 07864, all the four lines were GRD-resistant. Cluster analysis revealed seven clusters using disease reaction

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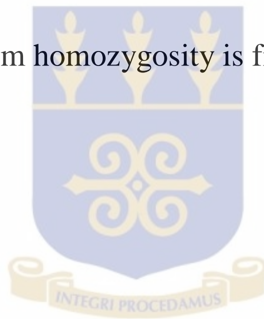
to GRD. The assessment of genetic diversity of GRD-resistant groundnut genotypes will help groundnut breeders to formulate crosses by choosing parents with different genetic backgrounds and in the development of gene-mapping populations with greater marker polymorphism. The 36 F_2 populations generated from 9 x 9 half diallel mating scheme were infested with veruliferous aphids, *Aphis craccivora* and scored three times fortnightly following inoculation. General combining ability (GCA) and specific combining ability (SCA) effects for GRD resistance were highly significant, indicating that both additive and non-additive gene effects govern the inheritance of GRD resistance. Low narrow sense heritability for Area Under Disease Progress Curve (29.29 %) along with high broad sense heritability (94.78 %) further highlight the influence of non-additive gene action in controlling resistance to GRD, suggesting effective selection of superior genotypes at advanced generations when maximum homozygosity is fixed.



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DEDICATION

To my mother, wife and children, brothers and sisters for their prayers and support throughout this study.

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LIST OF ABBREVIATIONS

100SKWT	One hundred sound kernel weight
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
ATA	Agricultural Transformation agenda
AUDPC	Area under disease progress curve
BW	Bacteria wilt
cDNA	Complementary Deoxyribo nucleic acid
CP	Coat protein
CTAB	Cetyl trimethyl ammonium bromide
DAPI	diamidino-2-phenylindole
DI	Aphid damage index
DNA	Deoxyribo nucleic acid
EST	Express sequence tag
F_1	First filial generation
F_2	Second filial generation
FAO	Food and Agricultural Organization
GCA	General combining ability
GD	Genetic distance
GEI	Genotype x environment interaction
GISH	Genomic *in situ* hybridization
GLM	Generalized linear model
GRAV	Groundnut rosette assistor virus

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GRD	Groundnut rosette disease
GRV	Groundnut rosette virus
HCL	Hydrochloric acid
He	Heterozygote percentage
HS	Half – sib
IC - RT – PCR	Immunocapture-reverse transcriptase-polymerase chain reaction
ICRISAT	International Crop Research Institute for Semi – Arid Tropics
ILRI	International Livestock Research Institute
IAP	Inoculation access period
IAR	Institute for Agricultural Research
LGA	Local Government Area
LRR	Leucine-rich repeats
LSD	Least significant difference
MAb	Monoclonal antibody
MAS	Marker assisted selection
MS	Mean square
MT	Metric ton
NAICPP	National Accelerated Industry Crop Production Program
NARP	National Agricultural Research Program
NBS	Nucleotide binding site
NBS – LRR	Nucleotide-binding-site leucine-rich repeat
NCBI	National Centre for Biotechnology Information
NID	Normally and independently distributed
NOPP	Number of pods per plant

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ORF	Open reading frame
PBNV	Peanut bud necrosis tospovirus
PCR	Polymerase chain reaction
PIC	Polymorphic information content
PRA	Participatory rural appraisal
PK	protein kinases
PWPT	Pod weight per plant
PWTON	Pod weight in tons per hectare
QTL	Quantitative trait loci
RAPD	Random amplified fragment length polymorphism
RGA	Resistant gene analogue
RNA	Ribose nucleic acid
RSI	Rank summation index
RT – PCR	Reverse transcriptase polymerase reaction
SAS	Statistical analysis software
Sat-RNA	Satellite – RNA
SCA	Specific combining ability
SCAR	Sequence characterized amplified region
SHP	Shelling percentage
SKWPT	Sound kernel weight per plant
SKWTTON	Sound kernel weight ton per hectare
SSA	Sub – Saharan Africa
SSR	Simple sequence repeat
Taq	*Thermos aquaticus*

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TAS – ELISA	Triple antibody sandwich - ELISA
TIR	Toll and interleukin-1 receptor
TSWV	Tomato Spotted Wilt Virus
UPGMA	Unweighted pair group method with arithmetic averaging.
USDA	United States Department of Agriculture
UV	Ultra violet
WACCI	West Africa Centre for Crop Improvement
WB	Wash buffer

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CHAPTER ONE

1 GENERAL INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food legume highly adapted to tropical and subtropical climates of the world. It is a key crop for small scale farmers especially in Africa and Asia where the crop serve as a valuable source of dietary protein, oil, and fodder for livestock. It contains 48-50% oil and 26-28% protein, and a rich source of dietary fibre, minerals, and vitamins (Janila *et al.,* 2013). In addition, groundnut has the ability to fix atmospheric nitrogen to the soil to help in the maintenance of soil fertility. This crop is cultivated annually on about 24.63 million hectares worldwide with annual production of 41.27 million tons in shell and productivity of about 1.85 t ha^{-1} (FAO, 2012). The global annual increase in production is 0.4% between 2009 and 2012 was attributed to both, an annual increase in yield by 0.1% and in area by 0.3% (Janila *et al.,* 2013). In West Africa, Nigeria is the largest producer of groundnuts with production of 3.07 million tons on about 2.4 million hectare (FAO, 2012). Groundnut is the fifth most important oilseed in the world in terms of volume of oil production and is widely grown in more than 100 countries of tropical, subtropical, and warm temperate regions of the globe (Upadhyaya *et al.,* 2012)

Despite the economic, social and cultural importance of groundnuts, its productivity is severely constrained by several biotic and abiotic factors. Drought is the major abiotic constraint affecting groundnut productivity and quality worldwide. Two thirds of the global production occurs in rain-fed regions of the semi-arid tropics where rainfall is generally erratic and insufficient, causing unpredictable drought stress (Reddy *et al.,* 2003). Groundnut yield and quality are severely constrained by a wide variety of fungal,

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bacterial, viral, and nematode pathogens. Among the fungal diseases, early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Cercosporidium personatum*) are most prevalent, and occur throughout groundnut growing regions (Liu *et al.,* 2013). Late leaf spot and rust (*Puccinia arachidis*) diseases often occur simultaneously and can cause 50–70% yield loss in India and some African countries (Khedikar *et al.,* 2010). Groundnut rosette disease (GRD) causes greater yield loss than any other viral disease affecting groundnut in the semi-arid tropics of the world (Naidu *et al*., 1999). The disease is caused by three interdependent viruses (causal agents) (see 2.3.3). It is the most destructive viral disease of groundnut in Africa (Naidu *et al*., 1999) resulting in sporadic yield loss of about 30% annually in farmers field. It is endemic to groundnut-growing regions of sub-Saharan Africa (SSA) and Madagascar (Yayock *et al*., 1976). The most serious yield losses were reported during 1975 when an epidemic in northern Nigeria destroyed approximately 0.7 million hectares of groundnut, with an estimated loss of US$250 million (Yayock *et al*., 1976). In Nigeria, farmers have identified groundnut rosette disease (GRD) as widespread and devastating, compelling some of them to abandon production in some areas. The disease is characterised by small, chlorotic, twisted and distorted leaflet with shortened internodes and thickened stems. Affected plants especially those infected at a young stage are severely stunted (Bock *et al.,* 1990). The disease also affects both quality of the haulm and the pod. This disease can cause up to 100% yield (Adu-Dapaah *et al.,* 2004; Waliyar, *et al.,* 2007).

Previous studies indicate that GRD could be managed by chemical control of the vector (Bock and Nigam, 1998). However, resource poor farmers seldom use chemical control measures due to lack of resources, labour constraints and cost (Dwivedi *et al.,* 2003; Adu-Dapaah *et al.,* 2004). The above factors coupled with health hazards associated with

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the use of insecticides make the use of host resistance the most cost effective and environmentally friendly alternative. Earlier attempts to control GRD using host resistance resulted in development of several resistant varieties across West Africa (Waliyar *et al.,* 2007). However, these varieties were only tolerant to one of the components of groundnut rosette virus (GRV) but susceptible to groundnut rosette assistor virus (GRAV) indicating lack of resistance to this component of the rosette complex that serves as a helper component for aphid transmission (Subrahmanyam *et al.,* 1998; Olorunju *et al.,* 2001). Furthermore, resistance to GRV is not immunity and seems to be overcome under high inoculum pressure and in adverse environmental conditions (Bock *et al.,* 1990). Therefore development of genotypes that are resistant to GRD virus is the most effective, economic and sustainable method of limiting virus inoculum build-up and spread of both the aphid and the virus.

The main objective of this study was to develop groundnut breeding lines with potential for resistance to groundnut rosette disease with acceptable farmers' preferred agronomic and yield traits

The specific objectives were to:

a. ascertain farmers' knowledge and preferences of rosette resistant genotypes;

b. assess molecular polymorphism among aphid and rosette resistant genotypes;

c. asses genotype x environment interaction towards improved selection efficiency to obtain high-yielding varieties; and

d. determine the mode of inheritance of resistance to groundnut rosette disease

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CHAPTER TWO

2 LITERATURE REVIEW

2.1 Groundnut (*Arachis hypogaea* L.)

Groundnut (*Arachis hypogaea* L.) is an annual or perennial plant that is distinguished from most other species by producing aerial flowers, but fruiting below the soil level. *Arachis* belongs to the family *Fabaceae,* tribe *Aeschynnomeneae,* sub-tribe *Stylanthinae*. *Arachis hypogaea* L. is the only domesticated species in the genus (Tillman and Stalker, 2009), and Krapovickas (1969) concluded that *A. hypogaea* var. *hypogaea* is likely the most ancient type because it has similar branching patterns as wild species, no compound florets, and a prostrate growth habit. The centre of origin for the genus *Arachis* is the Mato Grosso area of Brazil, but species evolved over a wide range of habitats in South America (Gregory *et al.,* 1980). Molecular data indicate that the centre of genetic variation also is the Mato Grosso area of Brazil to eastern Bolivia (Stalker *et al.,* 1994). Eighty species have been described (Krapovickas and Gregory, 1994; Valls and Simpson, 2005) which have been divided into nine sections based on morphology and cross-compatibility relationships. Smartt and Stalker (1982) proposed that the A and B genomes of section *Arachis* may be an A1 and A2 rather than being truly different based on chromosome pairing relationships. The species of different sections have overlapping distributions in many areas. Hybrids between species in different sections are difficult to produce and are usually sterile, while intrasectional hybrids can be fertile if they have similar genomic make-up (Stalker *et al.,* 1991). The species *A. hypogaea* has two subspecies *hypogaea* and *fastigiata* which are further divided into six botanical types *hypogaea* and *hirsute*, and *fastigiata, vulgaris, equatoriana* and *peruviana* botanical varieties. The subspecies are separated morphologically based on presence or absence of flowers on the main stem and regularly alternating vegetative and reproductive

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nodes on branches. The characteristics of these both botanical varieties shows that ***hypogaea*** has no floral axes or branches on main stem; alternating pairs of vegetative and reproductive axes on branches (alternate branching); inflorescence simple; vegetative branches moderate to profuse; primary branches longer than main stem; growth habit spreading, intermediate, or erect; usually two seeds per pod; pod beak not very prominent; seed size medium (runner market type) to large (Virginia market type); testa color generally tan (red, white, purple, or variegated also exist); cured seed dormancy moderate; maturity medium to late (Ntare *et al.,* 2007). The ***fastigiata*** has floral axes on main stem; irregular pattern of vegetative and productive branches with reproductive branches predominating on branches (sequential branching); inflorescence usually simple; vegetative branches sparse; primary branches shorter than main stem; growth habit upright; two to four seeds per pod; pod beak absent, slight, or prominent; seed size small to medium; testa color tan, red, white, yellow, purple, or variegated; cured seed dormancy little (Ntare *et al.,* 2007).

Cultivated groundnut has an allotetraploid genome (AABB, $2n = 4x = 40$). The low level of genetic variation within the cultivated gene pool and its polyploid nature limit the utilization of molecular markers to explore genome structure and facilitate genetic improvement. Nevertheless, a wealth of genetic diversity exists in diploid *Arachis* species ($2n = 2x = 20$), which represent a valuable gene pool for cultivated peanut improvement (Guo *et al.,* 2012). *Arachis hypogaea* is a recent allotetraploid (David *at al.,* 2012), most probably resulting from the hybridization of two wild species followed by natural chromosome duplication (Halward *et al.,* 1991; Young *et al*., 1996; Seijo *et al.,* 2007). The genome of *A. hypogaea* is large, being estimated at 2·8 Gb (Grilhuber, 2005) with a large repetitive fraction of approximately 64 % determined by DNA renaturation kinetics (Dhillon *et al.,* 1980)

Cytogenetic analyses in *A. hypogaea* have revealed two types of chromosomes: ten pairs of A-type chromosomes, with strongly 4', 6-diamidino-2-phenylindole (DAPI)-stained (and hence AT-rich) heterochromatin at the centromeres, including the smallest pair of all

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chromosomes (Robledo *et al.,* 2010), and another ten pairs of chromosomes with more weakly staining centromeric heterochromatin bands, designated B chromosomes (Seijo *et al.,* 2004; Robledo *et al.,* 2010). Studies comparing the chromosomal heterochromatic banding patterns together with evidence from positions of rDNA clusters (Robledo, *et al.,* 2010) and genomic *in situ* hybridization (GISH) (Seijo *et al.,* 2007) suggest that *A. hypogaea* A chromosomes are similar to those in the wild diploid *A. duranensis*, whilst the peanut B chromosomes are similar to those in the wild diploid *A. ipaënsis* Other evidence such as species geographic distribution (Robledo *et al.,*2010) and molecular phylogenies (Kochert *et al.,* 1996; Burow *et al.,* 2009; Moretzsohn *et al.,* 2013) corroborates that the most probable A and B genome donors to *A. hypogaea* are *A. duranensis* and *A. ipaënsis*.

2.2 Genetic Resources for Groundnut Rosette Disease

Over 3400 germplasms accessions evaluated for reaction to groundnut rosette disease at Chitedze Agricultural Research Station Lilongwe, Malawi, only 89 long duration Virginia types were identified as resistance to the disease. A high percentage (76%) of them originated in West Africa (Nigeria 39%, Burkina – Faso 13.9%, Cote d'voire 9.9%, Senegal 6.9%, Mali 30%, Gambia 2.0% and Equitoria Guinea 1.0%) and the rest were from Southern Africa (Subrahmanyam *et al.,* 1998). In addition, 11 short duration Spanish types were identified in Africa germplasm originating from West Africa especially Burkina – Faso. Out of a total of over 2000 accession evaluated in a preliminary screening trial in 1994/95 growing season at the same station, 15 were found to be rosette disease resistance (Subrahmanyam *et al.,* 1998). Additional resistant sources were sought from 2,301 accessions from different sources and 252 advanced breeding lines derived from crosses involving earlier identified sources of resistance to rosette (Olorunju *et al.,* 2001). The lines were evaluated in field screening trials using an infector row technique during 1996 and 1997 growing seasons at the Institute for

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Agricultural Research (IAR), Samaru, Nigeria (Olorunju *et al.,* 2001). Among the germplasm lines, 65 accessions were reported to show high levels of resistance while 134 breeding lines were resistant (Olorunju *et al.,* 2001).The report concluded that all rosette disease resistant lines were susceptible to groundnut rosette assistor virus (GRAV) and that the identified germplasm and breeding lines will contribute to an integrated management of groundnut rosette disease.

In addition to *A. hypogaea* collections, more than 1,300 *Arachis* species accessions have been collected (Stalker *et al.,* 2002), with about 800 *Arachis* entries being maintained by the USDA (Stalker and Simpson, 1995). Preservation of wild *Arachis* species is difficult for most accessions because the long, fragile pegs break during harvest and the soil must be sifted to recover pods. Stalker and Simpson (1995) reported that nearly 25% of the species from which seed can be obtained under nursery conditions have fewer than 50 seeds in storage. Additionally, at least 25% of the *Arachis* species accessions in germplasm nurseries are maintained vegetatively because of their poor seed production under cultivation. A large number of disease and insect resistance, and other agronomically useful traits are present in accessions of *Arachis* species, which makes them potentially valuable genetic resources for crop improvement (Stalker and Moss, 1987; Stalker and Simpson, 1995).

2.3 Groundnut Rosette Disease

Groundnut rosette disease is the most destructive viral disease of groundnut in Africa and can cause serious yield losses under favourable conditions. Groundnut production is constantly threatened by potential outbreaks of rosette disease epidemics. Improvement of host plant resistance to this disease provides the most effective control strategy (Olorunju *et al.,* 2001; Herselman *et al.,* 2004). ICRISAT and its partners have made significant contributions towards the understanding of the epidemiology of the disease and confirmation based on

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molecular diagnostic assays. This knowledge has provided a basis for development and utilization of groundnut cultivars with resistance to the groundnut rosette disease and impacted the lives of thousands of farmers in sub-Saharan Africa (Olorunju *et al.,* 2001).

2.3.1 Origin and occurrence of Groundnut Rosette Virus

Groundnut is the only known natural host of a complex of three agents of rosette disease (GRV sat-RNA and GRAV). It is likely that the viruses have evolved and survived in the host species native to Africa before the introduction of groundnut (Subrahmanyam *et al.,* 1998). After its introduction in the 16th century, groundnut became an accidental host of rosette disease representing a case of the "new – encounter" phenomenon (Buddenhagen and Ponti, 1984). It is possible that resistance came to Africa in some of the original introductions from South American centre (s) of origin and due to recurrent epidemics in West Africa (Olorunju *et al.,* 2001). It was concentrated to a greater degree by natural out crossing and recombination. Groundnut rosette disease was first reported in 1907 from Tanganyika (Waliyar *et al.*, 2007), now called Tanzania, and has since been reported in several other African countries south of Sahara. The major areas of disease occurrence include Burkina Faso, Ghana, Nigeria, Malawi, Mozambique and Uganda (Ntare *et al.*, 2002). Symptoms similar to groundnut rosette disease have been reported in some countries of Asia and South America, but diagnostic tests to unequivocally confirm the presence of the disease have not been conducted (Reddy, 1991). Thus, it is generally assumed that groundnut rosette disease is endemic to groundnut growing countries in Africa South of the Sahara and its off-shore islands such as Madagascar (Ntare *et al.*, 2002). During the course of evolution, as these genes did not possess any survival value in the absence of the disease, they may have been altered in the majority of genotypes (Reddy, 1991). One prerequisite for the loss of traits

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during 'evolution' is their simple inheritance and rosette resistance is governed by two independent major recessive genes (Nigam and Bock, 1990).

2.3.2 Symptoms of Groundnut Rosette Disease (GRD)

GRD occurs with two variant symptoms, chlorotic rosette and green rosette, with considerable variation within each type (Murant, 1989; Naidu *et al.,* 1999). Both forms of the disease cause plants to be severely stunted, with shortened internodes and reduced leaf size, resulting in a bushy appearance of plants (Naidu *et al.,* 1999). In chlorotic rosette, leaves are usually bright yellow with a few green islands and leaf lamina is curled. In the green rosette, leaves appear dark green, with light green to dark green mosaic (Naidu *et al*., 1999). Chlorotic rosette occurs throughout the Sub-Sahara Africa (SSA), whereas green rosette has been reported from East and West Africa (Naidu *et al*., 1999). A less common symptom variant, mosaic rosette, due to mixed infection of the plants by the Sat-RNA causing chlorotic variant and mottle variant, was reported from East Africa (Waliyar, *et al.,* 2007). Variability in Sat-RNA is mainly responsible for symptom variations (Murant and Kumar, 1990; Taliansky *et al.,* 1997). In addition, differences in genotypes, plant stage at infection, variable climatic conditions and mixed infections with other viruses also contribute to symptom variability under field conditions (Naidu *et al.,* 2007). Infection due to chlorotic or green rosette disease occurring in young plants (prior to flowering) usually results in 100% yield loss. In contrast, plants infected during later growth stages (between flowering and pod setting) may show symptoms only in some branches or parts of branches and yield loss depends on severity of infection (Naidu *et al.,* 2007). Infection after pod setting/maturation causes negligible effects on pod yield (Waliyar *et al*., 2007). An average annual yield loss due to GRD is estimated to be between 5 and 30% in non-epidemic years and epidemics often result in 100% yield loss (Waliyar *et al*., 2007). The deleterious impact of GRAV or GRV on

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host plant together with Sat-RNA in a synergistic manner is not known. Ansa *et al.* (1991) have reported that stunting is more severe in diseased groundnut plants containing all the three agents than in diseased groundnut plants containing only GRV and Sat-RNA. Other reports have suggested that GRAV or GRV infection alone in groundnut results in transient mottle symptoms with insignificant impact on the plant growth and yield (Taliansky *et al.,* 2000). These results have, however, been contradicted by Naidu and Kimmins (2007) who demonstrated that GRAV infection alone affects plant growth and contributes to significant yield losses in susceptible groundnut cultivars.

2.3.3 Causal agents of Groundnut Rosette Disease

Groundnut rosette disease is a viral disease, transmitted by an aphid, *Aphis craccivora* Koch (Insecta: *Homoptera*) in a persistent calculative manner (Waliyar, *et al.,* 2007). Three causal agents are involved in GRD etiology: *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and a Satellite-RNA (Sat-RNA) (Reddy *et al*., 1985; Murant *et al*., 1988; Taliansky *et al.,* 2000). The intimate interaction between GRAV, GRV, and sat-RNA is crucial to the development of the disease. GRV, a member of the genus *Umbravirus*, has a single-stranded, positive-sense RNA genome of 4,019 nt (Taliansky*et al.,* 1996) that contains four large open reading frames (ORFs). ORF 2 is a putative RNA-dependent RNA polymerase and is likely expressed as a fusion protein with the product of ORF1 by a –1 frameshift mechanism. The 3¢ ORFs (Bock *et al.,* 1990); Deom *et al.,* 2000) are almost completely overlapping. The protein encoded by ORF 3 was shown to be a *trans*-acting long-distance movement protein that can traffic nonrelated viral RNA systemically (Ryabov *et al.,* 1999), while analysis of the ORF 4 putative amino acid sequence suggests that it may be involved in cell-to-cell movement (Taliansky, *et al.,* 1996).

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GRAV is a member of the family *Luteoviridae*. It was first recognized as a component of groundnut rosette disease by Waliyar *et al.,* (2007). Casper *et al.* (1983) and Reddy *et al.* (1985) characterized the virus and identified it as a *luteovirus.* The virus replicates autonomously in the cytoplasm of phloem tissue. GRAV is transmitted by *A. craccivora* in a persistent manner, and experimentally by grafting, but not by mechanical sap inoculation, seed, and pollen or by contact between the plants (Taliansky *et al.,* 2000). Groundnut is the only known natural host of GRAV is reported to occur wherever GRD has been reported (Waliyar *et al*., 2007). The virus on its own causes symptomless infection or transient mottle, and can cause significant yield loss in susceptible groundnut cultivars (Naidu *et al*., 1999). There are no reports on occurrence of strains of GRAV causing the disease (Waliyar *et al*., 2007).

GRV belongs to the genus *Umbravirus*. It was first isolated and characterized by Reddy *et al.* (1985). The virus replicates autonomously in the cytoplasm of the infected tissues (Taliansky *et al.,* 2000). GRV on its own causes transient symptoms, but a Sat-RNA associated with GRV is responsible for rosette disease symptoms. GRV depends on GRAV for encapsulation of its RNA and transmission by *A. craccivora* in a persistent mode (Robinson *et al.,* 1999). Groundnut is the only known natural host, but several experimental hosts in the family *Chenopodiaceae* and *Solanaceae* have been reported (Murant *et al.,* 1998). No strains of GRV have been reported (Waliyar *et al*., 2007) and the virus is restricted to SSA and its offshore islands. GRV acts as a helper virus for replication of sat-RNA.

The Sat-RNA (sub-viral RNAs) of GRV belongs to the Subgroup-2 (small linear) satellite RNAs. It is a single-stranded, linear, non-segmented RNA of 895 to 903 nucleotides (Murant *et al*., 1988; Block *et al.,* 1994; Taliansky *et al.,* 2000). It totally depends on GRV for its replication, encapsulation and movement, both within and between the plants. Sat-RNA is responsible for rosette symptoms and plays a critical role in helper virus dependent

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transmission of GRV. Different variants of Sat-RNA have been shown to be responsible for different rosette symptoms, such as green rosette and chlorotic rosette (Murant and Kumar, 1990; Taliansky *et al.,* 1997). It is mechanically transmissible along with the GRV and is also transmitted by aphids in the presence of GRV and GRAV. A single aphid vector acquires GRAV, GRV, and sat-RNA; however, it does not always transmit the three disease agents together to a host plant (Naidu *et al.,* 1999). GRAV or GRV plus sat-RNA can be transmitted separately. However, for the disease to perpetuate in nature, all three agents must be transmitted by the aphid vector to a plant (Deom *et al.,* 2000). Phylogenetic analysis of the overlapping ORFs 3 and 4 shows that the GRV isolates cluster according to the geographic region from which they were isolated, indicating that Malawian GRV isolates are distinct from Nigerian GRV isolates (Deom *et al.,* 2000).

Phylogenetic analysis also showed clustering within the sat-RNA isolates according to country of origin, as well as within isolates from two distinct regions of Malawi Deom *et al.,* 2000). Because the GRAV CP sequence is highly conserved, independent of the geographic source of the GRAV isolates, the GRAV CP sequence represents the most likely candidate to use for pathogen-derived resistance in groundnut and may provide effective protection against groundnut rosette disease throughout SSA (Deom *et al.,* 2000).

Groundnut rosette disease has been reported in Angola, Burkina Faso, Côte d'Ivoire, Gambia, Ghana, Kenya, Madagascar, Malawi, Niger, Nigeria, Senegal, South Africa, Sudan, Swaziland, Tanzania, Uganda, and Zaire (now DR Congo) (Gibbons, 1977; Naidu *et al*., 1999). The agents of GRD have not been detected elsewhere in the world, despite the fact that groundnut is grown in more than 100 countries around the world (Upadhyaya *et al.,* 2012) and *A. craccivora* is found in almost all these groundnut growing regions (Naidu *et al*., 1999).

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2.3.4 Diagnosis of Groundnut Rosette Disease

Groundnut rosette disease can be tentatively diagnosed in the field based on the characteristic symptoms in groundnut or by mechanical inoculation onto a suitable indicator host such as *Chenopodium amaranticolor.* Symptom development on *C. amaranticolor* indicates the presence of GRV, but this test is not always reliable when the indicator plants are subjected to the widely fluctuating temperatures of SSA (Naidu *et al*., 1999). Improved diagnostic methods include a triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) for detection of GRAV (Rajeshwar *et al.,* 1987) and reverse transcription-polymerase chain reaction (RT-PCR) that allows detection of each of the three agents (Naidu *et al.,* 1999). The advantage of the RT-PCR method is that it concurrently detects all three groundnut rosette disease agents in plants and aphids (Naidu *et al.,* 1999).

2.3.5 Epidemiology of Groundnut Rosette Disease

The epidemiology of GRD is complex, involving interactions between and among two viruses and a Sat-RNA, the vector, and the host plant and environment (Naidu *et al.,* 1998). Since none of the causal agents is seed-borne, primary infection of crops depend on the survival of infected plants (virus sources) and vectors (aphids) (Naidu *et al.,* 1998). In the West, East and Southern Africa, *A. craccivora* maintains itself successfully through the dry and wet seasons on some crops and wild host plants. In Nigeria these hosts are found in the *Amaranthaceae, Asteraceae, Caesalpinaceae, Compositae*, *Euphorbiaceae, Fabaceae, Moringaceae, Nyctaginaceae, Papilionaceae, Portulacaceae, Solanaceae* and *Verbenaceae* (Alegbejo, 2000).

The epidemics of groundnut rosette virus disease that occurred in the main groundnut producing areas of Nigeria was speculated to be due to unusual combination of weather and groundnut sowing dates, which lead to massive build-up, early dispersal and survival of

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aphids in the wet season (Misari *et al.,*1988). Also intermittent wet and dry spells in the early part of the season, without heavy rainfall, were probably responsible for the development and successful dispersal of alate aphids (Yayock *et al.,* 1976).

2.3.6 Virus-vector interactions and transmission

Aphis craccivora, commonly known as the cowpea aphid is the principal vector involved in the transmission of all the GRD agents in a persistent and circulative manner (Waliyar, 2007). GRV and Sat-RNA must be packaged within the GRAV coat protein to be aphid transmissible. Studies have shown that all the GRAV particles whether they contain GRAV RNA or GRV RNA and Sat-RNA are acquired by the aphid vector from phloem sap in 4h and 8h acquisition access feeding for chlorotic and green rosette, respectively (Misari *et al.,* 1988). Then, there is a latent period of 26h 40min and 38h 40min for chlorotic and green rosette, respectively, and the inoculation access feeding period of 10min for both forms (Misari *et al.,* 1988). Once acquired, aphid can transmit virus particles for up to two weeks and beyond. All stages of the aphid can acquire and transmit the disease agents. Transmission rates of 26-31% have been reported with one and two aphids per plant, and 49% with five aphids per plant (Misari *et al.,* 1988).

Aphid vector does not always transmit all the three agents together (Naidu *et al.,* 1999). Under natural conditions, some GRD-affected plants (GRV and Sat-RNA positive) were found to be free from GRAV, and GRAV was detected in some non-symptomatic plants (no GRV and Sat-RNA) (Naidu *et al.,* 1999). This situation was due to difference in inoculation feeding behaviour of the vector leading to transmission of (i) all the three agents together, (ii) only GRAV or (iii) GRV and Sat-RNA, as demonstrated by the electrical penetration graph (EPG) studies of aphid stylet activities (Naidu *et al.,* 1999). This showed that during short inoculation feeding (test probe or stylet pathway phase) vector aphids probe groundnut leaves

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without reaching the phloem, transmitting only GRV and Sat-RNA, which multiply in the epidermal and mesophyll cells. Even if GRAV particles are deposited in the mesophyll cells, they cannot replicate, as they can replicate only in the phloem cells (Naidu *et al.,* 1999). However, vector aphids can transmit GRAV, and GRV, Sat-RNA when the stylets penetrate sieve elements (salivation phase) of the phloem cells. Therefore, the success of transmitting all the three agents together is high when inoculation feeding period is longer or increasing the number of aphids per plant (Misari *et al.,* 1988). Vector aphids fail to acquire or transmit GRV and Sat-RNA from diseased plants lacking GRAV and such plants become dead-end sources. However, if such plants receive GRAV later due to vector feeding, the plants again serve as source of inoculum (Waliyar *et al*., 2007).

2.4 Progress made in combating Groundnut Rosette Disease in Nigeria

In Nigeria, research on the development of groundnut cultivars with resistance to rosette was initiated in 1986 by Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria in collaboration with ICRISAT (Olorunju *et al.,* 1992). Concerted efforts were made to improve resistance to groundnut rosette viruses in the existing locally grown varieties. These earlier attempts resulted in the development of a number of rosette resistant varieties such as SAMNUT 10 (RMP 12), SAMNUT 11 (RMP 91), SAMNUT 16 (M554.76), SAMNUT 20 (M412.80I), and SAMNUT 21 (MDR-8-19).SAMNUT 22 and SAMNUT 23 and SAMNUT 24. Popularization of these varieties and availability of seed to farmers were made possible by the then Federal government projects (NAICPP and NARP), collaborative work between IAR and ICRISAT, ILRI and GGP/CFC using farmer participatory approach. There was a dramatic increase in production from 1.4 million MT to over 2 million MT from 1994 to 2003 in Nigeria because of the well-coordinated collaborative work between NARJs and IARCs in combating the groundnut virus disease in Africa (Ntare *et al.,* 2002). Despite these

attempts, sporadic occurrences of the disease of about 30 % from year to year and among fields were still observed in farmer's field (Waliyar *et al*., 2007). Control strategies adopted by most farmers have traditionally emphasized on vector control mainly by pesticide and cultural practices such as manipulating sowing dates and plant density (Subrahmanyam *et al.,* 1998). Chemical control methods have been only partially effective, since aphid populations can reach very high numbers, leading to intensive pesticide application in an attempt to eliminate the vector, and when accompanied with drought may lead to epidemics. Furthermore, there are concerns that the vector may develop pesticide resistance and the intense application may have deleterious effect on the environment. Therefore the development of genotypes that are resistant to both aphids and the virus is the most effective, economic and sustainable method of limiting virus inoculums build-up (Herselman *et al.,*2004).

2.5 Genetics of Resistance to Groundnut Rosette Disease

Breeding for resistance to groundnut rosette disease demands a good knowledge of the breeding methodologies as well as a good understanding of the disease and its causal organisms. Identification of sources of resistance and its efficient utilization require an understanding of the genetic control of resistance and knowledge of the amount of genetic variability available for selection. Determining the suitable parents to use for development of resistant genotype is particularly important. Early genetic studies on groundnut rosette disease showed that resistance was effective against GRV and its sat-RNA and was governed by 2 independent recessive genes (de Berchoux, 1960). He also stated that resistant lines were not immune and that individual plants could become infected when subjected to inoculation by massive number of aphids. This resistance was reported to operate equally against both chlorotic rosette (de Berchoux, 1960) and green rosette (Harkness, 1977). He attributed the

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low recovery of resistant plants from Virginia x Spanish crosses to heavy inoculum pressure at early stage of growth and suggested occurrence resistance breakdown from generation to generation. Nigam and Bock (1990) studied the inheritance of resistance to chlorotic rosette (GRV and its sat-RNA) in crosses involving botanical varieties of groundnut in Malawi and confirmed the findings of de Berchoux (1960) of two recessive genes governing the resistance in all the backgrounds. In resistant plants, the presence of GRAV was detected. Gene conferring resistance to GRV and its sat-RNA did not confer resistance to GRAV (Bock and Nigam, 1988; Bock *et al.,* 1990). Similar findings on the inheritance of resistance to green rosette using mixed infection in the field (GRV + and its sat-RNA + GRAV) and single GRV infection under greenhouse conditions were reported from Nigeria by Olorunju *et al.* (1992). There was one exception from RMP12 x M124.781 crosses, where in F_2 generation, the plant segregated into 1 susceptible: 3 resistant, suggesting dominant gene action governing rosette resistance (Olorunju *et al.,* 1992).

Amin (1985) reported high level of resistance to *A. cracivora* in some crosses under greenhouse conditions. Progenies of *A. chacoense* and *A. villas* interspecific derivatives with cultivated groundnut also showed high resistance to *A. crracivora*. Resistance to aphid vector identified in cultivated groundnut ICG 5240 [EC36892] (Padgham *et al.,* 1990) is reported to be controlled by single a recessive gene (van de Merwe, 2001; Herselman *et al.,* 2004)

2.6 Combining Ability for traits in Groundnut

Cultivar improvement for yield and stress resistance requires availability of genetic resources that could act as sources of genes conferring the desire traits that could be introgressed into the present cultivars (Sitaresmi *et al.,* 2010; Nsabiyera *et al.,* 2013). Gene introgression could be achieved through a combination of desired traits into target genotypes using recombination breeding under local conditions. This is essential to the generation of genetic diversity and

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fixing genes in the progeny (Marame *et al.,* 2009; Zecevic *et al.,* 2011). This, however, involves a lengthy and costly process of identifying and combining superior parents into superior hybrids (Rego *et al.,* 2009). Diallel mating systems provide plant breeders with estimates for general combining ability (GCA) and specific combining ability (SCA). The GCA effects reflect the parent's genetic ability to influence all of its progeny for a specific trait, which is an expression of additive genetic effects (Griffing, 1956). The SCA effects represent non-additive genetic effects such as intra-allelic (dominance) or inter-allelic (epistasis) interactions or multiplicative gene action, which can be viewed as a departure from performance, can be predicted in simple additive models (Henderson, 1952; Griffing, 1956). Breeders have largely used the diallel mating scheme to estimate the potential value of genotypes *per se*, their combining ability effects for resistance to foliar disease in groundnut from a fixed or randomly chosen set of parental lines (Adamu *et al.,*2008). The studies of combining ability provide a guideline for selecting elite parents or combiners which may later be hybridized to accumulate fixable genes through selection. Both SCA and GCA have been reported to be significant in conditioning resistance to foliar disease in groundnut (Vishnuvardhan *et al.,* 2011). Pensuk *et al.* (2002) from a 6 x 6 diallel cross of resistance to peanut bud necrosis tospovirus (PBNV) reported highly significant GCA effects for PBNV incidence in F_2 and F_3 generations. SCA was also significant, but the relative contribution to variation among crosses was much less than those of GCA effects. In an earlier study, Anderson *et al.* (1990) reported significant GCA and SCA effects for peanut stripe virus (PStV) and rust incidence from a study of diallel in groundnut. Makne (1992) and Dwivedi *et al.* (1994) found significant SCA for seed weight per plant, number of pods per plant and pod weight per plant and concluded that these traits are controlled by non-additive gene action. Adamu *et al.* (2008) recommended that selection for pod yield and resistance to groundnut rosette disease should be done among progenies from RMP12/ICGV87281 and

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RMP12/ICGV87018 since they depicted best general combiners for these traits. He also suggested that the significance of SCA mean squares for some of the traits is an indication that non-additive gene effects played an important role in their inheritance. SCA mean square was much smaller than GCA mean squares, which indicates that additive genetic variance was more important than non-additive genetic variance for these traits. Studies on combining ability in F_2 and F_3 crosses of Spanish and Virginia groundnut have shown that GCA and SCA were significant for almost all traits (Ali, et *al.,* 2001) with preponderance of SCA which implies that selection for pod yield would be more effective in later generations. However, greater magnitude of GCA effect over SCA have been reported (Dwivedi *et al.,* 1998) indicating the importance of additive genetic variance over non-additive variance. From the available reports it is evident that information on the precise nature of genetic control of GRD in groundnut is still lacking. Appropriate experimental design that includes the GRD resistant lines should provide additional information on the gene action involved in the expression of resistance. The knowledge on combining ability and type of gene action responsible for regulation of expression of GRD would certainly help in planning appropriate breeding strategies.

2.7 Genotype x Environment Interaction (GEI) in groundnut

To design an appropriate breeding program, it is important to know the proportion of phenotypic variation of a trait that is heritable (Kearsey and Pooni, 1996), since the efficiency of a selection program is mainly dependent on the magnitude of genetic variation and heritability of a trait (Falconer and Mackay, 1996). Apart from high haulm and kernel yield in groundnut, adaptation to specific environments has also been a major breeding goal for groundnut breeders. GEI is a major problem involving quantitative traits, complicates the interpretation of genetic experiments, makes predictions difficult, and reduces the efficiency

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of selection. For quantitative traits, this interaction can be caused by genotypic rank change or by changes in the absolute differences between genotypes without rank change (Cooper and DeLacy, 1994). Therefore, knowledge about the magnitude of GEI is important to develop cultivars with higher yields and stable performance over a wide range of environmental conditions. Studies and interpretations of GEI range from simple analysis of variance to more specific analyses of genotype performance (Amini *et al.,* 2013). The existence of GEI in groundnut breeding has been reported by Bentur *et al.* (2004); Senapathi *et al.* (2004) and Hariprasanna *et al.* (2008). The expectation has been the identification of suitable genotypes having maximum GEI with moderate level of resistance or susceptible to disease would be of immense benefit to improve the production of groundnut (Mothilal *et al.,* 2010). They further reported significant linear component of GEI for kernel yield and concluded that genotypes differed for their linear response to fluctuations in environments. The magnitude of variation due to environment for kernel yield was higher than G x E (linear) for the same trait which depicted the major part of the total variation and was considered a linear function of environment only (Mothilal *et al.,* 2010). In an earlier study of G x E interaction for PBNV, Buiel *et al.* (1995) reported that Genotype x environment interaction variance was significant but small. The field resistance of the genotypes studied was equally effective in all environments. Selection in any of these environments may be possible, but is more effective in environments which are favorable for disease development. Yan and Kang (2003) described the different types of G x E interactions and highlighted the implications of these in plant breeding and crop production. Crossover interactions (change in rankings of varieties across environments) are of greatest interest to breeders as these directly affect genotype selection in specific environments. Consequently, promising selections in one environment may perform poorly in another. Such crossover interactions often compel breeders to implement multiple selection programs within industries based on the

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homogeneity of regions, thereby utilizing greater resources. Ignoring significant G x E in favour of resource savings can lead to reduced genetic gains from selection (Ramburan *et al.,* 2011). Inaccurate characterization of genotype adaptability may lead to poor productivity in environments that interact negatively with specific genotypes and this has implications on industry sustainability. With regards to genetic gains from selection, large G x E interactions, as components of total phenotypic variance, affect heritability (proportion of total phenotypic variance that is due to genetic variance) negatively. The larger the G x E interaction component, the smaller the heritability estimate; thus, progress from selection would be reduced as well (Yan and Kang, 2003).

2.8 Genetic Diversity in Cultivated Groundnut Based on Molecular Markers

DNA-based markers provide a reliable means for estimating the genetic relationships among genotypes or taxonomic groups as compared to the morphological markers (Sajib *et al.,* 2012). Precise understanding of the degree of genetic relationships among genotypes, botanical varieties of peanut, and *Arachis* species could provide insights into the domestication and evolution of this crop. Furthermore, it would have a valuable impact on peanut improvement, through identification of appropriate parents, to ensure a broad genetic base by inter-variety and inter-species crosses. DNA-markers, such as, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR) have been used for cultivar discrimination and to study the botanical relationships among the cultivated peanut varieties (Subramanian, *et al.,* 2000; Raina *et al.,* 2001; He and Prakash, 2001; He *et al.,* 2003). AFLP and SSR techniques can be used to detect DNA polymorphism in the cultivated peanut (He and Prakash, 1997; Tang *et al.,* 2007). AFLP and SSR are two powerful DNA fingerprinting techniques. A number of loci can be analysed in an experiment and there is a higher reproducibility of banding patterns by

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AFLP. SSR markers have several advantages over other molecular markers for their codominant inheritance, large number of alleles per locus, and abundance in genomes (Sajib *et al.,* 2012). These characteristics have promoted the application of SSR as molecular markers in fingerprinting (Wang and Du, 2005), genome mapping (Yin *et al.,* 2006), phylogenetic and genetic relationship studies (Duan *et al.,* 2006), and marker assisted breeding (Yi *et al.,* 2004) in many crops. However, there are few reports concerning SSR and AFLP for evaluation of genetic diversity and relationships among the *Arachis* species, and much remains to be discovered (Tang *et al.,* 2007). He and Prakash (1997) reported considerable DNA polymorphism in *A. hypogaea* revealed by the AFLP approach, this assay has been used for molecular diversity studies in peanut by several researchers (Dwivedi *et al.,* 2001; He *et al.,* 2003; Jiang *et al.,* 2007). Comparing SSR and AFLP primers Jiang *et al.* (2007) reported that SSR primers amplified 91 polymorphic loci in total with an average of 3.14 alleles per primer, and the AFLP primers amplified 72 polymorphic loci in total with an average of 2.25 alleles per primer. Four SSR primers (14H06, 7G02, 3A8, 16C6) and one AFLP primer (P1M62) were found to be most efficient in detecting diversity. They also noted that genetic distance between pairs of Bacteria Wilt (BW) genotypes ranged from 0.12 to 0.94 with an average of 0.53 in the SSR data and from 0.06 to 0.57 with an average of 0.25 in the AFLP data. The SSR-based estimates of the genetic distance were generally larger than that based on the AFLP data. The genotypes belonging to subsp. *fastigiata* possessed wider diversity than that of subsp. *hypogaea*. The clustering of genotypes based on the SSR and AFLP data were similar but the SSR clustering was more consistent with morphological classification of *A. hypogaea* (Jiang *et al.,* 2007). Optimum diverse genotypes of both subsp. *hypogaea* and subsp. *fastigiata* can be recommended based on this analysis for developing mapping populations and breeding for high yielding and resistant cultivars.

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In a study of Phylogenetic Relationships in Genus *Arachis* based on SSR and AFLP markers, Tang *et al.* (2008) found genetic distance detected by the SSR markers ranged from 0.09 to 0.95, and the mean was 0.73; and the genetic distance detected by the AFLP markers ranged from 0.01 to 0.79 with an average of 0.42. They also reported that in all the tested BW resistant peanut genotypes, SSR primer pairs were multilocus ones, and the amplified fragments per SSR marker in each peanut genome ranged from 2 to 15 with a mean of 4.77. The peanut cultivars were closely related to each other, and shared a large number of SSR and AFLP fragments. Jiang *et al.* (2007) partitioned the BW resistant peanut genotypes into two main groups and four subgroups at the molecular level, and that *A. duranensis* is one of the wild ancestors of *A. hypogaea.* The lowest genetic variation was detected between *A. cardenasii* and *A. batizocoi,* and the highest was detected between *A. pintoi* and the species in the section *Arachis* (Tang *et al.,* 2007). Distinct clustering pattern of wild and cultivated genotypes was also reported in genetic diversity studies through SSR and EST – derived SRR maker systems (Moretzshon *et al.,*2005; Kottapalli *et al.,* 2007; Koppolu *et al.,* 2010). In a related study using single nucleotide polymorphism–based genetic diversity in the reference set of peanut (*Arachis* spp.) Khera *et al.* (2013) reported high level of diversity between wild and cultivated peanut and affirmed that grouping pattern exhibited discrete clustering of genotypes based on subspecies, botanical varieties and genome types. Mean genetic similarity between genotype pairs was found to be 0.13 and maximum between ICG 8200 and ICG 8206 at 0.4. They also reported the average major alleles was maximum in AA genome (0.81) and minimum in EE genome (0.56) while for BB and AABB genomes, it was found to be 0.71 and 0.63, respectively. The average PIC ranged from 0.21 (AA genome) to 0.38 (EE genome) while BB and AABB genomes recorded 0.31 and 0.32 respectively (Khera *et al.,* 2013). The narrow genetic base variation observed in cultivated tetraploid groundnut may be attributed to its very recent origin in its evolutionary time as compared to other crops

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and is a serious genetic bottle neck towards modern breeding effort (Khera *et al.,* 2013). Hence tapping the maximum genetic variation in the primary gene pool is vital to groundnut improvement. From the literature reviewed so far, the genetic background of parents in breeding programs is still narrow, which may have impeded the progress of breeding (Mace *et al.,* 2007). Hence, a better understanding of the genetic diversity amongst the available GRD resistant germplasm is a prerequisite for further efficient improvement of GRD resistance.

2.9 Participatory breeding and varietal selection in groundnut

Over the three past decades groundnut production in Nigeria has declined in importance both as food and cash crop for household and National economies (Ndjeunga *et al..,* 2010). Prospects for regaining production and market share lie in the adoption of improved varieties and crop management techniques that will significantly increase productivity and the improvement of quality standards. The key factors that constrain farmers' adoption of technologies are inappropriateness of the technologies, unavailability of required inputs and farmers' socio-economic conditions (KARI, 1996; Martins *et al.,* 2002). Technologies that do not meet farmers' preferences, objectives and conditions are less likely to be adopted (Upton, 1987). During priority setting within the KARI-Kisii mandate region, groundnut was ranked fourth in importance for arid and semi-arid areas (Andima *et al.,* 2006). Reasons for this included, lack of improved high yielding disease tolerant varieties, organized seed production system, poor agronomic practices, pests and diseases, low producer prices, lack of markets and market information and low adoption of developed technologies (Rachier *et al.,* 2006, Okoko *et al.,* 1998). The matrix ranking of the groundnut varieties conducted by 20 farmers in Suba District of Kenya indicated ICGV-SM 99568 was ranked first because it is early maturing, it is easy to shell, has tasty big seeds with good colour that has high market demand

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(Okoko *et al.,* 2010). They further concluded that although ICGV-SM 12991 had the highest yield it was ranked second because of its small seed size leading to low marketability. Homabay local and ICGVSM 90704 were ranked last because of their poor germination, growth vigour, late maturing. In a PRA study conducted on Bambara groundnut Alhassan and Egbe (2013) indicated that more males (52.91%) than females (47.08%) were engaged in the production of bambara groundnut. This contrasted the works of Gibbon and Pain (1985) and Mkandawire and Sibuga (2002).These reports indicated that bambara groundnut production was done mainly by female subsistence farmers. Many men might have gone into the production of bambara groundnut because the crop fetches higher income now than it did previously (Tanimu and Aliyu, 1995). Alhassan and Egbe (2013) also indicated that 83.33% of farmers in the study area planted Bambara groundnut on ridges and 65.83% of farmers intercropped cowpea and cassava. The growing of crops in mixed cropping is consistent with the goal of food security (Alhassan and Egbe, 2013). However, apart from participatory variety selection of tropical legume II project by ICRISAT, there is no information at the Institute for Agricultural Research on PRA to assess farmers' preferences with the view of involving them in groundnut improvement program. Thus, there is need for concerted efforts to study the problems through research and social motivation for improving sustainability of cropping system and for meeting the challenges of low adoption of improved varieties.

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CHAPTER THREE

3 FARMERS' PERCEPTION OF PRODUCTION CONSTRAINTS AND PREFERRED TRAITS FOR RESISTANT GROUNDNUT ROSETTE VARIETIES

3.1 Introduction

The groundnut improvement programme at the Institute for Agricultural Research, Samaru is currently developing high yielding, GRD resistant groundnut varieties that are acceptable to farmers using the participatory variety selection approach. However, this conventional breeding procedure has been cited to be more formal concentrating on researchers' objectives of solving problems without considering farmers' preferences and opinion (Assefa *et al.,* 2005). The consequences of neglecting farmers in setting up research and policy agenda are well documented (Gupta and Lagoke, 1999; Bänziger and Cooper, 2001; Snapp, 2002; Danial, 2003; Kamara *et al.*, 2006; Derera *et al.*, 2006; Ceccarelli and Grando, 2007). It is important for plant breeders to understand how and why farmers choose varieties of their crops, because this will ultimately determine whether a new or improved variety will be useful. Understanding farmers' choice and selection practices, their knowledge and goals underlying them, and the similarities and differences with plant breeders provides a means for the two groups to work together more effectively. This understanding and collaboration is critical for supporting long-term global food security (Makanda *et al.,* 2009). In order to ensure sustainable groundnut production, there is a need for combining farmers' and researchers' objectives. These combinations have significantly contributed to agricultural development (Bucheyeki *et al.*, 2011). Gathering groundnut production constraints and farmers' varietal preferences with the view to incorporating them into breeding objective was expected to contribute to increased rate of adoption, improved food security, and reduced poverty. The objectives of the participatory rural appraisal (PRA) were to:

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a. identify groundnut production constraints.

b. assess farmers' knowledge of groundnut rosette disease

c. appraise farmers' preference for rosette resistant varieties.

3.2 Materials and Methods

3.2.1 Description of the study areas

The study was conducted in Batsari Local Government Area (LGA) of Katsina State (12°45′10N 7° 14′31). Batsari LGA occupies a land area of 1,107 km^2 with population of 208, 978 people (Censor, 2006). Major Socio – economic activities of the inhabitants of this area are farming and livestock keeping. Majority of them are Hausa and Hausa – Fulani. The second location was Nasarawa – eggon LGA of Nasarwa state (8° 32′N, 8^0 17″ 58. 78′ E,). The LGA occupy land mass of 1,208 km^2 with population of 149, 129 inhabitants (Census, 2006). Major Socio – economic activities here are farming and trading. Majority of them are Eggon and Mada. Maps of these areas are shown in Figure 3.1. These areas represent the groundnut growing regions in sudano – Sahelian and Northern guinea savannah of Nigeria, respectively. These areas are characterised by mono-modal type of rainfall that falls between June and October in Batsari and April to October in Nasarawa eggon.

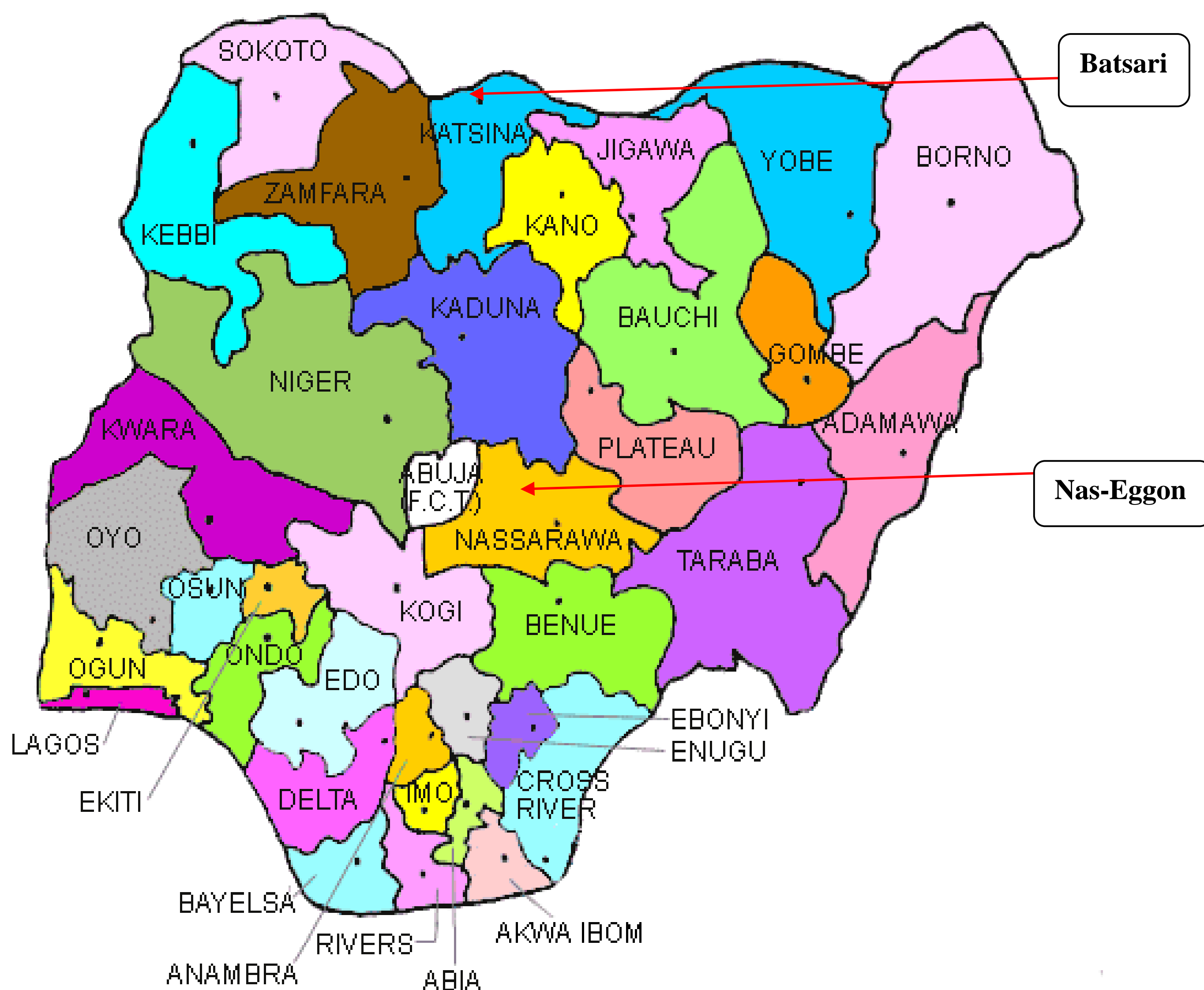

Figure 3. 1:Map of Nigeria showing Batsari (Katsina state) and Nasrawa-Eggon (Nasarawa state)

3.2.2 Farmer survey and data analysis

Preliminary visits were made to the two locations to discuss with farmers prior to the study. First visit was made on 15th and16th January 2010 to Bastari in Kastina state which was followed by visit to Nasarawa Eggon (Nasarawa state) on the 21st and 22nd February 2010. The visit provided opportunities for informal interactions with groundnut farmers and processors. During these initial visits, secondary data on groundnut production and

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constraints were obtained from local extension officers. In addition, enumerators, who spoke the local languages, were identified, trained and made to pre-test the questionnaires. The farmers within villages were randomly selected at different strata. In order to obtain information on specific issues covered under the PRA, a formal survey was then conducted during January to March 2011 using a structured questionnaire, and other participatory rural appraisal tools including focus group discussions and observations made during transects walks across the areas.

In both Batsari, and Nasarawa – eggon, 50 farmers were interviewed. Demographic information such as general household structure, education level, wealth status (as judged by property owned), cropping enterprises, and production constraints were obtained using the structured questionnaire (Appendix 1). Group discussions were done on completion of the questionnaire interviews to confirm data obtained and to solicit new information that was not captured during the formal process.

Both qualitative and quantitative data were subjected to statistical analysis using the statistical Package for Social Science (SPSS) version 15 (SPSS Inc., Chicago IL). Frequencies were determined for quality questions. Associations and t – test for comparison were determined for quantitative variables. Graphs were used to present results. Preferred and unfavoured traits, as well as the importance and severity of rosette diseases were ranked to highlight farmers' perceptions.

3.3 Results

3.3.1 Household and demographic information

Majority (36 %) of the farmers in Batsari were above 40 years while those of Nasarawa – eggon were younger (61 %) and ranged between 20 – 35 years (Table 3.1) and majority of them were married. The sex ratios of the household were not significantly different ($p \leq 0.05$)

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in the two districts (t = 6.31, P =0.33). The number of farmers who had tertiary education or belonged to an association did not differ in the two districts. However, the age of farmers was significantly different and so were married and single farmers across the districts. The numbers of farmers with primary and secondary education were significantly different (Table 3.1). In both areas, at least 80% of the farmers had lived in a village for more than 10 years practicing small – scale farming and had acquired experience in farming. Ninety – eight per cent of the farmers at Batsari village were males and in Nasarawa – eggon 7% were female. The majority of farmers in Batsari village were illiterate while 50% of the farmers in Nasarawa – eggon were literate. A proportion of the farmers (8%) in Batsari and 32 % in Nasarawa – eggon had primary education (Table 3.1). Farmers belonging to associations were 12.8% (Batsari) and 9.1% in Nasarawa eggon. Farmers' experience (farming for more than 10 years) significantly ($P < 0.05$) correlated with level of education (r = 0.51517**) and level of awareness of rosette diseases (r = 0.45602*) (Table 3.2)

Table 3. 1:Farmer and Household information for Batsari and Nasarawa – eggon LGA in Nigeria for the 2010 growing season

Variable	Local Government Area			t – value	Probability
	Batsari	Nasarawa	Average		
Gender of household head	(%)	(%)	(%)	6.31	0.50
Male head	98	93	95		
Female head	2	7	5		
Age of farmers				1.90	0.49
20 – 25	0	18	9		
26 – 30	0	23	12		
31 – 35	0	20	10		
36 – 40	16	14	15		
41 – 45	36	14	25		
46 – 50	24	7	16		
51 – 55	16	5	11		
> 56	8	0	4		
Marital status				2.35	0.03
Married	100	88.6	94**		
Single	0	6.8	3		
Widowed	0	1	1		
Divorce	0	1	1		
Over 10 year experience in farming	94	84	89		
Level of education acquired				2.35	0.50
Illiterate	88	50	69		
Primary	8	32	20		
Secondary	4	16	10		
Tertiary	0	2	1		
Membership of association					
Member	12.8	9.1	11	6.31	0.33
Non member	76.2	90.9	84		

* and ** significant and $P < 0.05$ and $P < 0.01$, respectively

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Table 3. 2: Pair wise correlation of some farmers' level of awareness of the groundnut rosette disease

	Gender	Age	Marital Status	Famer Experience	Membership of association	Level of education	Rosette disease awareness
Gender		0.202	0.992**	0.462	-0.897**	0.998**	0.999**
Age			0.321	0.962**	-0.615*	0.262	0.196
Marital Status				0.568	-0.945**	0.998*	0.992*
Famer Experience					-0.807**	0.515**	0.456*
Membership of association						-0.922	-0.894**
Level of education							0.562*

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Maize, sorghum, groundnut, yam, cowpea and sesame were mentioned as the most important crops grown for cash income and sources of food security, while cassava, tomatoes, water melon, onion and pepper were less cultivated by the sampled farmers. Groundnut was ranked as the most important cash crop in both Batsari and Nasarawa – eggon areas (98% and 82%, respectively). While sesame was ranked as the second most important crop in Batsari (83.7%), yam was ranked the second in Nasasrawa – eggon (86.4%) (Table 3.3). Ranking of groundnut as the most important cash crop showed farmers' strong interest in the crop. This was probably because of the increasing demand for cultivation to target markets, as well as alleviating poverty and food shortage at household level. The results of the study showed that farmers produced groundnut in association with other crops especially maize and sorghum. Groundnut is intercropped by most farmers and with only few farmers growing it as a sole crop.

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Table 3. 3: Pair-wise ranking of major crops grown by farmers in Batsari and Nasarawa – eggon in 2011

Crop	Districts				Average (%)
	Batsari (%)		Nasarawa – eggon (%)		
	Cash crop	Food crop	Cash crop	Food crop	
Rainy season					
Groundnut	98 **(1)**	40 (5)	86 (2)	43 (7)	92.0
Groundnut var:	SAMNUT 21 and 23		Maiyado" RRB		
Cowpea	75.5 (3)	30 (6)	64.6 (5)	51 (5)	70.1
Maize	63.4 (4)	100 **(1)**	75.8 (3)	93 (1)	59.6
Millet	75.3 (3)	98 (2)	53.7 (7)	23 (8)	64.5
Sesame	83.7 (2)	32 (7)	89.4 (1)	47 (6)	86.6
Yam	—		86.4 (2)	82 (3)	86.4
Sorghum	54.5 (5)	74 (3)	67.3 (4)	87 (2)	50.9
Cassava	25.2 (6)	51(4)	63.0 (6)	23 (8)	44.1
Rice	—		58.9 (7)	58 (4)	58.9
Dry season					
Tomatoes	56.8		56.5		56.7
Onion	78.0		53.6		65.8
Water – melon	78.5		45.3		61.9
Pepper	75.0		34.3		54.7

The figures are percentage responses, number in parenthesis = rank and — = crop not reported

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3.3.2 Characteristics of preferred groundnut varieties in Batsari and Nasarawa – eggon districts

The choice of a groundnut variety in rural areas is determined by some characteristics of the plant and the environment. Although farmers' criteria in choosing varieties were similar across the two study areas, there were marked differences in the characteristics of the varieties preferred by farmers. These differences varied from site to site (Fig. 3.3). Most of the major traits preferred were those associated with yield, market value and those that enabled the crop to escape or produce yield, even when attacked by pests and diseases. Pest and disease tolerance and high oil content (Fig. 3.3) were considered to be the most important desired characteristics in Batsari and Nasarwa – eggon with 21% and 28% of the respondents respectively while early maturity was most important in Batsari. The other important traits preferred by famers in both locations were the pod yield. In Batsari, 14% of the interviewed farmers considered haulm to be an important trait. This is not quite an important trait in Nasarawa – eggon as only 6% use haulm.

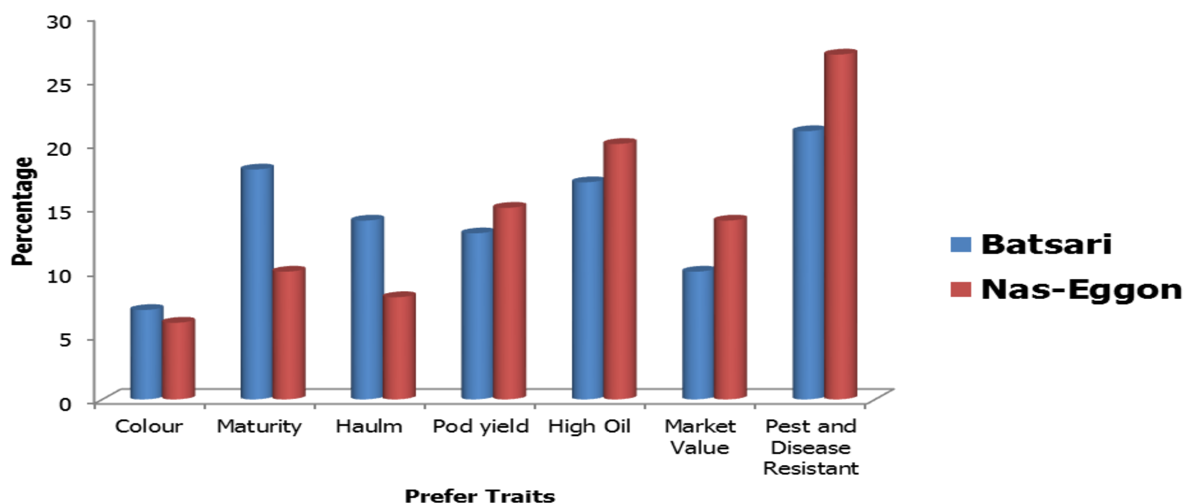

Figure 3. 2:Distribution of traits preferred by farmers

The results revealed that farmers were aware of groundnut rosette disease which was commonly known by various names such as “Kuturtan gyada” emphasizing the predominance of the disease in the area. The majority of the farmers reported the disease to be associated with insects and few of them recognized aphid as being responsible. Eighteen per cent of the respondents associated the disease with inadequate rainfall (Fig. 3.4).

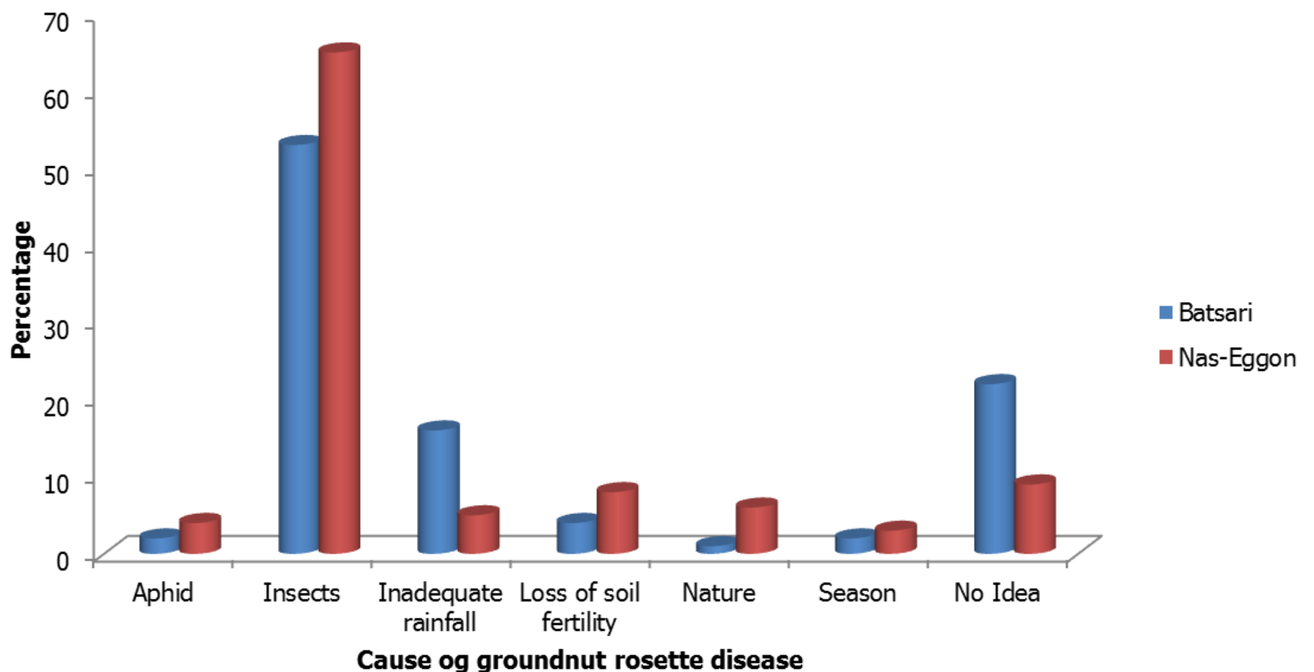

Figure 3. 3:Farmers perception on the causes of groundnut rosette disease

Most farmers recorded moderate (20 – 40%) to high (50 %) yield loss due to GRD (Fig. 3.5). A total yield loss (100 %) had been experienced by some farmers

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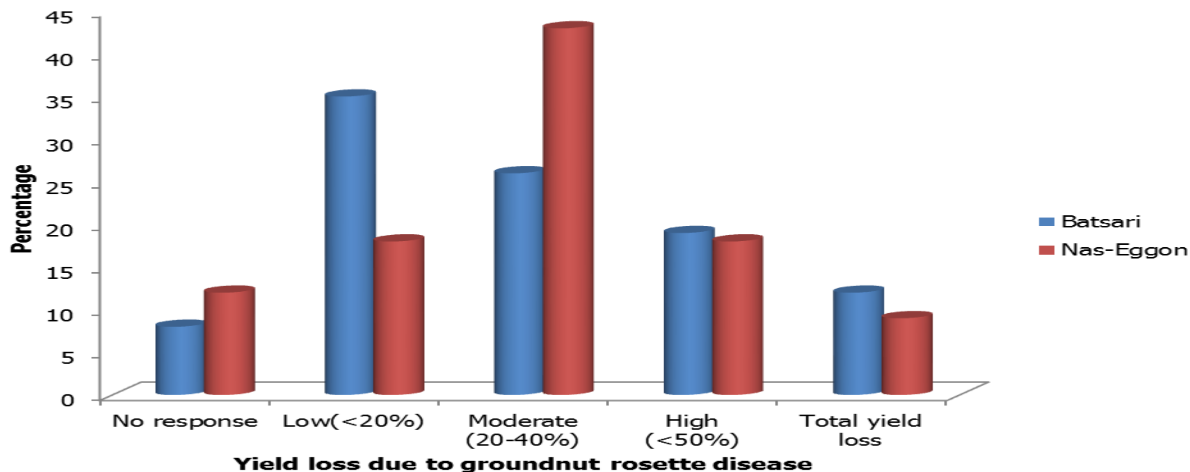

Figure 3. 4:Farmers perception of yield loss due to groundnut rosette disease

About 34 – 40 % of the respondents in both Batsari and Nasarawa – eggon did nothing to combat the menace of groundnut rosette disease, while many other farmers rogued out infected plants with some utilizing rosette resistant groundnut varieties (Figure 3.6)

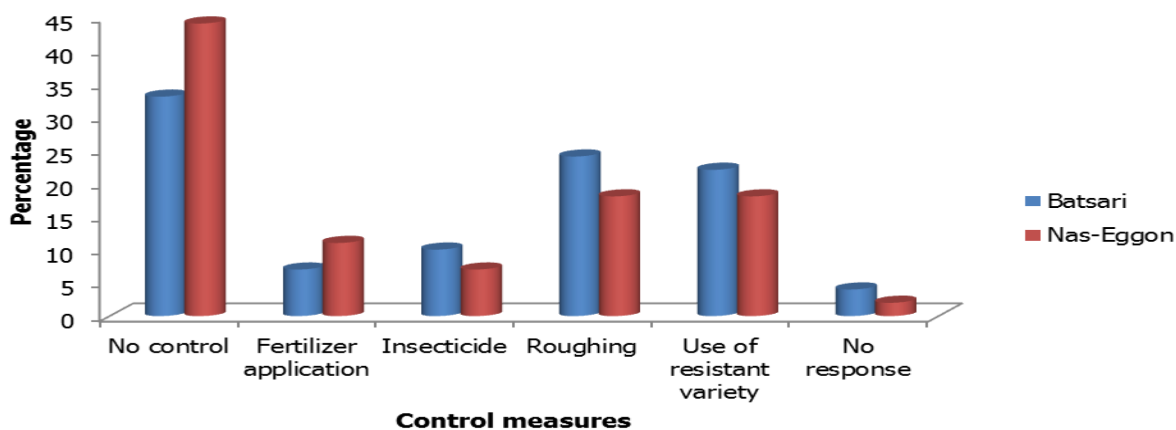

Figure 3. 5: Groundnut rosette disease control measures adopted by farmers

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3.3.3 Preferred groundnut varieties and associated characteristics

Farmers indicated that they selected groundnut varieties for commercial production based on consumer preference. Farmers also chose groundnut varieties for production on the basis of potential pod and haulm yield, oil quality and content, and market price. For instance, in Nasarawa – eggon, farmers ranked 'Maiyado' as the most preferred variety because of market demand, and high yield potential, although it is susceptible to groundnut rosette diseases and other foliar diseases, while 'SAMNUT21' and 'SAMNUT23' varieties were preferred in Batsari because of high pod yield, earliness, seed colour, market acceptance and tolerance to pest and foliar diseases.

3.3.4 Perception of farmers on constraints to groundnut production

Several constraints were mentioned and ranked by the farmers (Table 3.4). The most important constraints reported were pests and diseases and poor quality seeds and drought. The farmers recounted these limitations to severely reduced yields. Price fluctuation for groundnut was reported to constitute a problem. Whenever there was a bumper harvest groundnut prices dropped, so they kept their produce in storage, until such a time that the price increased in the off-season. Farmers differentiated between threats due to diseases from those caused by weeds and drought. The ranking of the frequent diseases or pests across villages revealed that GRD was the most threatening constraint. The symptoms observed by respondents to describe GRD were stunting, bushy and yellowing of leaves, where groundnut plants were not suffering from water shortage.

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Table 3. 4:Pair-wise ranking of the most important constraints in groundnut production in Batsari and Nasarawa – eggon

Constraint	Score by farmers			
	Batsari	Nass – eggon	Total Score	Ranking
Drought	2	4	6	3
Pest and Diseases	1	2	3	1
Weeds	3	4	7	4
High cost of insecticides	5	3	8	5
Poor quality seed	2	1	3	1
Price fluctuation	3	2	5	2

1 = very serious problem and 5 = minor

3.4 Discussion

A participatory rural appraisal was conducted to understand farmers' production systems and perceptions on groundnut rosette diseases across two locations in Nigeria. The PRA helped to obtain information on auxiliary data on socio – economic aspect of farmers. Farmers faced several constraints from seed through crop production, crop protection and marketing in groundnut production.

The majority of farmers were males and had been farming for more than 10 years. Most of them were in the age range of 25 – 30 years in Nasarawa-eggon but older in Batsari. The major source of income was from crop growing that accounted for more than 80% of their household income However, most farmers recorded low yield of groundnut crop owing to several constraints that called for intervention and strategies to enhanced productivity.

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Most farmers at Batsari location were aware of improved groundnut varieties and some grow them together with their landrace varieties. Nasarawa – eggon completely relies on their landrace variety 'Maiyado'. Reasons for poor adoption rate of improved varieties could be due to limitations of certified seeds, high prices and inadequate information on the improved varieties. The seeds of landrace varieties with farmers' preference traits were sold at reasonably affordable prices.

The farmers faced similar groundnut production constraint across Batsarit and Nasarawa – eggon despite differences in geographical locations. The major challenges were poor quality seeds and prevalence of groundnut rosette disease. The findings from this study further showed that groundnut rosette disease was probably associated with insects and drought. Adu – Dapaah *et al.* (2004) associated insect and drought as the favourable conditions for groundnut rosette disease from the PRA studies of groundnut. The Agricultural Transformation Agenda (ATA) programme of the Federal Government of Nigeria was designed to enhance the livelihood of farming community through improving productivity and hence raising their income through supply of agricultural inputs; fertilizers, good quality seeds and credit facility. These did not reach resource poor farmers because of the requirements that include detailed information on farming activities, and marketing. For farmers with limited education and understanding of the process, the requirements are unobtainable. Furthermore, majority of farmers were not members of any cooperative society. To derive maximum benefit from the ATA programme, the Federal government should improve the extension services to facilitate the formation of farmers' cooperative societies at grass root levels for coordinated agricultural activities.

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Genotype plays a very significant role in achieving higher productivity. In general, across the locations it was noticed that there was no efficient seed system or replacement mechanism for penetration of improved cultivars of groundnut. Most of the farmers use very old landrace 'Maiyado', demonstrating the poor rate of seed replacement in these parts of the country. The risk taking ability and openness of Batsari's farmers to new technologies (SAMNUT21 and SAMNUT23) made a big difference to their achieving high productivities, an approach that was relatively lacking in Nasarawa - eggon. 'Maiyado' was preferred by majority of farmers at both locations because of its colour, high oil contents and resistance to foliar disease. These are traits that most farmers and consumers look for in groundnut (Ndjuenga *et al.,* 2010). Until recently, researchers at IAR/ICRISAT focused mainly on earliness, yield and resistance to foliar diseases (Olorunju *et al.*, 2001) to improve groundnut production in sub Saharan Africa. The new focus is involvement of farmers through PRA to final production of improved seed which will ultimately, enhance rapid adoption. This agrees with the findings of Nkonya and Featherstone (2001) who found that varieties with farmers preferred traits were easily adopted. This was evident with SAMNUT21 and SAMNUT23 with a high adoption rate (> 70%) by farmers at Katsina state, Kano, Jigawa and Kaduna states because the background parents of these varieties are local varieties with farmers preferred traits (Ndjeunga *et al.,* 2003). However, the nonavailability of the two varieties in Nasarawa state, compelled the farmers to use the landrace they have and available in market as recycle seeds. The high price of certified seed if available was another reason for low adoption.

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3.5 Conclusions and Recommendations

This study identifies groundnut varieties grown by the farmers, criteria for choice of the varieties and constraints in production, thus providing the basis for formulation of farmer-oriented groundnut breeding programme. Farmers have diverse perceptions and complex combinations of criteria they use in selecting groundnut varieties. The key criteria include high yields, early maturity, tolerance to groundnut rosette disease drought and insect pests. Groundnut production in both Batsari and Nasarawa-Eggon is constrained by related factors. The most important constraints perceived by farmers are pest and rosette diseases, poor quality seeds and drought. Farmers in both locations were aware of groundnut rosette disease as it is called by various local names. For instance "Kutrtan-gyada" was the name given to GRD in Batsari.

To increase groundnut production, research should take into consideration the farmers' circumstances and preferences and develop varieties and crop management packages meet farmers demands. Incorporation of farmers' preferences in selection of groundnut varieties in breeding process would increase likelihood of adoption of the varieties. Whereas groundnut breeding cannot incorporate all the desired attributes, the key attributes should be included in particular varieties and many varieties should be bred focusing the demands of different groups of farmers. Considering that farmers prefer saved seeds of local varieties as a strategy for coping with cash flow constraints, effort should be made to breed varieties that are resistant to insect pests and disease. Such varieties are likely to be highly adopted by smallholder farmers, especially when the other key criteria they apply in variety selection are also incorporated.

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CHAPTER FOUR

4 ASSESSMENT OF GENETIC DIVERSITY OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) GENOTYPES FOR RESISTANCE TO ROSETTE DISEASE USING SSR MARKERS

4.1 Introduction

Breeding for foliar disease resistant genotypes is the ideal solution for reducing the crop losses. Identification and utilization of a broad spectrum of genetically diverse sources of GRD resistance is critical for the development of a new generation of broad-based high-yielding GRD-resistant groundnut cultivars. Limited knowledge about the genetic diversity of the GRD-resistant germplasm and deleterious linkage drag has impeded the utilization of a wide spectrum of GRD resistance donors. Diversity studies in groundnut have generally revealed extensive phenotypic variation amongst varieties (Upadhyaya *al.,* 2001, 2003) yet limited variation at the molecular level (Subramanian *et al.,* 2000; Moretzsohn *et al.,* 2004). Several approaches including molecular (Jiang *et al.,* 2007; Milla-Lewis *et al.,* 2010; Khera *et al.,* 2013) and morphological characterization have been used in assessing the genetic diversity of groundnut germplasm but results of morphological characterization are highly influenced by environmental factors (Shoba *et al.,* 2010). Molecular marker technologies are playing an increasingly important role in conservation and use of plant genetic resources in plant breeding programmes (Varshney *et al.,* 2009). Among the DNA markers, simple sequence repeat (SSR) markers are more preferable as it is more variable within genomes than other marker types (Belaj *et al.,* 2003). Additionally, SSRs have the advantage of being co-dominant, only requiring very small amounts of DNA and hence have been widely applied in many plant genetics studies, e.g. for evaluating genetic diversity (Zhebentyayaeva *et al.,* 2003; Fahima *et al.,* 1998).

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In Nigeria, extensive efforts have been made in groundnut breeding for GRD-resistance and several resistant cultivars have been released. However, these cultivars have been released 10 years ago have begun to show resistance breaking that is influenced by genetic variability in the pathogen population (Legrève and Duveiller, 2010). Only a few sources of GRD-resistance have been successfully used in breeding programs at the Institute for Agricultural Research (IAR) even though several resistant genotypes are available (Olorunju *et al.,* 2001). Most GRD-resistant cultivars released in IAR are based on just three sources of resistance (RMP12, MDR-8-19 and UGA2). Obviously, the genetic background of parents in IAR groundnut breeding programs is still narrow, which may have impeded the progress of breeding. Therefore, a better understanding of the genetic diversity amongst GRD-resistant germplasm is a prerequisite for further efficient improvement of GRD-resistance.

The objectives of the present study are to use SSR markers to detect DNA polymorphism among cultivated groundnut genotypes with differential levels of GRD resistance and for selecting parents for further breeding programmes.

4.2 Materials and methods

4.2.1 Plant material and DNA extraction

Fifty groundnut genotypes obtained from the IAR and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Mali, consisting of aphid and rosette resistant genotypes were assayed in this study (Table 4.1). Total genomic DNA was isolated from young leaves of 15 – 20 days old seedlings. Each sample consisted of about 5g of leaves pooled from 2-3 seedlings and DNA was extracted using a CTAB-based procedure, with 3% (v/v) b-mercaptoethanol in a 3% (w/v) CTAB buffer (Mace *et al.,* 2003). The quantity and quality of DNA were determined

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electrophoretically through comparison with known concentrations of uncut l DNA standards and spectrophotometric analysis at 260/280nm, and subsequently diluted to 5ng/ml. Laboratory analysis was done at Generation Challenge Program (GCG) Kenya between August, 2012 – November 2012.

4.2.1 SSR Analysis

Forty SSR primer pairs (Table 4.2) were used to amplify the genomic DNAs. PCR reactions were carried out in 10µL reaction volume using a GeneAmp PCR System 9700 (Applied Biosystems). The PCR reaction mixtures contained between 5 and 15ng of genomic DNA, 10–30 pmol of each primer, 100–125mM of dNTP, 0.6–1.2U/ml of Taq DNA polymerase (Amersham), 1 PCR buffer (10mM Tris–HCl pH 8.3, 50mM KCl) and 0.5–2.5mM $MgCl_2$. The fixed-temperature PCR programmes consisted of an initial denaturation step for 2 min at 94^0C, followed by 35 cycles of denaturation for 45 s (94^0C), annealing for 1 min (57–64^0C) and extension for 1 min 30s (72^0C). The PCR products were then incubated at 72^0C for a further 10 min to ensure complete extension. A second PCR programme using the touchdown approach was also used for selected SSRs with the following conditions: initial denaturation for 2 min at 94^0C, followed by 10 cycles: 94^0C for 45 s, 65^0C (21^0C/cycle) for 1 min and 72^0C for 1 min 30 s. This was then followed by 20 cycles of 94^0C for 45s, 55^0C for 1 min and 72^0C for 1 min 30s, followed by a final extension step of 10min (72^0C).

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Table 4. 1:Groundnut genotypes with different levels of resistance and susceptibility to groundnut rosette disease (GRD) included in the study

Groundnut genotypes	Seed source	Aphid/rosette resistant status
ICGV-IS 07812	ICRISAT	Rosette Resistant
ICGV-IS-07885	ICRISAT	Rosette Resistant
ICGV-IS-07886	ICRISAT	Rosette Resistant
ICGV-IS-07888	ICRISAT	Rosette Resistant
ICGV-IS-07890	ICRISAT	Rosette Resistant
ICGV-IS-07893	ICRISAT	Rosette Resistant
ICGV-IS-07894	ICRISAT	Rosette Resistant
ICGV-IS-07895	ICRISAT	Rosette Resistant
ICGV-IS-07899	ICRISAT	Rosette Resistant
ICGV-IS-07903	ICRISAT	Rosette Resistant
ICGV-IS-07904	ICRISAT	Rosette Resistant
ICGV-IS-07839	ICRISAT	Rosette Resistant
ICGV-IS-07842	ICRISAT	Rosette Resistant
ICGV-IS-07844	ICRISAT	Rosette Resistant
ICGV-IS-07850	ICRISAT	Rosette Resistant
ICGV-IS-07852	ICRISAT	Rosette Resistant
ICGV-IS-07859	ICRISAT	Rosette Resistant
ICGV-IS-07864	ICRISAT	Rosette Resistant
ICGV-IS-07865	ICRISAT	Rosette Resistant
ICGX – SM 00017/5/P10/P1	ICRISAT	Aphid Resistant
ICGX-SM 00020/5/P6/P2	ICRISAT	Aphid Resistant
ICGX-SM 00020/5/P9	ICRISAT	Aphid Resistant
ICGX – SM 00020/5/9	ICRISAT	Aphid Resistant
ICGX – SM 00020/5/P4/P1	ICRISAT	Aphid Resistant
ICGX-SM 00017/5/P1/P1	ICRISAT	Aphid Resistant
ICGX-SM 00017/5/P15/P2	ICRISAT	Aphid Resistant
ICGX-SM 00020/5/15/P2	ICRISAT	Aphid Resistant
ICGX-SM 00020/5/P2/P1	ICRISAT	Aphid Resistant
ICGX-SM 00020/5/P4/P1	ICRISAT	Aphid Resistant
ICGX-SM 00020/5/P4/P10	ICRISAT	Aphid Resistant
KWANKWASO	SAMARU	Rosette susceptible
MANIPENTA	SAMARU	Rosette susceptible
RS006F3B1-21	ICRISAT	ROSDOM

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34	RS006F4B1-25	Source	ROSDOM
35	RS006F4B1-31	ICRISAT	ROSDOM
36	RS006F4B1-35	ICRISAT	ROSDOM
37	RS006F3B1-27	ICRISAT	ROSDOM
38	RS006F3B1-53 (B)	ICRISAT	ROSDOM
39	RS006F3B1-57 (B)	ICRISAT	ROSDOM
40	RS006F3B1-59 (R)	ICRISAT	ROSDOM
41	RS006F4B1-10 (B)	ICRISAT	ROSDOM
42	RS006F4B1-13	ICRISAT	ROSDOM
43	RS006F4B1-2	ICRISAT	ROSDOM
44	RS006F4B1-22	ICRISAT	ROSDOM
45	SAMNUT10	IAR	Rosette Tolerant
46	SAMNUT14	IAR	Rosette susceptible
47	SAMNUT21	IAR	Rosette Tolerant
48	SAMNUT22	IAR	Rosette Tolerant
49	SAMNUT23	IAR	Rosette Tolerant
50	SAMNUT24	IAR	Rosette Tolerant

ROSDOM=Rosette disease resistant + dormancy, IAR=Institute for Agricultural Research, ICRISAT=International Crop Research Institute for Semi-Arid Tropics

4.2.2 Electrophoresis and data collection

PCR amplification products were separated on 6% nondenaturing polyacrylamide gels and revealed using a silver staining procedure based on ammoniacal solutions of silver, modified from Kolodny (1984). The size of the allele scored was determined through comparison with the 100 bp DNA ladder (Amersham) included on all gels. Estimates of similarity were based on Nei *et al.* (1983) definition of similarity as $S_{ij} = 2a/(2a+b+c)$, where S_{ij} is the similarity between two individuals, i and j, a is the number of bands present both in i and j, b is the number of bands present in i and absent in j, and c is the number of bands absent in i and present in j. Gene diversity (H_e) was estimated according to the formula of Nei *et al,* (1983) for each locus as $H_e = 1 - \sum P_{ij}^2$, where P_{ij} is the frequency of jth allele for i^{th} locus summed across all allele of

the locus. Nei's *et al.* (1983) genetic distance (GD) was calculated for each pair of population and for each pair of tested entries. Dendrogram based on Nei's genetic distances (Nei, *et al.,* 1983) were generated using the unweighted pair group method with arithmetic average (UPGMA). Polymorphism information content (PIC) as described by Torres *et al.* (2008) as follows: $PIC = 1 - \sum_{i=1}^{n} P_i^2$ Where P_i is the frequency of the jth allele for the ith marker, and summed over *n* alleles, it was used to represent the information value of a marker for detecting polymorphism within a population. It depends on the number of detectable alleles and their frequency distribution. All statistical analyses were conducted using PowerMarker 1.32 Window based computer package (Yeh and Yang, 2000).

4.3 Results

4.3.1 Allelic variation at SSR loci

Diversity assessment of the 50 groundnut genotypes was performed using 40 SSR primer pairs, of which 35 primers amplified polymorphic bands. A total of 166 polymorphic alleles were recorded among the groundnut genotypes tested (Table 4.2). The 35 polymorphic SSR primers each amplified 2 to 12 microsatellite loci, with an average of 4.74 loci per primer. Several primers including IPAHM103, IPAHM287 and IPAHM524 were more efficient than the rest in detecting the diversity among groundnut genotypes since each amplified 6 to 8 loci. An allele observed in less than 10% of the 50 accessions was considered to be rare. A total of 14 rare alleles were observed. Value of each marker PICs ranged from 0.19 for marker detected by IPAHM354 to 0.82 for the marker detected by IPAHM524 (Table 4.2). The PIC values greater than 0.5 are considered highly informative, however, markers with 0.5 PIC > 0.25 were also considered to be informative (Botstein *et al.,* 1980). The variation observed in this study was

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significantly associated with the number of alleles detected at each locus; hence the SSR marker revealed large amount of variation in the sampled groundnut genome. The observed frequency of the 166 alleles ranged from 0.25 for marker IPAHM524 to 0.89 for marker IPAHM345 with an average of 0.54 per marker.

Based on SSR analysis, the average genetic pairwise distance among the 50 genotypes was 0.31 (Table 4.3). The largest distance was 0.51 (between ICGV – IS – 07812 and RS006F4B1 – 31) and the shortest distance was 0.05 between ICGV – IS – 07865 and ICGV – IS – 07864, all the four lines were GRD-resistant. The distances among the susceptible accessions were relatively large, ranging from 0.35 (between KWANKWASO and SAMNUT14) to 0.39 (KWANKWASO and MANIPENTA). The average distance between the aphid resistant and susceptible genotype (KWANKWASO) was 0.37 with the greatest diversity between 'KWANKWASO' and (ICGX-SM 00020/5/P6/P_2 and ICGX-SM 00020/5/P_9) (0.41). A GRD-susceptible farmer variety (MANIPENTA) had an average distance of 0.38 from the rosette resistant genotypes with a range from 0.26 (between 'MANIPENTA' and ICGV – IS – 07894) to 0.45 (between 'MANIPENTA' and ICGV – IS – 07842). This susceptible farmers' variety had an average genetic distance of 0.37 from aphid resistant genotypes and ranged from 0.30 (between 'MANIPENTA' and ICGX – SM – 000020/5/P_4/P_{10}) to 0.41 ('MANIPENTA' and ICGX – SM 00017/5/P_{10}/P_1; 'MANIPENTA' and ICGX – SM 00020/5/9; 'MANIPENTA' and ICGX-SM 00020/5/P_2/P_1) . Similarly, 'MANIPENTA' had an average distance of 0.38 with RS0006F series with a range from 0.27 (between 'MANIPENTA' and RS0006F3B1 – 21) to 0.45 (between 'MANIPENTA' and RS0006F4B1 – 22). Finally, with rosette tolerant SAMNUT series, 'MANIPENTA' had an average genetic distance of 0.39 with a range of 0.13 (between 'MANIPENTA' and SAMNUT22) to 0.44 (between 'MANIPENTA' and SAMNUT24).

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Comparing the different GRD-resistant sources based on Nei, (1983) original genetic distance resulted in the dendrogram shown in Figure 4.2. The ICGV – IS series and ICGX – SM GRD-resistant sources showed the smallest genetic distant (0.06) and were grouped together. The largest genetic distances are found between Local variety and RS0006F series (0.20) and were well separated from other GRD-resistant sources (Fig 4.2). Large variation of gene diversity among loci was found.

4.3.2 Comparison of gene diversity

The statistics describing the genetic diversity found at each locus in each population was calculated. Considering the genetic background of experimental groundnut genotypes, the average gene diversity for the whole sample was 0.51. The lowest gene diversity (0.20) was detected in 4 loci for all genotypes. Gene diversity of > 0.50 in all the genotypes was observed for 25 loci. The highest gene diversity in each population was found at IPAHM524 (0.84), IPAHM108 (0.76), IPAHM229 (0.75), IPAHM23 (0.74) and IPAHM219 (0.74). Eighteen loci with gene diversity > 0.6 were found in GRD-resistance sources. (Table 4.2)

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Table 4. 2:Primers used in the study, gene bank ID, repeat motif, frequency and number of alleles as well as gene diversity, and polymorphic information contents (PIC) based on the analysis of 50 groundnut genotypes for 35 polymorphic SSR markers

Marker	Gene Bank Accession ID	Repeat Motif	Allele Frequency	No of Allele	No of rare allele	Gene Diversity	PIC
IPAHM23	ER974415	(CA)17(TA)3	0.43	4	1	0.63	0.56
IPAHM73	ER974423	(GA)13	0.62	4		0.54	0.48
IPAHM82	IPAHM 82	(GA)15	0.52	5		0.54	0.44
IPAHM92	ER974430	(GT)11	0.56	3		0.51	0.40
IPAHM93	ER974431	(CT)15	0.83	4		0.30	0.28
IPAHM103	ER974437	(CA)3(GA)17	0.69	8		0.50	0.48
IPAHM105	ER974438	(CT)18	0.43	6	1	0.68	0.62
IPAHM108	ER974439	(TC)18	0.29	8	1	0.76	0.72
IPAHM123	ER974446	(GA)18	0.39	6	1	0.71	0.66
IPAHM136	ER974452	(TC)2(CT)13	0.49	5	1	0.57	0.48
IPAHM164	ER974461	(GA)20	0.43	6	1	0.69	0.64
IPAHM165	ER974462	(GA)13	0.51	3		0.61	0.54
IPAHM171a	ER974466	(TC)7TGTT(TC)9	0.59	5		0.60	0.57
IPAHM171c	ER974468	(GA)16	0.52	2		0.50	0.37
IPAHM176	ER974469	(GA)18	0.57	6		0.59	0.54
IPAHM177	ER974470	(CA)11TA(CA)3 (TA)4	0.51	3		0.62	0.55
IPAHM219	ER974474	(TG)15	0.34	6	1	0.74	0.70
IPAHM229	ER974475	(CA)14TA(CA)3	0.32	6	1	0.75	0.71
IPAHM282	ER974488	(CA)14(TA)5	0.53	4		0.64	0.60
IPAHM283	ER974489	(TA)4(TG)26TTG (GT)2	0.56	3		0.58	0.51
IPAHM287	ER974492	(TG)16(AG)22	0.48	7	1	0.70	0.66
IPAHM288	ER974493	(GA)4AA(GA)2	0.86	5		0.24	0.23
IPAHM290	ER974494	(TA)3(CA)3CC(CA)5(TA)8	0.76	4		0.38	0.34
IPAHM354	ER974514	(GA)16	0.89	3		0.20	0.19
IPAHM356	ER974515	(GA)21G(GA)2	0.39	7	1	0.74	0.69
IPAHM37	ER974519	(CA)10(TA)7	0.72	3		0.44	0.40
IPAHM373	EE974518	(TTG)6CT(GTT)8	0.76	2		0.37	0.30
IPAHM395	ER974522	(GA)14	0.86	3		0.25	0.23
IPAHM407a	ER974525	(TC)7TGTT(TC)9	0.53	4		0.63	0.58
IPAHM455	ER974536	(TA)5(TG)16	0.51	4		0.66	0.61
IPAHM429	ER974534	(GT)17	0.45	3	1	0.65	0.57
IPAHM468	ER974542	(GA)15	0.60	2		0.48	0.37
IPAHM524	ER974548	(GA)20AA(GA)3	0.25	12	1	0.84	0.82
IPAHM531	ER974551	(TAC)7	0.49	4	1	0.52	0.40
IPAHM475	ER974545	(GT)7(GA)12	0.48	6	1	0.61	0.53
Total				166	14		
Mean			0.55	4.47		0.57	0.51

Table 4. 3:Pairwise genetic distance coefficients of 50 GRD-resistant genotypes using 36 SSR primer pairs combinations analyzed by PowerMarker software

	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
1	ICGV-IS 07812																																																	
2	ICGV-IS-07885	0.21																																																
3	ICGV-IS-07886	0.42	0.28																																															
4	ICGV-IS-07888	0.40	0.27	0.22																																														
5	ICGV-IS-07890	0.42	0.29	0.25	0.36																																													
6	ICGV-IS-07893	0.38	0.20	0.27	0.34	0.25																																												
7	ICGV-IS-07894	0.30	0.21	0.29	0.29	0.27	0.22																																											
8	ICGV-IS-07895	0.35	0.35	0.39	0.36	0.42	0.45	0.38																																										
9	ICGV-IS-07899	0.37	0.35	0.39	0.48	0.33	0.28	0.33	0.35																																									
10	ICGV-IS-07903	0.24	0.13	0.29	0.28	0.27	0.16	0.10	0.32	0.32																																								
11	ICGV-IS-07904	0.49	0.41	0.25	0.19	0.38	0.37	0.41	0.47	0.45	0.37																																							
12	ICGV-IS-07839	0.37	0.30	0.34	0.13	0.45	0.41	0.32	0.38	0.49	0.30	0.21																																						
13	ICGV-IS-07842	0.32	0.23	0.32	0.35	0.23	0.29	0.17	0.33	0.36	0.14	0.46	0.36																																					
14	ICGV-IS-07844	0.33	0.22	0.32	0.35	0.27	0.27	0.15	0.36	0.35	0.13	0.46	0.37	0.10																																				
15	ICGV-IS-07850	0.30	0.24	0.33	0.36	0.21	0.28	0.23	0.31	0.25	0.17	0.45	0.43	0.23	0.21																																			
16	ICGV-IS-07852	0.46	0.35	0.21	0.21	0.34	0.33	0.35	0.44	0.40	0.34	0.17	0.15	0.36	0.37	0.43																																		
17	ICGV-IS-07859	0.29	0.27	0.24	0.37	0.22	0.23	0.22	0.39	0.31	0.23	0.34	0.36	0.25	0.28	0.36	0.28																																	
18	ICGV-IS-07864	0.45	0.29	0.30	0.40	0.09	0.20	0.28	0.40	0.32	0.26	0.41	0.41	0.24	0.27	0.27	0.34	0.18																																
19	ICGV-IS-07865	0.41	0.29	0.31	0.42	0.10	0.21	0.30	0.44	0.35	0.28	0.43	0.45	0.25	0.29	0.28	0.35	0.20	0.05																															
20	ICGX – SM 00017/5/P10/P1	0.20	0.16	0.30	0.31	0.30	0.27	0.22	0.32	0.37	0.13	0.38	0.30	0.20	0.25	0.27	0.35	0.20	0.30	0.27																														
21	ICGX-SM 00020/5/P6/P2	0.36	0.33	0.32	0.36	0.34	0.25	0.31	0.49	0.25	0.32	0.36	0.42	0.32	0.32	0.33	0.33	0.27	0.36	0.37	0.34																													
22	ICGX-SM 00020/5/P9	0.32	0.27	0.31	0.37	0.26	0.29	0.21	0.37	0.41	0.18	0.38	0.37	0.19	0.23	0.31	0.35	0.22	0.25	0.23	0.16	0.39																												
23	ICGX – SM 00020/5/9	0.32	0.27	0.28	0.42	0.34	0.25	0.31	0.42	0.24	0.30	0.33	0.42	0.37	0.37	0.27	0.30	0.23	0.32	0.33	0.33	0.23	0.40																											
24	ICGX – SM 00020/5/P4/P1	0.24	0.24	0.36	0.39	0.31	0.33	0.17	0.36	0.27	0.18	0.44	0.37	0.19	0.18	0.27	0.40	0.23	0.31	0.28	0.17	0.33	0.21	0.36																										
25	ICGX-SM 00017/5/P1/P1	0.32	0.22	0.25	0.28	0.24	0.27	0.14	0.34	0.42	0.12	0.33	0.30	0.15	0.19	0.25	0.33	0.23	0.25	0.27	0.16	0.40	0.11	0.38	0.21																									
26	ICGX-SM 00017/5/P15/P2	0.32	0.21	0.25	0.27	0.25	0.27	0.18	0.32	0.40	0.14	0.35	0.28	0.18	0.21	0.26	0.31	0.22	0.26	0.26	0.16	0.42	0.19	0.38	0.21	0.14																								
27	ICGX-SM 00020/5/15/P2	0.29	0.23	0.32	0.35	0.28	0.29	0.18	0.36	0.43	0.14	0.39	0.35	0.17	0.21	0.28	0.39	0.25	0.27	0.25	0.14	0.42	0.05	0.43	0.18	0.08	0.16																							
28	ICGX-SM 00020/5/P2/P1	0.24	0.19	0.34	0.32	0.32	0.28	0.14	0.34	0.31	0.16	0.42	0.33	0.21	0.21	0.28	0.37	0.23	0.31	0.28	0.17	0.33	0.18	0.33	0.09	0.19	0.20	0.15																						
29	ICGX-SM 00020/5/P4/P1	0.24	0.24	0.36	0.39	0.31	0.33	0.17	0.36	0.27	0.18	0.44	0.37	0.19	0.18	0.27	0.40	0.23	0.31	0.28	0.17	0.33	0.21	0.36	0.00	0.21	0.21	0.18	0.09																					
30	ICGX-SM 00020/5/P4/P10	0.25	0.18	0.36	0.31	0.32	0.29	0.16	0.35	0.28	0.17	0.40	0.30	0.23	0.22	0.30	0.34	0.21	0.30	0.32	0.21	0.33	0.24	0.32	0.10	0.20	0.19	0.22	0.06	0.10																				
31	KWANKWASO	0.36	0.29	0.29	0.42	0.24	0.19	0.27	0.38	0.24	0.18	0.34	0.40	0.27	0.23	0.22	0.32	0.23	0.20	0.24	0.26	0.26	0.28	0.24	0.27	0.29	0.27	0.29	0.32	0.27	0.32																			
32	MANIPENTA	0.45	0.39	0.43	0.37	0.40	0.34	0.26	0.39	0.34	0.35	0.38	0.41	0.45	0.41	0.40	0.37	0.32	0.41	0.43	0.41	0.35	0.41	0.41	0.34	0.38	0.37	0.41	0.33	0.34	0.30	0.39																		
33	RS006F3B1-21	0.49	0.34	0.35	0.38	0.40	0.38	0.36	0.44	0.44	0.37	0.37	0.38	0.41	0.41	0.45	0.33	0.32	0.38	0.37	0.34	0.40	0.34	0.40	0.34	0.38	0.36	0.38	0.33	0.34	0.29	0.43	0.27																	
34	RS006F4B1-25	0.42	0.29	0.27	0.36	0.34	0.34	0.33	0.42	0.29	0.32	0.28	0.38	0.35	0.34	0.35	0.26	0.30	0.35	0.36	0.35	0.29	0.34	0.28	0.31	0.33	0.32	0.37	0.34	0.31	0.28	0.27	0.42	0.36																
35	RS006F4B1-31	0.39	0.38	0.42	0.41	0.45	0.39	0.34	0.39	0.30	0.35	0.44	0.46	0.36	0.35	0.32	0.45	0.43	0.46	0.51	0.40	0.21	0.41	0.35	0.35	0.37	0.44	0.40	0.35	0.35	0.33	0.36	0.30	0.50	0.33															
36	RS006F4B1-35	0.38	0.39	0.35	0.46	0.38	0.32	0.40	0.44	0.21	0.36	0.40	0.50	0.36	0.36	0.37	0.37	0.32	0.38	0.35	0.37	0.22	0.38	0.28	0.28	0.45	0.42	0.40	0.31	0.28	0.32	0.25	0.43	0.40	0.26	0.28														
37	RS006F3B1-27	0.37	0.31	0.44	0.36	0.43	0.39	0.29	0.44	0.46	0.30	0.38	0.35	0.35	0.34	0.41	0.40	0.36	0.44	0.45	0.34	0.40	0.34	0.41	0.30	0.28	0.35	0.32	0.28	0.30	0.22	0.45	0.40	0.26	0.33	0.43	0.45													
38	RS006F3B1-53 (B)	0.24	0.15	0.31	0.28	0.33	0.22	0.20	0.32	0.31	0.09	0.36	0.29	0.23	0.23	0.25	0.34	0.29	0.32	0.29	0.14	0.34	0.21	0.33	0.16	0.18	0.21	0.17	0.09	0.16	0.16	0.28	0.37	0.36	0.34	0.34	0.35	0.30												
39	RS006F3B1-57 (B)	0.28	0.27	0.41	0.38	0.44	0.42	0.32	0.35	0.36	0.32	0.44	0.40	0.32	0.32	0.34	0.42	0.40	0.45	0.42	0.30	0.42	0.29	0.39	0.24	0.30	0.31	0.26	0.20	0.24	0.18	0.43	0.40	0.34	0.32	0.36	0.32	0.28	0.25											
40	RS006F3B1-59 (R)	0.36	0.36	0.43	0.46	0.30	0.34	0.40	0.49	0.17	0.37	0.44	0.51	0.38	0.37	0.33	0.41	0.35	0.32	0.29	0.38	0.21	0.43	0.27	0.28	0.47	0.46	0.44	0.30	0.28	0.29	0.30	0.42	0.38	0.31	0.34	0.18	0.44	0.31	0.34										
41	RS006F4B1-10 (B)	0.41	0.38	0.36	0.44	0.33	0.29	0.35	0.48	0.21	0.35	0.41	0.49	0.36	0.35	0.30	0.39	0.31	0.33	0.35	0.40	0.13	0.45	0.22	0.33	0.42	0.44	0.47	0.32	0.33	0.30	0.28	0.37	0.39	0.29	0.24	0.24	0.42	0.33	0.42	0.15									
42	RS006F4B1-13	0.35	0.32	0.38	0.34	0.43	0.41	0.36	0.34	0.30	0.31	0.31	0.40	0.40	0.37	0.31	0.40	0.41	0.48	0.49	0.35	0.44	0.36	0.38	0.30	0.32	0.33	0.34	0.30	0.30	0.27	0.41	0.36	0.41	0.34	0.29	0.34	0.35	0.29	0.21	0.34	0.39								
43	RS006F4B1-2	0.27	0.26	0.39	0.31	0.37	0.40	0.31	0.38	0.35	0.30	0.37	0.32	0.32	0.31	0.31	0.35	0.38	0.43	0.40	0.28	0.25	0.40	0.30	0.27	0.38	0.37	0.39	0.27	0.27	0.28	0.33	0.39	0.38	0.31	0.28	0.25	0.35	0.27	0.28	0.23	0.30	0.34							
44	RS006F4B1-22	0.36	0.27	0.35	0.30	0.38	0.39	0.30	0.35	0.45	0.26	0.32	0.34	0.28	0.32	0.39	0.38	0.29	0.38	0.40	0.24	0.39	0.25	0.41	0.28	0.21	0.23	0.24	0.27	0.28	0.23	0.40	0.43	0.29	0.32	0.39	0.40	0.25	0.27	0.19	0.45	0.47	0.25	0.32						
45	SAMNUT10	0.35	0.32	0.38	0.49	0.34	0.31	0.36	0.44	0.19	0.36	0.46	0.51	0.38	0.36	0.28	0.42	0.30	0.35	0.35	0.38	0.20	0.46	0.17	0.36	0.44	0.42	0.48	0.39	0.36	0.38	0.29	0.37	0.40	0.24	0.35	0.33	0.45	0.40	0.46	0.26	0.20	0.41	0.33	0.48					
46	SAMNUT14	0.32	0.25	0.31	0.30	0.28	0.36	0.27	0.35	0.46	0.24	0.35	0.38	0.26	0.29	0.32	0.40	0.24	0.32	0.33	0.21	0.40	0.18	0.41	0.27	0.17	0.24	0.19	0.26	0.27	0.25	0.35	0.44	0.39	0.35	0.38	0.44	0.30	0.29	0.32	0.44	0.48	0.29	0.37	0.20	0.46				
47	SAMNUT21	0.31	0.30	0.36	0.44	0.32	0.28	0.34	0.43	0.19	0.34	0.42	0.47	0.35	0.34	0.26	0.38	0.26	0.31	0.32	0.37	0.15	0.45	0.13	0.35	0.42	0.41	0.46	0.37	0.35	0.35	0.27	0.37	0.40	0.28	0.31	0.30	0.43	0.39	0.42	0.23	0.16	0.39	0.29	0.45	0.05	0.43			
48	SAMNUT22	0.37	0.29	0.28	0.42	0.35	0.27	0.33	0.40	0.34	0.29	0.33	0.47	0.40	0.36	0.35	0.36	0.25	0.37	0.38	0.35	0.31	0.36	0.30	0.37	0.33	0.35	0.36	0.35	0.37	0.33	0.29	0.31	0.29	0.28	0.39	0.36	0.28	0.31	0.39	0.35	0.29	0.40	0.40	0.37	0.31	0.35	0.30		
49	SAMNUT23	0.36	0.31	0.37	0.40	0.37	0.32	0.43	0.42	0.24	0.37	0.40	0.43	0.39	0.37	0.35	0.34	0.31	0.38	0.38	0.39	0.24	0.47	0.27	0.40	0.44	0.41	0.49	0.41	0.40	0.37	0.32	0.38	0.43	0.32	0.39	0.33	0.45	0.37	0.36	0.28	0.28	0.39	0.29	0.41	0.23	0.45	0.19	0.38	
50	SAMNUT24	0.37	0.33	0.38	0.33	0.40	0.34	0.34	0.46	0.48	0.29	0.33	0.36	0.38	0.38	0.41	0.37	0.32	0.41	0.42	0.32	0.37	0.29	0.43	0.37	0.30	0.40	0.32	0.34	0.37	0.35	0.38	0.44	0.44	0.40	0.46	0.44	0.38	0.35	0.42	0.46	0.48	0.37	0.45	0.33	0.49	0.20	0.43	0.38	0.44

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The cluster analysis using UPGMA based on genetic distances from SSR marker analysis clustered the 50 genotypes into 7 groups (A to G) at a genetic distance of 0.16 (Fig. 4.1). Group A comprises of 18 GRD-resistant genotypes and could further divide into 2 sub-groups (sub-group I and II) at genetic distance of 0.1. Sub-group AI consist of four rosette resistant (ICGV – IS series) and 4 aphid resistant (ICGX – SM series) genotypes. Two other members each of this group also stand as subgroup. Sub group AII is made up of 2 rosette resistant (ICGV – IS series) and 5 aphid resistance (ICGX – SM series) with RS0006F series grouped into a sub-group. Group B included only four genotypes; two RS0006F series, one IAR rosette tolerant variety (SAMNUT22) and a farmer preferred variety 'MANIPENTA'. Group E is also made up of 2 sub – groupings, sub-group EI consist of 4 rosette resistance with dormancy (RS0006F series), 1 aphid resistant (ICGX – SM series) and 4 rosette resistant (ICGV – IS series). All the genotypes in this group are originated from ICRISAT breeding unit. Sub – group E2 comprised of 3 IAR GRD-tolerant genotypes (SAMNUT10, 21 and 23), 8 RS0006F series and 2 ICGX – SM series. Variation in group E was wider than all other groups.

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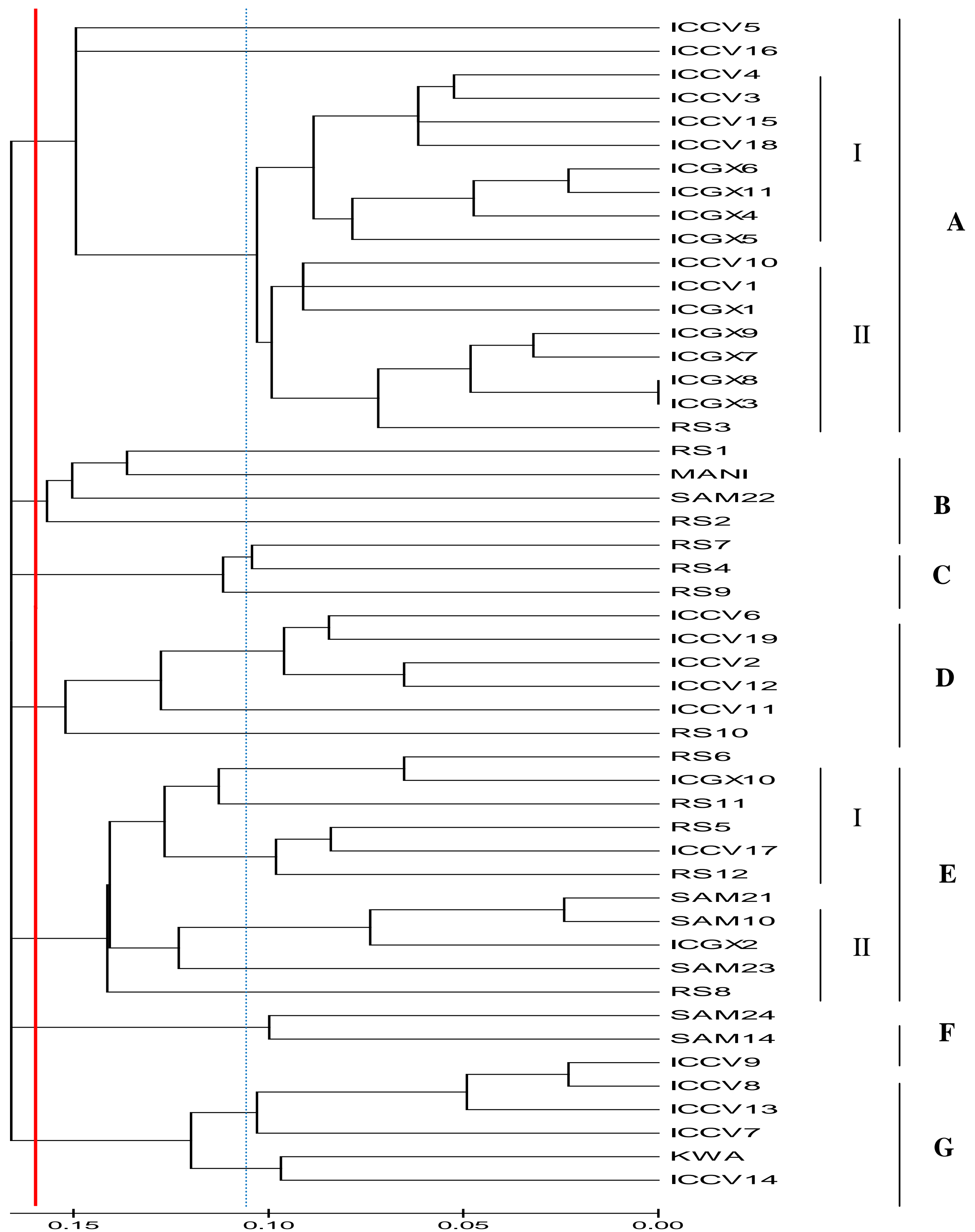

Figure 4. 1:Hierarchical dendrogram of 50 groundnut genotypes by using similarity coefficients based on the Nei's (1983) original genetic distance calculated from data of 166 SSR loci using the UPGMA method: Refer to Table 4.1 for names of the corresponding codes

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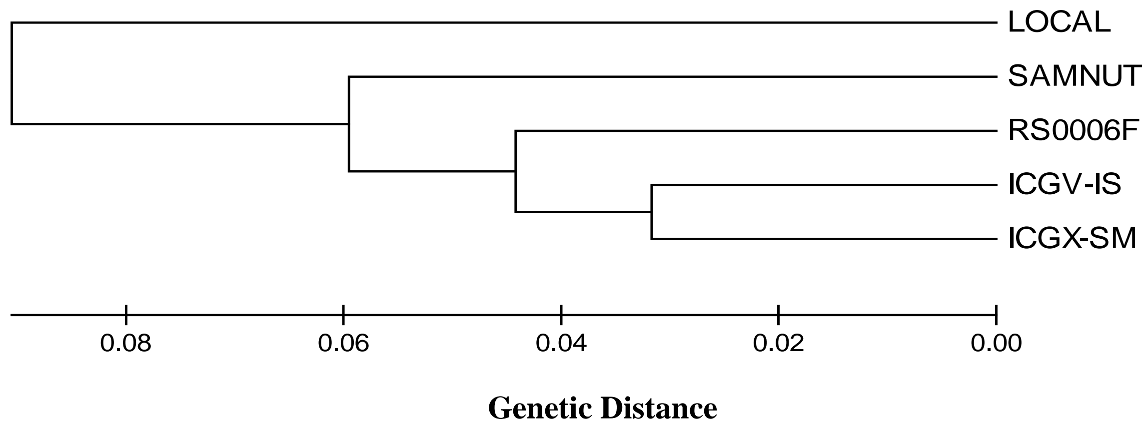

Genetic Distance

Figure 4. 2:The genetic relationships among the five groundnut populations calculated using UPGMA method based on the Nei's (1983) genetic distance

4.4 Discussion

The primary goal of this study was to elucidate the ability of SSR markers to detect molecular polymorphism among GRD-resistant cultivars of groundnut. The 35 SSR primer pairs detected a total number of 166 alleles. This support the findings of Milla-Lewis *et al.* (2010) who reported that for the first time that such a large number of alleles were used to describe genetic diversity in a small collection of cultivated varieties of groundnut. Despite the fact that some SSR primers had relatively low PIC values and an average pairwise genetic similarity at 0.32, the cultivars could be separated fairly well. The study demonstrates that microsatellite markers may be useful for detecting molecular variation among GRD-resistant groundnut cultivars. Although the cultivated groundnut germplasm exhibits a high level of morphological variation, the detectable level of DNA polymorphism in this species is relatively low when compared to other crops (Upadhyaya *et al.,* 2012). An average of 4.47 alleles per locus and a mean PIC value of 0.51 in the present study are comparable to those obtained for groundnut (Milla-Lewis *et al.,*2010) and

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in crops such as tobacco (Moon *et al.,* 2009) that also has a narrow genetic base. This finding illustrates the potential in introgressing genetic diversity from GRD resistance sources to broaden the genetic base of cultivated groundnut breeding. Wide variations were shown by clusters A and E along with occurrence of unrelated individuals in some sub-clusters. This SSR markers clustering pattern according to GRD reaction indicated a possible association between marker data and disease reactions of the genotypes. This supports the findings of Mondal and Badigannavar (2010), who found three and four SSR alleles to be associated with rust and late leaf spot resistance in groundnut, respectively. The highest genetic distance detected among GRD-resistant groundnut germplasm tested conformed with a wide variation of phenotype in the cultivated groundnut, which contradicted with some previous studies (Subramanian *et al.,* 2000; Tang *et al.,* 2004) but was consistent with He and Prakash (2001) research, indicating the great potential of its use in cultivated groundnut improvement program. This study detected relatively acceptable level of molecular diversity among groundnut genotypes with various levels of resistance to GRD although some markers were more informative than others. This result agrees with Jiang *et al.* (2007) who reported similar levels of resistance to bacterial wilt disease of groundnut using SSR markers. In this study it was shown that moderate levels of genetic variation could be detected effectively in groundnut using SSR markers. The grouping of the genotypes at molecular level indicated a clear distinction between subspecies and among groundnut with differential levels of GRD resistance. This molecular study has provided useful information toward parental selections and specific SSR marker that can be used for varietal identification.

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4.5 Conclusions and Recommendations

The SSR markers used in this study detected a high level of polymorphism and were successful in distinguishing groundnut genotypes with various levels of GRD resistance. Genetic distances effectively grouped the germplasm according to differential level of GRD resistance thus highlighting the potential value of genetic distances for preliminary classification of poorly characterized groundnut germplasm. The clustering pattern according to disease reaction may indicate a possible association between marker data and disease resistance. The assessment of genetic diversity of GRD-resistant groundnut genotypes present in the working germplasm collection will help groundnut breeders to formulate crosses by choosing parent with different genetic backgrounds and will assist in the development of gene-mapping populations with greater marker polymorphism. In view of the above and based on the study, resistant sources such as ICGV – IS – 0784, ICGX – SM – 00020/5/P_4/P_{10}, RS0006F4B1 – 22, SMANUT22 and SAMNUT24 could be recommended as parents in crosses with the GRD-susceptible breeding such as ‘MANIPENTA with high yield and high seed oil content.

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CHAPTER FIVE

5 INHERITANCE ON RESISTANCE TO GROUNDNUT ROSETTE DISEASE

5.1 Introduction

Breeding for resistance to diseases remains a principal focus in the groundnut breeding programme in Nigeria. Information about the mode of gene action conferring resistance to diseases is prerequisite to the development of a focused breeding programme. Information on the genetic structure of a set of parents and mode of gene action governing yield and its attributes is useful in designing suitable breeding procedures. To design an appropriate breeding programme, it is important to know the proportion of phenotypic variation of a trait that is heritable (Kearsey and Pooni, 1996) since the efficiency of a selection programme is mainly dependent on the magnitude of genetic variation and heritability of a trait (Falconer and Mackay, 1996). In breeding programmes for stress resistance, half-sib (HS) mating systems are commonly used to evaluate general combining ability of parental line development, recombine selected entries in recurrent selection programs, and obtain quantitative genetic information (Amini *et al.,* 2013). Estimation of heritability based on HS family evaluation gives a good prediction of narrow-sense heritability since genetic variance among HS families represents primarily the additive genetic variance contained in the phenotypic variance (Kearsey and Pooni, 1996; Wricke and Weber, 1986). In general, diallel mating designs provides information on genetic effects of a fixed set of parental lines or estimates of general combining ability (GCA) and specific combining ability (SCA), variance components as well as heritability. The use of F_2 progenies to study genetic analysis of GRD resistance has not been established so far. The results of this study is expected to provide detailed information on relevant quantitative genetic parameters such as GCA, SCA, the ratio of variance due to GCA and SCA and their interaction with environment. This

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information will provide the basis to establish breeding strategy toward genetic improvement of GRD resistance and broadening genetic base of GRD resistance breeding of the Institute for Agricultural Research (IAR), Ahmadu Bello University, Samaru, Nigeria.

Further, Genotype × environment (G×E) interaction, a major problem involving quantitative traits such as GRD, complicates the interpretation of genetic experiments, makes predictions difficult, and reduces the efficiency of selection. Therefore, knowledge about the magnitude of G×E interactions is important to develop cultivars with stable performance over a wide range of environmental conditions.

The objectives of this study were to:

a. estimate the variance components and test the significance of G x E interaction on groundnut rosette disease;

b. estimate their heritability and degree of association of GRD parameters with agronomic traits

c. determine the mode of inheritance of resistance to groundnut rosette disease

5.2 *Materials and Methods*

5.2.1 Population Development and Phenotype Evaluation

The study involved the use of nine experimental lines comprised of three aphid resistant (ICGX – SM 00020/5/9, ICGX – SM 00017/5/P_{10}/P_1 and ICGX – SM 00020/5/P_4/P_1 and three rosette resistant (ICGV IS 07890, ICGV IS 07899 and ICIAR-19BT) were obtained from the International Crop Research Institute for Tropical Agriculture (ICRISAT) Mali. These breeding lines were previously evaluated for three years (2008 – 2010) at the Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria, Nigeria and were confirmed to have field resistance to aphids and GRD, respectively. Three farmers preferred varieties (SAMNUT14,

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KWANKWASO, and MANIPENTA) were also included as parents in the population development. The pedigree descriptions of the nine genotypes are presented in Table 5.1. The genotypes were manually cross-pollinated in a half diallel mating scheme at the screen house of Institute for Agricultural Research (IAR) Samaru, ($11^0$10.00″N and $7^0$38.00″ E, 693 m), Ahmadu Bello University Zaria, Nigeria in 2011. Additional manual cross-pollinations were made at IAR research field during the 2011 rainy season. Seed limitations for multi-location evaluation were overcome by advancing F_1 seeds to next generation (F_2) as suggested by (Hallauer *et al.,* 2008)

The nine parental lines and 36 F_2 genotypes were evaluated for disease resistance using a 9 x 5 $\alpha-$lattice design with two replications at two locations (Samaru, Kaduna state, and Lafia, Nasarawa state ($8^0$32"N, $7^0$42"E) during the 2011/2012 growing seasons using an infector – row techniques as described by Olorunju *et al.* (2001) at the two locations. A system where infectors of susceptible genotypes (SAMNUT 14) were planted in an alternating rows with test materials. The infector rows were planted 2 weeks earlier before the test materials to allow the build-up of infestation. Two row plots 4.0 m in length with inter and intra-row spacing of 0.75 m x 0.25 m, respectively, were used.

5.2.1 Aphid and Rosette resistance evaluation

Viruliferous aphid *A. craccivora* colonies were collected from infested cowpea *Vigna unguiculata* L., and groundnut *A hypogaea* plants at different locations in groundnut producing area in Nigeria to cover the different isolates that may be present in the country.

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Table 5. 1: Pedigree, source, description and characteristics of parental genotypes used for population development

Genotype	Pedigree	Source	Description
ICGX – SM 00020/5/9	ICG 12991 x ICGV-SM 95713	ICRISAT	Resistant to *Aphis craccivora;* early maturing
ICGX – SM 00017/5/P_{10}/P_1	ICG 12991 x ICGV-SM 99529	ICRISAT	Resistant to *Aphis craccivora;* early maturing
ICGX – SM 00020/5/P_4/P_1	ICG 12991 x ICGV-SM 99574	ICRISAT	Resistant to *Aphis craccivora;* early maturing
ICGV IS 07890	ICG 12991 x ICGV-SM 95603	ICRISAT	Resistant to *GRD;* early maturing
ICGV IS 07899	ICG 12991 x ICGV-SM 95603	ICRISAT	Resistant to *GRD;* early maturing
ICIAR-19BT	KH 241D/ICGV 87922	ICRISAT	Resistant to *GRD;* early maturing
SAMNUT 14	55 – 437 ex –Dakar	IAR	Susceptible to GRD; late maturing
KWANKWASO	Local collection	SAMARU	Susceptible to GRD; late maturing
MANIPENTA	Local collection	SAMARU	Susceptible to GRD; late maturing

Source: Breeding Nurseries for Samanko Stations 2008 and Institute for Agricultural Research, Samaru – Zaria

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The colonies were each maintained on susceptible groundnut genotypes SAMNUT14 (susceptible farmers variety), in screen house. Two wingless (apterae) aphids were transferred onto 7 to 14-day-old seedlings of 9 parental lines and their 36 F_2s grown at IAR research field (S, field). Each genotype was observed for presence or absence of aphid colonies (adults as well as nymphs) 7 days after infestation. Plants with no aphid colonies were re-infested with viable aphids 7 days after the first infestation. It is rare to find plants without aphids in choice tests because the aphids are free to roam to find suitable plant hosts. Aphids that appeared to be transient, possibly probing for feeding sites, are often observed on resistant plants in choice tests, along with dead aphids. Sometimes several viviparous aptera, surrounded by a few nymphs, may be observed on resistant plants without the development of established colonies. Based on these observations, aphids were visually rated for each plant at two weeks intervals after infestation using a scale of 0 – 4 developed by Mensah *et al.* (2005, 2008), where

0 = No aphid

0.5 = fewer than 10 aphids per plant, no colony formed

1.0 = 11–100 aphids per plant, plants appear healthy

1.5 = 101–150 aphids per plant, plants appear healthy

2.0 = 151–300 aphids per plant, mostly on the young leaves or tender stems, plants appear healthy

2.5 = 301–500 aphids per plant, plants appear healthy

3.0 = 501–800 aphids per plant, young leaves and tender stems are covered with aphids, leaves appear slightly curly and shiny

3.5 = more than 800 aphids per plant, plants appear stunted, leaves appear curled and slightly yellow, no sooty mould and few cast skins

4.0 = more than 800 aphids per plant, plants appear stunted, leaves appear severely curled and yellow and are covered with sooty mould and cast skins

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An aphid damage index (DI) for each line was calculated by the following formula:

DI = ∑ (scale value x no. of plants in the category)/ (4 x total no. of plants) x 100.

The DI ranges between 0 for no infestation and 100 for the most severe damage (Mensah *et al.* , 2005). The DI was used as an indicator of aphid resistance and was applied in the analysis.

The disease severity was recorded as the amount of plant tissue that is diseased, green or chlorotic rosette. Reaction to rosette on a scale of 1 – 9 as described by GGP (2000) was used as follows:

1 = No apparent rosette symptoms

3 = 10 – 20% rosette symptoms

5 = 20 – 60% rosette symptoms

7 = 60 – 80% rosette symptoms

9 = 100% rosette symptoms

The results of these observations were transformed to compute infection responses as measured by Area Under Disease Progressive Curve (AUDPC) based on Moldovan *et al.* (2005) according to the following function:

$$\text{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i+1}}{2}\right)(t_i + 1 - t_i)$$

Where: y = disease severity at the ith observation (transformed),

t = time (days) of ith observation

n = Total number of assessment times

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5.2.2 Agronomic performance

The following traits were measured on five randomly selected plants per plot:

Plant height: This is the perpendicular height of the plant from the ground level to the end of the topmost leaf of the plant

Number of pods per plant: Total number of healthy filled pods picked from each plant

Number of pods per plot: Total number of healthy filled pods picked from each plot

Pod weight per plant (g): This the weight of the pods per plant following sun drying for 3 days

Pod weight per plot (g): This the weight of the pods per plot following sun drying for 3 days

Sound kernel weight per plant (g): This the weight of sound kernels recorded for each plant

Sound kernel weight per plot (g): This the weight of sound kernels recorded for each plot

100 – Sound kernel weight (g): This the weight of 100 – kernels for each plant

Shelling percentage (%): This is the ratio of sound kernel weight to dry pod weight expressed as a percentage

5.2.3 Data analysis

Analysis of variance for individual location and combined data computed to estimate the main effects of locations, genotypes, and their interaction. Genotypes were considered fixed effects, and replications and location were considered random effects. The analysis of variance using General Linear Model (GLM) of Statistical Analysis System (SAS) program were performed with the PROC MIXED procedure from SAS® 9.3.1 software (SAS Institute, 2013). Pairwise comparisons of means were made using Least Significant Differences (LSD) for multiple-means

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comparison method. A Type I error of 0.05 was used for all statistical comparisons when F value was significant. The analysis of the variance was done using the TYPE III model. Various variance components use derived from the expectation of means squares.

Table 5. 2:Format of ANOVA for individual location

Source of variation	Df	MS	EMS	F – test
Replication	$r-1$	MS_r		
Genotypes	$g-1$	MS_g	$\sigma_e^2 + r\sigma_g^2$	MS_g / MS_e
Error	$(g-1)(r-1)$	MS_e	σ_e^2	
Total	$gr-1$			

For individual location the variance components are computed from mean squares as follows:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

$$\sigma_g^2 = \frac{MS_g - MS_e}{r}$$

$$\sigma_e^2 = MS_e$$

Where:

MS_g = Genotypic means square

MS_e = Error mean square

σ_g^2 = Genotypic variance

σ_e^2 = Error variance

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Table 5. 3: Format of ANOVA for the combined locations

Source of variation	Df	MS	EMS	F – test
Replication	$r-1$	MS_b		
Replication (Location)	$r(l-1)$			
Location	$l-1$	MS_l	$\sigma_e^2 + r\sigma_{gl}^2 + rg\sigma_l^2$	MS_l / MS_{ge}
Genotype	$g-1$	MS_g	$\sigma_e^2 + r\sigma_{gl}^2 + rl\sigma_g^2$	MS_g / MS_e
Genotype x Location	$(g-1)(l-1)$	MS_{ge}	$\sigma_e^2 + r\sigma_{gl}^2$	MS_{ge} / MS_e
Error	$(gl-1)(r-1)$	MS_e	σ_e^2	
Total	$glr-1$			

Where:

$$\sigma_l^2 = \frac{MS_l - MS_{gl}}{rg}$$

$$\sigma_g^2 = \frac{MS_g - MS_{gl}}{rl}$$

$$\sigma_{gl}^2 = \frac{MS_{gl} - MS_e}{r}$$

$$\sigma_e^2 = MS_e$$

MS_l = Means square due Location

MS_g = Mean square due genotype

MS_{gl} = Mean square due Genotype x Location

MS_e = Error mean square

σ_l^2 Location variance

σ_g^2 = Genotypic variance

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σ^2_{gl} = Genotype x Location variance

σ^2_e = Error variance

r, l, g are number of replication, location, genotype respectively and e experimental error

5.2.3.1 Heritability estimates

Broad-sense heritability for each trait was calculated as follows:

$$h^2_b = \sigma^2_g / \sigma^2_P, \quad \sigma^2_P = \sigma^2_g + \frac{\sigma^2_{gl}}{l} + \frac{\sigma^2_e}{rl}$$ as described by (Littell *et al.,* 2006)

Heritability estimates were grouped as high (> 50%), moderate (20 – 50 %) and low (< 20 %) as suggested by Stansfield (1986).

Narrow-sense heritability estimates were computed using the variance of GCA and SCA as described Hallauer *et al.* (2008)

$$h^2_n = \frac{\sigma^2_A}{\sigma^2_{Ph}}, \quad \sigma^2_{Ph} = 2 * \sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_E$$

where h^2_n= the narrow sense heritability, $\hat{\sigma}^2_{GCA}$ component of variance estimates due to general combining ability which is a measure of additive effects, $\hat{\sigma}^2_{SCA}$ is the component of variance estimates due to specific combining ability, a measure of non-additive gene effects.

5.2.3.2 Expected genetic gain

Expected genetic gain as per percentage of population mean was estimated at 10% selection intensity as $\Delta G = i * h * GCV$ (Hallauer *et al.,* 2008) where *i* is standardized selection intensity,

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h^2 is the heritability in a broad sense and GCV is the genotypic coefficient of variation expressed as percentage of mean.

5.2.3.3 Rank Summation Index (RSI)

For the purpose of selection, an index, Rank Summation Index (RSI) Mulamba and Mock (1978) was generated from four traits namely pod weight and sound kernel weight per plant, aphid damage index and Area under Disease Progress Curve. The index was formed by ranking each trait according to the order of desired traits. Finally, the values assigned to each trait are added, obtaining the sum of the ranks, which indicates the classification of genotypes. The entry with good plant appeal and tolerance, and highest pod and sound kernel yield and low AUDPC ranked first, while the reverse ranked the last. Rank Summation Index (Mulamba and Mock, 1978) was summarized as follows;

$$RSI = \sum_{i=1}^{n} R_i s$$

Where,

RSI= Aggregate performance of a genotype using the ranking of each of the desired traits

R_i = the rank of the mean of each of the desired traits;

5.2.3.4 Genetic Analysis of Resistance to Groundnut Rosette Disease

Analysis of the diallel for general combining ability (GCA) and specific combining ability (SCA) effects for all traits were based on the Model I, Method 2 proposed by Griffing (1956). Parents and one set of F_2's but not reciprocal F_2's are included given $p(p+1)/2$ combinations, in which p is the number of parents used to derive the F_1 progeny. Trait values were predicted based on traits mean value to produce a balanced data set. Diallel data were analysed using the

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Diallel SAS-05 program (Zhang *et al.,* 2005). GCA and SCA effects were determined for parents and the 36F_2's, respectively.

The following linear mixed model was fitted to data to estimate variance components for single and multi-location diallel tests.

The model for the analysis of variance for single location was:

$$Y_{ijk} = \mu + r_k + g_i + g_j + s_{ij} + e_{ijk}$$

Where μ is the mean,

r_k is the replication effect,

g_i and g_j are the GCA effects,

s_{ij} is the SCA effect, and

e_{ijk} is the experimental error for the Y_{ijk} observation (k =1……… 36, r = 2, i = j = 9.

Model for the analysis of variance for multi-location was:

$$Y_{ijklm} = \mu + L_i + r_{j(i)} + GCA_k + GCA_l + SCA_{kl} + L*GCA_{ik} + L*GCA_{il} + L*SCA_{ikl} + E_{ijklm}$$

Where

Y_{ijklm} = the mth observation of the jth replication for k^{th} cross in i^{th} location;

μ = the overall mean;

L = the ith fixed (location) effect, $i = 1-2$;

$r_{j(i)}$ = the fixed effect of the jth replication within the ith location, $j = 1-2$;

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GCA_k, GCA_l = is the random general combining ability (GCA) effect of the kth female or the ith male ~Normally Independently Distributed (NID) $(0, \sigma_G^2)$;

SCA_{kl} = is the random specific combining ability (SCA) effect of the kth and the ith parents~ (NID) $(0, \sigma_S^2)$;

$L*GCA_{ik}, L*GCA_{ij}$ = is the random GCA by location Interaction effect ~ (NID) $(0, \sigma_{IG}^2)$;

$L*SCA_{ikl}$ = is the random SCA by location Interaction effect ~ (NID) $(0, \sigma_{IS}^2)$ and

E_{ijklm} = is the random error term ~ (NID) $(0, \sigma_E^2)$.

Table 5. 4: Format of Diallel analysis of variance for model I method II for groundnut progenies evaluated in one location

Source of variation	Df	MS	E(MS) Model I	E(MS) Model II
Replication	$r-1$			
Crosses	$[(p(p-1)/2]-1$	M_2	$\hat{\sigma}_e^2 + rK_C$	$\hat{\sigma}_e^2 + r\hat{\sigma}_C^2$
GCA	$p-1$	M_{22}	$\hat{\sigma}_e^2 + [r/(n-2)/(n-1)]K_{GCA}^2)$	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA}^2 + r(n-2)\hat{\sigma}_{GCA}^2$
SCA	$p(p-1)/2$	M_{21}	$\hat{\sigma}_e^2 + \{2r/[n(n-1)]\}K_{SCA}^2)$	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA}^2$
Error	$(r-1)\{[p(p-1)/2]-1\}$	M_1	$\hat{\sigma}_e^2$	$\hat{\sigma}_e^2$

r and *n* are number of replications and parents, respectively (Hallauer *et al.,* 2010)

The variance explained by the general combining ability effects of parents (half-sibs) is a half of additive genetic variance i.e. $\sigma_{GCA}^2 = 1/2 * \sigma_A^2$ while the variance explained by the female and male interactions (specific combining ability) is equal to dominance genetic variance. i.e.

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dominance genetic variance $\sigma^2_{SCA} = \sigma^2_D$. Phenotypic variance is the sum of the observational components of variance. $\sigma^2_{Ph} = 2 * \sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_E$

5.2.3.5 Baker's ratio

Prediction of progeny performance based on GCA and SCA was carried out by the use of Becker's ratio, which is the ratio of combining ability variance component described by Becker (1978) as follows: $\left[(2\sigma^2_{GCA})/(2\sigma^2_{GCA} + \sigma^2_{SCA})\right]$. The closer this ratio is to unity, the greater the predictability based on GCA alone.

Results

5.2.4 Variance components and heritability of traits

Highly significant ($(p < 0.01)$) differences were found around genotypes for all traits (Table 5.5). The same applied for location except for estimated shelling percentage and aphid damage index. The interaction of genotype and location (G x L) was also highly significant for most of the traits. However, there was no significant G x L interaction for one hundred sound kernel weight and aphid damage index. Estimates of variance components (Table 5.6) indicated that genetic components were a significant source of variation for all the traits except for sound kernel weight in ton per hectare. Unlike genotype, genotype x location variance was significant for traits such as plant height, number of pod per plant, sound kernel weight per plant and shelling percentage. Variance component due to location showed to be a highly significant source of variation for number of pods per plant and area AUDPC.

Broad-sense heritability was quite high for all the traits except for plant height and shelling percentage. Broad sense heritability (h^2_b) was estimated across locations for all traits considered

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in this study (Table 5.6). Estimates of broad-sense heritability from combined analysis ranged from 27.77% to 99.50%. The estimates was highest for 100 sound kernel weight (99.50%) followed by DI and AUDPC with estimates of 95.65 and 94.78 respectively. Most of the traits in this study had similar heritability estimates as to those reported by Sikinarum *et al.* (2007) and Puttha *et al.* (2008). Estimates of narrow sense heritability for use in selection among half – sib families and among individuals ranged from 0.90% for plant height to 67.54% for aphid damage index. A low estimate of narrow sense heritability estimate was obtained for AUDPC (29.29 %) with high estimate for DI (67.54). Genetic advance (GA) was relatively low for DI (5.88%) and AUDPC (3.75 %). This was coupled with low narrow sense heritability estimates. The estimate of GA for SKWPT was relatively low (Table 5.6).

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Table 5. 5:Mean squares of measured traits for 9 parents and 36 F2 half diallel progenies of groundnut evaluated over Samaru and Lafia Locations in 2012

Source of variation	PHT	NOPP	PWPT	SKWPT	100SKWT	PWTON	SKWTTON	SHP	DI	AUDPC
Replication	0.66	8.75	110.53	41.41	0.01	0.05	0.00	1.48	58.80	6.31
Rep(Location)	14.59	3.93	3.03	0.43	0.01	0.00	0.09	0.54	18.37	0.01
Location	757.39**	3020.08**	337.16	163.34**	189.32**	3.24**	1.77**	32.32	198.16	7865.90**
Genotype	261.18**	215.29**	1033.52**	283.06**	126.25**	2.07**	1.49**	144.70**	870.23**	780.01**
Genotype x Location	188.66**	67.75**	233.63**	97.15**	0.63	0.15**	3.07**	95.58*	37.82	40.72**
Residual	14.89	6.88	22.66	15.86	2.11	0.03	0.09	41.96	60.70	4.34
CV (%)	9.97	6.08	12.33	15.84	5.44	12.83	25.83	9.67	22.03	6.04
R^2	0.94	0.96	0.97	0.92	0.97	0.97	0.90	0.74	0.88	0.99

*and ** significance at P <0.05 and P < 0.01 respectively. PHT=Plant Height (cm), NOPP= Number of pod per plant, PWPT= Pod weight per Plant (g), SKWPT= Sound kernel weight per plant (g), 100SKWT= 100 sound kernel weight (g), PWTON= Pod weight ton ha^{-1}, SKWTTON= Sound kernel weight ton ha^{-1}, SHP= Shelling percentage (%), DI= Aphid damage Index and AUDPC = Area under disease progress curve

Table 5. 6: Variance components, Heritability estimates and expected gain for groundnut traits over combined Samaru and Lafia location in 2012

	Variance components of F_2 genotypes							
Traits	Genetic (σ_g^2)	Location (σ_l^2)	G x L (σ_{gl}^2)	Error (σ_e^2)	H_b^2 (%)	h_n^2 (%)	Genetic CV (%)	$\Delta G(\%\overline{X})$
PHT	18.13**	6.32	86.89**	14.89	27.77	0.90	10.80	0.18
NOPP	36.89**	32.80**	30.44**	6.88	68.53	29.48	14.09	1.34
PWPT	199.97**	1.15	105.49*	22.66	77.39	44.28	38.63	4.51
SKWPT	46.48**	0.74	40.65**	15.86	65.68	64.25	27.12	3.82
100SKWT	31.41**	2.10	-0.74	2.11	99.50		21.00	–
PWTON	0.48**	0.03	0.06	0.03	92.75	62.32	50.20	6.95
SKWTTON	-0.40	-0.01	1.49	0.09	–	67.07	–	–
SHP	12.28*	-0.70	26.81**	41.96	33.95	33.05	5.23	0.53
DI	208.10**	1.78	-11.44	60.70	95.65	67.54	40.80	5.88
AUDPC	184.82**	86.95*	18.19	4.34	94.78	29.29	39.45	3.75

PHT=Plant Height (cm), NOPP= Number of pod per plant, PWPT= Pod weight per Plant (g), SKWPT= Sound kernel weight per plant (g), 100SKWT= 100 sound kernel weight (g), PWTON= Pod weight ton ha^{-1}, SKWTTON= Sound kernel weight ton ha^{-1}, SHP= Shelling percentage (%), DI= Aphid damage Index and AUDPC = Area under disease progress curve.

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5.2.5 Performance of the groundnut genotypes grown at Samaru and Lafia, 2012

5.2.5.1 Sound Kernel weight per Plant (g) (SKWTPPT)

The mean SKWTPPT for genotypes over environmental index was 25.14 g and ranged from the lowest entry KWANKWASO (9.41g) to the highest ICGV IS 07890 X ICGV IS 07899 (48.90g) (Table 5.7). In Samaru, ICGV IS 07890 X ICGV IS 07899, ICGV IS 07890, ICGX – SM 00020/5/9 X ICGV IS 07899 and ICGX – SM 00020/5/9 X ICGV IS 07890 recorded the highest sound kernel yield of 57.05g, 47.50g, 41.93 g and 40.47g, respectively. The lowest performers for this trait were ICGX – SM 00017/5/P_{10}/P_1 X MANIPENTA, MANIPENTA and KWANKWASO with sound kernel yield per plant of 11.54g, 10.24g and 9.47g respectively. In Lafia however, ICGX – SM 00017/5/P_{10}/P_1, ICGV IS 07890 X ICGV IS 07899 and ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1 with sound kernel yield per plant of 42.30g, 40.76g and 39.90g respectively. The lowest yield were ICGX – SM 00020/5/9 X KWANKWASO, ICGX – SM 00020/5/9 X MANIPENTA and KWANKWASO. They have sound kernel per plant of 10.96g, 10.17 g and 9.36 g, respectively. Across the two locations, the best performing genotypes for sound kernel yield were ICGV IS 07890 X ICGV IS 07899, ICGV IS 07890 and ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1(48.90g, 43.46g and 38.72g, respectively). The lowest yielders were ICGX – SM 00020/5/9 X MANIPENTA, MANIPENTA and KWANKWASO with sound kernel yield per plant of 11.99 g, 11.03 g and 9.41g (Table 5.7)

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Table 5. 7: Performance of parents and their F_2 progenies for sound kernel weight per plant (g) over Samaru and Lafia environment in 2012

Genotypes	SKWTPPT (g)		
	Samaru	Lafia	Mean
ICGX – SM 00020/5/9	14.66	21.29	17.98
ICGX – SM 00017/5/P_{10}/P_1	34.49	42.30	38.40
ICGX – SM 00020/5/P_4/P_1	32.41	38.15	35.28
ICGV IS 07890	47.50	39.43	43.46
ICGV IS 07899	25.09	25.90	25.49
ICIAR-19BT	20.35	27.13	23.74
SAMNUT 14	17.99	22.54	20.26
KWANKWASO	9.47	9.36	9.41
MANIPENTA	10.24	11.82	11.03
ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P10/P1	37.54	39.90	38.72
ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P4/P1	37.70	38.42	38.06
ICGX – SM 00020/5/9 X ICGV IS 07890	40.47	21.92	31.20
ICGX – SM 00020/5/9 X ICGV IS 07899	41.93	16.25	29.09
ICGX – SM 00020/5/9 X ICIAR-19BT	31.77	26.16	28.97
ICGX – SM 00020/5/9 X SAMNUT 14	21.22	21.58	21.40
ICGX – SM 00020/5/9 X KWANKWASO	24.77	10.96	17.86
ICGX – SM 00020/5/9 X MANIPENTA	13.81	10.17	11.99
ICGX – SM 00017/5/P10/P1 X ICGX – SM 00020/5/P4/P1	22.39	39.41	30.90
ICGX – SM 00017/5/P10/P1 X ICGV IS 07890	32.67	31.40	32.03
ICGX – SM 00017/5/P10/P1 X ICGV IS 07899	36.32	27.24	31.78
ICGX – SM 00017/5/P10/P1 X ICIAR-19BT	36.80	25.09	30.94
ICGX – SM 00017/5/P10/P1 X SAMNUT 14	21.25	26.63	23.94
ICGX – SM 00017/5/P10/P1 X KWANKWASO	15.74	15.22	15.48
ICGX – SM 00017/5/P10/P1 X MANIPENTA	11.54	24.46	18.00
ICGX – SM 00020/5/P4/P1 X ICGV IS 07890	31.47	19.81	25.64
ICGX – SM 00020/5/P4/P1 X ICGV IS 07899	22.93	13.73	18.33

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ICGX – SM 00020/5/P4/P1 X ICIAR-19BT	33.65	17.31	25.48
ICGX – SM 00020/5/P4/P1 X SAMNUT 14	15.53	27.34	21.43
ICGX – SM 00020/5/P4/P1 X KWANKWASO	20.55	20.75	20.65
ICGX – SM 00020/5/P4/P1 X MANIPENTA	15.51	39.43	27.47
ICGV IS 07890 X ICGV IS 07899	57.05	40.76	48.90
ICGV IS 07890 X ICIAR-19BT	26.19	27.32	26.75
ICGV IS 07890 X SAMNUT 14	22.51	26.34	24.42
ICGV IS 07890 X KWANKWASO	21.32	18.95	20.14
ICGV IS 07890 X MANIPENTA	38.96	23.75	31.36
ICGV IS 07899 X ICIAR-19BT	26.97	27.53	27.25
ICGV IS 07899 X SAMNUT 14	27.41	18.08	22.74
ICGV IS 07899 X KWANKWASO	19.78	19.18	19.48
ICGV IS 07899 X MANIPENTA	28.94	11.85	20.39
ICIAR-19BT X SAMNUT 14	28.87	19.64	24.25
ICIAR-19BT X KWANKWASO	25.85	27.38	26.61
ICIAR-19BT X MANIPENTA	20.38	25.54	22.96
SAMNUT 14 X KWANKWASO	17.37	17.77	17.57
SAMNUT 14 X MANIPENTA	21.64	21.10	21.37
KWANKWASO X MANIPENTA	13.33	12.32	12.83
Mean	26.10	24.19	25.14
LSD	7.97	7.99	5.60

SKWTPPT= Sound kernel weight per plant

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5.2.5.2 Aphid Damage Index (DI)

The mean performance based on Aphid damage Index (DI) for genotypes over environmental index was 35.36 and ranged from the lowest entry ICGX – SM 00020/5/9 (17.30) to the highest KWANKWASO X MANIPENTA with DI of 64.97 (Table 5.8). Two entries; ICGX – SM 00020/5/9 (Aphid resistant lines) and ICGV IS 07890 (Rosette resistant line) had the lowest DI of less than 20. Sixteen entries had DI values of between 20 - 30 with ICGX – SM 00020/5/9 X ICGV IS 07890 having the lowest DI value of 20.45. Genotype MANIPENTA, ICGV IS 07899 X MANIPENTA and ICGX – SM 00020/5/9 X ICGV IS 07890 have significantly low DI values of 14.38, 15.18 and 16.98 respectively at Samaru and in Lafia, ICGX – SM 00017/5/P_{10}/P_1 X ICGX – SM 00020/5/P_4/P_1, ICGV IS 07890, ICGX – SM 00020/5/9 and ICGX – SM 00017/5/P_{10}/P_1 X ICIAR-19BTwere the genotypes with the lowest DI values of 14.38, 15.28, 16.22 and 16.98 respectively (Table 5.8). A highly significant variation ($P < 0.01$) was observed among the entries for DI values.

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Table 5. 8: Performance of parents and their F_2 progenies for aphid damage Index (DI over Samaru and Lafia environment in 2012

Genotypes	DI		
	Samaru	Lafia	Mean
ICGX – SM 00020/5/9	18.37	16.22	17.30
ICGX – SM 00017/5/P_{10}/P_1	34.17	22.66	28.41
ICGX – SM 00020/5/P_4/P_1	25.52	29.17	27.34
ICGV IS 07890	21.73	15.28	18.51
ICGV IS 07899	46.15	20.65	33.40
ICIAR-19BT	36.67	22.32	29.49
SAMNUT 14	44.27	52.60	48.44
KWANKWASO	44.46	55.58	50.02
MANIPENTA	14.38	65.87	40.12
ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P10/P1	33.85	18.37	26.11
ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P4/P1	21.02	34.17	27.60
ICGX – SM 00020/5/9 X ICGV IS 07890	16.98	25.52	21.25
ICGX – SM 00020/5/9 X ICGV IS 07899	19.18	21.73	20.45
ICGX – SM 00020/5/9 X ICIAR-19BT	17.99	46.15	32.07
ICGX – SM 00020/5/9 X SAMNUT 14	18.18	36.67	27.42
ICGX – SM 00020/5/9 X KWANKWASO	20.54	44.27	32.41
ICGX – SM 00020/5/9 X MANIPENTA	22.92	44.46	33.69
ICGX – SM 00017/5/P10/P1 X ICGX – SM 00020/5/P4/P1	43.75	14.38	29.06
ICGX – SM 00017/5/P10/P1 X ICGV IS 07890	21.54	33.85	27.70
ICGX – SM 00017/5/P10/P1 X ICGV IS 07899	50.52	21.02	35.77
ICGX – SM 00017/5/P10/P1 X ICIAR-19BT	43.99	16.98	30.48
ICGX – SM 00017/5/P10/P1 X SAMNUT 14	51.46	19.18	35.32
ICGX – SM 00017/5/P10/P1 X KWANKWASO	40.42	17.99	29.20
ICGX – SM 00017/5/P10/P1 X MANIPENTA	54.03	18.18	36.10
ICGX – SM 00020/5/P4/P1 X ICGV IS 07890	55.58	20.54	38.06
ICGX – SM 00020/5/P4/P1 X ICGV IS 07899	18.37	16.22	17.30

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ICGX – SM 00020/5/P4/P1 X ICIAR-19BT	53.46	22.92	38.19
ICGX – SM 00020/5/P4/P1 X SAMNUT 14	25.48	43.75	34.62
ICGX – SM 00020/5/P4/P1 X KWANKWASO	59.90	21.54	40.72
ICGX – SM 00020/5/P4/P1 X MANIPENTA	35.10	50.52	42.81
ICGV IS 07890 X ICGV IS 07899	38.90	43.99	41.44
ICGV IS 07890 X ICIAR-19BT	24.96	53.22	39.09
ICGV IS 07890 X SAMNUT 14	36.46	46.04	41.25
ICGV IS 07890 X KWANKWASO	39.90	54.03	46.97
ICGV IS 07890 X MANIPENTA	44.45	55.58	50.01
ICGV IS 07899 X ICIAR-19BT	38.64	53.46	46.05
ICGV IS 07899 X SAMNUT 14	21.43	25.48	23.45
ICGV IS 07899 X KWANKWASO	22.66	59.90	41.28
ICGV IS 07899 X MANIPENTA	29.17	35.10	32.13
ICIAR-19BT X SAMNUT 14	15.18	38.90	27.04
ICIAR-19BT X KWANKWASO	20.65	24.96	22.80
ICIAR-19BT X MANIPENTA	18.75	37.50	28.13
SAMNUT 14 X KWANKWASO	45.55	40.87	43.21
SAMNUT 14 X MANIPENTA	52.29	64.16	58.23
KWANKWASO X MANIPENTA	42.86	63.07	52.96
Mean	33.37	35.22	34.30
LSD	34.31	36.41	35.36

DI = aphid damage Index and LSD = least significant difference

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5.2.5.3 Area Under Disease Progress curve (AUDPC)

Area Under Disease Progress curve for genotypes over environmental index has mean and median 31.95 and 34.16 respectively, and ranged from 12.69 for genotype with lowest AUDPC value ICGX – SM 00020/5/9 X MANIPENTA to 49.23 for ICIAR-19BT X KWANKWASO with the highest AUDPC values. The value for AUDPC was smaller ($p < 0.01$) for the resistant (AUDPC < 30) and moderately resistant parents (AUDPC 31 – 50) compared to the moderately susceptible parents (AUDPC 51 – 60) and susceptible (AUDPC > 60) genotypes. Genotypes classified as moderately resistant and moderately susceptible differ ($P < 0.05$) statistically for AUDPC (Table 5.9). Based on AUDPC, eighteen genotypes were regarded as being the resistant genotypes with AUDPC values of < 30, five of these genotypes, ICGX – SM 00020/5/9 X MANIPENTA, ICGV IS 07890 X ICIAR-19BT IS 07899, ICGX – SM 00020/5/9 X ICGV IS 07890, ICGX – SM 00020/5/9 and ICGV IS 07890 have AUDPC of < 20 and considered to be resistant/tolerant to rosette disease infection.

At both Samaru and Lafia, ICGX – SM 00020/5/9 X MANIPENTA and ICGV IS 07890 X ICIAR-19BThave the lowest AUDPC value each with 10.94 and 14.44, respectively, and ICGX – SM 00020/5/9 X KWANKWASO and ICIAR-19BT X KWANKWASO were with the highest values of 43.14 and 43.96 respectively at Samaru. At Lafia, it was SAMNUT 14 and MANIPENTA that have the highest values of 58.25 and 58.50 respectively. The AUDPC values across the two environments indicated Lafia to have the highest AUDPC of 38.08 while the value at Samaru was 25.84, the differences were statistically significant ($P < 0.05$), a clear indication of genotype x Location interaction for SKWPPT, DI and AUDPC (Fig. 5.1).

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Table 5. 9: Performance of parents and their F_2 progenies for AUDPC over Samaru and Lafia environment in 2012

Genotypes	AUDPC		
	Samaru	Lafia	Mean
ICGX – SM 00020/5/9	14.44	18.75	16.59
ICGX – SM 00017/5/P_{10}/P_1	14.44	31.25	22.84
ICGX – SM 00020/5/P_4/P_1	14.44	25.75	20.09
ICGV IS 07890	14.81	19.75	17.28
ICGV IS 07899	11.31	33.00	22.16
ICIAR-19BT	11.31	57.00	34.16
SAMNUT 14	10.94	58.25	34.59
KWANKWASO	10.94	51.25	31.09
MANIPENTA	34.75	58.50	46.63
ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P10/P1	25.94	19.25	22.59
ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P4/P1	29.69	19.25	24.47
ICGX – SM 00020/5/9 X ICGV IS 07890	11.31	19.25	15.28
ICGX – SM 00020/5/9 X ICGV IS 07899	25.94	19.75	22.84
ICGX – SM 00020/5/9 X ICIAR-19BT	24.13	19.75	21.94
ICGX – SM 00020/5/9 X SAMNUT 14	22.83	19.75	21.29
ICGX – SM 00020/5/9 X KWANKWASO	43.14	19.25	31.19
ICGX – SM 00020/5/9 X MANIPENTA	10.94	14.44	12.69
ICGX – SM 00017/5/P10/P1 X ICGX – SM 00020/5/P4/P1	26.40	51.00	38.70
ICGX – SM 00017/5/P10/P1 X ICGV IS 07890	29.81	39.25	34.53
ICGX – SM 00017/5/P10/P1 X ICGV IS 07899	31.98	44.25	38.12
ICGX – SM 00017/5/P10/P1 X ICIAR-19BT	38.48	19.75	29.11
ICGX – SM 00017/5/P10/P1 X SAMNUT 14	33.60	39.25	36.43
ICGX – SM 00017/5/P10/P1 X KWANKWASO	29.31	39.50	34.41
ICGX – SM 00017/5/P10/P1 X MANIPENTA	29.31	40.50	34.91
ICGX – SM 00020/5/P4/P1 X ICGV IS 07890	29.12	37.38	33.25
ICGX – SM 00020/5/P4/P1 X ICGV IS 07899	14.44	18.75	16.59
ICGX – SM 00020/5/P4/P1 X ICIAR-19BT	38.87	37.38	38.12

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ICGX – SM 00020/5/P4/P1 X SAMNUT 14	30.51	46.00	38.25
ICGX – SM 00020/5/P4/P1 X KWANKWASO	30.51	51.25	40.88
ICGX – SM 00020/5/P4/P1 X MANIPENTA	30.51	48.00	39.25
ICGV IS 07890 X ICGV IS 07899	30.51	51.25	40.88
ICGV IS 07890 X ICIAR-19BT	28.95	45.00	36.97
ICGV IS 07890 X SAMNUT 14	10.94	14.44	12.69
ICGV IS 07890 X KWANKWASO	39.18	39.50	39.34
ICGV IS 07890 X MANIPENTA	34.36	39.25	36.81
ICGV IS 07899 X ICIAR-19BT	30.95	51.75	41.35
ICGV IS 07899 X SAMNUT 14	14.06	39.75	26.91
ICGV IS 07899 X KWANKWASO	19.94	39.75	29.84
ICGV IS 07899 X MANIPENTA	13.24	39.75	26.49
ICIAR-19BT X SAMNUT 14	13.91	39.75	26.83
ICIAR-19BT X KWANKWASO	25.24	37.75	31.5
ICIAR-19BT X MANIPENTA	43.96	54.50	49.23
SAMNUT 14 X KWANKWASO	34.36	57.50	45.93
SAMNUT 14 X MANIPENTA	34.94	58.25	46.59
KWANKWASO X MANIPENTA	37.38	53.00	45.19
Mean	40.97	54.50	47.73
LSD	25.84	38.08	31.95

AUDPC = Area under disease progress curve and LSD = least significant difference

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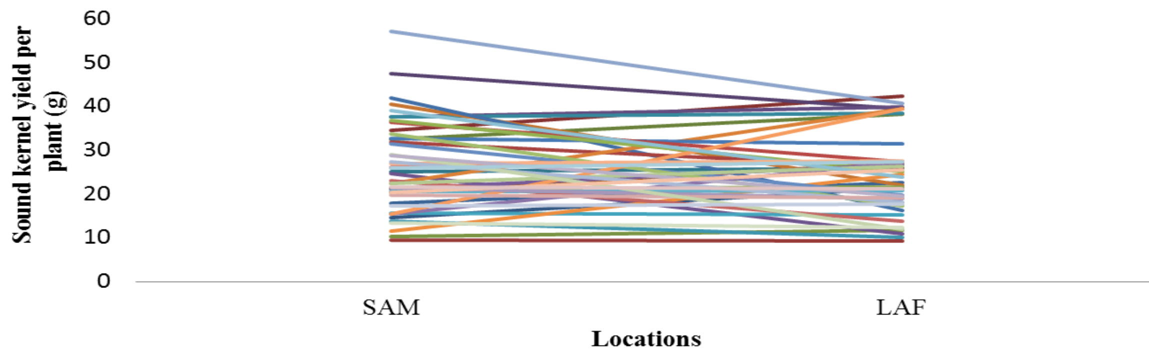

Figure 5. 1: Cross over Genotype x Location Interaction for sound kernel yield per plant across Batstari and Lafia Locations

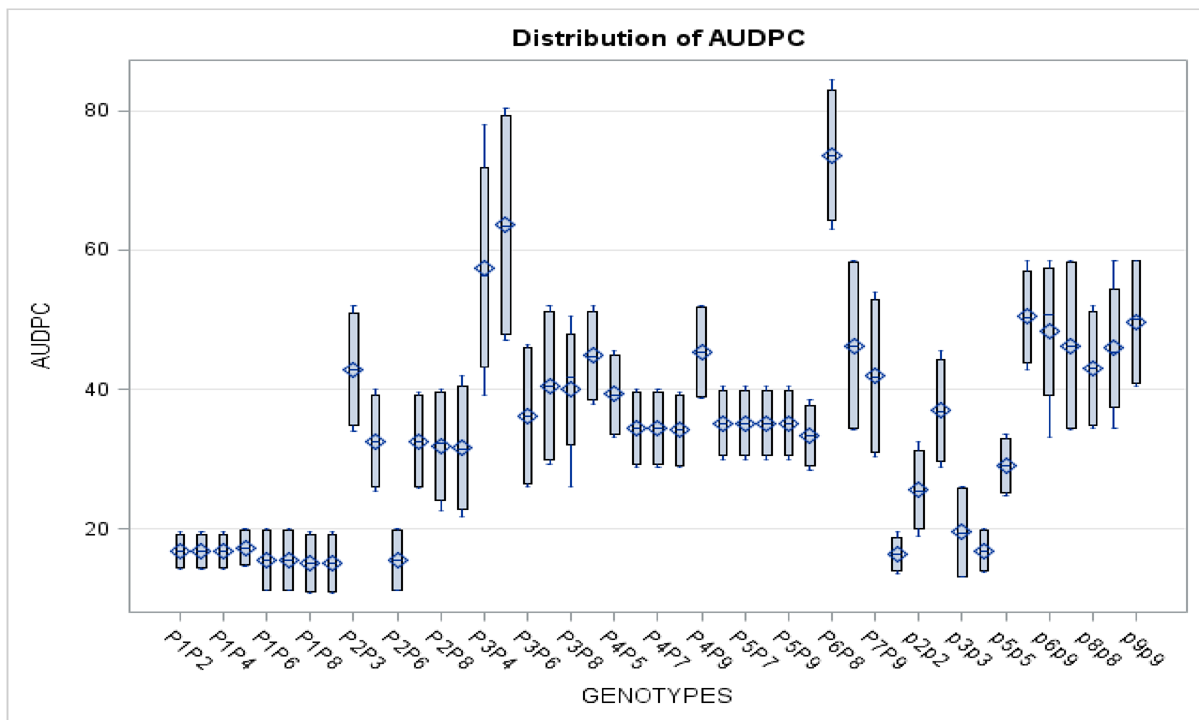

Figure 5. 2:Performance of F2 groundnut for sound kernel yield per plant and AUDPC

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5.2.5.4 Association between agronomic traits and rosette disease

Negative correlations between AUDPC and most of the yield parameters (Table 5.10). A weak negative correlations between AUDPC and plant height as ($r = -0.15$, $p > 0.05$), AUDPC and pod weight per plant ($r = -0.18$, $p > 0.05$), AUDPC and sound kernel weight per plant ($r = -0.26$, $p > 0.05$) were recorded. However, a highly significant negative correlations was observed for between AUDPC and pod weight ton ha^{-1} ($r = -0.45$, $p < 0.01$) and AUDPC and sound kernel weight ton ha^{-1} ($r = -0.46$, $p < 0.01$). AUDPC was positively correlated ($r = 0.63$, $p < 0.01$) with Aphid damage index. Nevertheless, genotypes classified as resistant generally had lower AUDPC ($p < 0.01$) than the susceptible genotypes (Table 5.10).

Table 5. 10: Correlations among area under the disease progress curve and agronomic traits in groundnut

	1	2	3	4	5	6	7	8	9	10
Plant Height (cm)		0.49**	0.27	0.15	0.43**	0.36*	0.49**	0.05	-0.20	-0.15
Number of pod per plant			0.45**	0.11**	0.36**	0.44	0.25	-0.16	0.13	-0.13
Pod weight per Plant (g),				0.24	0.59**	0.94**	0.55**	-0.58**	-0.34*	-0.18
100 sound kernel weight (g)					0.35**	0.26	0.36**	-0.08	-0.10	0.01
Pod weight per Plant (g)						0.62**	0.92**	-0.29	-0.36**	-0.46**
Sound kernel weight ton ha^{-1}							0.60**	-0.33**	-0.42**	-0.26
Sound kernel weight per plant (g)								-0.28	-0.44**	-0.46**
Shelling percentage (%),									0.09	-0.02
Aphid damage Index										0.62**
Area under disease progress curve										

* and ** significant at P < 0.05 and P < 0.01 levels of probability respectively
Data based on measured on 9 parents and 36 F_2 groundnut hybrids evaluated across Samaru and Lafia Location in 2012

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5.3 General and specific combining ability for traits

For all the traits studied, the mean square (MS) values of combining ability (GCA) were significant (P < 0.05). Similarly, the MS for specific combining ability were significant for traits except the MS of SHP. For DI and AUDPC, the SCA MS were higher the GCA MS. (Table 5.9). The magnitude of this interaction for most traits was however, small relative to the GCA main effect. Significant SCA×L interactions ($p < 0.05$) were observed for all traits. The estimates of $\hat{\sigma}^2_{GCA}$ and $\hat{\sigma}^2_{SCA}$ were significantly different from zero for all the traits except for pod weight in tons per hectare. To understand the relative importance of general and specific combining abilities for DI and AUDPC, estimates of components of GCA and SCA that approximates variances were estimated according Bakers ratio (Becker, 1978). The ratio was closer to unity (0.73) for aphid damage index and low value was obtained for AUDPC (0.30) (Table 5.11). The estimates of $\hat{\sigma}^2_A$ and $\hat{\sigma}^2_D$ showed that greater proportions of total genetic variance are attributed to non – additive (i.e. dominance and epistasis) for AUDPC (with $\hat{\sigma}^2_A$= 136.27 and $\hat{\sigma}^2_D$ = 323.08).

Partitioning of genotypes into genetic effects indicated significant GCA effects ($p < 0.01$) and SCA effects ($p < 0.01$) for all traits. The GCA effects for AUDPC ranged from – 10.61 for ICGX – SM 00020/5/9 to 5.44 in MANIPENTA (Table 5.12). Genotypes with the lowest desirable negative GCA effects were ICGX – SM 00020/5/9 (– 10.61), SAMNUT14 (– 2.04), ICGV IS 07890 (– 1.58) and ICGX – SM 00017/5/P_{10}/P_1 (–1.41). The highest GCA effects for this trait were exhibited by KWANKWASO (5.44) and MANIPENTA (3.58) which were the most susceptible genotypes in this study. The GCA effect for sound kernel weight per plant (g) was

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highest in ICGV IS 07890 (5.74) and lowest in MANIPENTA (- 9.25). The parents, ICIAR-19BT (4.69), ICGX – SM 00020/5/9 (3.78) and ICGX – SM 00017/5/P_{10}/P_1 (3.10) depicted significantly high positive GCA effects.

The specific combining ability effects for AUDPC ranged from – 15.65 to 35.96. Most crosses revealed positive SCA effect, 13 out of 36 crosses (36.11 %) had negative SCA effects (Table 5.13). The F_2 combinations, ICGX – SM 00017/5/P_{10}/P_1 X ICIAR-19BT (–15.65) had the best desirable negative SCA effects. Other crosses with desirable negative and significant SCA effects for this trait includes ICGX – SM 00020/5/P_4/P_1 X SAMNUT 14 (–9.66), ICGV IS 07899 X SAMNUT 14 (–8.73) and ICGX – SM 00017/5/P_{10}/P_1 X ICGV IS 07890 (–8.1). In contrast, ICGV IS 07890 X ICGV IS 07899 (-7.64), ICGX – SM 00020/5/9 X MANIPENTA (-7.22) and ICGX – SM 00020/5/9 X SAMNUT 14 (-5.52) had high negative but not significant SCA effects. The greatest SCA effect (35.96) was recorded for ICGX – SM 00020/5/P_4/P_1 X MANIPENTA.

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Table 5. 11: Mean squares of combined ANOVA for half 9 x 9 diallel analysis for general and specific combining abilities and their interactions with location for ten morphological traits of groundnut evaluated at two locations in 2012

Source of variation	Df	PHT	NOPP	PWPT	SKWPT	100SKWT	PWTON	SKWTTON	SHP	DI	AUDPC
GCA	8	961.23**	777.85*	4578.12**	2132.30**	187.92**	12.80**	9.43**	614.69*	5197.60**	165.90**
SCA	36	931.16**	311.86**	1194.09**	282.54**	241.50**	1.86**	1.09*	203.75	578.97*	652.03**
GCA X L	8	967.45**	226.85**	478.98**	67.13**	0.68	0.63**	1.44**	187.45**	530.68**	440.04**
SCA X L	36	175.90**	98.43**	459.05**	10.50**	0.61	0.22**	0.27**	218.19**	239.59**	67.57**
ERROR	88	655.12	301.74	997.02	698.01	2978.89	1.37	4.40	1846.13	2670.79	190.74

*and ** significant at $P < 0.05$ and $P < 0.01$ levels of probability respectively

PHT=Plant Height (cm), NOPP= Number of pod per plant, PWPT= Pod weight per Plant (g), SKWPT= Sound kernel weight per plant (g), 100SKWT= 100 sound kernel weight (g), PWTON= Pod weight ton ha^{-1}, SKWTTON= Sound kernel weight ton ha^{-1}, SHP= Shelling percentage (%), DI= Aphid damage Index and AUDPC = Area under disease progress curve

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Table 5. 12: Variance component for GCA, SCA and their interactions with location, Bakers ratio, additive and dominance variances considering random effect model for 9 parents and 36 F2 evaluated across Samaru and Lafia Locations in 2012

Traits	Variance components of F_2					Bakers ratio	(σ^2_A)	(σ^2_D)
	σ^2_E	σ^2_{GCA}	σ^2_{SCA}	σ^2_{GCA} x L	σ^2_{SCA} x L			
Plant Height (cm)	15.66	2.15	457.75	959.62	168.07	0.01	4.30	457.75
Number of Pod per plant	6.69	33.29	152.59	223.505	95.085	0.30	66.57	152.59
Pod weight per Plant (g)	22.41	241.72	585.84	467.775	447.845	0.45	483.43	585.84
Sound kernel weight per plant (g)	11.57	132.13	135.49	61.345	4.715	0.66	264.25	135.49
100 sound kernel weight (g)	2.15	–	119.68	–	–	–	–	119.68
Pod weight ton ha^{-1}	0.03	0.78	0.92	0.615	0.205	0.63	1.56	0.92
Sound kernel weight ton ha^{-1}	0.08	0.60	0.51	1.4	0.23	0.70	1.19	0.51
Shelling percentage (%)	34.11	29.35	84.82	170.395	201.135	0.41	58.71	84.82
Aphid damage Index	55.26	329.90	261.86	503.05	211.96	0.72	659.80	261.86
AUDPC	5.87	68.13	323.08	437.105	64.64	0.30	136.27	323.08

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Table 5. 13: Estimates of general combining ability (GCA) effects of 9 parental lines for four important morphological characters of groundnut evaluated across Samaru and Lafia Locations in 2012

PARENTS	PWPT	SKWPT	DI	AUDPC
ICGX – SM 00020/5/9	1.22	3.78**	-4.77**	-10.61**
ICGX – SM 00017/5/P_{10}/P_1	5.74**	3.10**	-15.33**	-1.41
ICGX – SM 00020/5/P_4/P_1	4.72**	2.77**	-4.66**	2.53**
ICGV IS 07890	12.64**	5.74**	3.50**	-1.58
ICGV IS 07899	3.96**	0.39	-2.55*	2.20
ICIAR-19BT	-0.45	4.69**	-1.69	1.89
SAMNUT 14	-6.69**	-4.68**	2.20	-2.04
KWANKWASO	-12.62**	-6.54**	11.73**	3.58**
MANIPENTA	-8.50**	-9.25**	11.57**	5.44**
SE±	1.13	0.59	1.12	1.41

*and ** significant at $P < 0.05$ and $P < 0.01$ levels of probability respectively

Other crosses depicting significantly positive SCA effects includes ICGX – SM 00020/5/P_4/P_1 X ICGV IS 07890 (12.56), ICGV IS 07890 X MANIPENTA (15.48), ICGX – SM 00017/5/P_{10}/P1 X KWANKWASO (15.84) and ICGX – SM 00017/5/P10/P1 X SAMNUT 14 (18.61) (Table 5.14).

The SCA effects for sound kernel weight per plant (g) was highest in ICGV IS 07890 X SAMNUT 14 (19.03) and lowest in ICGX – SM 00020/5/P_4/P_1 X ICGV IS 07890 (- 16.07). The other parents that recorded the significantly high SCA effects were ICGV IS 07899 X ICIAR-19BT (15.17), SAMNUT14 X KWANKWASO (13.94), ICIAR-19BT X SAMNUT14 (11.55),

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ICGX – SM 00020/5/9 X ICGV IS 07899 (7.88), ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P_4/P_1 (7.74), ICGX – SM 00020/5/9 X ICGV IS 07890 (7.22) and ICGX – SM 00017/5/P_{10}/P_1 X ICIAR-19BT (6.75). Whereas ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1 (-10.64), ICGV IS 07890 X ICGV IS 07899 (-13.84) and ICGX – SM 00020/5/P_4/P_1 X ICGV IS 07890 (-16.07) had the lowest SCA effects for sound kernel yield per plant (Table 5.14). However, the F_2s that combined significant and desirable SCA effects for SKWPT and AUDPC were ICGX – SM 00017/5/P_{10}/P_1 X ICIAR-19BT (6.75, –15.67), ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P_4/P_1 (7.74, –2.77), ICGX – SM 00020/5/P_4/P_1 X SAMNUT 14 (6.00, – 9.66) and ICGV IS 07899 X KWANKWASO (3.75, – 2.10).

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Table 5. 14: Estimates of specific combining ability (SCA) effects measured in the 36 F2 progenies evaluated across Samaru and Lafia Locations in 2012

CROSSES	PWPT	SKWPT	DI	AUDPC
ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P10/P1	-5.66	-10.64**	2.59	-4.82
ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P4/P1	12.30**	7.74**	2.84	-2.77
ICGX – SM 00020/5/9 X ICGV IS 07890	15.50**	7.22**	-8.39**	9.53**
ICGX – SM 00020/5/9 X ICGV IS 07899	-6.85**	7.88**	-5.86	1.74
ICGX – SM 00020/5/9 X ICIAR-19BT	-2.97	4.62**	12.98**	2.79
ICGX – SM 00020/5/9 X SAMNUT 14	0.70	-7.29**	1.09	-5.52
ICGX – SM 00020/5/9 X KWANKWASO	-0.93	-4.74**	1.50	-1.57
ICGX – SM 00020/5/9 X MANIPENTA	1.43	2.17	7.62	-7.22
ICGX – SM 00017/5/P10/P1 X ICGX – SM 00020/5/P4/P1	-7.85	3.69	-0.73	2.81
ICGX – SM 00017/5/P10/P1 X ICGV IS 07890	2.94	-4.75**	10.06**	-8.10
ICGX – SM 00017/5/P10/P1 X ICGV IS 07899	-0.31	-3.35	1.44	5.87
ICGX – SM 00017/5/P10/P1 X ICIAR-19BT	-1.86	6.75**	-1.97	-15.65**
ICGX – SM 00017/5/P10/P1 X SAMNUT 14	0.44	2.37	-4.02	18.61**
ICGX – SM 00017/5/P10/P1 X KWANKWASO	-4.17	-2.78	-12.33**	15.84**
ICGX – SM 00017/5/P10/P1 X MANIPENTA	-10.79**	-7.11**	-30.59**	7.97
ICGX – SM 00020/5/P4/P1 X ICGV IS 07890	-14.78**	-16.07**	-10.16**	12.56**
ICGX – SM 00020/5/P4/P1 X ICGV IS 07899	-17.51**	-1.22	0.97	9.57**
ICGX – SM 00020/5/P4/P1 X ICIAR-19BT	-20.29**	-5.21**	11.17**	0.71
ICGX – SM 00020/5/P4/P1 X SAMNUT 14	2.02	6.00**	-11.61**	-9.66**

*and ** significant at $P < 0.05$ and $P < 0.01$ levels of probability respectively

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ICGX – SM 00020/5/P4/P1 X KWANKWASO	-1.05	-8.59**	6.03	-4.18
ICGX – SM 00020/5/P4/P1 X MANIPENTA	-0.14	-0.24	2.57	35.96**
ICGV IS 07890 X ICGV IS 07899	-3.03	-13.84**	-3.86	-7.64
ICGV IS 07890 X ICIAR-19BT	52.39**	-1.52	16.45**	3.14
ICGV IS 07890 X SAMNUT 14	-11.99**	19.03**	6.65*	4.33
ICGV IS 07890 X KWANKWASO	-10.71**	0.98	4.75	-1.39
ICGV IS 07890 X MANIPENTA	-10.31**	-3.35	24.53**	15.48*
ICGV IS 07899 X ICIAR-19BT	2.07	15.17**	-8.96**	3.34
ICGV IS 07899 X SAMNUT 14	-5.79	-6.39**	6.99*	-8.73*
ICGV IS 07899 X KWANKWASO	-4.47	3.75*	6.37**	-2.10
ICGV IS 07899 X MANIPENTA	-2.79	5.69**	-10.45**	29.13**
ICIAR-19BT X SAMNUT 14	3.52	11.55**	-13.20**	3.43
ICIAR-19BT X KWANKWASO	1.91	-6.17**	-10.31**	9.46**
ICIAR-19BT X MANIPENTA	13.25**	1.43	9.46	4.05
SAMNUT 14 X KWANKWASO	11.79*	13.94**	7.99**	1.82
SAMNUT 14 X MANIPENTA	6.54*	3.30	8.45	0.81
KWANKWASO X MANIPENTA	8.40	5.10	14.00**	9.61
SE±	3.21	1.68	3.19	4.03

*and ** significant at $P < 0.05$ and $P < 0.01$ levels of probability respectively

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5.4 Selection for superior genotypes for resistance to groundnut rosette disease

Based on the estimates predicted for the selection index of Mulamba and Mock (1978) using as economic weights that includes pod and sound kernel weight per plant which are vital for groundnut improvement and with lower DI and AUDPC (measures of diseased parameter) are presented in Table 5.15. Based on the RSI, ICGX – SM 00020/5/9 X ICGV IS 07890 (RSI = 27), ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1 (RSI = 28) and ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P_4/P_1 (RSI = 34), the best three F_2 segregates. Among the parents ICGV IS 07890 (RSI = 13), ICGX – SM 00020/5/P_4/P_1 (RSI = 27) and ICGX – SM 00017/5/P_{10}/P_1 ((RSI = 36) were the best genotypes to deploy for development GRD-resistant.

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Table 5. 15: The top 10 and 4 poorest performing F_2 genotypes selected based on Rank summation Index of SKWT, PWT, DI and AUDPC

Genotypes	Response Status	RANKS				RSI
		SKWT	PWT	DI	AUDPC	
Top 10 F_2 progenies						
ICGX – SM 00020/5/9 X ICGV IS 07890	R x R	9	10	4	4	27
ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1	R x R	6	3	7	12	28
ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P_4/P_1	R x R	3	5	11	15	34
ICGX – SM 00020/5/9 X ICGV IS 07899	R x R	5	4	14	13	36
ICGX – SM 00017/5/P10/P1 X ICGV IS 07890	R x R	15	13	3	14	45
ICGX – SM 00020/5/9 X ICIAR-19BT	R x R	7	7	12	27	53
ICGV IS 07899 X ICIAR-19BT	R x R	16	14	19	10	59
ICGX – SM 00017/5/P10/P1 X ICIAR-19BT	R x R	21	16	6	18	61
ICGV IS 07890 X ICGV IS 07899	R x R	13	11	18	20	62
ICGV IS 07890 X ICIAR-19BT	R x R	1	1	30	31	63
Bottom 4						
SAMNUT 14 X MANIPENTA	S x S	25	30	43	41	139
ICGV IS 07890 X KWANKWASO	R x S	36	34	41	30	141
SAMNUT 14 X KWANKWASO	S x S	45	45	42	22	154
KWANKWASO X MANIPENTA	S x S	40	40	44	43	167
Parents						
ICGV IS 07890	R	2	2	2	7	13
ICGX – SM 00020/5/P_4/P_1	R	4	6	9	8	27
ICGX – SM 00017/5/P_{10}/P_1	R	5	4	14	13	36
ICGX – SM 00020/5/9	R	23	38	1	6	68
ICGV IS 07899	R	20	20	22	11	73
ICIAR-19BT	R	30	25	17	5	77
SAMNUT 14	S	37	33	40	3	113
MANIPENTA	S	44	44	31	19	138
KWANKWASO	S	45	45	42	22	154

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5.5 Discussion

Significant ($P < 0.05$) genetic variation and few transgressive segregates within the experimental population were identified for sound kernel yield and resistance to rosette disease. These data indicate that selection with significantly ($P < 0.05$) superior performance is possible within this population. Some resistant progenies from crosses between aphid and rosette resistant lines could be used in integrated management of groundnut rosette disease. These breeding lines are already in good agronomic background and can be used directly for commercial production following multi-locational evaluations and release. Out of the 36 progenies generated from aphid and rosette resistant sources, the best F_2 segregates were selected based on RSI of Mulamba and Mock (1978) and were also found to show field resistance across the two locations. The use of the area under disease progress curve (AUDPC) as a measure of disease severity and as a tool for plant resistance evaluation helps to describe disease progress throughout the whole growing season (Campbell and Madden, 1990). In this study, the highest AUDPC values were for breeding line with the highest disease infection. There were differences in the AUDPC values between breeding lines within location and between locations. The differences observed between AUDPC values of the breeding lines within location suggest differences in resistance of individual lines. On the other hand, the difference observed between locations could be explained by the differences in the environmental conditions. Although AUDPC and DI seem to be a better index for the whole disease progress, it would be wise to consider these traits together during the breeding progress. The 10 best crosses were competitive in both pod and sound kernel yield, DI and AUDPC as determined by RSI. This implies that selection based on several traits is necessary. As an index, AUDPC, not only provides adequate information on rate of GRD spread

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but also allows the comparison among genotypes in an accurate way, especially when compared to methods proposed in the literature. Moreover, this simple screening procedure can be utilized to integrate applied and basic groundnut research to increase knowledge (Yang *et al.,* 2010) while developing cultivars with a trait that has been very difficult to measure, rosette disease of groundnut. This tool can also be applied in both early- and late-maturing groundnut breeding programs for selecting desirable genotypes in target environments. It also provides a good phenotyping tool for genetic studies at the molecular and physiological levels (Simko and Piepho, 2012). With the AUDPC approach, more precise phenotyping can be possible allowing pathologist, geneticists and breeders to work together to accurately identify loci and genotypes with rosette disease properties and measure a low heritable trait that was often thought as difficult to measure.

The performance based on the degree of reaction to groundnut rosette disease of 9 parents and 36 F_1s differed significantly. Some of the genotypes showed good field resistance to GRD accompanied with reasonable sound kernel yield per plant. For example, among the parents ICGV IS 07890 (43.46 g) and ICGX – SM 00017/5/P_{10}/P_1 (38.40 g) were the most tolerant to GRD while among the crosses ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1 (38.72 g) and ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P_4/P_1 (38.06 g) were the most GRD tolerant genotypes. Most of the promising genotypes with regards to sound kernel yield and AUDPC were from a cross that involved at least one aphid or rosette resistant parent. The high levels of field resistance to GRD in these genotypes suggest that they could also be selected indirectly for aphid resistance because both *Aphid craccivora* and the presence of virus are required concurrently in the expression of GRD. This is agreement with the reports by Naidu *et al.* (1999)

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that both aphids and the virus responsible for the expression of GRD intricately depend on each other and play a crucial role in the biology and perpetuation of GRD disease. Because of the low frequency of GRD resistant plants with high yield per plant, early generation selection as part of pedigree selection program would be inefficient, hence inter-mating of GRD resistant progenies or retaining high yielding but susceptible progenies in bulk from which resistance forms could be selected in advance generation in GRD improvement program. However, studies have shown that high yield potential and high degree of resistance do not generally go together (Nigam *et al.,* 1990) while the breeding programs target them together. Therefore, in breeding for GRD resistant variety a balance has to be struck between the yield potential and level of resistance to avoid any possible yield drawback. As a consequence, several genotypes with high yield potential and moderate levels of GRD resistance were identified in this study

The significant variances due to genotypes x location interactions observed for aphid damage index and AUDPC demonstrate the inconsistent performances of the parents and the F_2 populations in the two locations, which was a result of the differences in the weather conditions. Similar findings were reported by Adamu *et al.* (2008), Bentur *et al.* (2004), Senapathi *et al.* (2004) and Heriprasanna *et al.* (2008), who concluded that identification of suitable genotypes having maximum G x E interaction with moderate levels of field resistance or susceptibility to disease would be needed to improve the production of groundnut. Mothilal *et al.* (2010) further reported significant linear component of G x E interaction for kernel yield and concluded that genotypes differed for their linear response to fluctuating environments. In a related study, Molken and Stuefer (2011) showed that climatic conditions have a strong influence on the severity of disease.

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Knowledge of magnitude of association between characters is useful in making simultaneous selection for more than one character. For improvement of groundnut rosette disease resistance and sound kernel yield, it is necessary to know the magnitude and direction of relationships among resistance parameters, sound kernel yield and some other important traits as this aids selection. In this study, yield is drastically reduced by GRD parameter (AUDPC) ($r = -0.2624$, $p > 0.05$). The significant reductions in sound kernel yield of groundnut due to GRD incidence which is in agreement Adamu *et al.* (2001) who reported reduction in pod and haulm yield due to rosette infection. Generally, negative correlations were obtained between sound kernel yield per plant and the AUDPC and DI suggesting that selecting for reduced levels of AUDPC, which is an index of GRD response, will improve sound kernel yield per plant in these genotypes. The significant negative correlation between AUDPC and plant height clearly suggest that excessive AUDPC could reduce groundnut height, which is common symptom of groundnut plant attacked with GRD, i.e. stunted growth, twisting and bushy nature are symptoms depicted by groundnut attacked by GRD virus (Olorunju *et al.,*2001).

In the present study, the values of narrow sense heritability for DI and AUDPC were low and high broad sense heritability. It follows that resistance to GRD was much more heritable in broad sense than in narrow sense and that the greater portion of heritable variation is of non-additive in nature. Therefore, the low Baker's ratio value and the relative importance of broad sense heritability relative to narrow sense heritability emphasized the preponderant role of non-additive gene action in controlling GRD. Broad-sense heritability provides information of genetic variation but does not provide indication for the progress expected from selection. Considering low heritability estimates and the presence of non-additive gene effects could hinder the progress

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of selection indicated by low genetic gain. Therefore, selection of superior genotypes in the early generation will be ineffective. Selection in more advanced generations based on progeny performance when homozygosity is fixed will be more effective.

Both general combining and specific combining abilities made a significant and important contribution to progeny variation for both DI and AUDPC. All the parents of the most disease-resistant crosses; ICGX – SM 00017/5/P_{10}/P_1, ICIAR-19BT, ICGX – SM 00020/5/9 and ICGX – SM 00020/5/P_4/P_1 had appreciable *per se* resistance and also favourable GCA resistance values. This suggests that, although resistance to GRD tends to be at least partly dominant (Clements *et al.,* 2004), optimal resistance in progenies will require crossing parental genotypes that both carry resistance, which support the finding of Loffler *et al.* (2011) and Hung and Holland (2012). Therefore, selection for resistance should not be confined to a single group but should be performed in parallel in all groups. The study did not observe any cross between two susceptible genotypes that resulted in progenies with good resistance GRD. The study however, observed that heterosis is critical for GRD resistance. In contrast, Loffler *et al.* (2011) observed that hybrids often had more disease than their parental inbreds, perhaps because they used quite susceptible tester lines and higher inoculum pressure for hybrids than parents. SCA was most important for determining progeny resistance, but GCA was also important for disease resistance. Significant SCA detected in 13 of 36 possible combinations indicate the presence of non-additive gene effect. Significant SCA effect were observed for the combinations ICGX – SM 00017/5/P_{10}/P_1 X ICIAR-19BT (aphid resistant//rosette resistant), ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P_4/P_1 (aphid resistant//aphid resistant), ICGX – SM 00020/5/P_4/P_1 X SAMNUT 14 (aphid resistant//rosette susceptible) and ICGV IS 07899 X KWANKWASO

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((aphid resistant//rosette susceptible). These results indicate resistance of these progenies was higher than would be expected from average of their expected parents based on AUDPC symptom rating. The largest positive SCA effects correspond to ICGX – SM 00020/5/P4/P_1 X MANIPENTA. This combination was more susceptible than predicted average parent performance indicating the importance of non-additive gene effect in this particular cross. Kenga *et al.* (2004) suggest that the difficulty in predicting the resistance level of the hybrid, on the basis of GCA alone should necessitate testing of specific male-female combinations. The SCA values provide important information about the performance of the hybrid relative to its parents. Arunga *et al*. (2010) found that the SCA effect alone has limited value for parental choice in breeding programs. They, therefore, suggested that the SCA effects should be used in combination with other parameters, such as hybrid means and the GCA of the respective parents such that a hybrid combination with both high mean and favourable SCA estimates and involving at least one of the parents with high GCA, would tend to increase the concentration of favourable alleles; which is desired by any breeder. Furthermore, it was observed that crosses involving one good combiner and one average or poor combiner showed negative SCA effects. For example, MANIPENTA and KWANKWASO had poor GCA values for GRD resistance, while their crosses with ICGV IS 07899 and ICGX – SM 00020/5/9, respectively, and had significant and desirable SCA effects. This is in agreement with Hannan *et al*. (2007) who observed that some parents exhibited a similar phenomenon in studies on tomato (*Lycopersicum esculentum* Mill.) while Habarurema *et al.* (2012) made similar conclusion in study on bacterial blight ((*Xanthomonas oryzae* pv.*oryzae*) in rice

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The combining ability ratio, also known as Baker's ratio, for resistance to GRD observed in this study was less than unity. According to Baker (1978), when combining ability ratio approaches unity, GCA alone cannot predict the performance of the parents. Thus, the GCA scores could not be used to predict the performance of the parents in the present study, because the value of Baker's ratio is much lower than the theoretical maximum of unity. Low Baker's ratio observed for AUDPC in this study highlighted the importance of SCA variance, and hence the importance of dominance and/or epistatic gene effects for increasing resistance to GRD. This implies that selection at latter generations would be done much more based on better hybrid combinations rather than the performance of the parents involved in crossing programs. Partitioning G x L into variance to GCA x L and SCA x L interaction effects indicate significant variances of both GCA x L and SCA x L effects. The significant of GCA x L variance implied that GRD symptom rating (AUDPC) was sensitive to environmental conditions and data from additional environments or seasons would make GCA effect more precise.

5.6 Conclusions and Recommendations

The nature of genetic variation for aphid damage index and AUDPC and its relationships with sound kernel yield are important for planning successful breeding program for GRD resistance. The breeding lines identified from this study as being resistant to GRD could complement already existing GRD resistant lines and could serve as new sources of resistance to GRD for IAR groundnut breeding unit. Significant G x E interaction and low narrow sense heritability estimates of GRD resistance and Non-additive gene effects confound the selection of superior genotypes, and there is a need to develop alternative selection strategies. Genetic relationships

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between GRD resistance traits and agronomic traits, especially sound kernel yield, have been established in this study. There is a high possibility to simultaneously improve pod yield and GRD resistance in groundnut populations evaluated in this study. Agronomic traits such as pod and sound kernel yield were closely related with low GRD infection. Therefore, they could be used as indirect selection tools for resistance to groundnut rosette disease Superior GRD resistant genotypes could only be identified at later generation when homozygosity is achieved. However, some few transgressive F_2 segregates with field resistance to GRD and appreciable sound kernel yield based on their performance and RSI across the two locations. ICGX – SM 00020/5/9 X ICGV IS 07890, ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P10/P1 and ICGX – SM 00020/5/9 X ICGX – SM 00020/5/P4/P1 with higher sound kernel yield and most field resistance to GRD pathogen were identified and could be used for commercial production in Northern ecological zones of Nigeria. ICGX – SM 00017/5/P_{10}/P_1 , ICIAR-19BT, ICGX – SM 00020/5/9 and ICGX – SM 00020/5/P_4/P_1 found to have good GCA for reduced GRD resistance should be deploy for groundnut breeding programme to improve the level resistance to the disease. The study recommends about evaluations at four locations that are clearly different in terms of altitude, vegetation, temperatures, soil types, rainfall to select for yield and GRD resistance. The use of GGBiplot tools could be used to select promising GRD resistant genotypes that are stable in a range of environments and also to identify environments that effectively discriminate genotypes based on GRD parameters.

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CHAPTER SIX

6 MOLECULAR CONFIRMATION OF ROSETTE RESISTANCE IN PROMISING GROUNDNUT GENOTYPES BY ONE-STEP REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT – PCR)

6.1 Introduction

Efforts in breeding for host–plant resistance with appreciable yield and superior seed quality have contributed to the development of several groundnut genotypes with acceptable levels of field resistance to rosette disease (Subrahmanyam *et al.,* 1998; Olorunju *et al.,* 2001; van der Merwe and Subrahmanyam, 2001). Efficient and accurate diagnosis is a key to mitigating the consequences associated with aphid transmission of viruses in groundnut. Currently, the most common diagnostic method adopted by groundnut breeders focuses largely on the monitoring of viral-like symptoms on plants. Other methods include determining the biological (mechanical or vector transmission, host-range test ⁄ infectivity assay, and in vitro features) and serological (Gillaspie *et al.,* 2007) properties of the viruses. Frequently, several of these methods are used in combination for greater effectiveness, thereby further intensifying existing efforts and resources devoted to this aspect of groundnut production (Naidu and Hughes, 2003). Thus, it is not surprising that despite the importance of GRD viruses and their crop-loss potential if left unchecked and accompanied with favourable conditions may lead to epidemics. Only limited attempts have been made to accurately screen field resistant genotypes with a more powerful diagnostic tool as RT-PCR.

In earlier breeding programmes for GRD, resistance was assessed by lack of symptom expression and therefore was largely due to GRV and sat RNA resistance (Bock *et al.,* 1990;

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Subrahmanyam *et al.,* 1998; Olorunju *et al.,* 2001) and this resistance to GRV resulted in indirect resistance to sat RNA and did not show symptoms. Although such groundnut rosette disease-resistant materials did not develop rosette disease symptoms in the field due to the absence of GRV and sat RNA, yield reduction in such plants was observed under both artificial and natural disease pressure presumably due to their susceptibility to groundnut rosette assistor virus (GRAV) (Subrahmanyam *et al.,* 1998; Olorunju *et al.,* 2001).

Several methods have been used for GRD virus detection in groundnut plants. GRAV were detected in plants and aphids by the triple-antibody sandwich form of enzyme linked immunosorbent assay (TAS-ELISA) using monoclonal antibodies (MAbs) raised to potato leaf-roll virus (Naidu *et al.,* 1998; Rajeshwari *et al.,* 1987; Scott *et al.,* 1996). The GRV RNA and sat RNA can be detected in plants by nucleic acid hybridization (Blok *et al.,* 1994). Due to their cross reactions with different luteoviruses, a panel of MAbs have to be used in TAS-ELISA to verify that a luteovirus detected in groundnut or in aphids is indeed GRAV (Scott *et al.,* 1996). Moreover, TAS-ELISA cannot provide information on whether or not the aphids carry particles containing GRAV-RNA, GRV-RNA or sat-RNA. Additionally, low concentrations of the rosette disease agents in vector aphids and host plant make it essential to develop a reliable and sensitive method for their detection. Studies have shown that viruses can be detected in individual groundnut plants and aphid using RT-PCR (Canning *et al.,* 1996; Singh *et al.,* 1996; Olmos *et al.,* 1997; Stevens *et al.,* 1997, Naidu *et al.,* 1998). The use of RT – PCR employed in a study could ensure that, GRD field resistance genotypes are free of contamination from any of GRAV, GRV and sat-RNA viruses. GRD field resistance may be symptomless (latent) and presence of any of the agents of GRD disease in the field resistant sources could prevent its use as new

sources of resistance for GRD breeding program. The rapid and accurate confirmation of the new sources of resistance is an essential prerequisite for the development of a breeding strategy towards controlling this disease. The objective of this study was to confirm the resistance status of identified promising GRD-resistant parents and F_2 progenies of groundnut genotypes by RT – PCR.

6.2 Materials and methods

6.2.1 Collection of plant samples

Two to three seeds of 14 GRD field resistant groundnut genotypes with appreciable sound kernel yield, along with susceptible checks MANIPENTA and KWANKWASO were planted in the greenhouse. They were further challenged with *veruliferous* aphids collected from heavily infested groundnut plant maintained on SAMNUT14 (susceptible control) and monitored for two weeks at IAR screen house. Leaf samples were collected randomly from each plant for each genotype labelled separately and tested at IAR Biotechnology Laboratory by RT-PCR to being free from any of GRAV, GRV and sat RNA.

6.2.2 RNA Extraction and Purification

Extraction was carried out using GeneJET Plant RNA Purification Mini kit #K0801# (Thermo Scientific). For each sample, about 200 mg of young leaves were ground into fine powder under liquid nitrogen with mortar and pestle. It was quickly transferred into RNase DNase free 1.5ml microcentrifuge tubes containing 500ul of plant RNA Lysis solution and vortexed for 20s, incubated for 3 min at 56^0C and centrifuged for 5 min at 14,000 rpm. The supernatant (550 µl) was transferred into a clean microcentrifuge tube and mixed with 250 µl of 96 % ethanol. The

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prepared mixture was then transferred into a purification column inserted in a collection tube, and the column was centrifuge for 1min at 11,000 rpm, and the flow through solution was discarded while the column and the collection tube was reassembled. 700 µl Wash buffer (WB1) was added to the column and centrifuged for 1min at 11,000 rpm, the flow through and collection tube was discarded and the purification column was placed into a clean 2ml collection tube. 500µl Wash Buffer (WB2) was added to the purification column and centrifuged for 1min at 11,000 rpm, the flow through was discarded while the column and collection tube was reassembled. Washing with WB2 was repeated but with centrifugation at 14,00rpm. The flow through in a collection tube was discarded. The purification column was inserted into RNase free 1.5ml tube. The RNA was eluted by adding 50µl nucleases free water to the center of purification column and centrifuged for 1min at 11,000rpm. This was repeated once to a total of two washes. The purification column was discarded and the RNA was stored at -20^{0}C for further downstream analysis.

6.2.3 Complementary DNA (cDNA) synthesis and Polymerase Chain Reaction (PCR)

Primers for specific amplification of nucleic acid sequences for each of the three agents of rosette disease were presented in the Table 6.1. RT-PCR reactions were set up separately for GRAV, GRV and sat RNA. cDNA was obtained by using M-MuLV Reverse Transcriptase #EP0352# (ThermoScientific). Total RNA as a template used in RT-PCR reaction was set up separately for GRAV, GRV and Sat-RNA in two different stage protocols: First Strand cDNA synthesis and PCR Reaction. 5 µl RNA Template was added into sterile nuclease free tube on Arctic Ice PCR PL* Temp Sensitive. It was followed by adding 20pmol primer (forward and

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reverse), the volume was made up to 11.5 µl DEPC-treated water. Subsequently, 4 µl 5X-reaction buffer, 0.5 µl nuclease free water, 2 µl (1mM final concentration) dNTP mix 25mM each and 2ul (40 µl) M-MuLV Reverse Transcriptase were added to a total volume of 20ul. It was mixed gently and centrifuged briefly. The reaction was incubated at 37^0C for 1hr and terminated by heating at 70^0C for 10min.

First Strand cDNA was amplified in PCR machine (PTC 200. MJ Research), using gene specific primers. The PCR reaction mixture 50 µl consists of: 2 µl cDNA, 10 pmol Primer Forward and Reversed each, 25 µl DreamTaq Green PCR Master Mix (2X) and 21 µl nuclease free water. (ThermoScientific). PCR amplification was set to run 30 cycles of 90^0C for 45s, 550C for 1min, 72^0C for 1min followed by extension cycle of 72^0C for 1hr and held at 4^0C. The PCR product was run on 2% Agarose gel in 0.5X TBE buffer, stained with Ethidium bromide, which was later visualized under UV transilluminator, and the images were captured with the aid of digital camera.

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Table 6. 1: Primers used in amplification of various regions of causal agents of groundnut rosette disease complex primers in the 100 series represent internal primers for specified regions

Primers	Sequence	Specific to	Source reference
HRP92	ATGAATACGGTCGTGGTTAGG	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*2000; Wangai *et al.,*2001
HRP93	TTTGGGGTTTTGGACTTGGC	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*2000; Wangai *et al.,*2001
HRP94	GGAAGCCGGCGAAAGCTACC	GRV ORF3P and 4P	Taliansky *et al.,* 1996
HRP95	GGCACCCAGTGAGGCTCGCC	GRV ORF3P and 4P	Taliansky *et al.,* 1996
HRP96	GGTTTCAATAGGAGAGTTGC	Sat-RNA	Naidu *et al.,* 1998; Deom *et al.,*2000; Scott *et al.,*1996
HRP97	AAATGCCTACTTTGGGCGTG	Sat-RNA	Naidu *et al.,* 1998; Deom *et al.,*2000; Scott *et al.,*1996
HRP110	GGAGGGTCTGGCGAAACATT	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*2000; Wangai *et al.,*2001
HRP111	CCCTTGTAAAGGAACCGGAAT	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*2000; Wangai *et al.,*2001
HRP104	CGAGGAGACCAAAGGGTGGT	GRV ORF 3P and 4P	Taliansky *et al.,* 1996; Wangai *et al.,*2001
HRP105	AGCTCCGACACAATAGCGAAG	GRV ORF 3P and 4P	Taliansky *et al.,* 1996; Wangai *et al.,*2001
HRP108	GAAAAGGTGAGGGGTGTGT	Sat-RNA	Naidu *et al.,* 1998; Deom *et al.,*2000; Wangai *et al.,*2001
HRP92	ATGAATACGGTCGTGGTTAGG	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*2000; Wangai *et al.,*2001
HRP109	TAGCTTGATTTCAAGCTCGC	Sat-RNA	Naidu *et al.,* 1998; Deom *et al.,*2000; Scott *et al.,*1996; Wangai *et al.,*2001

Source: Wangai *et al.,* 2001

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6.3 Results

Complementary DNA (cDNA) made from the total RNA preparations was subsequently used in PCR to amplify GRV and sat RNA-specific sequences using primers specific for GRAV and GRV, and sat-RNA specific primers respectively. A total of 16 groundnut genotypes comprising of 14 promising GRD resistance and 2 susceptible farmers' varieties (checks) (Table 6.1) were subjected to confirmatory test for the presence of the three agents of GRD; GRAV, GRV and sat-RNA. With primer GRAV-CP 400 nt fragment amplified 12 of the 16 genotype tested (Fig 6.1). For PCR fragment specific to GRV, primer also amplified products in 14 out of 16 genotypes. The sat-RNA on the other hand, amplified fragments in all the 16 genotypes, revealing the presence of sat-RNA in all the genotypes (Fig 6.1). ICIAR-19BT X MANIPENTA was the only genotype that is resistant to GRD as no amplification was observed with the two GRAV and GRV specific primers.

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Table 6. 2: Field resistance scored by DI and AUDPC and RT – PCR confirmation of GRD-resistance in some groundnut genotypes

Genotypes	SCORES				RT – PCR		
	DI	AUDPC	Yield	Field Status	GRAV	sat-RNA	GRV
ICGX – SM 00020/5/9	17.30	16.59	17.98	Resistance	+	+	–
ICGX – SM 00017/5/P_{10}/P_1	28.41	22.84	38.40	Resistance	+	+	+
ICGX – SM 00020/5/P_4/P_1	27.34	20.09	35.28	Resistance	+	+	+
ICGV IS 07890	18.51	17.28	43.46	Resistance	+	+	+
ICGV IS 07899	33.40	22.16	25.49	Resistance	+	+	+
ICIAR-19BT	29.49	34.16	23.74	Resistance	+	+	+
ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1	26.11	22.59	38.72	Resistance	+	+	+
ICGX – SM 00020/5/9 X MANIPENTA	33.69	10.94	11.99	Susceptible	+	+	+
ICGX – SM 00020/5/9 X ICGV IS 07899	20.45	22.84	29.09	Resistance	+	+	+
ICGX – SM 00017/5/P_{10}/P_1 X ICGV IS 07899	35.77	29.84	31.78	Resistance	+	+	+
ICGV IS 07899 X SAMNUT 14	41.28	31.50	22.74	Susceptible	+	+	+
ICIAR-19BT X MANIPENTA	43.21	26.83	20.39	Susceptible	+	–	–
ICIAR-19BT X SAMNUT 14	22.80	45.93	24.25	Susceptible	+	+	+
ICGV IS 07890 X MANIPENTA	46.05	41.35	31.36	Susceptible	+	+	+
MANIPENTA	40.12	34.59	11.03	Susceptible	–	+	+
KWANKWASO	50.02	31.09	9.41	Susceptible	+	+	+

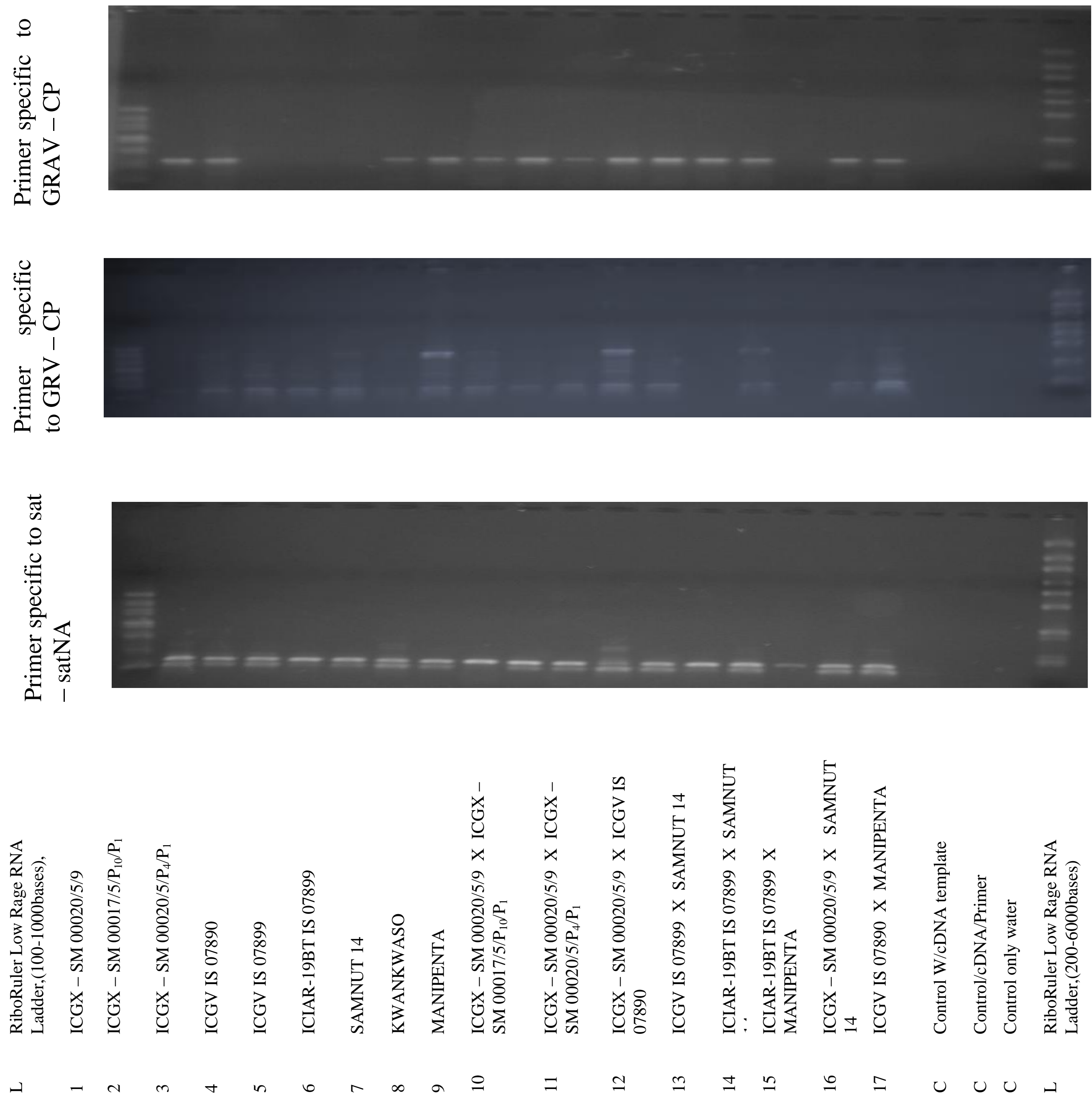

Figure 6. 1: Amplification banding pattern of GRAV – CP (HRP92/93), GRV – CP (OFR3P and 4P) and Sat-RNA markers in 16 groundnut genotypes

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6.4 Discussion

GRD has been a major viral disease of groundnut for decades. Sources of resistance have been previously detected by ELISA. However, as a phloem limited virus, it is present in low concentration hence required highly sensitive methods for its detection. Nucleic-acid based detection methods are recognized as being very powerful, rapid, specific and highly sensitive techniques for plant virus detection (James *et al.* 2006; Boonham *et al.* 2008; Olmos *et al.* 2008; Vincelli and Tisserat 2008). The use of RT-PCR in plant virus detection is well established, although studies on detection of *Potyviridae* are rare. Even the few studies that have investigated the detection of *Potyviridae* in plants with low luteovirus titre have relied on the use of species-specific-primers (Naidu *et al.,* 1998; Gibbs *et al.,* 2003; Gibbs *et al.,* 2008). RT-PCR detection of GRAV, GRV and sat-RNA was possible in all plant samples included in this study. The use of RT-PCR used in this study has facilitated the confirmation of resistance status of identified field GRD resistant groundnut genotypes and subsequent identification of different agents of GRD viruses. The molecular approach employed in this study, whilst not new to modern plant virus disease diagnostics, is unique to the investigation of GRD viruses associated with groundnut germplasm. As such, the findings and experimental methods will be substantially informative, from a phytosanitary perspective, to repositories around the groundnut regional bank which collect, maintain and distribute groundnut germplasm.

Some of the more conspicuous symptoms such as chlorosis, severe stunting and mosaic are advantageous from a disease- management standpoint because they increase the likelihood that groundnut plants with such symptoms will be identified and rogued out early in the growing season of production. This in turn significantly reduces the chances of subsequent virus

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dissemination by insect vectors (Hao *et al.,* 2003). However, it should be noted that although plants with symptoms were found to be infected with viruses in most cases, some infected GRD field resistant plants remained symptomless and did not induce any. These plants tested positive for at least one of the viruses (GRV, GRAV and sat-RNA) only after being screened by RT-PCR. Thus, as with other virus–plant interaction systems, some combinations of viruses and groundnut plants can give rise to asymptomatic or possibly latent infection (Odedara *et al.,* 2007; Salem *et al.,* 2010). Because these infected plants are not easily noticeable and can serve as virus reservoirs in the field, they are of serious concern in terms of biosecurity and virus disease control. These observations also confirmed the notion that symptomatology alone cannot be taken as a reliable basis for resistance to plant virus diseases; especially since symptom induction can be complicated by the occurrence of mixed virus infection, as demonstrated by this study. This is in agreement with Gillaspie (2006, 2007) and Salem *et al.* (2010), who observed that the use of symptom visualization in field plots improved screening for GRD-resistance in groundnut plants, but failed to improve the speed or accuracy of screening for GRD-resistance, because the GRD symptoms were apparently too mild in many groundnut genotypes for accurate diagnosis. It is important to note that GRD-induced symptoms can also occur in plants co-infected with satellite RNAs (Simon *et al.,* 2004; Salem *et al.,*2010), as observed for GRD-infected plants showing mild or no GRD symptoms observed in the present study.

The molecular diagnosis in this study clearly demonstrated that at least one of the three agents of GRD viruses, GRV, GRAV and sat-RNA, were present in all field resistant genotypes identified in this study. This is consistent with other studies reporting the occurrence of these viruses in groundnut (Naidu *et al.,* 1998) and Salem *et al.* (2010) for CABMV and CMV in cowpea as well

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as those describing the occurrence of GRV, GRAV and sat-RNA in groundnut germplasm maintained at IAR.

Since none of the genotypes tested has demonstrated resistance to all the three components based on RT – PCR assay and because GRAV is the main component involved in aphid transmission, genotypes such as ICGX-SM-000/20/5/P_4/P_1, ICIAR -19-BT and ICGV 07899 that showed a negative response to GRAV, could be exploited in resistance breeding programmes to restrict the spread of groundnut rosette disease. The GRV resistant genotypes ICG-IS-07899 X SAMNUT14 and ICIAR -19-BT X MANIPENTA could be used as sources of resistance to GRV and for commercial production under favourable conditions. ICG-IS-07899 X SAMNUT14 and ICIAR -19-BT X MANIPENTA are susceptible to the aphid vector but resistant to GRV and ICGX-SM-000/20/5/P_4/P_1, ICIAR -19-BT and ICGV 07899 are susceptible to rosette disease agents but resistant to feeding by the aphid vector, the two groups of genotypes offer a good source for molecular gene pyramiding.

6.5 Conclusions and Recommendations

The findings of the present study demonstrated the occurrence of most GRD viruses causing severe damage in groundnut in Northern Nigeria. Based on RT-PCR diagnostic test, it is further concluded that GRD appeared as a major devastating virus in the leading groundnut growing regions of Nigeria. This study has also shown the sensitivity of the use of RT-PCR to facilitate reliable diagnosis of viruses, and is particularly useful in cases where suspected viral symptoms are mild or absent. Although reverse transcription followed by PCR can be used to detect the presence of GRD in groundnut, the procedure provides little information regarding the relationship between the three agents of GRD. The intensity and appearance of GRD symptoms

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varies among genotypes, as does the efficiency of transmission by aphid vectors. Therefore, information on the relationship of these three agents of GRD is required to establish its distribution and epidemiology. It is evident from this study that the use of RT-PCR has successfully confirmed resistant status groundnut breeding lines with differential levels GRD resistance. Indeed, this study has allowed identification of at least one breeding line with combined resistance to GRAV and GRV. Further studies with multiplex RT-PCR, a method for detecting simultaneous infections by more than one viroid species in the same host is suggested. This could lead to a new era in the understanding of some of the unknowns in the often mysterious nature of this infection, and perhaps to better ways of disease management. The current work is the first attempt at using molecular approaches to confirm resistance status of groundnut genotypes at IAR in Nigeria.

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CHAPTER SEVEN

7 GENERAL DISCUSSION

7.1 Participatory rural appraisal (PRA)

A participatory rural appraisal conducted indicated that farmers recognized the groundnut production constraints that did not differ significantly between the two districts from different ecologies. Farmers in both Batsari and Nasarawa-eggon were aware of groundnut rosette disease which they called by various local names to be an important constraint to groundnut productivity in their locations. Management strategies for this disease ranged from doing nothing by the majority of farmers to the use of resistant varieties by a few farmers. Another constraint pointed out by farmers across locations was unavailability of improved seed. This implies that the breeding emphasis should focus on development of host resistant genotypes as the most important management strategy for GRD as most farmers across the studied areas were resource-poor who cannot afford the use of chemical control measures. The development of resistant varieties should involve a multidisciplinary team of breeders, pathologists, socio-economists, agronomist, soil scientists and farmers as earlier stipulated by several researchers (Witcombe *et al.,* 2005; Gyawali *et al.,* 2007; Muhinyuza *et al.,* 2012). They emphasized the need for active farmer participation in plant breeding as critical for successful adoption of improved varieties and their production. However, the link between research and farmers is still very weak or absent in the studied locations and even in most developing countries (Ortiz *et al.,* 2001). Failure of including farmers could result in low rate of adoption of improved varieties and persistent use of locally available GRD susceptible varieties. This probably has been the cause of low yield and sporadic occurrence of GRD disease in both areas. Efforts should therefore be concentrated on

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the breeding for high yielding GRD resistant varieties and their promotion. The study found that, community involvement in a breeding program for GRD was completely lacking. Few farmers were involved in participatory variety selection (PVS) i.e. the mother and baby trials of Tropical Legume II project leaving behind bulk of other stakeholders who played a great role in variety selection for sale and supply. Farmers have some special preferences that breeders normally do not select for in their programs. These preferences are part of the reasons farmers do not easily adopt improved cultivars even if they have important traits like resistance to diseases. These preferences are very diverse posing a big challenge to the breeders. However, with farmers' participation in the process of varietal development, breeders can appreciate and comprehend those preferences and include them in their selection. Since farmers have indicated their willingness to participate in varietal selection, this can be a means of ensuring early adoption of the cultivars selected by farmers themselves.

7.1.1 Epidemiology of GRD from the studied genotypes

The strict dependence on the presence of the agents of GRD for occurrence of the disease in farmers field probably explains why all the 9 parents and the field resistant progenies screened by RT – PCR were found to carry at least one of the three agents of GRD. The ability to detect each of the three agents of rosette disease in GRD field resistance genotypes is crucial in gaining an understanding of the ecology and epidemiology of the disease and to identify sources of resistance to the individual agents of the disease in groundnut. Like other persistently transmitted viruses, GRAV reported to be retained by the vector for periods of up to 14 days and possibly for life (Misari *et al.,* 1988, Waliyar *et al.*,, 2007). With these transmission characteristics, spatial spread of a persistently-transmitted rosette can be expected to be much more general and

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widespread than the more localized distribution of diseases caused by non-persistently or semi-persistently transmitted viruses (Duffus, 1973). Rosette symptoms may appear following the establishment of GRV and its sat-RNA in the absence of GRAV, but such plants will not serve as sources of inoculum for subsequent spread by aphids. Thus, long acquisition access feeding is essential to acquire GRAV, but the ability of the aphid vector to inoculate all three agents may depend on the inoculation period. The study indicated that some of the susceptible varieties e.g. KWANKWASO with typical chlorotic rosette symptoms did not contain GRAV, whereas some apparently symptomless field resistant genotypes contained GRAV (assayed by RT-PCR), this observation was also reported by Naidu and Kimmins (2007) with plant inoculated by single v*iruliferous* aphids under laboratory conditions. The reason for this observation was explained based on the transmission efficiency of the three agents of GRD which increases with an increase in inoculation access period (IAP).

The above observations show clearly that, from some F_2 lines containing both GRV and GRAV inoculated by aphids become infected with GRV and sat-RNA only. In the symptomless F_2 groundnut genotypes with particles of GRAV coat protein alone and available for acquisition by aphids may not be transmitted in sufficient quantity to cause infection hence, genotypes infected with GRAV alone are virtually symptomless as observed in this study. A separation of GRAV from GRV infective agents could result in either aphids acquiring or transmitting only one kind of particle from the susceptible plant or from inoculating a sufficient dose of only one kind of particle to the recipient plant. Further, Naidu *et al.* (2007) reported that GRV and its sat-RNA may not always occur in the same tissue together with GRAV which explain the transmissions of GRAV alone. Aphids normally inoculate phloem-limited viruses immediately after sieve element penetration through a brief period of salivation in the sieve elements (Prado and Tjallingii, 1994),

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which is followed by phloem ingestion. On a susceptible host, ingestion may be sustained and uninterrupted for several hours. In this situation, a single aphid may not release enough virus particles containing GRAV RNA into the plant to initiate GRAV replication. However, sufficient particles containing GRV RNA and its sat RNA may have been deposited in mesophyll tissue during exploratory probes to establish these agents (Ntare and Olorunju, 2001). In the light of this GRD epidemiology, ICGX – SM 00020/5/9, ICGX – SM 00017/5/P_{10}/P_1, ICGX – SM 00020/5/P_4/P_1, ICGV IS 07890, ICGV IS 07899 and ICIAR-19BT with remarkably higher field resistance to GRD (symptomless) would be recommended for use as breeding sources of resistance to GRD and could be recommended for commercial production. It is, however, suggested that the segregates are advanced to F_6 generations to ascertain their true status before multi-location testing and recommendation for release and for commercial production.

7.1.2 Performance of the genotypes across the two contrasting locations

The study demonstrated that GRD infections substantially shifted the ranking of genotypes with respect to relative fitness (in terms of pod weight and sound kernel weight) between the two locations. Based on this finding and on general predictions from evolutionary theory, it is suggested that GRD might have played an important yet unrecognized role in the long-term maintenance of genotypic diversity in groundnut through variable selection and G × E interactions. For pathogen-caused G × E interactions to occur, Weijschede´ *et al.* (2006) opined that infections should significantly affect plant performance and fitness. In this study, GRD compromised sound kernel weight, retarded vegetative propagation and curtailed the spatial expansion capabilities of infected as compared with field resistant groundnut genotypes. These

findings are in accordance with other studies reporting negative effects of virus infection on plant performance (Molken and Steufer, 2011).

The effects of GRD infection on the genotypes showed conspicuous levels of genotypic variation for most development and growth-related traits recorded in this study. Consequently, the genotypes which performed best in the one location did not occupy high ranks in the other location, and vice versa. This suggests that GRD infections cause significant alterations in genotype frequencies within groundnut breeding lines used. The observed G × E interactions indicated genotypic dissimilarities in sensitivity of groundnut to GRD infection, which may be caused by variation in virulence levels of the three agents of GRD. Mitchell-Olds (1992) postulated three conditions for the maintenance of genotypic variation through G × E interactions as it affect fitness. The first condition demands genotypic variation in fitness which is clearly demonstrated by this study that showed strong genotypic variation in closely fitness-related traits such as *per se* performance for pod and sound kernel weight, which are the most important economic traits in groundnut. These results are consistent with other studies showing genotypic variation for many fitness-associated traits in *T. repens* (Turkington, 1989; Weijschede´ *et al.,* 2006). The second condition requires genotype fitness to vary between environments, again in this study, the performance of genotypes differed greatly between the two locations studied, resulting in a marked shift in the ranking of genotypes between these environments. A similar conclusion was reported by Pagan *et al.* (2008) who showed genetic variations between different accessions for disease reaction across environments. Such negative frequency-dependent selection occurs when common genotypes as compared with less common genotypes suffer from a fitness disadvantage in virus-prone environments (Brunet and Mundt, 2000; Rueffler *et al.,* 2006). The apparent negative effects of GRD on plant performance, significant G × E interaction

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for GRD and evident repercussions for relative fitness reported in this study clearly stressed the significance of GRD infections for ecological and evolutionary processes and identified virus agents of this disease as possible key factors for driving population dynamics and selection gain.

7.1.3 Inheritance of resistance to groundnut rosette disease

The significant and preponderance of SCA for GRD parameters and low narrow sense heritability estimates denotes the importance of non-additive gene action in governing the expression GRD resistance in groundnut. This implies selection of the superior genotypes will be possible only at later generations to allow fixation of maximum homozygosity. Genotypes, ICGX – SM 00020/5/9, ICGX – SM 00017/5/P_{10}/$P_1$and ICGV IS 07890 with significant positive GCA effects for sound kernel yield accompanied desirable general combining ability for reducing the GRD severity in groundnut serve as good sources of resistance for GRD breeding . Segregates; ICGX – SM 00017/5/P_{10}/P_1 X ICIAR-19BT, ICGX – SM 00020/5/P_4/P_1 X SAMNUT, ICGV IS 07899 X SAMNUT14 and ICGX – SM 00017/5/P_{10}/P_1 X ICGV IS 07890 could be recommended for utilization as sources of resistance and for commercial groundnut production, because they exhibit favourable SCA estimates for yield and GRD resistance.

Accurate identification and detection of the GRD free groundnut genotype is the first steps in successful management of the GRD disease. The complete sequence, similarity and phylogenetic relationships among the three agents of GRD had been completed. This information has helped in the confirmatory test of GRD resistant lines. This study has shown that GRD virus infection of groundnut genotypes is common and RT-PCR has facilitated reliable diagnosis of each agent of GRD, and was particularly useful in this study, where suspected viral symptoms were mild or absent. The confirmatory test showed none of the field resistance genotypes were free of all the

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three agents of GRD, confirming the earlier reports. The current work represents a first attempt at using these molecular approaches to confirm resistance status of field resistance GRD groundnut genotypes at the Institute for Agricultural Research, Samaru, Nigeria. This will pave way for quick and reliable identification of resistance sources for management of groundnut rosette disease in Nigeria.

7.2 Challenges

Conventional selection has been the method of selection over the years, and has produced tangible results and appreciable genetic gains. Many high-yielding GRD resistant groundnut cultivars have been developed and released to farmers in Nigeria. The success of conventional selection for GRD resistance depends on environmental factors due to unpredictable and sporadic occurrence of GRD. To enhance natural virus infection, additional resources are required for rearing veruliferous aphids that act as vector for GRD, and inoculating each plant or establishing infector rows in the nurseries. Besides, the disease might not be uniformly distributed; for natural virus enhancement through spreader rows, disease might not develop early in the season to allow effective screening. Moreover, such techniques are laborious and increase costs of field screening.

It is acknowledged that the number of loci screened in the current study is low, particularly for a tetraploid species with 20 linkage groups and hence a close linkage between markers/alleles and loci controlling disease resistance cannot not be expected. Moreover, trait data are limited and are from 2 locations in 1 year only and may introduce bias

Accurate selection is also complicated because development of symptoms depended on time of infection. In such a case, MAS would be ideal to facilitate selection as it does not rely on

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symptoms or field conditions. It is suggested that, when the laboratory facilities are established and the appropriate molecular markers are available, use of MAS in breeding for GRD resistance would be recommended.

7.3 Conclusions and recommendations

The genetic diversity analysis undertaken has presented a valuable opportunity to additionally identify loci putatively linked to GRD resistance. The SSR results of this study provide critical information to breeders for planning future breeding strategies. It will also enable plant breeders to make informed decisions about parental selection for developing mapping populations; This type of analysis also offers a mechanism for breeders to counteract further genetic impoverishment of the cultivated groundnut gene pool.

The two groups of new sources of resistance could offer a good source for molecular gene pyramiding for GRV and vector resistance genes into a single genotype to achieve broad-based genetic resistance for developing sustainable crop management strategies against groundnut rosette. From a practical plant breeding perspective, utilization of more than one form of resistance should be expected to reduce the frequency of appearance of virus strains able to infect groundnut varieties that are released to farmers for production over large acreages.

It is hoped that in the near future teams of breeders at IAR will have a much more sophisticated understanding of the mechanisms of virus replication and movement and of factors controlling host range. It is anticipated that the knowledge from this study will be utilized in developing viable strategies that can expand our repertoire of methods available to protect groundnut plants against GRD disease for the benefit of groundnut farmers in Africa and Nigeria in particular.

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APPENDIX 1: Participatory Rural Appraisals questionnaire

A. Household and Socio – Economic Characteristics

Name of respondent	……………………………………………	
Age of Household head	……………………………………………	
Sex of household head	1 = Male	2 = Female
District	……………………………………………	
Local government Area	……………………………………………	
State	……………………………………………	

1. Formal Education (highest level attain)
   i. Illiterate
   ii. Primary school
   iii. Secondary school – SS / JSS
   iv. Technical school
   v. Poly/Mono-technic/College of Education/University

2. Marital status
   i. Single
   ii. Married
   iii. Others (specify)

3. Size of production unit
   i. Total acreage…………………….. acres
   ii. Cultivated area…………………….. acres
   iii. Grazing area…………………….. acres
   iv. Area under fallow……………….. acres

4. Tenure of land (Give acreage)
   i. Communal…………………………………….. acres
   ii. Private……………………………………….. acres
   iii. Government / Institutional…………………….. acres
   iv. Family ……………………………………….. acres

5. How long have you been farming…………………….. (Years)

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6. Do you farm part – time or full time
   i. Part – time
   ii. Full – time

7. If you are not full time farmer, how much of your time do you devote to farming operation (Tick the appropriate)
   i. Less than half
   ii. Half
   iii. More than half

8. Is any member of your household involved in any off – farm activities? (i) Yes (ii) No. if yes please specify the activity (ies)
   i. Formally employed
   ii. Making basket, winnowers
   iii. Trading / business
   iv. Hiring out oxen / farm implements / labour
   v. Others (specify)

9. Is anyone else in the household who does not live there involved in any off – farm activities

Livestock Type	**Ownership (Male / Female)**
Cattle	…………………………………..
Goats	…………………………………..
Sheep	…………………………………..
Donkeys	…………………………………..
Chickens	…………………………………..
Pigs	…………………………………..
Turkeys	…………………………………..
Others (specify)	…………………………………..

10. Give 5 of the major crops that are usually grown in first (rainy) season, who grows it and the purpose of production

	Crop	Acreage	Gender	Purpose of production
1				

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2				
3				
4				
5				

11. How much of the crop listed above did you sell last year?

Crop	Quantity produced (bags/tins/basins/kg		Amount sold (bags/tins/basins/kg		Price (Bushy/kg	
	Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season

B. LABOUR

1. What family labour is available for production activities

Age group	Full time participating in farming activities		Part time participating in farming activities	
	Male	Female	Male	Female

2. Do you use hired labour? (i) Yes (ii) No

3. If yes, specify for which crops…………………………………………………………..

4. What kind of hired labour do you use per season (on average)

Rainy season

Type of hired labour	Number of males	Number of females
Casual		
Permanent		
Village labour exchange		

Dry season

Type of hired labour	Number of males	Number of females
Casual		
Permanent		

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Village labour exchange		

5. For which activities do you hired labour?

	Activity	Casual	Permanent	village
1	Land preparation			
2	Planting			
3	Weeding			
4	Harvesting			
5	On – farm transport			
6	Others (specify)			

C. Groundnut Production

1. Give constraints / problems affecting your groundnut production (Tick whichever is appropriate and mention the control strategy)

Constraints	**Tick as appropriate**	**Control strategy**
Diseases (Specify)		
Field pest (Specify)		
Shortage of land		
Shortage of labour		
Poor quality seed		
Storage pest		
Low output price		
Low/reduced soil fertility		
Others (Specify)		

2. Give acreage under improved and local groundnut variety

Groundnut varieties	**Acreage**
Improved varieties	
Local variety	

3. Which variety of groundnut do you grow?

	Variety	**Year 1st planted**	**Initial seed source**	**Current seed source**
1	SAMNUT21			
2	SAMNUT22			
3	SAMNUT23			
4	SAMNUT10			
5	Maiyado			
6	Others (specify)			

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7				

4. Why do you prefer these varieties?

	Variety	Reasons (use code below)
1	SAMNUT21	
2	SAMNUT22	
3	SAMNUT23	
4	SAMNUT10	
5	Maiyado	
6	Others (specify)	
7		

Codes: 1 = high yield; 2 = disease resistance; 3 = early maturity; 4 = good taste; 5 = colour 6 = drought tolerance; 7 = easy to pound; 8 = good storability; 9 = weed suppression; 10 = uniform maturity; 11 = field pest resistance; 12 = big seeded; 13 = ready market; 14 = fetches higher price

5. What don't you like about these varieties? (see code)

	Variety	Reasons (use code below)
1	SAMNUT21	
2	SAMNUT22	
3	SAMNUT23	
4	SAMNUT10	
5	Maiyado	
6	Others (specify)	
7		

Codes: 1 = poor yield; 2 = susceptible to groundnut rosette disease; 3 = late maturity; 4 = bitter taste; 5 = colour 6 = drought susceptibility; 7 = hard to pound; 8 = easily affected by storage pest; 9 = difficult to harvest; 10 = non- uniform maturity; 11 = small seeded; 12 = restricted market

6. Desirable characteristics of a good groundnut variety in order of preference.

	List characteristics in order of importance
1	
2	
3	
4	
5	

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7. Which variety would demand more labour, explain why?

	Variety	Reasons
1	SAMNUT21	
2	SAMNUT22	
3	SAMNUT23	
4	SAMNUT10	
5	Maiyado	
6	Others (specify)	
7		

8. Which colour of groundnut do you prefer, give reason (s) for your preference.
   i. Red
   ii. Light tan
   iii. No preference
   iv. Others (specify)

9. Groundnut production systems

Activity	Months	Who performs?		
		Men	Women	children
Field selection				
Bush clearing				
Ploughing				
Planting				
Weeding				
Harvesting				
Transportation				
Drying				
Shelling				
Sorting				
Marketing				
Others (specify)				

D. Perception of groundnut rosette disease (use photo)

1. Do you know groundnut rosette disease? (i) Yes (ii) No
   i. What name do you call this disease?
   ii. What do you think causes this disease
   iii. In your own view how is this disease transmitted?
   iv. How do you try to control the disease?

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2. What is the loss in yield do to groundnut rosette disease?
   i. Low (less than 20 %)
   ii. Moderate (21 – 40 %)
   iii. High (over 50 %)
   iv. Total loss (100 %)

3. Do you know any variety (s), which is not affected by groundnut rosette disease? (i) Yes (ii) No
   i. ---
   ii. ---
   iii. ---
   iv. ---

4. In you view what is the trend of occurrence of this disease over the years?
   i. Increasing
   ii. Same
   iii. Decreasing

5. Do you plant groundnut in lines/rows? (i) Yes / (ii) No (specify spacing)

6. Do you grow groundnut in pure stand?

   i. Pure stand / sole
   ii. Mixed / intercropped

7. How many times do you weed groundnut?
   i. Once
   ii. Twice
   iii. Thrice

8. At what stage do you weed the groundnut?

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9. What purchased input do you use in production of groundnut?

Input	Purchased, borrow or hired	Approximate cost

10. How easy is it for you to obtain the relevant inputs for production? (use codes below)

Type of input	Input availability			
Seed				
Hoes				
Fertilizers				
Herbicide				
Insecticide				
Fungicides				
Others (specify)				
Code: 1 = very easy; 2 = easy; 3 = not easy and 4 = others (specify)				

E. Use, Marketing and Decision Making

1	What are your uses of groundnut?	
2	What proportion do you sell?	
3	What proportion do you retain for seed?	
4	If sold where you do sell?	
5	When do you sell?	
6	Sold sell or unshelled?	
7	Do you sell at once?	
8	Do you store any of these groundnut	

Who make the following decisions?

	Decision	Who makes?
1	How much to plant	
2	How much seed to retain	
3	How much to eat	
4	How much to sell	
5	When to sell	
6	Where to sell	

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F. Institutions

1. Is any member of a household a member of any group / association? Yes/No. if yes, specify the kind of group (Name the group

Extension contact group	Farmer association	Others (specify)

2. What are the major functions of the group/association?
   i. When did you become a member of the group /association? (give year)
   ii. Why do you become a member of the group (any benefit)?
   iii. Does the group/association address agricultural issues? Yes / No
   iv. If yes, enumerate the agricultural issues address

3. What are the major sources of information about agricultural activities?
   i. Government extension staff
   ii. NGO (specify)
   iii. Radio
   iv. Neigbour
   v. School
   vi. Parents
   vii. Training workshop
   viii. On – farm research / demonstration
   ix. Exchange visit/ field tours
   x. Visiting researcher
   xi. Newspaper/Newsletter/pamphlet
   xii. Others (specify)

4. Do you have a radio in your house? Yes / No

5. Do you listen to agricultural educational programs? Yes / No if yes, (Name the program)

6. If yes, is the coverage of the programs satisfactory? Yes / No

7. Did extension agents visit you last year? Yes / No

8. If yes, what time of the year or during which operation?

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i. Ploughing-------------------------- 1, Number of visit ---------------------------
ii. Planting----------------------------- 2, Number of visit ---------------------------
iii. Weeding --------------------------- 3, Number of visit ---------------------------
iv. Harvesting-------------------------- 4, Number of visit ---------------------------

9. Have you ever attended field day or demonstration trials? Yes / No

10. Have you ever attended a farmers training course? Yes / No

11. Please give any comment/suggestions relating to agriculture and groundnut production in particular.

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